Spread of Carbapenem-Resistant *Klebsiella pneumoniae* Clinical Isolates Producing NDM-Type Metallo-β-Lactamase in Myanmar

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ABSTRACT

A total of 38 isolates of carbapenem-resistant *Klebsiella pneumoniae* harboring *bla*NDM were obtained during surveillance of 10 hospitals in Myanmar. Of these 38 isolates, 19 (50%) harbored genes encoding 16S rRNA methylases, such as *armA* or *rmtB*. The *K. pneumoniae* strains tested belonged to 17 sequence types (STs), including the high-risk clonal lineages ST101 and ST147. The ST101 and ST147 isolates carried IncFII plasmids harboring *bla*NDM-5 and IncFIB(pQil) plasmids harboring *bla*NDM-1, respectively. These results indicate that IncFII plasmids harboring *bla*NDM-5 and IncFIB(pQil) plasmids harboring *bla*NDM-1 have been spreading in *K. pneumoniae* ST101 and ST147 isolates, respectively, in Myanmar.

IMPORTANCE

The emergence of carbapenem-resistant *K. pneumoniae* has become a serious problem in medical settings worldwide. The present study demonstrated that carbapenem-resistant *K. pneumoniae* strains have been spreading in medical settings in Myanmar. In particular, plasmid genes encoding NDMs and 16S rRNA methylases have been spreading in *K. pneumoniae* high-risk clones.

KEYWORDS
carbapenemase-producing Enterobacteriaceae, *Klebsiella pneumoniae*, NDM-type metallo-β-lactamase, 16S rRNA methylase
RESULTS

Clinical features of carbapenem-resistant *K. pneumoniae* complex isolates. The whole genomes of 46 isolates of the *K. pneumoniae* complex were sequenced using MiSeq. Average nucleotide identity (ANI) and Type (Strain) Genome Sever (TYGS) analyses revealed that 38 were *K. pneumoniae* subsp. *pneumoniae*, 7 were *K. quasipneumoniae* subsp. *similipneumoniae*, and 1 was *K. quasipneumoniae* subsp. *quasipneumoniae*. The 46 carbapenem-resistant *K. pneumoniae* complex strains were isolated from clinical samples obtained from patients hospitalized at 10 hospitals in Myanmar from December 2015 to September 2017. Of the 46 isolates, 30 were from six hospitals in the Yangon region, 14 were from three hospitals in the Mandalay region, and 2 were from one hospital in Kachin State (see Fig. S1 in the supplemental material). The susceptibilities of these isolates to various antibiotics were tested by the microdilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (5). All 46 isolates were resistant to aztreonam (AZT), ceftazidime (CAZ), meropenem (MEM), and tigecycline (TGC); 43 (94%) were resistant to imipenem (IPM); 39 (85%) were resistant to ciprofloxacin (CIP); 27 (59%) were resistant to amikacin (AMK); and 1 (2%) was resistant to colistin (CST) (Table 1).

Drug resistance genes of carbapenem-resistant *K. pneumoniae* complex isolates. All 38 isolates of *K. pneumoniae* subsp. *pneumoniae* harbored *bla*NDM genes, with 22 harboring *blaNDM-1*, 1 harboring *blaNDM-4*, 11 harboring *blaNDM-5*, and 4 harboring *blaNDM-7*, as well as *blaCTX-M* genes, including *blaCTX-M-15* or *blaCTX-M-14*. Nineteen (50%) isolates also harbored genes encoding 16S rRNA methylases, including *armA* or *rmtB*, making them highly resistant to aminoglycosides. Thirty-three isolates (87%) harbored *aac(6′)-Ib-cr*, which is the most common plasmid-mediated quinolone resistance gene (6). The 26 quinolone-resistant isolates (68%) with MICs of $\geq 1 \text{ mg/mL}$ had point mutations at quinolone resistance-determining regions, including GyrA and ParC (Table 2). A summary of the characteristics of the 8 carbapenem-resistant *Klebsiella* species, including *K. quasipneumoniae* subsp. *similipneumoniae* and *K. quasipneumoniae* subsp. *quasipneumoniae*, is shown in Table S1. Of them, 6 isolates harbored *blaNDM-1*, *blaCTX-M-15*, and *armA*, and 2 harbored *blaNDM-7* and *blaCTX-M-15*.

MLST and phylogenetic analyses of carbapenem-resistant *K. pneumoniae* complex isolates. Multilocus sequence typing (MLST) analysis revealed that 10 isolates (26%) belonged to sequence type 147 (ST147); 11 (29%) belonged to ST101; 2 each (5%) belonged to ST16, ST17, and ST4029; and 1 each (3%) belonged to ST15, ST36, ST42, ST273, ST394, ST401, ST420, ST534, ST1655, ST4030, and ST5912. A phylogenetic tree revealed three clades, designated clades A, B, and C (Fig. 1). Clade A consisted of isolates belonging to ST15, ST36, ST42, ST101, ST401, ST420, ST1655, and ST4029 and the *K. pneumoniae* reference strain; clade B consisted of isolates belonging to ST16, ST17, ST534, ST4030, and ST5912; and clade C consisted of isolates belonging to ST147, ST273, and ST394. The high-risk clonal lineages ST101 and ST147 belonged to clades A and C, respectively. The other high-risk clonal lineage, ST15, of one isolate

### Table 1: Drug susceptibility profiles of carbapenem-resistant *K. pneumoniae* complex isolates in 10 hospitals in Myanmar (n = 46)

| Antibiotic       | Breakpoint for resistance (µg/mL) | % resistant isolates | MIC (µg/mL) | Range | MIC<sub>50</sub> | MIC<sub>90</sub> |
|------------------|-----------------------------------|----------------------|-------------|-------|-----------------|-----------------|
| Amikacin         | ≥64                               | 50                   | 1 to >1,024 | >1,024 | >1,024          |                 |
| Aztreonam        | ≥16                               | 100                  | 16 to >1,024 | 256   | 512             |                 |
| Ceftazidime      | ≥16                               | 100                  | 512 to >1,024 | >1,024 | >1,024          |                 |
| Ciprofloxacin    | ≥1                                | 92                   | 0.25 to >1,024 | 64    | 256             |                 |
| Colistin         | ≥4                                | 3                    | 0.0625 to 4 | 0.25  | 2               |                 |
| Imipenem         | ≥4                                | 92                   | 0.5 to 256  | 8     | 64              |                 |
| Meropenem        | ≥4                                | 100                  | 4 to 128    | 32    | 128             |                 |
| Tigecycline      | ≥0.5                              | 100                  | 0.5 to 4    | 1     | 2               |                 |

*The breakpoint for tigecycline was determined according to EUCAST guidelines.*
| MLST type | No. of isolates | Hospital(s) | Carbapenemase genes(s) | Extended-spectrum β-lactamase-encoding gene(s) | 16S rRNA methylase gene(s) | Aminoglycoside acetyltransferase-encoding gene(s) | Mutation(s) in DNA gyrase |
|-----------|----------------|-------------|-----------------------|-----------------------------------------------|-------------------------|-----------------------------------------------|-------------------------|
| ST15      | 1              | A           | blaNDM-1              | blaCTX-M-15, blaSHV-106                      | armA                    | aac(6')-Ib-cr, aac(3')-Id, aadA2, aadA16     | S83F, D87A             |
| ST16      | 2              | B (1/2), F (1/2) | blaNDM-1, blaNDM-1 (1/2) | blaCTX-M-15, blaSHV-26, blaSHV-79, blaSHV-98 | armA, rmtB               | aac(6')-Ib-cr, aadA2, aac(6')-Ib3 (1/2), aac(3')-Id, aadA16 (1/2) | S83F, D87N, E84K      |
| ST17      | 2              | G           | blaNDM-1              | blaCTX-M-14                                   | —                       | aac(3')-Id, aac(6')-Ib-cr, aac(6')-Ib3        | —                      |
| ST36      | 1              | J           | blaNDM-1              | blaCTX-M-15, blaSHV-1-14, blaSHV-70          | armA                    | aac(3')-Id, aadA16                            | —                      |
| ST42      | 1              | A           | blaNDM-4              | blaCTX-M-15, blaSHV-26, blaSHV-79, blaSHV-98 | —                       | aac(3')-Id, aac(6')-Ib-cr, aadA16             | S83L, D87Y, S80I       |
| ST101     | 11             | A (8/11), E (2/11), I (1/11) | blaNDM-1, blaNDM-5 (3/11), blaNDM-5 (8/11) | blaCTX-M-15 | mrtB (1/10)                                   | aac(3')-Id, aac(6')-Ib-cr, aadA16, aph(3')-I, aph(6')-Id | S83I (9/10), S83Y (1/10), D87A (1/10) |
| ST147     | 10             | A (8/10), C (1/10), G (1/10) | blaNDM-1, blaNDM-3 (9/10), blaNDM-3 (1/10) | blaCTX-M-15, blaSHV-11 | —                                   | aac(3')-Id, aac(6')-Ib-cr, aadA16, aph(3')-I, aph(6')-Id | S83I             |
| ST273     | 1              | H           | blaNDM-7              | blaCTX-M-15, blaSHV-11                        | —                       | aac(3')-Id, aac(6')-Ib-cr, aadA16, aph(3')-I, aph(6')-Id | S83I             |
| ST394     | 1              | C           | blaNDM-5              | blaCTX-M-15                                   | mrtB                    | aadA2, aadA16                                | —                      |
| ST401     | 1              | J           | blaNDM-1              | blaCTX-M-15                                   | armA                    | aac(6')-Ib-cr, aadA16                         | —                      |
| ST420     | 1              | E           | blaNDM-1              | blaCTX-M-15, blaSHV-75                        | armA                    | aac(3')-Id, aadA16                            | —                      |
| ST534     | 1              | A           | blaNDM-1              | blaCTX-M-15, blaSHV-26, blaSHV-79, blaSHV-98 | —                       | aac(3')-Id, aac(6')-Ib-cr, aadA16             | S83I, S80I             |
| ST1655    | 1              | G           | blaNDM-1              | blaCTX-M-15, blaSHV-26, blaSHV-79, blaSHV-98 | armA                    | aac(3')-Id, aac(6')-Ib-cr, aadA16             | —                      |
| ST4039    | 2              | G           | blaNDM-1              | blaCTX-M-15, blaSHV-26, blaSHV-79, blaSHV-98 | —                       | aac(3')-Id, aac(6')-Ib-cr, aadA16             | —                      |
| ST4030    | 1              | G           | blaNDM-1              | blaCTX-M-15, blaSHV-1-87                      | —                       | aac(3')-Id, aac(6')-Ib-cr, aadA16             | S83I, S80I             |
| ST5912    | 1              | D           | blaNDM-1              | blaCTX-M-15, blaSHV-26, blaSHV-79, blaSHV-98 | —                       | aac(6')-Ib-cr, aadA16                         | S83F, D87N, E84K       |

Numbers in parenthesis indicate that the number of isolates with resistant genes or amino acid mutations per each ST isolate. No parenthesis indicate that all each ST isolate had resistant genes or amino acid mutations.

*— means that the isolate has no mutation, i.e. S83S.
belonged to clade A (7). MLST showed that carbapenem-resistant *K. quasipneumoniae* subsp. *similipneumoniae* belonged to ST705, ST1473, ST3590, and ST5967, and *K. quasipneumoniae* subsp. *quasipneumoniae* belonged to ST3866 (Table S1).

As shown in Fig. 1, isolates of the high-risk clonal lineage ST101 in clade A were from hospitals A, E, and I, whereas isolates of the high-risk clonal lineage ST147 in clade C were from hospitals A, C, and G. It is difficult to reveal the relationship between the other STs and hospitals.

**FIG 1** Phylogenetic tree of 38 carbapenem-resistant *K. pneumoniae* complex isolates obtained from clinical samples at 10 hospitals in Myanmar. The tree was constructed by the maximum likelihood method based on core-genome SNPs.
Eight isolates belonging to ST101 from hospital A had numbers of single nucleotide polymorphisms (SNPs) ranging from 68 to 126, two ST4029 isolates from hospital G had 68 SNPs, two ST17 isolates from hospital G had 95 SNPs, and five ST147 isolates from hospital A had numbers of SNPs ranging from 93 to 29,684. Of the five ST147 isolates, three isolates (MyNCGM201, MyNCGM225, and MyNCGM528) had numbers of SNPs ranging from 90 to 93.

**Plasmids carrying bla_{NDM}** All bla_{NDM} genes, including bla_{NDM-1}, bla_{NDM-4}, bla_{NDM-5}, and bla_{NDM-7}, were located on plasmids ranging in size from 45,321 bp to 176,315 bp. These plasmids belonged to eight types of plasmid incompatibility complexes, including IncC (5 isolates), IncFII (6 isolates), IncFIB(pQil) (9 isolates), IncFIB(pQil)/IncFII/K (3 isolates), IncFII(K)/IncFII/IncFII(pKP91) (1 isolate), IncM2 (4 isolates), IncR (1 isolate), and IncX3 (5 isolates) (Table 3). The remaining four plasmids did not belong to any Inc type.

These plasmids belonged to eight types of plasmid incompatibility complexes, including

- IncC-type, IncFIB(pQil)/IncFII(K), IncM2, and IncR-type plasmids; bla_{NDM-1} was located on IncC plasmids; bla_{NDM-4} was located on IncX3 plasmids; bla_{NDM-5} was located on IncFII and IncFIB(K)/IncFII/IncFII(pKP91) plasmids; and bla_{NDM-7} was located on IncX3 plasmids (Table 3).

Of the 38 plasmids carrying bla_{NDM}, 15 (39%) harbored genes encoding both NDMs and 16S rRNA methylases. These 15 plasmids included 7 that harbored armA on IncC- or IncM2-type plasmids and 8 that harbored rmtB on IncFII-, IncFIB(K)/IncFII/IncFII(pKP91)-, or IncR-type plasmids (Table 3).

The IncFIB(pQil)-type plasmids carrying bla_{NDM}, were detected in isolates from three regions in Myanmar, Kachin, Mandalay, and Yangon, whereas the IncC-type, IncFIB(pQil)/IncFII/IncFII(pKP91), IncM2, and IncR-type plasmids; bla_{NDM-4} was located on IncX3 plasmids; bla_{NDM-5} was located on IncFII and IncFIB(K)/IncFII/IncFII(pKP91) plasmids; and bla_{NDM-7} was located on IncX3 plasmids (Table 3).

**Genetic environments surrounding bla_{NDM} and 16S rRNA methylases.** Assessment of the genomic environments surrounding bla_{NDM} revealed 10 types of genetic structures, including bla_{NDM-1} (Fig. 2A to E), bla_{NDM-4} (Fig. 2F), bla_{NDM-5} (Fig. 2G to I), and bla_{NDM-7} (Fig. 2F).

The genetic structure surrounding bla_{NDM-1} could be divided into five types (Fig. 2A to E). The structure of type A was rmtB-bla_{TEM-1}-tnpA-rstA-AplII-bla_{NDM-1}-ble_{MBL}-trpF-dsbC-trpA. The structure tnpA-AplII-bla_{NDM-1}-ble_{MBL}-trpF-dsbC-trpA was identical to those of plasmids in other types of *Enterobacteriaceae*, including *Escherichia coli* pC06114.1 (GenBank accession no. CP016035) detected in 2015 in Germany and *K. pneumoniae* pM941-NDM5 (GenBank accession no. AP023454) detected in 2018 in Myanmar. The structure of types B and C was tnpA-rstA-bla_{NDM-1}-ble_{MBL}-trpF-dsbC-cutA-trpA, which was identical to the structures of plasmids of *K. pneumoniae* AATZP (GenBank accession no. CP014757) detected in 2014 in the United States; *K. pneumoniae* K66-45 (GenBank accession no. CP020902) detected in 2010 in Norway; and *K. pneumoniae* C435, C069, and C070 (GenBank accession no. LC521845, LC613144, and LC521839, respectively) detected in Thailand in 2016. The structure of type D was orf-oftnA-bla_{NDM-1}-ble_{MBL}-bla_{DHA-1}-gcvA-hybF, which was identical to the structures of plasmids of *E. coli* Es_ST2350_SE1 (GenBank accession no. CP031322), first detected in 2018 in the United Kingdom, and *K. pneumoniae* 33476891 (GenBank accession no. CP071086), first detected in 2020 in Switzerland. The structure of type E was aac(6’)-Ib3-qacE-oftnA-bla_{NDM-1}-ble_{MBL}-orf-oftgcvA, which was identical to the structure of a plasmid in *E. coli* Carbenemenase (NDM-1)-IncA/C2 (GenBank accession no. CP050162), first detected in 2012 in Hong Kong.

The genetic structure surrounding bla_{NDM-4} was tnpA-isdH6-bla_{NDM-4}-ble_{MBL}-trpF-dsbC-orf1-AplII-bla_{NDM-1}-ble_{MBL}-trpF-dsbC-orf1AplII (Fig. 2F), which was identical to those of plasmids of *E. coli* M2-16 (GenBank accession no. AP018146) in 2015 in Myanmar and *E. coli* TUM18530 (GenBank accession no. AP023454) in 2018 in Japan.

Three genetic structures were observed to surround bla_{NDM-5} (Fig. 2G to I). The structure of type G was dfrA12-qacE3-sul1-tnpA-orf-bla_{NDM-5}-ble_{MBL}-trpF-dsbC-trpA, which was identical to those of plasmids of *E. coli* isolated from 2013 to 2019 in China, Malawi, Myanmar, South Korea, Thailand, and the United States. The structure of type H was intT2-tnpA-orf-bla_{NDM-5}-ble_{MBL}-trpF-dsbC-trpA, which was identical to that of a
| Inc type                      | No. of isolates | Hospital(s) | MLST type(s)                  | Plasmid size (bp) | Carbapenemase- and ESBL-encoding gene(s) | Aminoglycoside resistance gene(s) |
|------------------------------|-----------------|-------------|-------------------------------|-------------------|-----------------------------------------|-----------------------------------|
| IncC                         | 5               | A (1/5), B (1/5), G (3/5) | ST15 (1/5), ST16 (1/5), ST17 (2/5), ST1655 (1/5) | 158,959–176,315   | bla NDm-1                                | armA (3/5), aac(6’)-lb-cr, aac(6’)-lb3 (3/5), aadA2 (1/5) |
| IncFl                        | 6               | A (5/6), C (1/6) | ST101 (4/6), ST147 (1/6), ST394 (1/6) | 94,549–94,603     | bla NDm-5                                | mtb, aadA2                        |
| IncFIB(pQil)                 | 9               | A (7/9), C (1/9), G (1/9) | ST147 | 51,716–87,316 | bla NDm-5, bla CT X M-15 |                                |
| IncFIB(pQil)/IncFII(K)       | 3               | G (2/3), I (1/3) | ST101 (1/3), ST4029 (2/3) | 119,263–126,228   | bla NDm-5, bla CT X M-15 |                                |
| IncFII(K)/IncFII(IncFII(KP91)| 1               | A | ST101 | 199,295 | bla NDm-5, bla CT X M-15 |                                |
| IncM2                        | 4               | E (1/4), I (1/4), J (2/4) | ST36 (1/4), ST101 (1/4), ST401 (1/4), ST420 (1/4) | 80,663–80,798     | bla NDm-1                                |                                |
| IncR                         | 1               | E | ST101 | 67,399 | bla NDm-1                                | mtb, aac(6’)-lb-cr, aadA16            |
| IncX3                        | 5               | A (2/5), D (1/5), H (1/5), G (1/5) | ST42 (1/5), ST273 (1/5), ST534 (1/5), ST4030 (1/5), ST5912 (1/5) | 45,321–46,161     | bla NDm-4 (1/5), bla NDm-3 (4/5) |                                |
| —                            | 4               | A (3/4), F (1/4) | ST16 (1/4), ST101 (3/4) | 10,494–122,000    | bla NDm-5                                | aadA2 (3/4), aac(6’)-lb-cr (1/4), aadA16 (1/4) |

*Numbers in parenthesis indicate that the number of isolates with resistant genes or amino acid mutations per each ST isolate. No parenthesis indicate that all each ST isolate had resistant genes or amino acid mutations.

*— means that the isolate has no mutation, i.e. 583S.
plasmid of *Enterobacter hormaechei*, p388, isolated in 2017 in the United States (GenBank accession no. CP021168). The structure of type I was *bla*<sub>TEM-1</sub>-rmtB1-nhaA-groEL-tnpA-ISApII-bla<sub>NDM-3</sub>-ble<sub>MBL</sub>-trpF-dsbC-tnpA, which was identical to those of *E. coli* plasmids pM214_FII and pM105_mF (GenBank accession no. AP018144 and AP018137, respectively), isolated in 2015 in Myanmar.

The genetic structure surrounding *bla*<sub>NDM-7</sub> (Fig. 2J), tnpA-isnH6-bla<sub>NDM-7</sub>-ble<sub>MBL</sub>-trpF-dsbC-orf-tnpA, was similar to that surrounding *bla*<sub>NDM-4</sub>, with the latter being identical to those of plasmids of *E. coli* M2-16 (GenBank accession no. AP018146) in 2015 in Myanmar and *E. coli* TUM18530 (GenBank accession no. AP023194) in 2018 in Japan.

The structures of the genomic environments surrounding *armA* and *rmtB* are shown in Fig. 3. The structure surrounding *armA* of type A was detected in four of seven isolates and was identical to those in *E. coli* isolated from 2003 to 2018 in China, Hong Kong, India, Norway, and Poland (GenBank accession no. CP072463, HQ451074, CP030858, CP020902, and CP058363, respectively). The structure surrounding *armA* of type B was detected in three of seven isolates and was identical to those in *K. pneumoniae* isolated in Oman, Japan, and South Africa (GenBank accession no. JX988621, AB759690, and CP023488, respectively). The structure surrounding *rmtB* of
type C was detected in 11 of 12 isolates and was identical to those in a strain of *K. pneumoniae* isolated in 2018 in the Czech Republic (GenBank accession no. CP050367) and strains of *E. coli* isolated from 2012 to 2019 in India, Italy, and Switzerland (GenBank accession no. CP033159, MN007141, and CP048368, respectively). The structure surrounding *rmtB* of type D was detected in 1 of the 12 isolates.

Of the 38 isolates, 7 harbored both *bla*NDM-1 and *armA* on the same plasmids, including four IncM2 and three IncC plasmids; 6 harbored both *bla*NDM-5 and *rmtB* on the same plasmids, including plasmid type IncFII or IncFIB(K)/IncFII/IncFII (pKP91); and 1 harbored both *bla*NDM-1 and *rmtB* on the same plasmid belonging to IncR.

The plasmid structures belonging to IncFIB(pQil), IncFII, IncX3, IncC, IncM2, and IncFIB(pQil)/IncFII(K) are compared in Fig. 4. Five of nine plasmids belonging to IncFIB (pQil), IncFII, IncX3, IncC, IncM2, and IncFIB(pQil)/IncFII(K) had structures identical to that of a plasmid in *K. pneumoniae* in 2015 in Myanmar (GenBank accession no. AP018834). All six plasmids belonging to IncFII had structures 92% identical to that of a plasmid in *E. coli* isolated in 2015 in Myanmar (GenBank accession no. AP018138). Six of seven plasmids belonging to IncX3 had structures 97% identical to that of a plasmid in *E. coli* isolated in 2015 in Myanmar (GenBank accession no. AP018141). On the other hand, *K. quasipneumoniae* subsp. *similipneumoniae* harbored IncX3, IncC, or IncM2 plasmids, and *K. quasipneumoniae* subsp. *quasipneumoniae* harbored an IncX3 plasmid (Fig. 4).

**DISCUSSION**

The present study suggests that *K. pneumoniae* ST101 isolates harboring *bla*NDM-5 on IncFII plasmids and ST147 isolates harboring *bla*NDM-1 on IncFIB(pQil) plasmids have spread in three regions in Myanmar in recent years. IncFII plasmids harboring *bla*NDM-5 in ST101 isolates and IncFIB(pQil) plasmids harboring *bla*NDM-1 in ST147 isolates seem to be horizontally spreading in hospital A. IncFII and IncFIB(pQil) plasmids harboring *bla*NDM were detected in *E. coli* ST354 and *K. pneumoniae* ST147 strains isolated in Myanmar (8), the United States (GenBank accession no. CP014757), Norway (GenBank accession no. CP020902), and Thailand (GenBank accession no. LC521839).

IncX3 plasmids harboring *bla*NDM*5* will be spreading among *Enterobacteriaceae*, including *Citrobacter* sp., *Enterobacter* sp., *E. coli*, and *K. pneumoniae* subspecies in Myanmar. In this study, we revealed that *K. pneumoniae* subsp. *pneumoniae*, *K. quasipneumoniae* subsp. *similipneumoniae*, and *K. quasipneumoniae* subsp. *quasipneumoniae* had IncX3 plasmids harboring *bla*NDM*5* or *bla*NDM*7* (Table 3; see also Table S1 in the supplemental material). Sugawara et al. reported that *Citrobacter amalonaticus*, *Citrobacter freundii*, *Enterobacter asburiae*, *Enterobacter xiangfangensis*, *E. coli*, *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae*, *Leclercia adecarboxylata*, and *Lelliottia nimipressuralis* harbored IncX3 plasmids harboring *bla*NDM*4*, *bla*NDM*5*, or *bla*NDM*7* in Myanmar (8). Another study showed that IncX3 plasmids harboring *bla*NDM*5* have spread in *K. pneumoniae* isolates in China (9).
FIG 4 Comparison of the plasmid sequences of IncFIB(pQil), IncFII, IncX3, IncC, IncM2, and IncFIB(pQil)/IncFII(K). The images were generated using BLAST Ring Image Generator software (https://sourceforge.net/projects/brig/files/BRIG-0.95-dist.zip/download). Plasmid sequences belonging to each Inc type were compared with plasmids of MyNCGM111 for IncFIB(pQil) (A), MyNCGM439 for IncFII (B), MyNCGM036 for IncX3 (C), MyNCGM076 for IncC (D), MyNCGM127 for IncM2 (E), and MyNCGM143 for IncFIB(pQil)/IncFII(K) (F).
Carbapenem-resistant Enterobacteriaceae are a significant public health concern in Myanmar (8). *K. pneumoniae* ST101 and ST147 isolates producing NDM-1 caused outbreaks in Spain (10), and ST101 isolates producing extended-spectrum β-lactamases (ESBLs) were also reported in Tanzania (11). Previous studies in Myanmar revealed that *K. pneumoniae* ST101 and ST147 strains were detected in samples from patients in medical settings and environments, including foodstuff in the Yangon region (12, 13). In addition to ST101 and ST147, other high-risk clones, ST11, ST15, ST14, and ST48, were reported in Myanmar (14), Bangladesh (7), Saudi Arabia (15), and China (16), respectively.

The results of SNP analysis of closely related isolates (Fig. 1) suggested that eight ST101 isolates from hospital A, two ST4029 isolates from hospital G, two ST17 isolates from hospital G, and three ST147 isolates from hospital A represented outbreaks.

Strains of Enterobacteriaceae containing plasmids carrying genes encoding NDMs and 16S rRNA methylases, making these bacteria resistant to carbapenems and aminoglycosides, will likely spread throughout medical settings in Myanmar. Isolates of the *Enterobacter cloacae* complex coproducing NDM-1/4 and ArmA/RmtC/RmtE have been detected in five regions in Myanmar (17), and other species of Enterobacteriaceae, including *E. coli* and Citrobacter freundii, resistant to carbapenems and aminoglycosides and producing NDM-1/4/5 and ArmA/RmtB/RmtC/RmtE have been detected in environments as well as medical settings in Yangon, Myanmar (13). These findings emphasize the need to monitor Enterobacteriaceae in Myanmar for the presence of plasmid-borne genes encoding carbapenemases and 16S rRNA methylases.

In conclusion, this is the first report describing the molecular epidemiology of carbapenem-resistant *K. pneumoniae* isolates in medical settings in three regions of Myanmar. The incidence of multidrug-resistant (MDR) *K. pneumoniae* clinical isolates in hospitals differed regionally, being 57.9% in the Yangon region (8) but 39.5% in the three regions included in the present study. Epidemiological surveillance is required to prevent the emergence and spread in Myanmar of MDR Enterobacteriaceae harboring genes encoding enzymes associated with drug resistance.

### MATERIALS AND METHODS

**Bacterial strains.** Forty-six clinical isolates of the carbapenemase-resistant *K. pneumoniae* complex, defined as strains showing resistance to imipenem or meropenem (MICs ≥ 4 μg/mL), were obtained between December 2015 and September 2017 from patients treated at 10 hospitals in Myanmar. Of these 46 isolates, 20, 1, 2, 1, 4, 1, 8, 5, 2, and 2 were from hospitals A through J, respectively. Bacteria were identified using the Vitek 2 system (bioMérieux, Marcy l’Etoile, France), with identities confirmed by sequencing of 16S rRNA. Of the 46 isolates, 22 were from blood, 10 were from tracheal aspirates and sputum, 7 were from pus and wounds, and 7 were from urine. As the situation in Myanmar has become increasingly uncertain in recent months, it is difficult to update the clinical information on the 46 *K. pneumoniae* isolates tested.

**Drug susceptibility testing.** Drug susceptibility was tested according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (5). The ranges of antibiotic concentrations tested were 0.5 to 1,024 μg/mL amikacin (AMK), 0.5 to 1,024 μg/mL aztreonam (AZT), 0.5 to 1,024 μg/mL ceftazidime (CAZ), 0.25 to 1,024 μg/mL ciprofloxacin (CIP), 0.0625 to 8 μg/mL colistin (CST), 0.5 to 1,024 μg/mL imipenem (IPM), 0.5 to 1,024 μg/mL meropenem (MEM), and 0.5 to 1,024 μg/mL tigecycline (TGC) (Table 1). The MICs of each antimicrobial agent were determined by broth microdilution methods using Mueller-Hinton broth and 96-well microtiter plates (Kohjin Bio Co., Ltd., Saitama, Japan).

**Whole-genome sequencing and genomic analysis.** Genomic DNAs of the 46 isolates were extracted using DNeasy blood and tissue kits (Qiagen, Tokyo, Japan) or 20-gauge genomic tips (Qiagen), and their complete genomes were sequenced using the MiSeq platform (Illumina, San Diego, CA) and MiONiN (Oxford Nanopore Technologies, Oxford, United Kingdom). Raw reads of each isolate were assembled using CLC Genomic Workbench version 10.0.1 (CLC Bio, Aarhus, Denmark). Species identities of these isolates were determined using an ANI calculator (18) or the Type (Strain) Genome Sever (TYGS) (https://tygs.dsmz.de). The sequences of drug resistance genes were determined using ResFinder 4.1, and plasmids were typed using Plasmid finder 2.1, both from the Center for Genomic Epidemiology (CGE) (https://www.genomic epidemiology.org/). The sequences of plasmids were annotated using the DDBJ Fast annotation and submission tool (https://dfast.ddbj.nig.ac.jp). Fluoroquinolone resistance has been associated with mutations in the quinolone resistance-determining region, which includes the *gyrA* and *parC* genes that encode DNA gyrase and topoisomerase IV, respectively. The *gyrA* and *parC* genes were detected in *silo* using CLC Genomics Workbench v.11.0.1 (CLC Bio, Denmark) (19). Comparative analysis of plasmid sequences surrounding *bla* genes was performed using BLAST and visualized using in silico molecular cloning (In Silico Biology Inc., Kanagawa, Japan). Imaging of plasmid similarity was performed using the BLAST Ring Image Generator (https://sourceforge.net/projects/brig/files/BRIG-0.95-dist.zip/download).
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