Novel Levofloxacin-Resistant Multidrug-Resistant *Streptococcus pneumoniae* Serotype 11A Isolates, South Korea

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Of 608 *Streptococcus pneumoniae* clinical strains isolated at a hospital in South Korea during 2009–2014, sixteen (2.6%) were identified as levofloxacin resistant. The predominant serotype was 11A (9 isolates). Two novel sequence types of multidrug-resistant *S. pneumoniae* with serotype 11A were identified, indicating continuous diversification of resistant strains.

*S. pneumoniae* is a common respiratory pathogen that is the leading cause of community-acquired pneumonia (1). Although β-lactam antibiotics have long been used for the treatment of respiratory diseases, the increasing prevalence of antibiotic-resistant *S. pneumoniae* strains has hampered treatment in recent decades (2,3). Resistance to fluoroquinolones has emerged in *S. pneumoniae* and is caused by mutations within short DNA sequences of *gyrA* and *parC* genes that encode the type II topoisomerase subunits known as quinolone-resistance determining regions (QRDRs) (1). Previous studies have shown that most of the *S. pneumoniae* strains with reduced susceptibility to the fluoroquinolone levofloxacin exhibit a multidrug-resistant (MDR) phenotype (2,4). Levofloxacin resistance was closely associated with epidemic MDR clones (3). Although fluoroquinolone resistance rates remain low in *S. pneumoniae* in most countries, some extensively drug-resistant (XDR) *S. pneumoniae* isolates have emerged; this resistance is defined as nonsusceptibility to ≥1 agent in all but ≤2 antimicrobial categories (2,4). We examined *S. pneumoniae* isolates from patients in South Korea to determine antimicrobial resistance. We found novel sequence types (STs) of MDR serotype 11A *S. pneumoniae* that exhibit resistance to second-line antibiotics such as levofloxacin, ceftriaxone, and meropenem.

The Study

During January 2009–December 2014, we isolated 608 *S. pneumoniae* clinical strains at a 698-bed, university-affiliated hospital in South Korea. We determined MICs by using the broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (5). We performed antimicrobial resistance tests for levofloxacin, ofloxacin, ciprofloxacin, penicillin, amoxicillin, ceftriaxone, meropenem, erythromycin, clindamycin, vancomycin, linezolid, tetracycline, and tigecycline. We used *S. pneumoniae* ATCC 49619 as a control strain. We defined MDR as resistance or intermediate resistance to ≥3 antimicrobial agents.

We determined serotypes by using the multiplex PCR assay recommended by the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/pcr.htm). Reactions also included an internal positive control targeting all known pneumococcal *cpsA* regions (6). We sequenced QRDRs of the *gyrA*, *gyrB*, *parC*, and *parE* genes in each isolate (7). We performed multilocus sequence typing to investigate the genetic backgrounds of fluoroquinolone-resistant pneumococci (8) and assigned allele numbers and STs by using the PubMLST database (http://pubmlst.org/spneumoniae).

Of the 608 clinical *S. pneumoniae* isolates, 16 (2.6%) were levofloxacin resistant (MIC ≥8 μg/mL). We collected 1 resistant isolate in 2009, 3 in 2012, 5 in 2013, and 7 in 2014. Thirteen isolates were from sputum, and 3 isolates were from bronchial lavage. The mean age of patients was 71 years; 14 were male, and 2 were female.

Serotype 11A (n = 9) was most common among the levofloxacin-resistant isolates, followed by serotypes 13 (n = 2), 19F (n = 2), 23F (n = 2), and 6B (n = 1) (Table 1). The most common STs were ST9875 (n = 5), ST8279 (n = 3), and ST9876 (n = 3), which together accounted for 11 of the 16 levofloxacin-resistant isolates. Nine isolates of ST9875, ST9876, and ST10300 were novel STs and had not been identified before this study.

All 16 levofloxacin-resistant isolates contained at least 2 amino acid alterations in the QRDRs of the *gyrA*, *parC*, and *parE* genes. Four QRDR mutations occurred with high frequency: Ser81Phe in *gyrA* was present in all 16 isolates; Ser79Phe and Lys137Asn in *parC* were present in 14 and 11 isolates, respectively; and Ile460Val in *parE* was found in 15 isolates. However, Lys137Asn in *parC* and Asp435Val and Ile460Val in *parE* are mutations not involved in resistance, according to previous reports (9,10). Isolate HM-854, which was penicillin susceptible, had Ser81Phe in *gyrA* and Asp79Phe in *parE* mutations.

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All isolates had ≥1 mutation in parC. The 2 isolates without the Ser79Phe mutation in parC instead carried AmpC83Gly or AmpC83Asn. The 4 isolates without the Lys137Asn mutation in parC instead carried the Asn91Asp mutation. Isolate HM-1017 (serotype 11A, ST-8279) had 7 QRDR mutations and exhibited the highest resistance against all antimicrobial agents, including levofloxacin (MIC 64 µg/mL). ST-8279 was associated with 2 different serotypes, 11A (n = 2) and 13 (n = 1). The 3 isolates of novel ST-9876 had the same QRDR amino acid changes but had different serotypes, 19F (n = 2) and 23F (n = 1).

The 16 levofloxacin-resistant isolates were also resistant to ofloxacin (MIC ≥8 µg/mL) and ciprofloxacin (MIC ≥8 µg/mL) (Table 2). All isolates except 3 had MICs ≥16 µg/mL against amoxicillin and ceftriaxone. Fourteen isolates were meropenem-resistant (MIC ≥1 µg/mL); all these isolates were susceptible to vancomycin and linezolid. Only 3 STs (ST-99, ST-189, and ST-3173) exhibited the lowest levofloxacin MIC (8 µg/mL); all these isolates were susceptible to amoxicillin (MIC ≤2 µg/mL).

Most of the 16 isolates in our study were of serotype 11A (n = 9): 5 isolates of ST-9875, 2 of ST-8279, and 1 each of ST-10300 and ST-99. An XDR ST-8279 (serotype 13) clone described in 2014 (2) was closely related to the 9 serotype 11A isolates in our study. ST-8279 is a double-locus (aroE and xpt) variant of ST-156, which is closely related to global clone Spain9V-3 (2). Spain9V-3 is related to 3 ST-3642 isolates (serotype 11A) reported in Taiwan in 2014.

Table 2. Antimicrobial susceptibilities of 16 levofloxacin-resistant *Streptococcus pneumoniae* clinical isolates identified from patients at a hospital in South Korea, 2009–2014*

| Strain | LEV | OFL | CIP | PEN | AMX | CRO | MER | ERY | CLI | VAN | LZD | TET | TIG |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| HM-646 | 16 (R) | 32 (R) | 32 | >16 (R) | >16 (R) | >16 (R) | 16 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-669 | 16 (R) | 32 (R) | 32 | >16 (R) | >16 (R) | >16 (R) | 8 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-683 | 8 (R) | 16 (R) | 16 | 4 (I) | 2 (S) | 2 (I) | 1 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-688 | 16 (R) | 32 (R) | 32 | >16 (R) | >16 (R) | >16 (R) | 8 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-705 | 8 (R) | 16 (R) | 8 | 2 (S) | Pneumonia | 0.5 (I) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-762 | 32 (R) | 64 (R) | 32 | >16 (R) | >16 (R) | >16 (R) | 16 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.015 |
| HM-781 | 16 (R) | 32 (R) | 16 | 16 (R) | 16 (R) | 16 (R) | 8 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-787 | 16 (R) | 32 (R) | 64 | 16 (R) | 16 (R) | 16 (R) | 8 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-809 | 16 (R) | 32 (R) | 64 | 16 (R) | 16 (R) | 16 (R) | 4 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-854 | 8 (R) | 16 (R) | 16 | 0.06 (S) | 0.06 (S) | 0.5 (S) | <0.015 (S) | 8 (R) | 0.06 (S) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-878 | 16 (R) | 32 (R) | 32 | 16 (R) | 16 (R) | 16 (R) | 8 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-953 | 16 (R) | 32 (R) | 16 | >16 (R) | >16 (R) | >16 (R) | 16 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-970 | 8 (R) | 16 (R) | 8 | >16 (R) | >16 (R) | >16 (R) | 16 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-1017 | 16 (R) | 32 (R) | 64 | >16 (R) | >16 (R) | >16 (R) | 16 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |
| HM-1055 | 16 (R) | 32 (R) | 128 | >16 (R) | >16 (R) | >16 (R) | 16 (R) | >16 (R) | >16 (R) | 0.5 (S) | 1 (S) | >16 (R) | 0.03 |

*AMX, amoxicillin; CIP, ciprofloxacin; CLI, clindamycin; CRO, ceftriaxone; ERY, erythromycin; I, intermediate; LEV, levofloxacin; LZD, linezolid; MER, meropenem; OFL, ofloxacin; PEN, penicillin; R, resistant; S, susceptible; TET, tetracycline; TIG, tigecycline; VAN, vancomycin.

†No susceptibility breakpoints are established for ciprofloxacin and tigecycline.
2010 (11) and to 3 MDR ST-166 isolates (serotype 11A) reported in South Korea in 2013 (12). In our study, 3 novel STs of MDR S. pneumoniae were identified (ST-9875, ST-9876, and ST-10300). All the ST-8279, ST-9875, and ST-10300 isolates in our study were serotype 11A, with the exception of 1 of the ST-8279 isolates. The ST-9875 and ST-10300 isolates were single-locus variants (in the spi and gki genes, respectively) of ST-8279. ST-9876 is a 1-locus (aroE) variant of an ST-3384 (serotype 9V) clone registered in the PubMLST database.

Serotypes 19F and 23F are included in the 13-valent pneumococcal conjugated vaccine (PCV13), but serotype 11A is not included in PCV13. Serotype 11A is, however, included in the 23-valent pneumococcal polysaccharide vaccine (PPSV23). The US CDC currently recommends the PPSV23 for all adults ≥65 years of age and all persons 2–64 years of age who are at high risk for pneumococcal disease (13). Through national vaccine programs in South Korea, since 2013, PPSV23 has been provided to all adults ≥65 years of age, and since 2014, 10-valent pneumococcal conjugated vaccine or PCV13 have been provided to young children free of charge (14).

Conclusions
In South Korea, serotype 11A was the most predominant serotype of the 16 levofloxacin-resistant and XDR S. pneumoniae isolates we found. Seven levofloxacin-resistant S. pneumoniae strains were isolated in 2014 alone; the dominant serotype was again 11A (n = 5). All except 1 of these 7 serotype 11A isolates were resistant to the 9 different antimicrobial agents tested. We identified 3 novel STs of MDR serotype 11A S. pneumoniae in our study. S. pneumoniae serotype 11A isolates with novel STs require careful monitoring to combat the increasing prevalence and diversification of MDR pneumococcal strains, especially those with resistance to fluoroquinolones, β-lactams, and third-generation cephalosporins.

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