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# Molecular Analysis and Activity Profiles of Fluoroquinolone-Resistant *S. pneumoniae* Isolates from North America, Latin America and Europe: Results From the SENTRY Antimicrobial Surveillance Program (2001-2002)

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SENTRY ANTIMICROBIAL SURVEILLANCE

## AMENDED ABSTRACT

**Background:** Newer FQs are increasingly used for community-acquired respiratory tract infections (CARTI) and there is an increased effort to detect FQ-R strains. The SENTRY Program collected a large sample of recent FQ-R-SPN isolates worldwide to determine the patterns of QRDR gene mutations responsible for a high FQ MIC.
**Methods:** Over 4,200 SPN isolates from CARTI patients were collected during 2001 - 2002. Ciprofloxacin (CIP), levofloxacin (LEV), moxifloxacin (MOX), gatifloxacin (GAT) and garenoxacin (GRN) MICs were determined by NCCLS methods. A very large subset (126) with CIP MICs 2 - >4 μg/ml from 50 different medical centers had PCR products sequenced for *gyrA*, *B*, and *parC*, *E* mutations.
**Results:** The overall rate of FQR-SPN was higher in EU (5.2%) compared to NA or LA (3.4 - 3.5%), increasing each year. The FQ potency ($MIC_{90}$, μg/ml) for strains with CIP MICs 2 or 4 μg/ml was: GRN (0.12) > MOX (0.25-0.5) > GAT (0.5 - 1) > LEV (2). Isolates with CIP MIC > 4 μg/ml were commonly R to LEV, MOX and GAT ($MIC_{90}$s, ≥ 4 μg/ml) compared to GRN (1 μg/ml). Strains with CIP MIC at ≤ 2 μg/ml generally had no QRDR alteration or a single *par E* mutation (67%), but those 4 μg/ml showed multiple mutations as did 85% of strains with CIP MICs > 4 μg/ml (34% had a mutation in *gyrA*, *parC* and *E* compared to < 2% with a MIC ≤ 4 μg/ml). The most common amino acid substitutions were S81F (83%; *gyrA*); S79F or Y (60%) and K137N (36%; *parC* ); and I460V (96%; *parE*). Mutations in *gyrB* were rare. Drug efflux produced FQR in strains with no detected QRDR changes.
**Conclusions:** The FQR rate among SPN causing CARTI has become the highest in EU compared to NA and LA. GRN retained greatest potency (≤ 1 μg/ml) against many CIP-R isolates compared to other FQs. High-level R was usually associated with *gyrA* mutations at position 81 and/or *parC* mutations at positions 79 and 137.

## INTRODUCTION

The SENTRY Antimicrobial Surveillance Program has been monitoring the antimicrobial susceptibility of the three most common causes of community-acquired respiratory infections since 1997. Only sporadic isolation of *Haemophilus influenzae* and *Moraxella catarrhalis* that have elevated or resistant fluoroquinolone MIC results have been detected since the inception of the program. There has, however, been a noticable trend of increasing fluoroquinolone-resistant *Streptococcus pneumoniae* over the first six years of the program. The incidence of multiple-drug-resistant *S. pneumoniae* is increasing worldwide and the use of fluoroquinolones to treat outpatient respiratory infections has also escalated. Based on these events, it is apparent that active surveillance is necessary for this commonly isolated pathogen that can lead to serious disseminated infection.

The most commonly documented fluoroquinolone resistance mechanisms have been related to mutations in the quinolone resistance-determining region (QRDR) of the genome or the presence of efflux pumps in the bacterial membrane. The effect of these resistance mechanisms varies according to the molecular structure. The newer and broader-spectrum compounds with enhanced activity against Gram-positive species, including *S. pneumoniae*, often will retain some degree of potency, even when multiple mutations are present in the QRDR. In addition, efflux pumps have variable effects on the MIC for different fluoroquinolones and the use of efflux pump inhibitors does not always provide an understanding of the level of gene expression. In the present study, we evaluated the fluoroquinolone resistance rates among *S. pneumoniae* collected by the SENTRY Program in North America, Latin America and Europe during 2001 and 2002. We also evaluated the resistance mechanisms involved and their effect on the MIC of several newer fluoroquinolones with enhanced Gram-positive activity.

## MATERIALS AND METHODS

A total of 74 medical centers forwarded *S. pneumoniae* isolates as part of the community-acquired respiratory tract infection objective for the SENTRY Program (2001 - 2002). In North America, 30 sites in the United States and five in Canada sent 2,099 isolates. Latin American sites (nine) forwarded 384 viable isolates. Thirty sites in Europe, Israel and Turkey sent 1,726 isolates representing 12 countries. The majority of isolates (63.6%) were from high quality sputa and invasive pulmonary samples. Significant numbers of isolates were also collected from sinus and upper airway (11.8%), middle ear (7.2%), blood (5.0%) and eye/conjunctiva (4.9%). Isolates were subcultured twice prior to testing for antimicrobial susceptibility and examined for bile solubility using the plate method. Bile-resistant streptococci were not tested. Purified colonies were suspended into Mueller-Hinton broth to a 0.5 McFarland standard and diluted using 100 μl into 10 ml of 3 - 5% lysed horse blood supplemented Mueller-Hinton broth. Isolates were tested by the NCCLS broth microdilution method using validated dry-form panels (TREK Diagnostics, Inc., Cleveland, OH). Among the antimicrobials tested, fluoroquinolones were represented by ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin and garenoxacin. Minimum inhibitory concentration (MIC) results were determined by visual inspection for growth and interpreted using NCCLS guidelines.

## MATERIALS AND METHODS (Continued)

A subset of 126 isolates with ciprofloxacin MIC results of 2 μg/ml (24), 4 μg/ml (49) and > 4 μg/ml (53) were selected from 50 different medical centers to have PCR products sequenced for *gyrA*, *gyrB*, *parC* and *parE* mutations.

The quinolone resistance determining regions (QRDRs) of genes *parC, parE, gyrA* and *gyrB* were amplified by PCR using primers and cycling conditions previously described. Sequencing was performed in both strands by the dideoxy-chain termination method with a Perkin Elmer Biosystems 377 DNA sequencer and sequence analysis performed using the Lasergene software package (DNASTAR, Madison, WI).

## RESULTS

- The prevalence of *S. pneumoniae* isolates with intermediate ciprofloxacin and levofloxacin MIC results (2 and 4 μg/ml, respectively) was similar among regions at rates of 24.5 - 29.9% and 0.0 - 0.3%, respectively. Higher rates were noted in Canada and Europe.
- The region with the highest prevalence of strains with a ciprofloxacin MIC results of ≥ 4 μg/ml was Europe (5.2%). However, North American centers reported the highest rates of levofloxacin-resistant pneumococci (1.1%) during 2001 - 2002 (Table 1).
- All *S. pneumoniae* isolates with ciprofloxacin MIC results of 2 or 4 μg/ml had MIC values for gatifloxacin, moxifloxacin and garenoxacin still in the susceptibility range, but one isolate showed an intermediate MIC for levofloxacin.
- Garenoxacin was the most active quinolone tested against this group of strains with the highest MIC being 0.25 μg/ml. Significantly lower potencies of levofloxacin, gatifloxacin and moxifloxacin ($MIC_{90}$, > 4 μg/ml) were found among strains with a ciprofloxacin MIC of > 4 μg/ml. The des-F(6)-quinolone, garenoxacin, retained a potency of 1 μg/ml. The rank order of in vitro activity against this group of strains was: garenoxacin ($MIC_{90}$, 1 μg/ml; 98.2% activity at ≤ 2 μg/ml) > moxifloxacin ($MIC_{90}$, 2 μg/ml; 38.6% activity at ≤ 1 μg/ml) > gatifloxacin ($MIC_{90}$, 4 μg/ml; 29.8% activity at ≤ 1 μg/ml) > levofloxacin ($MIC_{90}$, > 4 μg/ml; 29.8% activity at ≤ 2 μg/ml).
- *S. pneumoniae* isolates with a ciprofloxacin MIC result of 2 or 4 μg/ml most commonly had mutations in *parC* (29.2 - 53.1%) and/or *parE* (51.0 - 70.8%) encoding the A and B subunits of DNA topoisomerase IV, respectively (Table 3). Between 14% and 25% of strains had no QRDR mutations evident and DNA gyrase mutations were infrequent (0 - 10.2%).
- Nearly 90% of strains with a > 4 μg/ml ciprofloxacin MIC result (Table 3) had *parC* mutations, and *gyrA* mutations were much more common (64.2%) among these strains than among those with a MIC result of 2 - 4 μg/ml. In addition, less than 2% of the strains with high ciprofloxacin MICs (> 4 μg/ml) had no detectable mutation and a higher number of isolates (5.7%) had *gyrB* mutations compared to strains with the lower ciprofloxacin MIC results (2 - 4 μg/ml).

**Table 1.** Distribution of fluoroquinolone-intermediate and -resistant MIC results for levofloxacin and ciprofloxacin among *S. pneumoniae* isolates from American and European centers participating in the SENTRY Antimicrobial Surveillance Program (2001 - 2002).

| | No. (%) of strains at MIC value (μg/ml) | | | | |
|---|---|---|---|---|---|
| | Ciprofloxacin | | | Levofloxacin | |
| Region (no.) | 2 | 4 | >4 | 4 | >4 |
| North America | | | | | |
| United States (1,744) | 461(26.4) | 26(1.5) | 24(1.4) | 0(0.0) | 17(1.0) |
| Canada (355) | 106(29.9) | 15(4.2) | 9(2.5) | 1(0.3) | 7(2.0) |
| Latin America (384) | 94(24.5) | 10(2.6) | 3(0.8) | 0(0.0) | 2(0.5) |
| Europe (1,726) | 510(29.5) | 68(3.9) | 21(1.2) | 2(0.1) | 12(0.7) |

## RESULTS

**Table 2.** Comparison of fluoroquinolone MIC values for *S. pneumoniae* strains with elevated ciprofloxacin MIC results.

| | | MIC (μg/ml) | | |
|---|---|---|---|---|
| Ciprofloxacin MIC (no.) | Fluoroquinolone | 50% | 90% | Range |
| 2 (1,171) | Levofloxacin | 1 | 2 | 1-2 |
| | Gatifloxacin | 0.5 | 1 | 0.25-1 |
| | Moxifloxacin | 0.25 | 0.5 | 0.12-0.5 |
| | Garenoxacin | 0.06 | 0.12 | ≤0.03-0.25 |
| 4 (119) | Levofloxacin | 1 | 2 | 0.5-4 |
| | Gatifloxacin | 0.5 | 0.5 | 0.25-2 |
| | Moxifloxacin | 0.25 | 0.25 | 0.12-1 |
| | Garenoxacin | 0.06 | 0.12 | ≤0.03-0.25 |
| >4 (57) | Levofloxacin | >4 | >4 | 1->4 |
| | Gatifloxacin | 4 | >4 | 0.5->4 |
| | Moxifloxacin | 2 | >4 | 0.12->4 |
| | Garenoxacin | 0.5 | 1 | ≤0.03->4 |

**Table 3.** Mutation rates in the quinolone-resistance determining region (QRDR) for *S. pneumoniae* isolates with ciprofloxacin MICs ≥ 2 μg/ml.

| | | No. of strains with QRDR mutations (%)/Amino acid substitutions[a] | | | | |
|---|---|---|---|---|---|---|
| Ciprofloxacin MIC (μg/ml) | No. tested | *gyr A* | *gyr B* | *par C* | *par E* | None |
| 2 | 24 | 2 (8.3)/<br>2 Ser81Phe | 0 (0.0) | 7 (29.2)/<br>6 Lys137Asn<br>1 Ser79Tyr<br>1 Ser52Gly | 17 (70.8)/<br>16 Ile460Val<br>1 Asn377Lys | 6 (25.0) |
| 4 | 49 | 5 (10.2)/<br>4 Ser81Phe<br>1 Ala17Thr | 1 (2.0)/<br>1 Asp435Glu | 26 (53.1)/<br>11 Ser79Phe<br>9 Lys137Asn<br>3 Asp83Asn<br>3 Ser52Gly<br>2 Asn91Asp<br>1 Asp83Tyr<br>1 Glu135Asp<br>1 Ala142Ser<br>1 Ser79Tyr | 25 (51.0)/<br>23 Ile460Val<br>1 Asp435Asn<br>1 Glu494Asp | 7 (14.3) |
| > 4 | 53 | 34 (64.2)/<br>28 Ser81Phe<br>3 Ser81Tyr<br>3 Glu85Lys<br>1 Val71Ile<br>1 Ala17Thr | 3 (5.7)/<br>2 Asp435Ile<br>1 Val432Asp | 46 (86.8)/<br>28 Ser79Phe<br>13 Lys137Asp<br>6 Ser79Tyr<br>3 Asn91Asp<br>3 Asp83Asn<br>1 Ser52Gly<br>1 Glu135Asp<br>1 Asp83Tyr<br>1 Gly77Glu | 33 (62.3)<br>33 Ile460Val<br>4 Asp435Asn<br>1 Pro454Ser | 1 (1.9) |

a. Multiple mutations in the same gene region were found for some isolates.

- Although it is not clearly evident in Table 3, *S. pneumoniae* with lower-level ciprofloxacin resistance were less likely to have multiple mutations in topoisomerase IV and DNA gyrase genes (30.0%). These multiple mutations were usually associated with amino acid substitutions at positions 79 and 137 (*parC*) and 460 (*parE*). In contrast, 84.9% of strains with a ciprofloxacin MIC result of > 4 μg/ml had more than one mutation in the QRDR and 35.9% had more than two. This higher level of resistance was most commonly due to a *gyrA* mutation at position 81 and mutations in one or both of the two DNA topoisomerase IV subunits at positions mentioned above.

## CONCLUSIONS

- The prevalence of fluoroquinolone resistant *S. pneumoniae* strains causing CARTI remains relatively low, but has been continuously increasing in the last few years. The rate of levofloxacin resistance has increased from 0.2% in 1997 - 1998 to 0.8, 0.5, 0.7 and 1.1% over the following four years. *S. pneumoniae* strains with this phenotype were twice as common in Canada compared to the United States and about four times as common compared to Latin America and Europe during 2001 - 2002.
- *S. pneumoniae* isolates with ciprofloxacin MIC values of 2 - 4 μg/ml generally remain susceptible to levofloxacin, gatifloxacin, moxifloxacin and garenoxacin. However, only garenoxacin retains activity against high-level fluoroquinolone-resistant strains with multiple QRDR mutations.
- Isolates with ciprofloxacin MIC results of 2 - 4 μg/ml were more likely to have mutations in *parC* and *parE*. On the other hand, most high-level resistant strains showed mutations in *gyrA* and *parC* and/or *parE*.
- *S. pneumoniae* isolates with elevated or resistant fluoroquinolone MICs with no detectable gyrase or topoisomerase mutations may be due to a yet uncharacterized efflux pump.

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