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JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
ph. 319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

# Fluoroquinolone-Resistant *Haemophilus influenzae* Continue to be Infrequently Isolated, Within Worldwide Surveillance Networks (1997-2008)

**DJ BIEDENBACH, RN JONES, HS SADER, M CASTANHEIRA, RE MENDES**
**JMI Laboratories, North Liberty, IA, USA**

SENTRY ANTIMICROBIAL SURVEILLANCE

## Abstract

**Background**: Fluoroquinolone (FQ) use for treatment of bacterial respiratory infections has increased, and rare outbreaks of fluoroquinolone-resistant (FQR) *Haemophilus influenzae* have been observed, particularly among older patients. This study determines the FQ activity against *H. influenzae* collected in North and Latin America, Europe and Asia-Pacific regions over 12 years of resistance surveillance.

**Methods**: 25,683 clinical isolates were collected from respiratory or blood cultures at 142 medical centers in 35 countries (SENTRY). Data on FQR isolates from 1997-2001 were published previously and data from more recent collections during 2002-2008 (12,621 strains) were compared. All strains were susceptibility tested against ciprofloxacin (CIP), levofloxacin (LEV) during all study years by CLSI broth microdilution methods. Etest was used to determine the potency of CIP, LEV, gemifloxacin (GEM), gatifloxacin (GAT) and moxifloxacin (MOX) against 7 previously unreported strains with CIP MIC values ≥0.12 µg/ml. These isolates were examined for GyrA and ParC mutations in the quinolone resistance determining region (QRDR).

**Results**: During 1997-2001 and 2002-2008, the rate of *H. influenzae* with a CIP MIC ≥0.12 µg/ml was only 0.23 and 0.17%, respectively. True CIP non-susceptible (NS; MIC ≥2) was only 0.04% over all studied years. All 7 CIP NS isolates (MIC, 0.125 to >32 µg/ml) had GyrA (S81) mutations and 5 strains had additional ParC (S79 or G77) alteration. Overall, GEM and GAT were most potent, followed by LEV and MOX against these FQR isolates. 75% of the patients with documented CIP NS *H. influenzae* were adults (≥20 years).

**Conclusions**: The isolation of FQR-*H. influenzae* remain extremely rare in medical centers throughout the world. The selective pressure produced by increased use of FQ class agents appears to have had limited adverse effect on QRDR mutations for *H. influenzae* over the past 12 years of resistance surveillance.

## Introduction

Community-acquired bacterial pneumonia (CABP) is a significant burden to the medical community and patients with suspected or documented disease are increasingly being treated with fluoroquinolones. One of the most common offending pathogens associated with CABP is *Haemophilus influenzae*. Fluoroquinolone resistance in this species has been documented in surveillance study data and in case reports beginning in the early 1990s. However, the data has been sporadic and it can be difficult to ascertain the true prevalence of resistance to fluoroquinolone agents amongst *H. influenzae* unless well structured surveillance data is available for analysis.

The SENTRY Antimicrobial Surveillance Program has been collecting susceptibility data on *H. influenzae* since 1997. This study reports on the fluoroquinolones included in the SENTRY Program during 2002-2008 to supplement previously published data (SENTRY 1997-2001). The previous study documented a very low incidence of resistance (0.15%) to this class of agents from medical centers in North America, Latin America and Europe. The SENTRY surveillance data from Asia and the Western Pacific documented a higher rate of fluoroquinolone-resistant *H. influenzae* (0.82%) during this same time period with an overall worldwide resistance rate of only 0.23%. We conclude that the rate of fluoroquinolone-resistant *H. influenzae* has not been escalating and that only sporadic events of this resistance phenotype occur, with the exception of some documented epidemiological outbreaks.

## Methods

Bacterial isolates. A total of 12,621 isolates used in the analysis of this study had a MIC value for levofloxacin with slightly smaller numbers tested against ciprofloxacin and moxifloxacin. Twenty two *H. influenzae* strains (0.17%) had elevated ciprofloxacin MIC values (≥0.12 µg/ml; susceptible breakpoint at ≤1 µg/ml). Among these non-wildtype strains, seven isolates with confirmed elevated fluoroquinolone MIC values (0.12 - >32 µg/ml) were available for further evaluation.

Isolates were identified by the local site and confirmed by the reference laboratory (JMI Laboratories, North Liberty, Iowa, USA). Confirmation of suspected "fluoroquinolone-resistant" strain identifications was performed using patterns of growth around X and V factor disks (Remel, Lenexa, Kansas, USA) and the use of Vitek NHI identification cards (bioMerieux, Inc., Durham, North Carolina, USA).

Antimicrobial susceptibility testing. Ciprofloxacin, levofloxacin and moxifloxacin were routinely tested by the broth microdilution method during all study years. Gemifloxacin and gatifloxacin were only tested during earlier years (2002-2006). Limited surveillance of *H. influenzae* was performed during 2005 (bloodstream isolates only). The isolates were tested for susceptibility by reference broth microdilution methods using the Clinical Laboratory Standards Institute (CLSI) recommendations (M07-A8, 2009). Haemophilus Test Media in validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA) were utilized.

Quality assurance of test results was performed using quality control strain *H. influenzae* ATCC 49247 (CLSI M100-S20, 2010) and the inoculum density was confirmed using regular colony counts during each testing event.

Confirmation of fluoroquinolone MIC values and resistance mechanisms. Seven isolates available for further evaluation had the elevated fluoroquinolone MIC values (0.12 - >32 µg/ml) confirmed by the Etest method (bioMerieux, Inc., Durham, North Carolina, USA). These strains were susceptibility tested against ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin and gemifloxacin. These isolates were also screened for the presence of mutations in GyrA and ParC using PCR and sequencing.

## Results

- The twenty-two *H. influenzae* isolates with elevated fluoroquinolone MIC values were isolated from patients in several countries including Canada, Japan, Hong Kong, Australia, France, Korea, Germany, Taiwan, Sweden, USA and the People's Republic of China.
- Overall, ciprofloxacin and levofloxacin inhibited ≥99.2% of *H. influenzae* isolates at ≤0.03 µg/ml during several study years (Tables 1 and 2). Moxifloxacin was slightly less active inhibiting 96.5 – 98.6% of the isolates at ≤0.03 µg/ml (Table 3).
- During the years that gatifloxacin and gemifloxacin were routinely tested, these fluoroquinolones inhibited >99.9% of the *H. influenzae* isolates at ≤0.5 µg/ml (data not shown).
- Among the isolates with elevated fluoroquinolone MIC values, gatifloxacin was the most active agent overall, followed by gemifloxacin and moxifloxacin and levofloxacin (Table 4).
- All isolates with elevated fluoroquinolone MIC values had Ser81 alterations in GyrA. In addition, all strains with a ciprofloxacin MIC ≥1 µg/ml exhibited concurrent mutations in ParC (Table 4).
- Three strains from a single medical site (113) in Germany were likely clonally related based upon the similarity of the antibiogram, identical mutations in the quinolone resistance determining region (QRDR) region and PFGE patterns (Table 4).

**Table 1**. Activity of ciprofloxacin tested against 12,605 isolates of *H. influenzae* collected during 2002-2008 from the SENTRY Antimicrobial Surveillance Program.

| | Cum. % inhibited at MIC (µg/ml): | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Year (no. tested) | ≤0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | ≥4 |
| 2002 (3,199) | 99.3 | 99.8 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 100.0 |
| 2003 (2,931) | –[a] | –[a] | 99.9 | 99.9 | >99.9 | 100.0 | | |
| 2004 (2,842) | 99.4 | 99.8 | 99.9 | >99.9 | 100.0 | | | |
| 2005 (142) | 99.3 | 100.0 | | | | | | |
| 2006 (1,202) | 99.6 | 99.9 | 100.0 | | | | | |
| 2007 (1,233) | 99.6 | 99.9 | 100.0 | | | | | |
| 2008 (1,056) | 99.2 | 99.6 | 99.6 | 99.6 | 99.6 | 99.7 | 99.7 | 100.0 |

a. Concentrations not tested.

**Table 2**. Activity of levofloxacin tested against 12,621 isolates of *H. influenzae* collected during 2002-2008 from the SENTRY Antimicrobial Surveillance Program.

| | Cum. % inhibited at MIC (µg/ml): | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Year (no. tested) | ≤0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | ≥4 |
| 2002 (3,212) | 99.4 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 100.0 |
| 2003 (2,934) | 99.3 | 99.8 | 99.9 | >99.9 | 100.0 | | | |
| 2004 (2,842) | 99.2 | 99.8 | 99.9 | >99.9 | 100.0 | | | |
| 2005 (142) | –[a] | – | – | – | 100.0 | | | |
| 2006 (1,202) | – | – | – | – | 100.0 | | | |
| 2007 (1,233) | – | – | – | – | 100.0 | | | |
| 2008 (1,056) | – | – | – | – | 99.7 | 99.7 | 99.7 | 100.0 |

a. Concentrations not tested.

**Table 3**. Activity of moxifloxacin tested against 12,096 isolates of *H. influenzae* collected during 2002-2008 from the SENTRY Antimicrobial Surveillance Program.

| | Cum. % inhibited at MIC (µg/ml): | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Year (no. tested) | ≤0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | ≥4 |
| 2002 (2,692) | 98.0 | 99.7 | 99.9 | >99.9 | >99.9 | >99.9 | >99.9 | 100.0 |
| 2003 (2,929) | 96.5 | 99.5 | 99.9 | >99.9 | 100.0 | | | |
| 2004 (2,842) | 96.7 | 99.4 | 99.8 | 100.0 | | | | |
| 2005 (142) | 98.6 | 100.0 | | | | | | |
| 2006 (1,202) | –[a] | – | – | – | 100.0 | | | |
| 2007 (1,233) | – | – | – | – | 100.0 | | | |
| 2008 (1,056) | – | – | – | – | 99.6 | 99.7 | 99.7 | 100.0 |

a. Concentrations not tested.

**Table 4**. Fluoroquinolone MIC values and analysis of the QRDR region of seven *H. influenzae* isolates with elevated ciprofloxacin MIC results.

| | | Etest MIC (µg/ml) | | | | | Mutation(s) | |
|---|---|---|---|---|---|---|---|---|
| Site-strain# | Year | Cipro | Levo | Moxi | Gati | Gemi | GyrA | ParC |
| 032-1723B | 2002 | >32 | >32 | >32 | >32 | 8 | S81R; D85N | S79R; E83K |
| 112-3717A | 2004 | 0.5 | 0.38 | 0.25 | 0.19 | 0.19 | S81F | WT[b] |
| 117-52C | 2007 | 0.125 | 0.19 | 0.19 | 0.19 | 0.19 | S81L | WT |
| 002-1635B | 2008 | 1 | 0.75 | 1 | 0.5 | 1 | S81L; E139K | S79I |
| 113-3369B | 2008 | >32 | 8 | 8 | 2 | 6 | S81F; D85A | G77D |
| 113-3372B | 2008 | >32 | 8 | 8 | 2 | 8 | S81F; D85A | G77D |
| 113-3375B | 2008 | >32 | 8 | 16 | 2 | 6 | S81F; D85A | G77D |

a. WT, wildtype.

## Conclusions

- There have been reports of colonization or emergence of *H. influenzae* with elevated fluoroquinolone MIC values, and these have usually been among elderly patients, particularly associated with long-term care facilities.
- This study documents the continuing low occurrence of fluoroquinolone-resistant *H. influenzae* in the general population of hospitalized patients, sampled on a global scale by the SENTRY Program.
- Fluoroquinolone-resistant strains of *H. influenzae* remain rarely isolated, and it appears that large surveillance programs may be needed to detect the initial evolution of this unusually encountered phenotype.

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