Multiple Mutations in the Quinolone Resistance Determining Regions (QRDR) Detected Among Fluoroquinolone-Resistant *Haemophilus parainfluenzae* Collected in the USA

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### ABSTRACT

Background: *Haemophilus parainfluenzae* (HPAR) is an opportunistic pathogen that can colonize the nasopharynx of healthy individuals and is frequently isolated from respiratory infections, for example those related to *Streptococcus pneumoniae* (SPN). Resistance to fluoroquinolones (FQs) remains a significant problem, since SPN and HPAR are often coinhabiting in the upper respiratory tract of the same individual. In a recent study [1], we detected two HPAR isolates with elevated MICs against moxifloxacin and ciprofloxacin respectively. To further examine microbial resistance, we collected HPAR isolates from 2012 collected from 56 USA hospitals and used molecular methods to determine the presence of mutations in antibiotic resistance genes.

Methods: Among the isolates collected in 2012, we selected 56 that were resistant to at least one FQ. The MIC results were based on the ApaI restriction pattern only. The EUCAST method (MH-F) was used for susceptibility testing and the CLSI method was used for quality control. Isolates were compared to sequence data of *HPAR ParC* and *GyrB* using the MALDI Biotyper (Bruker Daltonics, Billerica, MA). Sequences were compared to those of *Escherichia coli* and the nucleotide differences were used to determine the amino acid changes.

Results: A total of 123 HPAR isolates were sequenced to determine the presence of mutations in the QRDR for each isolate. Of these isolates, 37 were resistant to moxifloxacin and 10 to ciprofloxacin, one to levofloxacin, and 7 to both moxifloxacin and ciprofloxacin. The sequencing results were based on the ApaI restriction pattern only. To determine the presence of mutations, we used the MALDI Biotyper (Bruker Daltonics, Billerica, MA) and compared the sequences for mutations in the QRDR for each isolate.

Conclusions: Our study showed that HPAR is frequently isolated from the upper respiratory tract of individuals and has a high level of resistance to FQs. This highlights the need for further studies to determine the impact of FQ resistance on the treatment of respiratory infections.

### MATERIALS AND METHODS

**Electrophoresis:** A total of 123 HPAR isolates were sequenced on both strands and the nucleotide differences were used to determine the amino acid changes.

**Results:** The MIC test for FQs was performed using the EUCAST method. The EUCAST methods were performed using the MALDI Biotyper (Bruker Daltonics, Billerica, MA).

**Susceptibility Testing:** Isolates were initially susceptibly tested by the reference broth dilution method in accordance with CLSI guidelines. All panels were performed using CLSI reference strains.

**Genomic DNA was prepared in agarose blocks, digested with enzymes and sequenced on both strands using the MALDI Biotyper (Bruker Daltonics, Billerica, MA).**

**FQs were tested using Haemophilus testing media (HTM) or Mueller-Hinton agar (MH).**

**• EUCAST and CLSI methodologies generated similar MIC results for ciprofloxacin and levofloxacin.**

**The authors wish to thank P. R. Rhomberg and K. K. Simpson for their assistance and support.**

### RESULTS

**Fourteen H. parainfluenzae isolates were collected in 12 USA hospitals and each isolate exhibited elevated MIC values.**

Table 1: FQ susceptibility results of 14 fluoroquinolone-resistant *H. parainfluenzae* isolated during 2012.

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Moxifloxacin MIC (µg/mL)</th>
<th>Ciprofloxacin MIC (µg/mL)</th>
<th>Levofoxacin MIC (µg/mL)</th>
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</tbody>
</table>
| *Figure 1:* PFGE restriction patterns of *H. parainfluenzae* strains digested with ApaI (a) or SmaI (b). Two enzymes were used due to non-typable isolates with one or another method.

### CONCLUSIONS

**• Susceptibility results ranged for moxifloxacin from 2-64 µg/mL and ciprofloxacin and levofloxacin from 2-16 µg/mL tested according to CLSI guidelines.**

**• Fourteen H. parainfluenzae isolates were collected in 12 USA hospitals and each isolate exhibited elevated MIC values.**

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### REFERENCES


### CLINICAL USEFULNESS

**• Resistance to FQs is increased in the presence of mutations in the QRDR.**

**• The EUCAST method (MH-F) was used for susceptibility testing and the CLSI method was used for quality control.**