# Genetic detection of quinolone resistance in *Haemophilus parainfluenzae*: Mutations in the quinolone resistance-determining regions of *gyr*A and *par*C

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The quinolone resistance-determining regions of *gyr*A and *par*C of both quinolone-sensitive and quinolone-resistant *Haemophilus parainfluenzae* strains were amplified and sequenced. Similar to *Haemophilus influenzae*, resistance to quinolones in *H parainfluenzae* is associated with mutations in the quinolone resistance-determining regions of both *gyr*A and *par*C. The present study discusses the importance of this finding.

**Key Words:** *Genetic detection;* Haemophilus parainfluenzae; *Quinolone resistance*

## Le dépistage génétique de la résistance à la quinolone en cas d'*Haemophilus parainfluenzae* : Les mutations dans les régions du *gyr*A et du *par*C déterminant la résistance à la quinolone

Les auteurs ont amplifié et séquencé les souches d'*Haemophilus parainfluenzae* sensibles à la quinolone et résistantes à la quinolone des régions du *gyr*A et du *par*C déterminant la résistance à la quinolone. À l'instar de l'*Haemophilus influenzae*, la résistance du *H parainfluenzae* aux quinolones s'associe à des mutations des régions de *gyr*A et de *par*C déterminant la résistance à la quinolone. La présente étude expose l'importance de cette découverte.

Although generally not regarded as a pathogen, *Haemophilus parainfluenzae* does occasionally cause infections in humans, including pneumonia (1), peritonitis (2,3), biliary tract infection (4), endocarditis (5) and urinary tract infection (6). More recently, its role in chronic obstructive pulmonary disease has been discussed (7,8). Due to its potential role in the infectious process, susceptibility testing of *H parainfluenzae* is important to guide antibiotic treatment when required. This is especially relevant because beta-lactamase-mediated resistance to ampicillin has been observed more frequently in *H parainfluenzae* than in *Haemophilus influenzae* (9). Furthermore, fluoroquinolone-resistant *H parainfluenzae* has recently been reported in Japan (10). Of the 920 *H parainfluenzae* isolates recovered from respiratory specimens of patients admitted to outpatient clinics in Japan between 1998 and 2000, 31 isolates or 3.4% were resistant to fluoroquinolones based on the standard microbroth dilution method, and had a minimum inhibitory concentration (MIC) breakpoint of greater than 2 µg/mL for levofloxacin, according to the Clinical and Laboratory Standards Institute (USA) guidelines (11). The MIC required to inhibit the growth of 90% of organisms for five fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, sitafloxacin and sparfloxacin) for the fluoroquinolone-sensitive *H parainfluenzae* isolates ranged from 0.015 µg/mL or less to 0.06 µg/mL, and was 100-fold higher for the fluoroquinolone-resistant *H parainfluenzae* isolates. However, the molecular basis of this resistance was not studied.

Mutations in both *gyr*A and *par*C have been documented to be associated with quinolone resistance in *H influenzae* and a number of other Gram-negative bacteria (12-15).

## METHODS

In the present study, the quinolone resistance-determining regions (QRDRs) of *gyr*A and *par*C for both quinolone-sensitive and quinolone-resistant *H parainfluenzae* strains were amplified by polymerase chain reaction (PCR) and then sequenced. PCR primers described for amplifying *gyr*A and *par*C of *H influenzae* (14) did not amplify the corresponding genes of *H parainfluenzae*. Based on the complete genome sequence of *H parainfluenzae* strain T3T1 (www.sanger.ac.uk/Projects/H_parainfluenzae/), primers were designed to target the QRDRs of both *gyr*A and *par*C of this organism. For *gyr*A, the forward and reverse primers GYRAF2 (5′-TCG ACG TGT GCT TTT CTC CAT G-3′) and GYRAR1 (5′-GTT GCC ATC CCC ACC GCA ATA CCG-3′), respectively, amplified a 405 base pair gene fragment. For *par*C, the forward and reverse primers PARCF1 (5'-TCT GAA CTG GGT TTA AAT GCC-3') and PARCR1 (5'-ACC ACG ACC GGT TTC ATA CAT-3'), respectively, amplified a 564 base pair gene fragment.

The PCR mixture consisted of 2.5 mM magnesium chloride, 0.2 mM deoxyribonucleotide triphosphates, 10 mM primers and 1.25 units of HotStarTaq DNA polymerase (Qiagen, Canada). The PCR cycle consisted of an initial 15 min at 95°C, followed by 35 cycles at 95°C for 1 min, 54°C for 1 min and 72°C for 1 min and a final phase of 72°C for 10 min. The PCR amplicons were purified with the QIAquick PCR purification kit (Qiagen, Canada) before being sequenced by the DNA analyzer 3730x1 (Applied Biosystems, USA). The sequences of both strands were edited, assembled and aligned for comparison with published sequences obtained from

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**TABLE 1**
**Amino acid compositions of the *gyr*A and *par*C quinolone resistance-determing regions (QRDRs) in fluoroquinolone-sensitive and quinolone-resistant *Haemophilus parainfluenzae* strains**

| | Fluoroquinolone MIC values by Etest (µg/mL) | | | Amino acid composition | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Strain number | Levofloxacin | Ciprofloxacin | Moxifloxacin | QRDR of *gyr*A* | | | | QRDR or *par*C† | | |
| ATCC 33392‡ | <0.03 | Not done | Not done | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 87-0092 | 0.016 | 0.023 | 0.125 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 88-0043 | 0.012 | 0.008 | 0.032 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 88-0194 | 0.023 | 0.012 | 0.064 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 88-0283 | 0.016 | 0.006 | 0.032 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 89-0518 | 0.012 | 0.006 | 0.094 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 89-0796 | 0.064 | 0.047 | 0.250 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 90-0572 | 0.032 | 0.008 | 0.094 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 95-0376 | 0.032 | 0.016 | 0.125 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 95-0377 | 0.023 | 0.016 | 0.094 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 08-013 | 0.023 | 0.016 | 0.125 | Asp | Ser | Asp | Gly | Asp | Ser | Tyr |
| 07-020 | 2.0 | 4.0 | >32 | Asp | Phe§ | Tyr§ | Gly | Asp | Phe§ | Tyr |
| 07-028 | 12 | 12 | >32 | Asp | Phe§ | Tyr§ | Gly | Asp | Phe§ | Tyr |

*Etest (bioMerieux, Canada). The minimum inhibitory concentration (MIC) of levofloxacin was determined by Sensititre plates (Trek Diagnostic Systems Inc, USA). *QRDR of gyrA at amino acid positions 83, 84 and 88 (14); †QRDR of parC at amino acid positions 82, 83, 84 and 88 (14); ‡ATCC 33392 is a* H parainfluenzae*-type strain sensitive to fluoroquinolones; §Mutations in QRDRs. Asp Aspartate; Gly Glycine; Phe Phenylalanine; Ser Serine; Tyr Tyrosine*

quinolone-sensitive and quinolone-resistant *H influenzae* strains (14) by using software from DNAStar Inc (USA).

## RESULTS AND DISCUSSION

Two *H parainfluenzae* isolates recovered from respiratory specimens were referred to the National Microbiology Laboratory (NML; Winnipeg, Manitoba) for further characterization: isolate 1, NML# 07-028, from the pleural fluid of a 52-year-old woman from British Columbia; and isolate 2, NML# 07-020, from the sputum of an 83-year-old man from Quebec. Both isolates were confirmed as *H parainfluenzae* by biochemical tests and 16S ribosomal RNA sequencing. Isolate 1 belonged to biotype I, and isolate 2 belonged to biotype II. Detection of beta-lactamase was performed using the BBL DrySlide nitrocefin (Becton Dickinson, Canada). Antibiotic susceptibility testing by the disk-diffusion method was performed according to the Clinical and Laboratory Standards Institute guidelines (16). Etest (bioMérieux, Canada) was used to determine the MIC of fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin) to *H parainfluenzae*. Microbroth dilution method using the Sensititre plates (Trek Diagnostic Systems Inc, USA) was also performed to test the MIC of levofloxacin. Both isolates did not produce beta-lactamase and were found by disk-diffusion susceptibility testing to be resistant to ciprofloxacin, moxifloxacin, clarithromycin and azithromycin, but sensitive to ampicillin, amoxicillin-clavulanic acid, cefaclor, ceftriaxone, trimethoprim-sulfamethoxazole and chloramphenicol. In addition, 11 quinolone-sensitive *H parainfluenzae* isolates (sensitive to ciprofloxacin, moxifloxacin and levofloxacin) were included for comparison. These 11 isolates included ATCC 33392, three from respiratory sources, three from stool/appendix, and one each from cerebral spinal fluid, eye, gallbladder and one of unknown source.

Table 1 compares the MICs of fluoroquinolones and the amino acid compositions of the QRDRs of *gyr*A and *par*C in the *H parainfluenzae* strains examined in the present study. Similar to *H influenzae* (14), resistance to quinolones in *H parainfluenzae* is associated with mutations in both *gyr*A and *par*C. In the two resistant strains examined, the mutations involved substitutions of Ser84Phe and Asp88Tyr of *gyr*A, and Ser84Phe of *par*C. The difference in the MICs (2 µg/mL versus 12.0 µg/mL) of levofloxacin in these two resistant strains suggest that strain 07-028 might possess additional resistance mechanisms, such as additional mutations in the drug targets GyrB and ParE, decreased permeation of the drug, or presence and/or overexpression of an endogenous efflux pump system that causes reduced accumulation of the drug in the target site. These possibilities have not been examined in the strains.

Besides being capable of causing a variety of infections, *H parainfluenzae* has been reported to be associated with acute exacerbations of chronic bronchitis (AECB) (17). Empirical antibiotic treatment of patients with AECB has been proven to be beneficial in those with severe airflow obstruction and presenting with at least two or all three cardinal symptoms (increased dyspnea, increased sputum volume and increased sputum purulence) (18). With increasing reports of resistance of many bacteria to multiple antibiotics including the beta-lactams, fluoroquinolones have become the antibiotic of choice to treat AECB (19).

## SUMMARY

We report new primers for PCR amplification of the QRDRs of *gyr*A and *par*C of *H parainfluenzae* for molecular detection of quinolone resistance. We confirm that quinolone resistance in *H parainfluenzae*, similar to that reported for *H influenzae* and other Gram-negative bacteria, is associated with genetic mutations that lead to amino acid substitutions in the QRDRs of both *gyr*A and *par*C. Because these two random isolates were identified at the local hospital or provincial public health laboratories, the prevalence of quinolone-resistant *H parainfluenzae* strains in the general population is not available from the present study. Further studies are required to understand the extent of fluoroquinolone resistance in *H parainfluenzae*, and the potential involvement of other DNA gyrase and topoisomerase genes, such as *gyr*B and *par*E, in the resistance of these organisms to quinolones.

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