P1755 Prevalence of fluoroquinolone resistance and QRDR mutations in *S. pneumoniae* in Toronto, Canada, 1995-2006

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Abstract

Background: The emergence of fluoroquinolone resistance (FQR) in S. pneumoniae (SP) is a concern. We examined FQ use, FQR and QRDR mutations in pneumococci in Toronto, Canada.

Methods: From 1995-2006, the Toronto Invasive Bacterial Diseases Network (TIBDN) collected all sterile site isolates of pneumococci isolated from residents of Toronto/Peel, CA (pop 4M); from 2002-2006, all respiratory tract isolates were also collected. Broth microdilution susceptibility testing to CLSI standards is performed on all isolates, sequencing of QRDR regions was performed on all FQR isolates, and a representative sample of FQ susceptible isolates. Pop'n FQ use was obtained from IMS Health Canada. Demographic and medical data were collected for all patients.

Results: From 1995 to 2006, overall FQ use increased from 67 to 94 scripts/1000pop/yr; levofloxacin use increased from 0 to 12.6 scripts/1000pop/yr, and moxifloxacin use increased from 0 to 15.3 scripsts/1000 population per year. 7038/7909 (89%) of case isolates were available for testing. Ciprofloxacin non-susceptibility rates increased from 0.9% in 1995 to 4.1% in 2002, then decreased to 2.7% in 2006. Levo R rates increased from 0 in 1995 to 3.0% in 2002, then decreased to 1.8% in 2006. Moxi R rates have increased slowly, to 1.3% in 2005, then decreased to 1.1% in 2006. In cipro susceptible isolates (MIC<4), the prevalence of parC mutations was 18/1053 (1.7%) and the prevalence of gyrA mutations was 1/1053 (0.09%). The prevalence of parC and gyrA mutations in isolates with levo MIC<2ug/ml was 0.6% (18/3058) and 0.03%.(1/3058). The prevalence of parC and gyrA mutations in isolated with moxi MIC <.25ug/ml was 0.5% (14/2952) and 0 (0/2952). There was no increase over time in the prevalence of mutations in isolates susceptible to FQ. Isolates mutations in gyrA but not in parC were first detected in 2002 (a single isolate), and remain rare (2 isolates in 2006).

Conclusions: Despite increasing use of FQ antibiotics FQR is stable or decreasing in Toronto. Isolates with mutations in gyrA and parC remain very rare in FQ susceptible isolates, Similarly, mutations in gyrA alone remain exceedingly uncommon, suggesting the moxifloxacin exerts minimal selective pressure for resistance.

Introduction:

The Toronto Invasive Bacterial Diseases Network (TIBDN) has performed population-based surveillance for invasive pneumococcal disease in metropolitan Toronto and Peel region (pop'n 4M) since January 1, 1995. The emergence of fluoroquinolone resistance in *S. pneumoniae* and its impact on empiric therapy for pneumococcal disease continues to be a concern. We examined isolates and data from this surveillance to determine the prevalence of fluoroquinolone resistance and QRDR mutations in these pneumococcal isolates, and the relationship between the emergence of resistance and fluoroquinolone use in the human population.

Figure 1. TIBDN population area

Acknowledgements:

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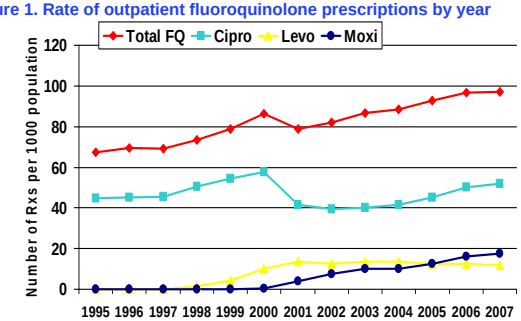
Methods:

From 1995 to 2006, all sterile site isolates of pneumococci identifed through population-based surveillance for pneumocosal disease in Metropolitan Toronto and Peel region were collected. From 2002 to 2006, all respiratory site isolates from hospital laboratories were also collected. Broth microdilution antimicrobial susceptibility testing was performed to CLSI standards. The quinolone resistance determining regions (QRDR) of *parC* and *gyrA* were sequenced in all fluoroquinolone resistant isolates and a representative sample of fluoroquinolone susceptible isolates. Population fluoroquinolone use was obtained from IMS Health Canada. Demographic and clinical data were collected from review of health records and interviews with patients and attending physicians.

Results:

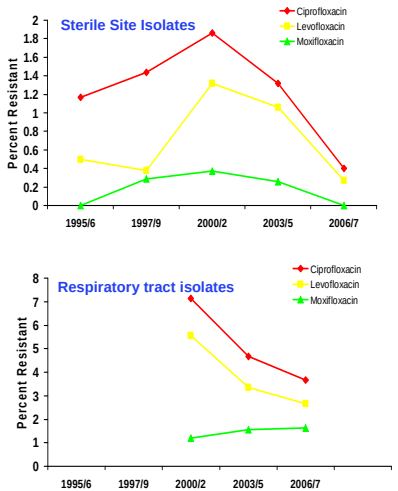
Per capita out-patient fluoroquinolone use during the study period is shown in Figure 1. Formulary restrictions in the provincial drug benefit formulary resulted in a decrease in ciprofloxacin use between 2000 and 2001. Total FQ use increased by 44% from 1995 to 2007.

Figure 1. Rate of outpatient fluoroquinolone prescriptions by year



During the surveillance period 5388 cases of invasive pneumococcal disease and 2634 patients with isolates from respiratory tract specimens were identifed. 86% (2267) of respiratory and 89% (4819) of sterile site isolates were available for susceptibility testing. Evolution of resistance over time is shown in Figure 2.

Figure 2. Resistance of pneumococci to fluoroquinolones over time, TIBDN



Results (cont'd)

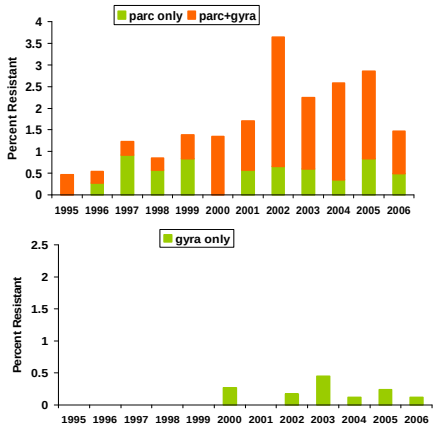
The prevalence of mutations in gyrA and parC by fluoroquinolone susceptibility is shown in Table 1. This table demonstrates that our sampling strategy likely missed no more than 2 isolates with gyrA mutations (2% of such isolates), and 20-30 parC mutations (about 15% of such isolates). Thus, these data can be used to examine the incidence of mutations over time in our isolates.

Table 1: Prevalence of QRDR mutations by fluoroquinolone susceptibility

Isolate susceptibility	Number sequenced/Total (1995-2006)	Number (%) with parC mutation	Number (%) with gyrA mutation
Levo MIC>1ug/ml or Moxi MIC>.25ug/ml	157/157 (100%)	124 (79%)	98 (62%)
Levo MIC<2 ug/ml and Moxi MIC<.5ug/ml	3331/7649 (44%)	20 (0.6%)	1 (0.03%)

Figure three shows the annual prevalence of parC and gyrA mutations in surveillance isolates. Use of ciprofloxacin and levofloxacin should result in primary mutations in parC; these isolates may then acquire secondary mutations in gyrA. Use of moxifloxacin should select for mutations in gyrA primarily. The first single gyrA mutation was detected prior to the introduction of moxifloxacin onto the market, and there has been no increase in the prevalence of strains with single gyrA mutations, despite increasing use of moxifloxacin over the last seven years.

Figure 3. Prevalence of isolates with QRDR mutations over time, TIBDN



Antibiotic histories were available for 7 of 9 patients with single gyrA mutations. In the three months prior to their infection, 4 had been exposed to levo, 2 had been exposed to cipro, and one had had no fluoroquinolone exposure. In patients who had been exposed to fluoroquinolones in the three months prior to their infection, those who had received moxifloxacin were somewhat less likely to have strains that contained QRDR mutations conferring resistance to fluoroquinolones (3/90, 3.3% of patients who had received moxi had mutations, compared to 27/425 6.4% of patients, OR 0.54, 95% CL 0.10, 1.8)

Conclusions:

Despite increasing use of fluoroquinolone antibiotics, both fluoroquinolone resistance and the prevalence of QRDR mutations conferring reduced MICs to fluoroquinolones are stable or decreasing in Toronto. Moxifloxacin appears to apply less selective pressure for fluoroquinolone resistance than other quinolone antibiotics.