Prior use of fluoroquinolones (FQ), fluoroquinolone resistance and *gyrA*/*parC* mutations in *Streptococcus pneumoniae* (SPN) in Ontario

A. McGeer[1]*, R. Melano[2], S. Patel[2], S. Drews[3], K. Green[1], D.E. Low[1], the Toronto Invasive Bacterial Diseases Network,

[1]Mount Sinai Hospital, Toronto, Ontario, Canada, [2]Ontario Agency for Health Promotion and Protection, Toronto, Ontario, Canada, [3]Provincial Laboratory for Public Health, Calgary, Alberta, Canada

Contact: Karen Green tel: 1.416.586.5105 kgreen@mtsinai.on.ca

P1370

Abstract (revised)

Objectives: Use of FQs is known to be associated with selection for FQ resistance (FQR) in SPN. We assess the extent to which prior FQ use was associated not only with increases in FQR but also with increases in *parC* and *gyrA* mutations in FQ susceptible SPN.

Methods: TIBDN performs population-based surveillance for invasive pneumococcal disease (IPD) in Toronto/Peel (pop. 4M); respiratory isolates are also collected. Prior antibiotic use is collected via chart review and patient/physician interview. Broth microdilution susceptibility testing to CLSI standards is performed. QRDR were sequenced for all FQR isolates (Cip mic>2ug/ml or Lev R or Moxi R), all SPN isolates from patients (PTs) with FQ exposure (FQEXP=any FQ in the prior 3 months), and a sample of other isolates from 2000 to 2007. Population FQ use was obtained from IMS Brogan.

Results: From 2000-2009, FQ use increased from 68-97 scripts/1000pop/yr; Levo from 0-9.3 scripts/1000pop/yr, Moxi from 0-18 scripts/1000pop/yr. 4171 respiratory isolates and 4795 IPD cases were identified. FQR decreased significantly from 2002 to 2007, then remained stable; Levo R for IPD was 1.1% (2002), 0.5% (2007), 0.8% (2010) and for respiratory isolates was 5.9% (2002), 1.9% (2007), 1.3% (2010). Overall, Moxi R was 0.49% (2002), 0.47% (2007), 0.46% (2010). Of 107 Levo R isolates with QRDR sequencing, 99 had mutations in gyrA and parC, 6 in gyrA only and 2 in par C only. Of the 2695 Levo S/I isolates sequenced, 3 had both *parC* and *gyrA* mutations, 55 a *parC* only mutation, 6 *gyrA* only and 2631 had no mutations. Complete data on prior FQ use was available for 5588 (69%) of adult cases. 948 (17%) adult PTs reported FQEXP in the previous three months. Of the 107 Levo R isolates, 50 (47%) occurred in PTs with FQEXP; while 23 (21%) occurred in PTs without FQEXP. FQEXP data was unknown for the remaining 34 (32%). Of the 55 Levo S/I isolates with QRDR mutations, 33 (60%) occurred in 533 isolates from PTs with FQEXP; and 22 (40%) from 1568 PTs without FQEXP (P<.001). Moxi represented none of FQEXP before 2002, and 42% in 2007/8. Among Levo S isolates, at least one mutation in QRDR was found in 18/203 (8.9%) from PTs with Cipro EXP, compared to mutations being identified in 7/209 (3.3%) isolates from PTs reporting Levo EXP and 4/94 (4.3%) isolates from PTs reporting Moxi EXP (P=.04).

Conclusions: Despite increased use of FQs, FQR decreases. Most FQ isolates with QRDR mutations are found in PTs with recent FQEXP. Exposure to more active FQs may be associated with reduced risk of development of QRDR mutations.

Background

Respiratory fluoroquinolones are recommended as first line monotherapy for community acquired pneumonia and invasive pneumococcal disease in adults. Exposure to fluoroquinolones in vivo and in vitro is known to select for strains with mutations in the QRDR regions of *gyrA* and *parC* genes which have increased MICs to fluoroquinolone antibiotics. We sequenced a sample of pneumococal isolates from population–based surveillance in a region of Ontario to determine the extent to which prior FQ use was associated with FQR and increases in *parC* and *gyrA* mutations in FQ susceptible isolates.

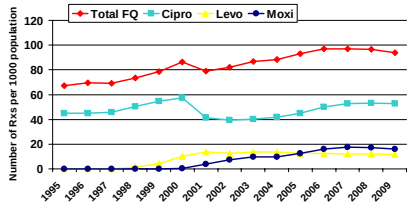
Methods

The Toronto Invasive Bacterial Diseases Network (TIBDN) performs-population based surveillance for invasive (IPD) and respiratory pneumococal disease in Toronto/Peel (pop. 4M). Details of previous antibiotic exposure has been collected since 2000. Prior antibiotic use is collected via chart review and patient/physician interview. Broth microdilution susceptibility testing to CLSI standards is performed. The quinolone resistance-determining regions (QRDR) of the *parC* and *gyrA* genes were sequenced for all FQR isolates (Cip mic>2ug/ml or Lev R or Moxi R), all SPN isolates from patients (PTs) with FQ exposure (FQEXP=any FQ in the prior 3 months), and a sample of other isolates from 2000 to 2007. The QRDR were amplified in standard conditions using following primers: for *parC* (forward: CGGTTCAACGCCGTATTCTT, reverse: ATCCCAGTCGAACCATTGAC; 394 bp), *gyrA* (forward: TGTTCACCGTCGCATTCTCT, reverse: ATACCAGTTGCTCCATTAACC, 393 bp). Amplicons were sequenced at Agencourt Bioscience Corporation (Beverly, MA). Multiple nucleotide sequences were performed with the ClustalW2 program (http://www.ebi.ac.uk/Tools/clustalw2/index.html).
Population FQ use was obtained from IMS Brogan.

Results

0utpatient fluoroquinolone use increased from 67 to 97 prescriptions/1000pop/year between 1995 and 2007, and has remained stable since (Figure 1). Restrictions in the provincial drug benefit formulary resulted in a decrease in ciprofloxacin use between 2000 and 2001; use has since increased to pre-restriction levels. In 2009, levofloxacin usage was 11.7 prescription/1000 pop., and moxifloxacin use was 16.9 prescriptions/ 1000 population.

Figure 1. Rate of outpatient fluoroquinolone prescriptions by year

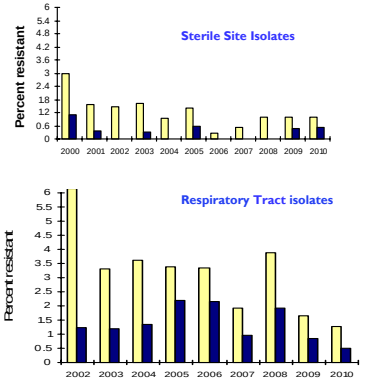


Between 2000 and 2010, there were 4798 cases of invasive pneumococcal disease (e.g. bacteremia, meningitis, empyema) and 4172 patients with respiratory isolates of *S. pneumoniae* reported to the study. 8226 of these 8970 isolates (91.7%) were available for susceptibility testing.

There were 1008 pediatric cases (914 IPD and 94 respiratory isolates). No isolates were resistant to any fluoroquinolone. Only three patients were known to have prior exposure to fluoroquinolones (1 each in 2008, 2009 and 2010), and only one of the 494 with QRDR sequenced had a mutation potentially affecting fluoroquinolone resistance: an Asn-Asp change at positiion 91 in *parC*.

Among the adult cases, levofloxacin resistance rates have decreased significantly since 2000 in both IPD cases (P=.06) and respiratory isolates (P<.001). Moxifloxacin resistance rates for all isolates remain low. In 2010, moxifloxacin resistance rates were 0.5% for both sterile site and respiratory isolates.

Figure 2. Levofloxacin (yellow) and moxifloxacin (blue) resistance in respiratory and sterile site isolates of *S. pneumoniae*

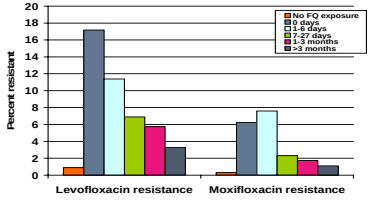


Results (con't)

Of the 6838 cases with data regarding antibiotic use at the time the culture yielding *S. pneumoniae* was taken, 113 (1.7%) were receiving a fluoroquinolon, including 58 of 3437 (1.7%) cases of IPD and 55 of 3401 (1.6%) patients with respiratory isolates. Of the 5588 cases with complete data on fluoroquinolone exposure in the prior 3 months, 948 (17%) had received a fluoroquinolone (436/2876 (15%) of IPD cases and 512/2712, (19%)) of patients with respiratory isolates.

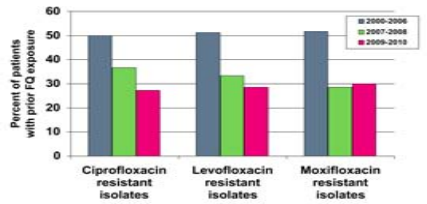
Rates of fluoroquinolone resistance were significantly higher in patients who had more recently been exposed to fluorquinolones; however, FQ exposure more than three months prior to infection was still associated with significantly higher rates of resistance (Figure 3).

Figure 3: Rates of levofloxacin and moxifloxacin resistance in patients with previous exposure to fluoroquinolones, by number of days from last dose of fluoroquinolone to date culture obtained



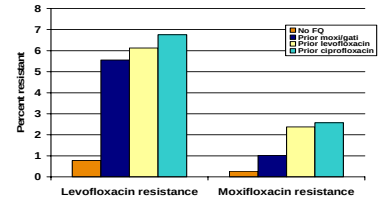
Among fluoroquinolone resistant isolates, the proportion that could be directly attributable to prior use in that individual decreased over time, from approximately 50% in 2000-2006 to about 30% in 2007-2010 (Figure 4).

Figure 4. Proportion of fluoroquinolone resistant isolates attributable to recent use of fluoroquinolone in the same individual, over time



There are no statistically significant differences between levofloxacin and moxifloxacin resistance rates based on the specific fluoroquinolone to which the patient had been exposed.

Figure 5: Rates of levofloxacin and moxifloxacin resistance in patients with previous exposure to fluoroquinolones, by type of FQ exposure



Results (con't)

For episodes of disease occurring between 2000 and 2007, sequencing of QRDR regions was conducted on all FQR isolates, all isolates from patients exposed to fluoroquinolones, and a representative sample of other isolates. 2802/5872 (48%) of identified cases had sequencing of their QRDR regions. The prevalence of mutations in *gyrA* and *parC* by fluoroquinolone susceptibility is shown in Table 1.

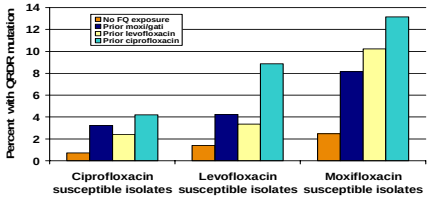
Table 1: Prevalence of QRDR mutations by fluoroquinolone susceptibility

Isolate susceptibility	Number (%) wild type	Number (%) with *parC* mutation only	Number (%) with *gyrA* mutation only	Number (%) with both *parC* and *gyrA* mutations
Cipro resistant	7 (4.8%)	28 (19%)	8 (5.5%)	102 (70%)
Levo resistant	1(0.9%)	1 (0.9%)	6 (5.6%)	99 (93%)
Moxi resistant	0	0	1 (2.4%)	41 (98%)
Cipro susceptible	2625 (99%)	28 (1.1%)	4 (0.3%)	0
Levo susceptible	2631 (98%)	55 (2.0%)	6 (0.2%)	3 (0.1%)
Moxi susceptible	2632 (95%)	56 (2.0%)	11 (0.4%)	61 (2.2%)

As expected, patients failing ciprofloxacin were somewhat more likely to have an isolate with a mutation in *parC* only than patients failing levofloxacin or moxifloxacin (16/219 (7.3%) of ciprofloxacin failures with *parC* only mutation, compared to 4/207 (2.0%) for levofloxacin and 2/96 (2.1%) for moxifloxacin failures, P=.01).

Among fluoroquinolone susceptible isolates with QRDR sequencing performed, patients with prior exposure to fluoroquinolones were significantly more likely to have isolates with mutations in their QRDR regions. Isolates from patients with levofloxacin susceptible isolates who were exposed to ciprofloxacin were more likely to have a QRDR mutation than isolates from patients exposed to levofloaxcin or moxifloxacin (P=.04).

Figure 6: Proportion of isolates with at least one mutation conferring decreased susceptibilty to fluoroquinolones among fluoroquinolone susceptible isolates, by exposure to different fluoroquinolones



Conclusions

- Despite increasing use of fluorquinolones, resistance to fluoroquinolones in *S. pneumoniae* has been decreasing.
- In our population, no fluoroquinolone resistance has occurred in pediatric pneumococcal isolates.
- Moxifloxacin remains the most active fluoroquinolone. Overall resistance to moxifloxacin remains less than 1%.
- Recent fluoroquinolone exposure is associated with a significantly increased risk of fluoroquinolone resistance; and the more recent the exposure, the greater the increased risk of resistance.
- There is evidence that more active fluoroquinolones are less likely to be associated with selection for QRDR mutations than less active fluoroquinolones.

Acknowledgements

We are grateful to the TIBDN investigator and staff, to the many patients and physicians who contribute to TIBDN network surveillance, and to Bayer Schering Pharma for financial support for surveillance and QRDR sequencing.