Interactions between serotype and mutations in quinolone-resistance determining regions of *Streptococcus pneumoniae*

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Abstract (revised)

Background: Mutations in QRDR regions are the primary cause of fluoroquinolone resistance (FQR) in *S. pneumoniae* (SPN). It is not known whether the distribution of mutations and/or the association of QRDR with increasing MIC is similar in different SPN serotypes (STs).

Methods: The Canadian Bacterial Surveillance Network (CBSN) has been monitoring trends in antimicrobial resistance in SPN in Canada. From 1995-2006, 27,951 isolates from across Canada have been collected. Serotyping and sequencing of the *gyrA* and *parC* regions was performed for all FQR isolates FQ (N=451), and a sample of others (N=3438).

Results: The most common parC mutations were: K137N (N=870), S79F (293), S79Y (106), D83N (36), D83Y (17), D83G (9), S52G (6), N91D (6), R95C (5), Y129S (5), D78N (4), S79A (2), A115P (2). 14 other mutations were identified once. S79F, S79Y, D83N/Y/G, D78N/A, and A115P were associated with increased MICs to FQ; other mutations were not. K137N was present in <5% of isolates of SGs 3,4,10,11,15,18,20,22,33,35,38, >85% of isolates of SGs 12 and 9, and an intermediate percentage of other SGs. Among FQR, S79Y is significantly more common in isolates of ST 22F and 6A, and mutations at position 83 are more common in ST 9N and 9V (P<.01). Of isolates with parC mutations, those of ST 12F had significantly lower MICs to all FQ than those of other STs. 27% of FQS SPN with a parC mutation at position 79 were ST 12F, compared to 3% of FQR isolates. The most common gyrA mutations were S81F (N=179), S81Y (33), E85K (33), S114G (14), E85G (4). 12 other mutations were identified once. Only mutations at position 81 and 85 were associated with increased FQ MICs. The prevalence of gyrA mutations in the presence of parC mutations varied significantly with ST; eg. 10/12 (83%) 19A and 32/40 (80%) 19F strains with a parC mutation also had a gyrA mutation, compared to 9/24 (38%) ST 3 and 6/17 (35%) ST 12F (P<.001). When isolates were categorized by whether or not their serotypes were included in 7- or 23-valent pneumococcal vaccines, there were no significant differences in the prevalence of parC mutations. Isolates of STs in the 23-valent vaccine with a parC mutation were somewhat less likely to have a gyrA mutation than other isolates (66% vs 55%, P=.07).

Conclusion: Mutations in QRDR determining regions occur with differing frequencies in isolates of different ST. Changing ST distributions associated with pneumococcal vaccination programs may have an impact on FQR rates.

Introduction:

Respiratory fluoroquinolones are recommended as first line monotherapy for community acquired pneumonia and invasive pneumococcal disease in adults. Exposure to fluoroquinolones *in vitro* and *in vivo* is known to select for strains with mutations in the QRDR region of gyrA and parC genes which have increased MICs to fluoroquinolone antibiotics.

Resistance to fluoroquinolones in pneumococci was first identified in Canada in the mid 1990s, a few year after the introduction of ciprofloxacin. Rates of resistance increased from 1995 to 2000, but have since been stable or decreasing, despite increasing use of fluoroquinolone antibiotics.

We hypothesize that the stabilization of resistance to fluoroquinolone antibiotics has occurred because the preferential use of more active respiratory fluoroquinolones for the treatment of respiratory tract infections reduces selective pressure for resistance. However, there have been a number of other changes in the epidemiology of pneumococcal disease in the last decade. The greatest change in Canada has been the introduction of public vaccination programs for the 23-valent polysaccharide pneumococcal vaccine for adults, and the 7-valent conjugate vaccine for children. If different serotypes are more or less likely to develop mutations in QRDR regions, then the change in serotype distribution of pneumococcal infections associated with the introduction of vaccination programs might affect fluoroquinolone resistance rates.

We sequenced a sample of pneumococcal isolates from a Canadian surveillance program to assess the extent to mutations in the QRDR region parC and gyrA are different in different pneumococcal serotypes.

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Methods:

CBSN is a collaborative group of hospital and private microbiology laboratories from all 13 provinces and territories of Canada. Each year, laboratories, based on their size, are asked to submit either 20 or 100 consecutive isolates of *S. pneumoniae* from all sites, as well as all sterile site isolates for the year. Only one isolate per patient is included. A total of 186 laboratories have participated since 1993, including 19 private laboratories and 164 laboratories serving hospitals ranging in size from 18 to 1325 beds. Participation is voluntary, and varies from year to year; however, a representative core group of 50 laboratories have submitted for the entire surveillance period. Since 1995, approximately 30% of the isolates have been derived from 19 laboratories collaborating in population-based surveillance for pneumococcal disease in south-central Ontario (TIBDN: the Toronto Invasive Bacterial Diseases Network).

Isolates are shipped to the central study laboratory at the Mount Sinai Hospital, where they are confirmed as *S. pneumoniae* and frozen. Broth microdilution susceptibility testing is performed and interpreted using CLSI standards. Serotyping is performed on all isolates from TIBDN, all isolates resistant to fluoroquinolones, penicillins or trimethoprim-sulfamethoxazole, and a sample of other isolates.

For this study, the QRDR regions of parC and gyrA genes were sequenced for all FQ resistant isolates, all TIBDN sterile site isolates obtained after 1997, and a sample of other isolates.

Results:

From 1995 to June 2006, 27,951 isolates of *S. pneumoniae* were collected in this surveillance system. Of these, 9483 were serotyped. Sequencing of the QRDR region was performed for 4319 isolates.

Among these 4319 isolates, the QRDR mutations identified, and their impact on MICs to fluoroquinolone antibiotics are shown in Tables 1 and 2. Isolates with a parC mutation at position 79 were more likely than those with a mutation at position 83 to also have a gyrA mutation (210/372, 56% vs. 12/51, 24%, P<.0001)

Table 1: Prevalence of QRDR mutations by fluoroquinolone MIC

	Mutations (N, %) in:	
	ParC	GyrA
Moxifloxacin MIC <0.5ug/ml and Levofloxacin MIC<2ug/ml	188/4027 (4.6%)*	2/4027 (0.5%)
Moxifloxacin MIC>=0.5ug/ml or Levofloxacin MIC>=2ug/ml	268/292 (92%)	253/292 (87%)

*Of the 188, 114 (61%) were non-susceptible to ciprofloxacin

Table 2: Effect of QRDR mutations on fluoroquinolone MICs

	Mutation effect on fluoroquinolone MIC		
	Increased	No change	Uncertain
parC	S-79-F (N=293), -Y (N=106) D-83-N (N=36), -Y (N-17), D-83 -G (N=9) D-83-A,H,V (N=1 each) D-78-N (N=4), -A (N=1) G-128-S (N=1)	K137N (N=870) R95C (N=5) Y129S (N=5) E100A (N=2) M85I, E99AG106S, S107F, M108I,G128S,L130F (N=1 ea)	S52G (N=6) N91D (N=6) S79A (N=2) S79I (N=1) T138I (N=1)
gyrA	S-81-F (N=179), -Y (N=33), S-81-C,A,L (N=1 each) E-85-K (N=33), -G (N=4) E-85-A,D (N=1 each)	S114G (N=14) P162? (N=2) T56I,R97C,M99I,M132I (N=1 each)	D-80-A (N=1)

Results (cont'd)

The distribution of common mutations in parC varies by serotype (Table 3). Isolates with mutations only in gyrA were uncommon (N=17): they occurred in 9 different serotypes, with no one serotype represented more than twice. In isolates with parC mutations, gyrA mutations were more common in isolates of serogroup 19 (Table 4).

Isolates of conjugate vaccine serotypes were somewhat less likely to have mutations associated with reduced susceptibility to fluoroquinolones.

Table 3: Prevalence of parC mutations by serotype

Serotype	No strains typed and sequenced	No (%) K137N	No (%) S79F	No (%) S97Y	No (%) D83N/Y
All	3873	680 (18%)	262 (6.8%)	93 (2.4%)	54 (1.4%)
11A	131	1 (0.8%)	**23 (18%)**	5 (3.8%)	4 (3.1%)
12F	102	**97 (95%)**	**25 (25%)**	0	0
14	569	51 (8.9%)	28 (4.9%)	2 (0.4%)	5 (0.9%)
15A,15B,15C	90	3 (3.3%)	12 (13%)	3 (3.3%)	2 (2.2%)
18C	159	1 (0.6%)	3 (1.9%)	1 (0.6%)	0
19A	118	11 (9.3%)	10 (8.5%)	1 (0.9%)	1 (0.9%)
19F	303	17 (5.6%)	26 (8.9%)	8 (2.6%)	5 (1.7%)
22F	180	0	6 (3.3%)	9 (5%)	2 (1.1%)
23F	260	38 (15%)	32 (12%)	9 (3.5%)	3 (1.1%)
3	294	3 (1.0%)	10 (3.4%)	9 (3.1%)	5 (1.7%)
4	253	3 (1.2%)	*2 (0.8%)*	1 (0.4%)	2 (0.8%)
6A	190	27 (14%)	13 (6.8%)	**11 (5.8%)**	1 (0.5%)
6B	324	13 (4.0%)	21 (6.5%)	7 (2.2%)	5 (1.5%)
7F	107	**52 (49%)**	1 (0.9%)	1 (0.9%)	1 (0.9%)
9A,9L,9N,9V	335	**298 (89%)**	21 (6.3%)	8 (2.3%)	**12 (3.6%)**
PPV23 type	3271	529 (18%)	215 (6.6%)	*63 (1.9%)*	47 (1.4%)
PCV7 type	2104	*328 (16%)*	126 (5.9%)	*34 (1.6%)*	27 (1.3%)

Table 4: Prevalence of gyrA mutations in strains with parC mutations, by serotype

Serotype	Number strains with parC mutation	Number (%) of those with mutation in gyrA
All	398	228 (57%)
12F	24	6 (25%)
14	31	**18 (58%)**
19A	12	10 (83%)
19F	40	32 (80%)
22F	17	6 (35%)
3	23	9 (39%)
19F	303	17 (5.6%)
22F	180	0
PPV23 type	316	174 (55%)
PCV7 type	180	*112 (62%)*

Conclusions:

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