

1 **Tracking Longitudinally Fluoroquinolone Resistance and their determinants in**
2 **Penicillin susceptible and non-susceptible *Streptococcus pneumoniae* in Hong Kong, 2000**
3 **to 2005.**

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20 *Running title: Fluoroquinolone Resistance in Streptococcus pneumoniae in Hong Kong*

1 **ABSTRACT**

2 10.5% of 1388 *Streptococcus pneumoniae* (SPNE) isolated during 2000-2005 had
3 ciprofloxacin MIC \geq 4.0 μ g/ml, and levofloxacin MIC \geq 4.0 μ g/ml of 1.6% (0.8%-4.3%).
4 Molecular characterization indicated fluoroquinolone resistance occurred independently in
5 our prevalent Spain^{23F}-1 clone, expressing 23F, 19F and 14. Resistance rates to levofloxacin
6 in SPNE have remained stable at a Hong Kong hospital.

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1 **TEXT**

2 Drug-resistant *Streptococcus pneumoniae* (SPNE) remains a major concern in Hong
3 Kong. Fluoroquinolone resistance in SPNE had been reported as one of highest in the world,
4 with a levofloxacin resistance rate (MIC $\geq 4\mu\text{g/ml}$) of 13.3% [7]. Among invasive isolates, a
5 resistance rate of 3.8% was documented, although it increased to 15.2% in PRSP[8]. These
6 strains had been linked to the penicillin-nonsusceptible pneumococcal (PNSP) clones [8,16].
7 In Canada, ciprofloxacin-resistant SPNE increased from $<1\%$ in 1997 to 4.2% in 2005, with a
8 rise in levofloxacin resistance rate to 1.1% [1]. In USA, levofloxacin resistance increased
9 from 0.6% to 1.3% [18]. Among those with levofloxacin MIC $2\mu\text{g/ml}$, first step mutations
10 were present in 16.2% isolates recovered from nursing home residents compared to 6.4%
11 from others [17]. Such strains had been considered precursors of fully resistant strains [3, 21].
12 Treatment failure with fluoroquinolones had been reported due to selection with second step
13 mutation [5, 14]

14 At the Prince of Wales Hospital (PWH), a 1350-bed teaching hospital in Hong Kong, we
15 tracked longitudinally the prevalence of fluoroquinolone resistance in SPNE for the period
16 2000 to 2005. The clonal relationship of SPNE isolates with ciprofloxacin MIC $\geq 4\mu\text{g/ml}$ by
17 serotyping, pulsed field gel electrophoresis (PFGE), and the mutations at the quinolone
18 resistance determining region (QRDR) of *gyrA*, *B*, *parC* and *E* genes were studied.

19 **Bacterial Isolates and Susceptibility Tests.** 1388 non-duplicate SPNE isolated from blood
20 and sputa of patients admitted to PWH were examined. The MICs of penicillin, cefotaxime,
21 ciprofloxacin, and levofloxacin were determined by broth dilution method described by
22 Clinical and Laboratory Standards Institute [2].

23 **PFGE and Serotyping.** 108 isolates with MIC to ciprofloxacin $\geq 4\mu\text{g/ml}$ were examined by
24 PFGE, as described [6,11]. Serotyping was performed using Pneumotest antisera (Statens
25 Serum Institute, Copenhagen, Denmark). Representative isolates of Spain^{23F}-1 clone which
26 produced indistinguishable PFGE pattern type A were included. Other PFGE types/subtypes

1 were assigned as for Tenover *et al.* [23].

2 **Analysis of Fluoroquinolone Resistance Genes.** The mutations in the QRDRs of *gyrA*, *gyrB*,
3 *parC* and *parE* genes were examined by PCR-RF-SSCP, as described [13]. Some of these a.a.
4 substitutions had previously been published [13].

5 The overall levofloxacin resistance rate was 1.6% (ranged 0.8% to 4.3%) (Table 1). The
6 mean percentage of isolates with ciprofloxacin MIC $\geq 4.0\mu\text{g/ml}$ was 10.5% (ranged 7.4% to
7 24.5%). Between 2001-2005, 55.6% (720/1294) isolates were PNSP (ranged 49.2%-64.5% in
8 the five years). The percentages of ciprofloxacin MIC $\geq 4.0\mu\text{g/ml}$ isolates which were
9 penicillin-susceptible and penicillin-non-susceptible were 10% (58/579) and 11.2% (91/809)
10 respectively, whilst for levofloxacin resistance, the rates were 0.5% and 2.2% respectively.
11 The MIC₅₀ and MIC₉₀ of levofloxacin in both penicillin-susceptible and PNSP were 1.0 and
12 2.0 $\mu\text{g/ml}$ respectively. The MIC₅₀ of ciprofloxacin was 1.0 $\mu\text{g/ml}$ in penicillin-susceptible but
13 2.0 $\mu\text{g/ml}$ in PNSP, whilst the MIC₉₀ were both at 4.0 $\mu\text{g/ml}$.

14 The characteristics of SPNE with ciprofloxacin MIC $\geq 4.0\mu\text{g/ml}$ are listed in Table 2. The
15 commonest serotype was 23F (35 isolates), followed by 19F (25 isolates). Other serotypes
16 included 4, 6B, 6(non-B), 8, 9, 14, 17, 19(non-F) and 22, and the remainder nontypable. The
17 commonest PFGE pattern type A, which was indistinguishable to isolates of Spain^{23F}-1 clone,
18 was present in 46 of 108 isolates. The majority of these isolates expressed capsular types 23F
19 or 19F, were penicillin-nonsusceptible, either susceptible or intermediate to cefotaxime, and
20 have levofloxacin MICs ranging 0.5 to $>8.0\mu\text{g/ml}$. All but 3 isolates with a.a. substitution at
21 *gyrA* gene (S81F) were isolates with PFGE type A pattern. These isolates had one or more
22 mutations at the *parC* gene (S79F, D83N, and/or K137N) and a mutation at the *parE* gene
23 (I460V). Of the remaining isolates belonging to PFGE type A that did not have a.a.
24 substitution at GyrA, 36/39 isolates already had a.a. substitution at ParC (K137N). Overall,
25 7.2% (63/881) SPNE from 2000 – 2003 with ciprofloxacin MIC $\geq 4.0\mu\text{g/ml}$ had one or more
26 a.a. substitution(s) at GyrA and/or ParC, whilst 6.0% (52/870) SPNE had an a.a. substitution

1 at ParC alone. Besides PFGE type A, other PFGE patterns were mostly distinct and diverse
2 (PFGE patterns not shown). These isolates mainly had a single ParE a.a. substitution (I460V).

3 PNSP rates rose rapidly in Hong Kong in 1990s [10,12] and reached 61% by 2000 [7].
4 A gradual reduction in PNSP was noted since 2003. The pneumococcal conjugate vaccine was
5 not made available in Hong Kong until mid-2006, and thus unlikely due to its effect.
6 Levofloxacin resistance rates remained low at PWH, contrary to high rates previously
7 reported in other Hong Kong hospitals [7]. Levofloxacin-resistant SPNE had been associated
8 with nosocomial origin, older patients ≥ 75 yrs old with chronic obstructive airway disease,
9 nursing home residents and prior fluoroquinolones [9]. A study on the susceptibility of
10 community SPNE isolates from Hong Kong in 2003 yielded 0% resistance to respiratory
11 fluoroquinolones [15], indicating that levofloxacin-resistant SPNE are likely confined to
12 specific risk groups. High levofloxacin-resistance rates might have been partially contributed
13 by varying dose regimens using 100mg-levofloxacin tablets that was used locally.

14 The majority of levofloxacin-resistant SPNE originated from Spain^{23F}-1 clone; but
15 expressed varying capsular types [8,16]. Penicillin-susceptible fluoroquinolone resistant
16 strains were also noted. With the heterogeneity on the mutations at the QRDRs, it is likely
17 fluoroquinolone resistance occurred independently and less from dissemination of a
18 fluoroquinolone-resistant variant [7]. An estimated mutation rate to fluoroquinolone resistance
19 in Hong Kong was 2.9% (2 in 70) [16] and appeared compatible in our setting. However,
20 levofloxacin-susceptible isolates of Spain^{23F}-1 clone with a ParC a.a. substitution (K137N)
21 and a ParE a.a. substitution (I460V) are not infrequently seen in this study, and may only
22 require a second step mutation to become resistant [4,22]. These strains may not be readily
23 detected by current susceptibility breakpoints to respiratory fluoroquinolones [19,20] and will
24 necessitate detection of these mutations. Adequate fluoroquinolone doses to achieve high
25 AUC/MIC breakpoints for levofloxacin (200) and moxifloxacin (400) had been suggested to
26 ensure eradication of SPNE [3].

1 Fluoroquinolone resistance is likely to have occurred independently, in our prevalent
2 Spain^{23F}-1-23F,-19F, and -14 clones. Resistance rates to levofloxacin in SPNE have remained
3 stable at a Hong Kong teaching hospital in the last six years.
4

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8

9 **References**

- 10 [1] **Adam, H. J., K. N. Schurek, K. A. Nichol, C. J. Hoban, T. J. Baudry, N. M. Laing, D.**
11 **J. Hoban, and G. G. Zhanel.** 2007. Molecular characterization of increasing fluoroquinolone
12 resistance in *Streptococcus pneumoniae* in Canada, 1997-2005. *Antimicrob. Agents*
13 *Chemother.* **51**: 198-207.
- 14 [2] Clinical Laboratory Standards Institute. Performance standards for antimicrobial
15 susceptibility testing, M100-S14. 2004. Clinical Laboratory Standards Institute, Wayne, Pa.
16 (previously known as, National Committee for Clinical Laboratory Standards).
- 17 [3] **DeRyke, C. A., X. Du, and D. P. Nicolau.** 2006. Evaluation of bacterial kill when
18 modeling the bronchopulmonary pharmacokinetic profile of moxifloxacin an levofloxacin
19 against *parC*-containing isolates of *Streptococcus pneumoniae*. *J. Antimicrob. Chemother.* **58**:
20 601-609.
- 21 [4] **Doern, G.V., S. S. Richter, A. Miller, N. Miller, C. Rice, K. Heilmann and S.**
22 **Beekmann.** 2005. Antimicrobial resistance among *Streptococcus pneumoniae* in the United
23 States: Have we begun to turn the corner on resistance to certain antimicrobial classes? *Clin.*
24 *Infect. Dis.* **41**:139-148.
- 25 [5] **Fuller, J.D., and D.E. Low.** 2005. A review of *Streptococcus pneumoniae* infection
26 treatment failures associated with fluoroquinolone resistance. *Clin. Infect. Dis.* **41**:118-21.

- 1 [6] **Hall, L. M. C., R. A. Whiley, B. Duke, R C. George, and A. Efstratiou.** 1996. Genetic
2 relatedness within and between serotypes of *Streptococcus pneumoniae* from the United
3 Kingdom: analysis of multilocus enzyme electrophoresis, pulsed-field gel electrophoresis, and
4 antimicrobial resistance patterns. *J. Clin. Microbiol.* **34**:853-859.
- 5 [7] **Ho, P.L., R. W. Yung, D. N. Tsang, T. L. Que, M. Ho, W. H. Seto, T. K. Ng, W. C. Yam,**
6 **and W. W. Ng.** 2001. Increasing resistance of *Streptococcus pneumoniae* to fluoroquinolones:
7 results of a Hong Kong multicentre study in 2000. *J. Antimicrob. Chemother.* **48**:659-65.
- 8 [8] **Ho, P. L., W. C. Yam, T. K. Cheung, W. W. Ng, T. L. Que, D. N. Tsang, T. K. Ng, and**
9 **W H. Seto.** 2001. Fluoroquinolone resistance among *Streptococcus pneumoniae* in Hong
10 Kong linked to the Spanish 23F clone. *Emerg. Infect. Dis.* **7**:906-8.
- 11 [9] **Ho, P.L., W. S. Tse, K. W. T. Tsang, T. K. Kwok, T. K. Ng, V. C. C. Cheng, and R. M.**
12 **T. Chan.** 2001. Risk factors for acquisition of levofloxacin-resistant *Streptococcus*
13 *pneumoniae*: a Case-Control Study. *Clin. Infect. Dis.* **32**:701-7.
- 14 [10] **Ip, M., D. J. Lyon, and A. F. Cheng.** 2001. Patterns of antibiotic resistance, serotype
15 distribution, and patient demographics of *Streptococcus pneumoniae* in Hong Kong. *Chemother.*
16 **47**:110-116.
- 17 [11] **Ip, M., D. J. Lyon , R.W. Yung, C. Chan, and A. F. Cheng.** 1999. Evidence of clonal
18 dissemination of multidrug-resistant *Streptococcus pneumoniae* in Hong Kong. *J. Clin.*
19 *Microbiol.* **37**:2834-9.
- 20 [12] **Ip, M., D. J. Lyon, R. W. Yung, L. Tsang, and A. F. Cheng.** 2002. Introduction of new
21 clones of penicillin-nonsusceptible *Streptococcus pneumoniae* in Hong Kong. *J. Clin.*
22 *Microbiol.* **40**:1522-5.
- 23 [13] **Ip, M., S. S. L. Chau, F. Chi, A. Qi, and R. W. M. Lai.** 2006. Rapid Screening of
24 Fluoroquinolone-Resistance Determinants in *Streptococcus pneumoniae* by PCR-Restriction
25 Fragment and Single-Strand Conformational Polymorphism. *J. Clin. Microbiol.* **44**:970-975.
- 26 [14] **Kays, M. B., G. G. Zhanel, M. A. Reimann, J. Jacobi, G. A. Denys, D. W. Smith, and**

- 1 **M F. Wack.** 2007. Selection of a *gyrA* mutation and treatment failure with gatifloxacin in a
2 patient with *Streptococcus pneumoniae* with a preexisting *parC* Mutation. *Pharmacother.*
3 **27:221-6.**
- 4 [15] **Ling, J. M., A. W. Lam, E. W. Chan and A. F. Cheng.** 2003. What have we learnt from
5 community-acquired infections in Hong Kong? *J. Antimicrob. Chemother.* **51:895-904.**
- 6 [16] **Morrissey, I., D. J. Farrell, S. Bakker, S. Buckridge, and D. Felmingham.** 2003.
7 Molecular characterization and antimicrobial susceptibility of fluoroquinolone-resistant or
8 susceptible *Streptococcus pneumoniae* from Hong Kong. *Antimicrob. Agents Chemother.*
9 **47:1433-1435.**
- 10 [17] **Pletz, M. W. R., A. P. Shergill, L. McGee, B. Beall, C. G. Whitney, and K. P.**
11 **Klugman,** for the Active Bacterial Core Surveillance Team. 2006. Prevalence of first-step
12 mutants among levofloxacin-susceptible invasive isolates of *Streptococcus pneumoniae* in the
13 United States *Antimicrob. Agents Chemother.* **50:1561-1563.**
- 14 [18] **Richter, S. S., K. P. Heilmann, S. E. Beekmann, N. J. Miller, C. L. Rice, and G. V.**
15 **Doern.** 2005. The molecular epidemiology of *Streptococcus pneumoniae* with quinolone
16 resistance mutations. *Clin. Infect. Dis.* **40:225-35.**
- 17 [19] **Schurek, K. N., H. J. Adam, D. J. Hoban, and G. G. Zhanel.** 2006. Call for the
18 international adoption of microbiological breakpoints for fluoroquinolones and *Streptococcus*
19 *pneumoniae*. *Int. J. Antimicrob. Agents* **28:266-269.**
- 20 [20] **Schurek, K. N., H. J. Adam, C. G. Siemens, C. J. Hoban, D. J. Hoban, and G. G.**
21 **Zhanel.** 2005. Are fluoroquinolone-susceptible isolates of *Streptococcus pneumoniae* really
22 susceptible? A comparison of resistance mechanisms in Canadian isolates from 1997 and
23 2003. *J. Antimicrob. Chemother.* **56:769-772.**
- 24 [21] **Smith, H. J., M. Walters, T. Hisanaga, G. G. Zhanel, and D. J. Hoban.** 2004. Mutant
25 prevention concentrations for single-step fluoroquinolone-resistant mutants of wild-type,

1 efflux-positive, or parC or gyrA mutation-containing *Streptococcus pneumoniae* isolates.
2 Antimicrob. Agents Chemother. **48**:3954-8.

3 [22] Sun, H. K., X. Du, C. A. DeRyke, G. V. Doern, and D. P. Nicolau. 2007. parE
4 mutations in *Streptococcus pneumoniae* may reduce bactericidal activity of respiratory
5 fluoroquinolones. Int. J. Antimicrob. Agents. **29**:223-232.

6 [23] Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H.
7 Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns
8 produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin.
9 Microbiol. **33**:2233-9.

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1 **Table 1 Percentage of Fluoroquinolone Resistance in *Streptococcus pneumoniae* at the**
 2 **Prince of Wales Hospital, 2000-2005.**

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Year	No. of Isolates (N=1388)	% Levofloxacin MIC \geq 4.0 μ g/ml (No. of Isolates)	% Ciprofloxacin MIC \geq 4.0 μ g/ml (No. of Isolates)	% Penicillin MIC \geq 0.12 μ g/ml (No. of isolates)
2000	94	4.3% (4)	24.5% (23)	-* (89)
2001	195	3.0% (6)	14.7% (29)	63.6% (124)
2002	336	0.9% (3)	7.7% (25)	64.5% (217)
2003	256	1.2% (3)	12.4% (31)	49.2% (126)
2004	242	1.7% (4)	7.4% (18)	46.7% (113)
2005	265	0.8% (2)	7.5% (20)	52.8% (140)

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* Mainly PNSP isolates from sputum specimens were included.

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1 **Table 2. Characteristics of *Streptococcus pneumoniae* with CIP ≥ 4.0 $\mu\text{g/ml}$ in relation to the PFGE types, Serotypes, Antibiotic**
 2 **Susceptibilities and Amino acid substitutions at QRDR regions of Fluoroquinolone resistance determinants.**
 3

Gene with Amino Acid Substitution	YEAR				gyrA		parC				parE	No. of Isolates, N=108	PFGE Type		SEROTYPE					CIP [†]		LEV*				PEN*			CTX*			
	2000	2001	2002	2003	S81	S494	S79	D83	E96	K137			I460	A	Other	23F	6B	19F	14	others	4	≥ 8	0.5-1	2	4	≥ 8	≤ 0.06	0.12-1	≥ 2	≤ 0.5	1	≥ 2
	None	2	1	1										4	1	3				1	3	4	2	1	3			3	1		3	1
ParE	3	11	8	19						V	41	2	39	1	1	6	1	32	39	2	16	23	1	1	27	7	7	31	8	2		
ParC ParE			2				F			V	2	2		1	1				2		2			2			1		1			
	16	13	10	7			Y			V	1	1			1				1	5	21	23			1			1		2		
			1	1			N			V	46	34	6	27		12		7	41	1	2			2	5	29	13	14	30	2		
							N			V	2	1	1	1			1		1	1		2		1	1		1	1		1		
ParC	1						N			V	1	1			1				1		1						1		1			
GyrA ParE		1				F				V	1		1			1			1				1		1				1			
GyrA ParC ParE			1	1		F	F			V	2		2			2			2				2		2			2		2		
	1	1		1		F				N	3	3		3					3			3		3	2	1		3				
			1			F	F			N	2	2		1		1			2			2		2		2			2			
						F		N		N	1	1		1					1			1		1		1			1			
				1		F	F	D		N	1	1		1		1			1			1		1	1			1		1		
All	1				F	T	N			N	1	1		1					1			1		1	1			1		1		

4 *MIC values ($\mu\text{g/ml}$) for CIP ciprofloxacin, LEV levofloxacin, PEN penicillin, CTX cefotaxime. NT non typeable using the Pneumotest antisera (Statens SerumInstitute, Copenhagen),
 5 Amino acids D = Asp, E=Glu, F = Phe, I=Ile, K=Lys, N= Asn, S=Ser, T= Thr, V = Val, Y = Tyr.