L-945

Activity of Gemifloxacin Tested Against Historical and Contemporary Isolates of *Neisseria gonorrhoeae* (NG) Including Fluoroquinolone-Resistant Strains (QRNG)

DJ BIEDENBACH, L DESHPANDE, G TILLOTSON, RN JONES
JMI Laboratories, North Liberty, IA; Oscient Pharmaceuticals, Waltham, MA

ICAAC 2005
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

AMENDED ABSTRACT

Background: Uncomplicated *Neisseria gonorrhoeae* (NG) infections have routinely been treated with single-dose fluoroquinolones (FQ), and oral or parenteral cephalosporins. However, escalating occurrence of ciprofloxacin (CIP)-resistant (MIC, ≥ 1 µg/ml) NG has compromised STD therapy and promoted the search for more active FQs or alternative agents. Gemifloxacin (GEMI), a proven potent FQ, was re-evaluated as a candidate for QRNG therapy.
Methods: 250 NG strains were tested by CLSI agar dilution methods. 50 QRNG from the Far East, Europe and USA (1990s) were compared to 47 QRNG strains isolated in 2004 (6 USA states plus blood cultures; 24). Other R phenotypes were: Pencillinase-producing (PPNG; 28), penicillin (PEN)-intermediate (I)/R (158)/78, tetracycline-R (TET-R; 59) and QRNG (97). The higher FQ MIC values were associated with QRDR mutations of *gyrA* (Ser91Phe, Asp95Asn or Gly) and *parC* (Ser88Phe, Glu91Gly). PEN, TET, CIP, levofloxacin (LEV) and ceftriaxone (CTX) were comparator agents.
Results: GEMI was 4-fold more active than CIP or LEV against a collection that had 20.0% QRNG, 31.2% PEN-R, and 23.6% TET-R. The CTX MIC_{90} was 0.06 µg/ml (100.0% S). Elevated QRNG rates were detected in Hawaii (64.0%), Washington (32.9%), California (20.0%) and Oregon (13.3%). Co-R trends were found for FQs, TET and PEN. QRNG rates are increasing in the USA and isolates have also progressed from I to fully R levels in the last decade, similar to that in the Far East.

S patterns		GEMI MIC (µg/ml)		
CIP	PEN (no.)	50%	90%	Range
S	S (12)	≤0.008	≤0.008	≤0.008-0.016
	I (117)	≤0.008	0.016	≤0.008-0.016
	R (24)	≤0.008	0.016	≤0.008-0.016
I	S (2)	0.06	-	0.06
	I (11)	0.06	0.06	0.03-0.06
	R (34)	0.06	0.25	0.016-0.25
R	S (0)	-	-	-
	I (30)	1	1	0.06-2
	R (20)	1	2	0.12-2

Conclusions: GEMI remains active (MIC, ≤ 0.25 µg/ml) versus CIP-S and -I NG strains. Clinical trials with GEMI are warranted to determine single- or multi-dose regimens to address treatment of QRNG infections.

INTRODUCTION

Over four decades of directed research and development in the field of quinolones have led to the discovery of a new group of "novel", or "respiratory" fluoroquinolones. These new compounds developed for the 21st century, follow a better understanding of structure-activity relationships and are characterized by enhanced activity against Gram-positive cocci, especially *Streptococcus pneumoniae*, as well as many intracellular pathogens, whilst retaining excellent activity against Gram-negative organisms. Gemifloxacin (formerly SB265805, LB20304) represents one such synthetic broad-spectrum fluoronaphthyridone with high affinity for both fluoroquinolone target enzymes (DNA gyrase and topoisomerase IV) at therapeutically achievable drug concentrations. This "dual-targeting" action of gemifloxacin confers higher *in vitro* activity against first- and second-step fluoroquinolone-resistant mutants of pneumococci compared to the other marketed fluoroquinolones analyzed.

Studies conducted in the late 1990s testing clinical strains of *Neisseria gonorrhoeae*, including strains with reduced susceptibility to quinolones (QRNG), established the higher potency of gemifloxacin. Testing 150 clinical strains of gonococci, 33.3% of which had an elevated ciprofloxacin MIC (43 intermediate-susceptibility [MIC 0.12-0.5 µg/ml] and 7 resistant [MIC, ≥ 1 µg/ml] obtained from Japan, United States [USA] and The Netherlands), the authors found a gemifloxacin MIC_{90} result of 0.12 µg/ml that was lower than those recorded for trovafloxacin (0.25 µg/ml) and for moxifloxacin or grepafloxacin (0.5 µg/ml). Similarly in Japan, testing 94 consecutive clinical isolates of *N. gonorrhoeae* from 1992-93 and 100 isolates from 1996-97 (including 31 ciprofloxacin-resistant strains), Tanaka et al. substantiated the higher potency of gemifloxacin with MIC_{90} values of 0.03 and 0.12 µg/ml, respectively. This was in contrast to the MIC_{90} values of 0.12 to 0.25 µg/ml (for 1992-93 isolates) and from 0.5 to 2 µg/ml (for 1996-97 isolates) of the other fluoroquinolones analyzed (sparfloxacin, trovafloxacin, tosufloxacin and levofloxacin). However, the authors also noted that the gemifloxacin MIC_{90} value for the 1996-97 isolates was four-fold higher than that for the 1992-93 isolates (0.12 versus 0.03 µg/ml), and that the MIC_{90} values for the comparator fluoroquinolones were two- to eight-fold greater for the 1996-97 isolates, reiterating the need for continued resistance surveillance among gonococci.

To evaluate if gemifloxacin has retained its superior potency among fluoroquinolones against gonococcal isolates, which have since evolved further and developed higher levels of resistance to fluoroquinolones, we tested a collection of 250 *N. gonorrhoeae* isolates. The collection was chosen to represent well-characterized genotypes of QRNG, multiple phenotypes of ß-lactam, tetracycline and QRNG resistance, and a sample of recent clinical isolates (2004) of *N. gonorrhoeae* isolated from blood cultures and genital specimens and also from six distinct geographic locations in the USA, where QRNG has been reported to be endemic/epidemic.

MATERIALS AND METHODS

The selected collection of 250 *N. gonorrhoeae* clinical isolates included: 176 (70.4%) recent strains isolated from clinical specimens in 2004 from geographic sites in the USA; 24 recent clinical bacteremic strains from the SENTRY Antimicrobial Surveillance Program; and 50 historic QRNG strains from the Jones Microbiology Institute laboratories' collection (JMI; North Liberty, Iowa, USA). The clinical strains were obtained from six geographically distinct sites in the USA where QRNG has been reported to be endemic/epidemic, which includes: California (five strains; 2.8% of recent clinical isolates); Hawaii (25; 14.2%); New York (25; 14.2%); Ohio (36; 20.5%); Oregon (15; 8.5%) and Washington (70; 39.8%). More than one-fourth of the strains from the entire collection (74; 29.6%) had well-characterized resistance phenotypes by mechanisms analysis: 50 QRNG isolates; 14 strains resistant to penicillin and tetracycline; and 10 isolates with intermediate susceptibility to penicillin. The QRNG strains were isolated in the Far East and characterized in Japan. More than half (29; 58.0%) of these QRNG strains have documented mutations in the quinolone-resistance-determining-regions (QRDR) with: 1.) a single aminoacid substitution in *gyrA* (19 strains); 2.) single amino acid substitutions in both *gyrA* and *parC* (seven strains); and 3.) double amino acid substitution in *gyrA* and a single mutation in *parC* (3 strains).

Minimal inhibitory concentrations (MICs) of six antimicrobial agents were determined by the reference agar dilution method using GC agar base supplemented with a defined supplement. The antimicrobial agents tested were: gemifloxacin (Oscient Pharmaceuticals Corp., MA), ciprofloxacin, levofloxacin, penicillin, tetracycline and ceftriaxone. The results were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]). The following quality control strains were used to validate the study: *N. gonorrhoeae* ATCC 49226 and *Staphylococcus aureus* ATCC 29213.

RESULTS

- Gemifloxacin MIC results were compared to five antimicrobials commonly used as reference agents (Table 1). The rank order of potency of these antimicrobials for the entire collection (250 isolates) was: ceftriaxone (MIC_{90}, 0.06 µg/ml) > gemifloxacin (1 µg/ml) > tetracycline (2 µg/ml) > ciprofloxacin = levofloxacin = penicillin (4 µg/ml). Using MIC_{90} for comparison, gemifloxacin was consistently four-fold more potent than the other quinolones tested (1 versus 4 µg/ml) and the highest gemifloxacin MIC recorded was only 2 µg/ml.
- Penicillinase-producing *N. gonorrhoeae* (PPNG) constituted 11.2% of the collection tested (Table 2). Production of penicillinase did not result in any adverse changes in the MIC results of the fluoroquinolones or the enzyme-stable ß-lactam tested. In fact, the MIC_{90} was ≥ two-fold lower for the penicillinase-positive compared to the penicillinase-negative strains for all four agents; gemifloxacin, ciprofloxacin, levofloxacin and ceftriaxone.
- The vast majority of the gonococcal isolates (94.4%) had reduced susceptibility to penicillin (MIC, ≥ 0.12 µg/ml; Table 2). Increasing resistance to penicillin was associated with elevated MIC_{50} and MIC_{90} values for gemifloxacin and the five-comparators analyzed. Gemifloxacin MIC_{90} values increased from 0.06 µg/ml for penicillin-susceptible to 1 µg/ml for penicillin-intermediate and -resistant strains.
- More than one-third of the isolates (38.8%; 97) had reduced susceptibility to ciprofloxacin, with 18.8% of strains having intermediate-susceptibility (0.12-0.5 µg/ml) and 20.0% resistant (≥ 1 µg/ml; see Table 2). Activity of gemifloxacin was negatively affected by increasing ciprofloxacin and levofloxacin MICs.

Table 1. Gemifloxacin activity compared to reference agents when tested against 250 strains of *N. gonorrhoeae*.

	MIC (µg/ml)			
Antimicrobial agent	50%	90%	Range	%susceptible/resistant[a]
Gemifloxacin	0.016	1	≤0.008-2	-/-
Ciprofloxacin	≤0.008	4	≤0.008->4	61.2/20.0
Levofloxacin	0.016	4	≤0.008->4	-/-
Penicillin	1	4	0.016->4	5.6/31.2
Tetracycline	0.5	2	0.06->4	19.6/23.6
Ceftriaxone	≤0.008	0.06	≤0.008-0.12	100.0/-

a. Interpretive criteria as published by the CLSI. - = no interpretive criteria have been determined for this agent.

Table 2. Comparative gemifloxacin activity tested against *N. gonorrhoeae* isolates having defined resistance mechanisms or interpretive categories for ß-lactams and ciprofloxacin.

		MIC (µg/ml)			
Organism group (no. tested)	Antimicrobial agent	50%	90%	Range	%susceptible/resistant[a]
Penicillin					
Susceptible (14)	Gemifloxacin	≤0.008	0.06	≤0.008-0.06	-/-
	Ciprofloxacin	≤0.008	0.12	≤0.008-0.12	85.7/0.0
	Levofloxacin	0.016	0.12	≤0.008-0.25	-/-
	Tetracycline	0.25	0.5	0.06-0.5	85.7/0.0
	Ceftriaxone	≤0.008	≤0.008	≤0.008	100.0/-
Intermediate (158)	Gemifloxacin	≤0.008	1	≤0.008-2	-/-
	Ciprofloxacin	≤0.008	4	≤0.008->4	74.1/19.0
	Levofloxacin	0.016	4	≤0.008->4	-/-
	Tetracycline	0.5	1	0.12->4	22.8/7.0
	Ceftriaxone	≤0.008	0.016	≤0.008-0.12	100.0/-
Resistant (78)	Gemifloxacin	0.06	1	≤0.008-2	-/-
	Ciprofloxacin	0.25	>4	≤0.008->4	30.8/25.6
	Levofloxacin	0.25	>4	≤0.008->4	-/-
	Tetracycline	2	4	0.25->4	1.3/61.5
	Ceftriaxone	0.03	0.12	≤0.008-0.12	100.0/-
ß-lactamase-positive (28)	Gemifloxacin	0.06	0.5	≤0.008-1	-/-
	Ciprofloxacin	0.25	2	≤0.008->4	32.1/25.0
	Levofloxacin	0.25	2	≤0.008->4	-/-
	Tetracycline	2	>4	0.25->4	3.6/64.3
	Ceftriaxone	≤0.008	0.03	≤0.008-0.06	100.0/-
ß-lactamase-negative (222)	Gemifloxacin	≤0.008	1	≤0.008-2	-/-
	Ciprofloxacin	≤0.008	4	≤0.008->4	64.9/19.4
	Levofloxacin	0.016	4	≤0.008->4	-/-
	Tetracycline	0.5	2	0.06->4	21.6/18.5
	Ceftriaxone	≤0.008	0.06	≤0.008-0.12	100.0/-
Ciprofloxacin					
Susceptible (153)	Gemifloxacin	≤0.008	0.016	≤0.008-0.016	-/-
	Levofloxacin	0.016	0.03	≤0.008-0.06	-/-
	Penicillin	0.25	2	0.016->4	7.8/15.7
	Tetracycline	0.5	2	0.06->4	31.4/14.4
	Ceftriaxone	≤0.008	0.03	≤0.008-0.06	100.0/-
Intermediate (47)	Gemifloxacin	0.06	0.12	0.016-0.25	-/-
	Levofloxacin	0.25	0.5	0.12-0.5	-/-
	Penicillin	2	>4	0.03->4	4.3/72.3
	Tetracycline	1	2	0.5->4	0.0/31.9
	Ceftriaxone	0.03	0.12	≤0.008-0.12	100.0/-
Resistant (50)	Gemifloxacin	1	2	0.06-2	-/-
	Levofloxacin	4	>4	0.06->4	-/-
	Penicillin	1	>4	0.25->4	0.0/40.0
	Tetracycline	1	4	0.12->4	2.0/44.0
	Ceftriaxone	0.016	0.03	≤0.008-0.06	100.0/-
Non-susceptible (50)[b]	Gemifloxacin	0.06	0.25	0.03-1	-/-
	Ciprofloxacin	0.25	1	0.12->4	0.0/12.0
	Levofloxacin	0.25	1	0.12-4	-/-
	Penicillin	2	>4	0.03->4	4.0/70.0
	Tetracycline	1	2	0.5-4	0.0/36.0
	Ceftriaxone	0.03	0.12	≤0.008-0.12	100.0/-

a. Interpretive criteria as published by the CLSI. - = no interpretive criteria have been determined for this agent.
b. Fifty Far East clinical isolates from 1994 - 1997 with defined QRDR changes.

- Analysis of 200 recent clinical isolates from 2004 (Table 3; 176 from six states and 24 bacteremic strains) revealed that gemifloxacin had an identical MIC_{90} value (1 µg/ml) to that obtained for the entire collection. The potency of gemifloxacin (MIC_{90}, 1 µg/ml) was between that of the ceftriaxone (0.03 µg/ml) and older fluoroquinolones (4 µg/ml).
- The antibiograms of the 176 clinical strains from six USA states and the 24 bacteremic isolates are listed in Table 3. Penicillin susceptibility rates for the isolates from all six states were ≤ 20.0%, and ranged from 0.0% (New York) to a maximum of 20.0% (California and Oregon); and tetracycline susceptibility rates varied from 0.0% (New York) to 52.8% (Ohio). Ciprofloxacin susceptibility rates varied enormously, ranging from 28.0% (Hawaii) to greater than 90.0% (New York and Ohio).
- The 50 QRNG strains isolated in the Far East (Table 2; 29 with documented mutations in the QRDR region) were analyzed independently. The gemifloxacin $MIC_{50/90}$ values were 0.06/0.25 µg/ml and the highest MIC was only 1 µg/ml. These strains isolated in the 1990's generally had an intermediate range of MIC results to the monitored fluoroquinolones; and only 12.0% of the 50 strains had a resistant ciprofloxacin MIC. This is in clear contrast to the recent QRNG isolates, which were predominantly high-level resistant to ciprofloxacin (MIC, ≥ 1 µg/ml).

Table 3. Gemifloxacin and five comparison agents tested against recent (2004) genital and blood culture isolates listed by geographic area (United States) or specimen source.

		MIC (µg/ml)			
Organism group (no. tested)	Antimicrobial agent	50%	90%	Range	%susceptible/resistant[a]
California (5)	Gemifloxacin	≤0.008	-	≤0.008-0.5	-/-
	Ciprofloxacin	≤0.008	-	≤0.008-2	80.0/20.0
	Levofloxacin	0.016	-	0.016-2	-/-
	Penicillin	1	-	0.06->4	20.0/20.0
	Tetracycline	0.5	-	0.25->4	20.0/20.0
	Ceftriaxone	≤0.008	-	≤0.008-0.016	100.0/-
Hawaii (25)	Gemifloxacin	0.5	1	≤0.008-1	-/-
	Ciprofloxacin	4	4	≤0.008->4	28.0/64.0
	Levofloxacin	2	4	≤0.008->4	-/-
	Penicillin	1	>4	0.06->4	8.0/44.0
	Tetracycline	1	>4	0.25->4	12.0/20.0
	Ceftriaxone	≤0.008	0.016	≤0.008-0.03	100.0/-
New York (25)	Gemifloxacin	0.016	0.016	≤0.008-1	-/-
	Ciprofloxacin	≤0.008	0.016	≤0.008->4	92.0/8.0
	Levofloxacin	0.016	0.03	≤0.008->4	-/-
	Penicillin	0.25	1	0.12-2	0.0/8.0
	Tetracycline	0.5	1	0.5-2	0.0/8.0
	Ceftriaxone	≤0.008	0.016	≤0.008-0.03	100.0/-
Ohio (36)	Gemifloxacin	≤0.008	≤0.008	≤0.008-0.03	-/-
	Ciprofloxacin	≤0.008	≤0.008	≤0.008-0.12	97.2/0.0
	Levofloxacin	0.016	0.016	≤0.008-0.25	-/-
	Penicillin	0.25	2	0.016-2	8.3/16.7
	Tetracycline	0.25	2	0.06-2	52.8/16.7
	Ceftriaxone	≤0.008	0.016	≤0.008-0.06	100.0/-
Oregon (15)	Gemifloxacin	≤0.008	1	≤0.008-1	-/-
	Ciprofloxacin	≤0.008	>4	≤0.008->4	86.7/13.3
	Levofloxacin	0.016	>4	≤0.008->4	-/-
	Penicillin	0.25	4	0.03-4	20.0/13.3
	Tetracycline	0.5	2	0.12-2	46.7/13.3
	Ceftriaxone	≤0.008	0.03	≤0.008-0.06	100.0/-
Washington (70)	Gemifloxacin	≤0.008	1	≤0.008-2	-/-
	Ciprofloxacin	≤0.008	>4	≤0.008->4	67.1/32.9
	Levofloxacin	0.016	>4	≤0.008->4	-/-
	Penicillin	0.5	2	0.03->4	2.9/14.3
	Tetracycline	0.5	2	0.12->4	22.9/21.4
	Ceftriaxone	≤0.008	0.03	≤0.008-0.06	100.0/-
Bacteremia (24)	Gemifloxacin	≤0.008	0.016	≤0.008-0.016	-/-
	Ciprofloxacin	0.016	0.03	≤0.008-0.03	100.0/0.0
	Levofloxacin	0.016	0.03	≤0.008-0.06	-/-
	Penicillin	1	4	0.03-4	4.2/45.8
	Tetracycline	1	2	0.12->4	12.5/41.7
	Ceftriaxone	0.016	0.06	≤0.008-0.06	100.0/-
All clinical cases (200)	Gemifloxacin	≤0.008	1	≤0.008-2	-/-
	Ciprofloxacin	≤0.008	4	≤0.008->4	76.5/22.0
	Levofloxacin	0.016	4	≤0.008->4	-/-
	Penicillin	0.5	2	0.016->4	6.0/21.5
	Tetracycline	0.5	2	0.06->4	24.5/20.5
	Ceftriaxone	≤0.008	0.03	≤0.008-0.06	100.0/-

a. Interpretive criteria as published by the CLSI. - = no interpretive criteria have been determined for this agent.

CONCLUSIONS

- Our results confirm that gemifloxacin continues to be most active in vitro among fluoroquinolones when tested against recent gonococcal isolates (MIC_{50}, 0.016 µg/ml and MIC_{90}, 1 µg/ml). Despite the pre-selection bias towards antimicrobial-resistant gonococcal isolates (entire collection QRNG rate of 38.8%, and high-level penicillin and tetracycline rates of 31.2 and 23.6%, respectively), the potency of gemifloxacin was competitive and positioned between that of ceftriaxone and older quinolones.
- Increasing penicillin MIC was associated with ≥ eight-fold increase in the MIC_{90} value of gemifloxacin and its comparators (ciprofloxacin, levofloxacin, ceftriaxone and tetracycline), but the highest gemifloxacin MIC was only 2 µg/ml.
- While the MIC_{90} value of gemifloxacin was 128-fold higher for the ciprofloxacin-resistant compared to the –susceptible strains (2 versus 0.016 µg/ml), the gemifloxacin MIC_{90} value was ≥ two-fold lower than that for the older quinolones analyzed for the QRNG strains.
- Fifty historic QRNG with QRDR changes in which quinolone resistance was first demonstrated, had predominantly intermediate-level susceptibility to ciprofloxacin (88.0%) and correspondingly the majority of the strains had a single mutation in *gyrA* (19 of 29 strains with QRDR changes; 65.5%). This was in contrast to the 200 contemporary strains from 2004 analyzed in this study, where 93.6% (47 of 200 strains) of the QRNG strains were fully resistant to ciprofloxacin. This shift in the degree of reduced susceptibility to ciprofloxacin, resulted in a corresponding four-fold increase in the MIC_{90} value of gemifloxacin over 10 years (1 versus 0.25 µg/ml; Tables 2 and 3).
- Our results confirm the high activity of gemifloxacin in comparison to the other fluoroquinolones analyzed against the collection of contemporary strains of *N. gonorrhoeae*, irrespective of the resistance mechanism or phenotype. Gemifloxacin with its documented potency, should be considered for further development as an anti-gonococcal agent to treat emerging strains that are resistant to penicillins, tetracycline and some older fluoroquinolones or have become less responsive to azithromycin.

SELECTED REFERENCES

Centers for Disease Control and Prevention. (2002). Discontinuation of cefixime tablets-United States. *Morbidity and Mortality Weekly Report* 51:1052.

Centers for Disease Control and Prevention. (2002). Sexually transmitted diseases treatment guidelines 2002. *Morbidity and Mortality Weekly Report* 51(RR-6):1-78.

Centers for Disease Control and Prevention. (2004). Increases in fluoroquinolone-resistant *Neisseria gonorrhoeae* among men who have sex with men – United States, 2003, and revised recommendations for gonorrhoea treatment, 2004. *Morbidity and Mortality Weekly Report* 53:335-338.

Centers for Disease Control and Prevention. (2004). Sexually Transmitted Disease Surveillance 2003 Supplement: Gonococcal Isolate Surveillance Project (GISP) Annual Report -2003. Atlanta, Georgia: *U.S. Department of Health and Human Services, November 2004.*

Clinical and Laboratory Standards Institute. (2005). *Performance standards for antimicrobial susceptibility testing, 15th informational supplement M100-S15.* Wayne, PA:CLSI.

Deguchi T, Yasuda M, Nakano M, Ozeki S, Ezaki T, Saito I, Kawada Y. (1996). Quinolone-resistant *Neisseria gonorrhoeae*: Correlation of alterations in the GyrA subunit of DNA gyrase and the ParC subunit of Topoisomerase IV with antimicrobial susceptibility profiles. *Antimicrobial Agents and Chemotherapy* 40:1020-1023.

Jones RN, Deshpande LM, Erwin ME, Barrett MS, Beach ML. (2000). Anti-gonococcal activity of gemifloxacin against fluoroquinolone-resistant strains and a comparison of agar dilution and Etest methods. *Journal of Antimicrobial Chemotherapy* 45:67-70.

Jones RN, Erwin ME, The Quality Control Study Group. (2000). Development of gemifloxacin in vitro susceptibility test methods for gonococci including quality control guidelines. *Diagnostic Microbiology and Infectious Disease* 37:195-201.

Lowe MN, Lamb HM. (2000). Gemifloxacin. *Drugs* 59:1137-1148.

National Committee for Clinical Laboratory Standards. (2003) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A6.* Wayne, PA:NCCLS.

Ruiz J, Jurado A, Garcia-Mendez E, Marco F, Aguilar L, Jimenez De Anta MT, Vila J. (2001). Frequency of selection of fluoroquinolone-resistant mutants of *Neisseria gonorrhoeae* exposed to gemifloxacin and four other quinolones. *Journal of Antimicrobial Chemotherapy* 48:545-548.

Tanaka M, Nakayama H, Notomi T, Irie S, Tsunoda Y, Okadome A, Saika T, Kobayashi I. (2004). Antimicrobial resistance of *Neisseria gonorrhoeae* in Japan, 1993 - 2002: Continuous increasing of ciprofloxacin-resistant isolates. *International Journal of Antimicrobial Agents* 24S:515-522.

Tanaka M, Tunoe H, Mochida O, Kanayama A, Saika T, Kobayashi I, Naito S. (2000). Antimicrobial activity of gemifloxacin (SB-265805), a newer fluoroquinolone, against clinical isolates of *Neisseria gonorrhoeae*, including fluoroquinolone-resistant isolates. *Diagnostic Microbiology and Infectious Disease* 38:109-113.

Tapsall J. (2001). Antimicrobial resistance in *Neisseria gonorrhoeae*. Geneva, Switzerland: *World Health Organization, 2001*; WHO/CDS/CSR/DRS/2001.3:16.

World Health Organization Western Pacific Gonococcal Antimicrobial Surveillance Programme. (2003). Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in the WHO Western Pacific Region, 2002. *Communicable Disease Intelligence* 27:488-491.