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Antimicrobial Activities of Garenoxacin (BMS 284756) against Asia-Pacific Region Clinical Isolates from the SENTRY Program, 1999 to 2001

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Between 1999 and 2001, 16,731 isolates from the Asia-Pacific Region were tested in the SENTRY Program for susceptibility to six fluoroquinolones including garenoxacin. Garenoxacin was four- to eightfold less active against *Enterobacteriaceae* than ciprofloxacin, although both drugs inhibited similar percentages at 1 μg/ml. Garenoxacin was more active against gram-positive species than all other fluoroquinolones except gemifloxacin. For *Staphylococcus aureus*, oxacillin resistance was high in many participating countries (Japan, 67%; Taiwan, 60%; Hong Kong, 55%; Singapore, 52%), with corresponding high levels of ciprofloxacin resistance (57 to 99%) in oxacillin-resistant *S. aureus* (ORSA). Of the ciprofloxacin-resistant ORSA isolates, the garenoxacin MIC was >4 μg/ml for only 9% of them. For *Streptococcus pneumoniae*, penicillin nonsusceptibility and macrolide resistance were high in many countries. No relationship was seen between penicillin and garenoxacin susceptibility, with all isolates being susceptible at <2 μg/ml. There was, however, a partial correlation between ciprofloxacin and garenoxacin MICs. For ciprofloxacin-resistant isolates for which garenoxacin MICs were 0.25 to 1 μg/liter, mutations in both the ParC and GyrA regions of the quinolone resistance-determining region could be demonstrated. No mutations conferring high-level resistance were detected. Garenoxacin shows useful activity against a wide range of organisms from the Asia-Pacific region. In particular, it has good activity against *S. aureus* and *S. pneumoniae*, although there is evidence that low-level resistance is present in those organisms with ciprofloxacin resistance.

Garenoxacin is a novel des-F(6) quinolone that lacks a fluorine at the C-6 position but has fluorine incorporated through a C-8 difluoromethyl ether linkage. It has been shown to have activity against a wide range of clinical isolates (1, 7, 12, 34), and in particular, garenoxacin has been shown to have good activity against *Staphylococcus aureus*, both methicillin sensitive and resistant (6), and the respiratory pathogens *Streptococcus pneumoniae* (26), *Haemophilus influenzae*, and *Moraxella catarrhalis* (8). The activity of garenoxacin has been further assessed against strains of *S. aureus* with specific topoisomerase mutations (21), and more recently, it has been shown that garenoxacin has similar potency against both topoisomerase IV and gyrase (16) (dual-targeting quinolone), thus requiring mutations in both topoisomerases for resistance to occur (29). Although horizontal transfer is a major reason for the spread of ciprofloxacin-resistant strains, the role of antimicrobial selection may also play an important role. As single *parC* or *gyrA* mutations in *S. aureus* (31) confer resistance to ciprofloxacin, the widespread use of this antibiotic may have already selected a population of organisms requiring only one further mutation for resistance to the new fluoroquinolones to occur. Similarly, with *S. pneumoniae*, mutations within the quinolone resistance-determining region (QRDR) for both topoisomerases can lead to resistance for the new fluoroquinolones (18). Within the Asia-Pacific region, multidrug resistance and, specifically, fluoroquinolone resistance for both gram-positive and gram-negative organisms is relatively high (33). Rapid development of ciprofloxacin resistance in oxacillin-resistant *S. aureus* (ORSA) was reported in Hong Kong with 9% resistance in 1988 and 82% resistance in 1993 (27). Similar high levels of resistance have been reported for ORSA from other countries in the region, 66% for Korea (22), 97% for Taiwan (12% in oxacillin-susceptible *S. aureus* [OSSA]) (23), and >50% for Japan (36). Resistance to fluoroquinolones has been reported for *S. pneumoniae* with 13.3% (27.3% for non-penicillin-susceptible isolates) resistance to levofloxacin in a recent study in Hong Kong (15) and up to 12% resistance in Korea (22). It is therefore important to determine the comparative activities of the newer fluoroquinolones, including garenoxacin, against recent clinical isolates in the Asia-Pacific region. An earlier study presented data on respiratory pathogens in the region for 1998 to 1999 (2). This present SENTRY (Asia-Pacific Region including South Africa) study gives an updated report on comparative fluoroquinolone susceptibilities against both gram-positive and gram-negative organisms within the region and analyzes in more detail the activities of garenoxacin against *S. pneumoniae* and *S. aureus*.

MATERIALS AND METHODS

Bacterial isolates. Clinically significant strains from the SENTRY surveillance program were collected by 17 hospitals from eight countries (Japan, Taiwan, Mainland China, Hong Kong, Philippines, Singapore, Australia, and South Africa) over defined intervals between 1999 and 2001. Repeat isolates from patients were excluded. Isolates were from cases of bacteremia (n = 7,793), lower respiratory infections in hospitalized patients (n = 2,124), upper respiratory tract

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TABLE 1. Fluoroquinolone activity against *Enterobacteriaceae*, nonfermentative gram-negative bacilli, and gram-positive organisms from the Asia-Pacific region, 1999 to 2001[a]

Organism	No. of strains	Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Susceptible at concn (μg/ml) of:				
					0.25	0.5	1	2	4
Enterobacteriaceae									
Citrobacter freundii	58	Garenoxacin	1	>4	34	39	50	63	77
	58	Ciprofloxacin	≤0.25	>2	70	72	74	84	NT
	58	Levofloxacin	0.25	>4	67	70	75	84	89
	58	Gatifloxacin	0.25	>4	51	69	70	84	89
	58	Gemifloxacin	0.25	>4	63	70	77	82	84
Enterobacter aerogenes	119	Garenoxacin	0.12	1	84	88	91	94	95
	119	Ciprofloxacin	≤0.25	0.25	90	95	97	99	NT
	119	Levofloxacin	0.06	0.25	89	94	97	99	99
	119	Gatifloxacin	0.06	1	87	89	95	99	99
	119	Gemifloxacin	≤0.03	0.5	89	92	97	97	98
Enterobacter cloacae	451	Garenoxacin	0.12	>4	72	76	78	80	84
	451	Ciprofloxacin	≤0.25	>2	81	86	87	89	NT
	451	Levofloxacin	0.06	2	77	86	87	90	91
	451	Gatifloxacin	0.06	2	76	81	87	90	92
	451	Gemifloxacin	≤0.03	2	77	81	88	90	92
Escherichia coli	2,411	Garenoxacin	≤0.03	>4	80	82	83	84	85
	2,411	Ciprofloxacin	≤0.25	>2	82	84	84	84	NT
	2,411	Levofloxacin	≤0.03	>4	81	84	84	85	87
	2,411	Gatifloxacin	≤0.03	>4	81	84	84	85	87
	2,411	Gemifloxacin	≤0.03	4	83	84	84	85	90
Klebsiella oxytoca	167	Garenoxacin	0.12	2	88	89	89	91	94
	167	Ciprofloxacin	≤0.25	≤0.25	91	94	95	97	NT
	167	Levofloxacin	≤0.03	0.25	91	93	96	97	97
	167	Gatifloxacin	0.06	0.5	89	92	96	97	97
	167	Gemifloxacin	≤0.03	0.5	89	91	95	96	98
Klebsiella pneumoniae	1,053	Garenoxacin	0.12	>4	77	80	83	84	85
	1,053	Ciprofloxacin	≤0.25	>2	81	84	87	88	NT
	1,053	Levofloxacin	0.06	4	80	85	87	89	91
	1,053	Gatifloxacin	0.06	4	80	83	87	88	91
	1,053	Gemifloxacin	≤0.03	4	82	84	87	88	91
Morganella morganii	86	Garenoxacin	1	>4	30	46	61	66	76
	86	Ciprofloxacin	≤0.25	>2	67	68	83	83	NT
	86	Levofloxacin	0.06	>4	65	68	83	87	89
	86	Gatifloxacin	0.12	>4	64	67	73	83	86
	86	Gemifloxacin	0.06	4	66	67	68	74	90
Proteus mirabilis	211	Garenoxacin	0.5	>4	27	70	82	84	84
	211	Ciprofloxacin	≤0.25	2	84	85	87	91	NT
	211	Levofloxacin	0.06	1	84	85	91	95	98
	211	Gatifloxacin	0.25	4	76	84	85	88	95
	211	Gemifloxacin	0.12	4	83	84	85	85	91
Serratia marcescens	233	Garenoxacin	2	>4	1	4	21	58	73
	233	Ciprofloxacin	≤0.25	>2	65	71	75	80	NT
	233	Levofloxacin	0.25	>4	62	70	76	80	85
	233	Gatifloxacin	0.5	>4	35	62	73	78	86
	233	Gemifloxacin	0.25	>4	56	69	76	77	87
Nonfermentative gram-negative bacilli									
Acinetobacter baumanii	340	Garenoxacin	0.06	>4	57	58	60	62	75
	340	Ciprofloxacin	0.25	>2	51	56	58	59	NT
	340	Levofloxacin	0.12	>4	56	58	59	61	77
	340	Gatifloxacin	0.12	>4	58	58	60	61	79
	340	Gemifloxacin	0.03	>4	59	61	62	62	82
Pseudomonas aeruginosa	1,236	Garenoxacin	2	>4	2	9	40	69	79
	1,236	Ciprofloxacin	≤0.25	>2	66	79	84	87	NT
	1,236	Levofloxacin	0.5	>4	20	60	75	83	87
	1,236	Gatifloxacin	1	>4	5	32	64	79	85
	1,236	Gemifloxacin	0.25	4	51	72	82	85	89
Stenotrophomonas maltophilia	132	Garenoxacin	2	>4	5	11	38	65	84
	132	Ciprofloxacin	>2	>2	0	1	18	49	NT
	132	Levofloxacin	1	4	3	25	68	87	93
	132	Gatifloxacin	1	4	7	34	70	87	91
	132	Gemifloxacin	1	4	15	45	77	86	90
Gram-positive bacteria									
Enterococcus faecalis	511	Garenoxacin	0.25	4	64	81	83	86	93
	511	Ciprofloxacin	1	>2	0	11	64	80	NT
	333	Levofloxacin	1	>4	0	9	75	82	82

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TABLE 1—*Continued*

Organism	No. of strains	Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Susceptible at concn (μg/ml) of:				
					0.25	0.5	1	2	4
	511	Gatifloxacin	0.5	>4	17	73	81	82	83
	511	Gemifloxacin	0.06	>1	82	83	85	88	93
Enterococcus faecium	148	Garenoxacin	>4	>4	9	10	11	16	41
	148	Ciprofloxacin	>2	>2	1	4	9	25	NT
	92	Levofloxacin	>4	>4	0	4	13	42	48
	148	Gatifloxacin	>4	>4	5	8	18	33	41
	148	Gemifloxacin	4	>4	10	16	35	41	59
OSSA	1,516	Garenoxacin	≤0.03	≤0.03	98	98	99	99	99
	1,516	Ciprofloxacin	≤0.25	0.5	76	95	97	98	NT
	1,007	Levofloxacin	0.12	0.25	96	97	97	98	99
	1,516	Gatifloxacin	0.06	0.12	98	98	98	99	99
	1,516	Gemifloxacin	≤0.03	≤0.03	98	99	99	99	99
ORSA	1,146	Garenoxacin	1	4	23	38	61	84	92
	1,146	Ciprofloxacin	>2	>2	12	14	15	15	NT
	760	Levofloxacin	4	>4	15	17	17	22	66
	1,146	Gatifloxacin	2	>4	15	18	31	65	89
	1,145	Gemifloxacin	1	4	30	47	77	85	94
Staphylococcus epidermidis	429	Garenoxacin	0.25	2	50	54	80	94	99
	429	Ciprofloxacin	2	>2	46	48	49	51	NT
	204	Levofloxacin	2	>4	46	47	47	60	88
	429	Gatifloxacin	0.5	2	49	50	66	97	99
	429	Gemifloxacin	0.06	1	70	88	97	99	99
Streptococcus agalactiae	302	Garenoxacin	0.06	0.12	98	98	99	99	100
	302	Ciprofloxacin	1	1	0	40	94	97	NT
	264	Levofloxacin	0.5	1	0	59	95	97	98
	302	Gatifloxacin	0.25	5	83	98	98	99	99
	302	Gemifloxacin	≤0.03	0.06	98	99	100	100	100
Streptococcus pyogenes	352	Garenoxacin	0.06	0.12	100	100	100	100	100
	352	Ciprofloxacin	0.5	1	16	87	94	99	100
	313	Levofloxacin	0.5	1	12	88	95	100	100
	352	Gatifloxacin	0.25	0.25	90	100	100	100	100
	352	Gemifloxacin	≤0.03	≤0.03	100	100	100	100	100
Streptococcus pneumoniae	1,486	Garenoxacin	0.06	0.06	98	99	100	100	100
	1,486	Ciprofloxacin	1	2	0	13	72	96	97
	1,353	Levofloxacin	1	1	0	31	97	98	98
	1,486	Gatifloxacin	0.25	5	81	98	98	98	99
	723	Moxifloxacin	0.12	0.25	97	98	98	98	100
	807	Gemifloxacin	≤0.03	≤0.03	99	100	100	100	100

[a] MIC_{50}, MIC at which 50% of the isolates tested are inhibited; NT, not tested.

infections (n = 2,799), wound or soft tissue infections (n = 1,069), and urinary tract infections (n = 1,223). All strains were sent to a central reference laboratory (Women's and Children's Hospital, Adelaide, Australia).

Susceptibility testing. MICs were obtained for all isolates by a broth microdilution technique (TREK Diagnostic Systems Limited, East Grinstead, United Kingdom) according to NCCLS standards (24). Isolates were tested against more than 26 antimicrobial agents, including 5 fluoroquinolones, ciprofloxacin, levofloxacin, gatifloxacin, gemifloxacin, and garenoxacin. Garenoxacin, levofloxacin, and gatifloxacin were tested against all isolates over the concentration range of ≤0.03 to 4 μg/ml, except that gram-positive isolates were not tested against levofloxacin in 1999. Ciprofloxacin was tested against nonfastidious pathogens over the concentration ranges of ≤0.25 to 2 μg/ml (1999 and 2000) and ≤0.015 to 2 μg/ml (2001). The ranges used for fastidious isolates were ≤0.015 to 2 μg/ml (1999 and 2000) and ≤0.03 to 4 μg/ml (2001). Gemifloxacin was tested against nonfastidious pathogens over the range of ≤0.03 to 4 μg/ml (1999 and 2000), and the range used for all isolates in 2001 was ≤0.008 to 1 μg/ml. Moxifloxacin was only tested against fastidious isolates in 2000 and 2001, over the range of ≤0.03 to 4 μg/ml. For *S. pneumoniae* MIC determination, cation-adjusted Mueller-Hinton broth containing 5% lysed horse blood was used. The same medium but without the lysed horse blood was used for *M. catarrhalis*, and for *H. influenzae*, *Haemophilus* test medium broth was used as the growth medium. The concentration of the final inoculum was 5×10^5 CFU/ml. The trays were incubated for 20 to 24 h at 35°C in ambient air. MICs were defined as the lowest concentration of drug that yielded no visible growth of the test organism. Control strains were *S. pneumoniae* ATCC 49619, *H. influenzae* strains ATCC 49247 and 49766, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213.

PCR and DNA sequencing. Primers as previously described (25) were used to amplify the QRDRs of *gyrA*, *gyrB*, *parC*, and *parE*. DNA sequencing was performed by ABI PRISM Big Dye terminator cycle sequencing (Applied Bioscience) with the ABI Prism 3700 (Applied Biosystems) automated sequencer. DNA sequences were confirmed by using products of independent PCRs to determine the sequence of each strand. DNAMAN (Lynnon BioSoft) sequence analysis software was used for alignment of DNA sequences and deduced amino acid sequences.

Active efflux. Isolates of *S. pneumoniae* for which ciprofloxacin MICs were >2 μg/ml were examined for active efflux. MICs of ciprofloxacin were determined by agar dilution with Mueller-Hinton agar supplemented with 5% sheep blood with or without 10 μg of reserpine per ml and an inoculum of 10^4 CFU/spot (4, 24). Strains for which there was a fourfold or greater decrease in the ciprofloxacin MIC in the presence of reserpine were considered to be positive for reserpine-inhibited efflux.

TABLE 2. *S. aureus* and *S. pneumoniae* susceptibility in the SENTRY Asia-Pacific region countries

Parameter	Result for country or region							
	Australia	Hong Kong	Japan	Singapore	South Africa	Taiwan	Mainland China	Philippines
S. aureus								
No. of isolates	1,138	302	570	195	163	207	23	64
% OSSA	73	45	32	48	66	40	87	92
% Ciprofloxacin resistant	1	7	3	2	1	0	0	3
% ORSA	27	55	67	52	34	60	13	8
% Ciprofloxacin resistant	57	99	97	97	96	78	100	60
S. pneumoniae								
No. of isolates	676	81	357	54	135	163	10	10
% Penicillin:								
Susceptible	81	37	39	46	30	23	90	80
Intermediate	10	9	24	11	25	16	10	20
Resistant	9	54	37	43	45	61	0	0
% Erythromycin:								
Susceptible	82	27	27	54	47	11	30	100
Resistant	17	73	73	46	53	88	70	0
% Clindamycin:								
Susceptible	93	72	62	89	54	45	30	100
Resistant	7	28	38	11	46	54	70	0

RESULTS

During the period 1999 to 2001, a total of 16,731 isolates were received from Australia (6,271 isolates), Hong Kong (1,587 isolates), Japan (3,106 isolates), Mainland China (357 isolates [1999 only]), Philippines (1,336 isolates), Singapore (1,052 isolates), South Africa (1,344 isolates), and Taiwan (1,678 isolates).

Table 1 shows the activities of six fluoroquinolones, including garenoxacin, against *Enterobacteriaceae*, nonfermenting gram-negative bacilli, enterococci, coagulase-negative staphylococci, and beta-hemolytic streptococci groups A and B. Garenoxacin was more active against gram-positive species than any other fluoroquinolone tested except gemifloxacin. All streptococcal strains were inhibited at concentrations of 1 μg/ml or less. Garenoxacin was generally less active than all other fluoroquinolones against *Enterobacteriaceae*. Compared to ciprofloxacin, garenoxacin was about four- to eightfold less active against *Enterobacteriaceae*, although similar percentages of strains were inhibited by both drugs at 1 μg/ml. Garenoxacin had activities similar to those of ciprofloxacin against *Acinetobacter* and *Stenotrophomonas* but was less active against *P. aeruginosa*.

Table 2 shows the percentage of ORSA and OSSA for each of the participating countries. Ciprofloxacin resistance in ORSA was high in all countries. Of the ORSA isolates for which the garenoxacin MIC was >4 μg/ml, most came from just two countries, Japan (80%) and Hong Kong (17%). The percentage of garenoxacin resistance for ciprofloxacin-resistant OSSA was 3.3%, with the one isolate coming from Japan.

S. pneumoniae susceptibilities to penicillin, erythromycin, and clindamycin for the region are shown in Table 2. The activities of the fluoroquinolones against the common pathogens causing community-acquired upper and lower respiratory tract infections are shown in Table 3. No quinolone resistance

TABLE 3. Fluoroquinolone activity against gram-negative respiratory tract pathogens from the Asia-Pacific region, 1999 to 2001

Organism	No. of strains	Antibiotic	MIC_{50}[a] (μg/ml)	MIC_{90} (μg/ml)	% Susceptible at concn (μg/ml) of:				
					0.06	0.12	0.25	0.5	1
H. influenzae	1,341	Garenoxacin	≤0.03	≤0.03	99	99	99	99	99
	1,341	Ciprofloxacin	≤0.03	≤0.25	84	85	99	99	99
	1,341	Levofloxacin	≤0.03	≤0.03	99	99	99	99	99
	1,341	Gatifloxacin	≤0.03	≤0.03	99	99	99	99	99
	819	Moxifloxacin	≤0.03	≤0.03	99	99	99	100	100
	745	Gemifloxacin	≤0.008	≤0.03	99	99	99	99	99
M. catarrhalis	600	Garenoxacin	0.03	0.03	99	99	99	100	100
	600	Ciprofloxacin	0.03	≤0.25	89	89	99	99	100
	600	Levofloxacin	≤0.03	0.06	99	99	99	99	100
	600	Gatifloxacin	≤0.03	≤0.03	99	99	99	100	100
	372	Moxifloxacin	0.06	0.06	99	99	99	99	100
	284	Gemifloxacin	≤0.008	≤0.03	99	100	100	100	100

[a] MIC_{50}, MIC at which 50% of the isolates tested are inhibited.

TABLE 4. Distribution of garenoxacin MICs for *S. pneumoniae* according to penicillin susceptibility

Penicillin MIC (μg/ml)	*n*	No. of strains for which garenoxacin MIC (μg/ml) is:					
		≤0.03	0.06	0.12	0.25	0.5	1
≤0.03	775	149	538	85	1		2
0.06	63	7	48	7			1
0.12	54	9	43	1	1		
0.25	61	25	35		1		
0.5	41	13	26	2			
1	73	28	40	3	1	1	
2	255	91	140	11	2	5	6
≥4	164	36	108	15	2	1	2
Total	1,486	358	978	124	8	7	11

was detected in *M. catarrhalis* ($n = 600$), and the ciprofloxacin MIC was 2 μg/ml for only one (0.07%) *H. influenzae* isolate from Japan. Table 4 shows the distribution of MICs of garenoxacin compared with those of penicillin for *S. pneumoniae*. Analyzing the data with two different cutoff values for garenoxacin (≤0.12 and ≤0.25 μg/ml) and penicillin (≤0.06 and ≤1 μg/ml) will return highly significant *P* values with the chi-square test ($P < 0.001$). Therefore, elevated MICs of garenoxacin are associated with reduced susceptibility and resistance to penicillin. We hypothesize that there is possible linkage with epidemic serotypes. For ciprofloxacin-resistant strains, there was a corresponding shift in garenoxacin MICs; however, the garenoxacin MIC was not >1 μg/ml for any isolate. Mutations in topoisomerase IV and DNA gyrase were demonstrated for all ciprofloxacin-resistant isolates for which the garenoxacin MIC was ≥0.25 μg/ml (Table 5). No reserpine-inhibited efflux was demonstrated for these isolates.

DISCUSSION

Garenoxacin has been shown to have broad-spectrum activity in studies from other regions of the world (3, 11, 20, 36). This study provides confirmation for isolates from the Asia-Pacific region (Table 1), although the activities against *Enterobacter* spp., *Morganella* spp., *P. aeruginosa*, and *Serratia* spp. are less than those of ciprofloxacin. Good streptococcal activity is evident. The main advances of the newer fluoroquinolones, including garenoxacin, compared to the earlier agents have been against gram-positive organisms, in particular *S. aureus* and *S. pneumoniae*, with the development of resistance requiring mutations in both topoisomerase II and IV, unlike ciprofloxacin, for which changes in only one topoisomerase are necessary. For *S. aureus*, ciprofloxacin resistance occurs mostly in ORSA and is due to mutations in either *parC* or *gyrA*. Thus, the prevalence of ORSA and resistance to ciprofloxacin in the region is an indication of the presence of one-step mutants. The percentage of ORSA isolates was high in Japan (67%), Taiwan (60%), Hong Kong (55%), and Singapore (52%). As expected from data elsewhere in the world (10), ciprofloxacin resistance was common in these isolates (Table 2). When mutations occur in both *gyrA* and *parC*, markedly increased MICs

TABLE 5. Mutations in topoisomerase IV and DNA gyrase for ciprofloxacin-resistant isolates of *S. pneumoniae* for which MICs of garenoxacin are elevated (0.25 to 1 μg/ml)

Country	MIC (μg/ml)	Serotype	Efflux[a]	Mutation in QRDR of:							
				ParC			ParE		GyrA		GyrB
				Ser79	Asp83	Lys137	Ile460	Asp435	Ser81	Glu85	
Hong Kong	1	14	1	Phe	—[b]	Asn	Val	—	Phe	—	—
Hong Kong	1	14	2	Phe	—	Asn	Val	—	Phe	—	—
Hong Kong	1	14	2	Phe	—	Asn	Val	—	Phe	—	—
Hong Kong	1	14	2	Phe	—	Asn	Val	—	Phe	—	—
Hong Kong	1	14	2	Phe	—	Asn	Val	—	Phe	—	—
Hong Kong	1	14	2	Phe	—	Asn	Val	—	Phe	—	—
Hong Kong	1	14	2	Phe	—	Asn	Val	—	Phe	—	—
Japan	1	6B	2	Phe	—	Asn	Val	—	—	Lys	—
Japan	1	22F	2	Phe	—	—	—	—	Phe	—	—
Hong Kong	1	14	1	Phe	—	Asn	Val	—	Phe	—	—
Japan	1	6A	1	Phe	—	—	Val	—	—	Lys	—
Japan	0.5	19F	2	—	Asn	—	Val	—	Phe	—	—
Australia	0.5	14	1	—	Asn	Asn	Val	—	Phe	—	—
Australia	0.5	14	1	—	Asn	Asn	Val	—	Phe	—	—
Hong Kong	0.5	23F	2	Phe	—	Asn	Val	Asn	Tyr	—	—
Australia	0.5	NT[c]	2	—	Asn	Asn	Val	—	Phe	—	—
Australia	0.5	14	2	—	Asn	Asn	Val	—	Phe	—	—
Hong Kong	0.5	23F	2	—	—	Asn	Val	Asn	Tyr	—	—
Hong Kong	0.25	23F	1	—	—	Asn	Val	Asn	Tyr	—	—
Japan	0.25	NT	2	Phe	—	—	Val	—	—	—	—
Hong Kong	0.25	23F	2	Tyr	—	Asn	Val	Asn	Tyr	—	—
Hong Kong	0.25	23F	2	Phe	—	Asn	Val	Asn	Tyr	—	—
Hong Kong	0.25	14	2	—	—	Asn	Val	Asn	Phe	—	—
Australia	0.25	14	2	—	Asn	Asn	Val	—	Phe	—	—

[a] Efflux is expressed as the reduction (*n*-fold) in ciprofloxacin MIC when performed in the presence of reserpine.
[b] —, no difference from *S. pneumoniae* ATCC 49619.
[c] NT, nontypeable.

of ciprofloxacin and a shift in MICs of moxifloxacin have been demonstrated, although not necessarily to resistant levels (30). Analysis of the Asia-Pacific data shows that of the 9% of the ORSA isolates resistant to ciprofloxacin were also resistant to 4-μg/ml garenoxacin. As garenoxacin has been shown not to be a substrate for the *norA* efflux pump (28), this resistance is most likely due to mutations in both topoisomerases. Interestingly, almost all of these resistant isolates came from Japan and Hong Kong. For the methicillin-susceptible *S. aureus*, ciprofloxacin resistance was uncommon (2%) and there was only 1 isolate that was also garenoxacin resistant, which again, came from Japan. Garenoxacin has been shown in vitro to have increased activity compared with moxifloxacin and gatifloxacin against *S. aureus* with mutations in both the *parC* and *gyrA* genes (28), and this was confirmed in our study for gatifloxacin. The occurrence of resistance to the newer fluoroquinolones in ORSA is disappointing and indicates that these agents may not be long-term alternatives for the treatment of ORSA infections.

For the respiratory pathogens, garenoxacin had good activity against *H. influenzae* and *M. catarrhalis*, as did all other fluoroquinolones (Table 3). These organisms do not pose any real therapeutic problem, and it is, therefore, the data for *S. pneumoniae* that are of interest. Within the region, penicillin nonsusceptibility is common, with rates exceeding 70% in some countries (Table 2). In addition, macrolide resistance is widespread, reaching a high of 88% in Taiwan. The clindamycin data indicate that *mef* efflux pump-mediated resistance (M phenotype) accounts for close to 50% of the resistance in most countries in the region, except for South Africa and China, where the *erm* gene pattern predominates. With these significant levels of β-lactam and macrolide resistance, the clinical role of the fluoroquinolones assumes greater importance. Despite the previously reported study (8), penicillin and fluoroquinolone resistance in the pneumococci appears to be related ($P < 0.001$) (Table 4). QRDR sequencing analysis (Table 5) showed that isolates for which the MIC is 0.25 or 0.5 μg/ml had various mutations in both ParC and GyrA. Most of the 11 isolates for which the MIC was 1 μg/ml, however, had similar double mutations in ParC (Ser-79 to Phe and Lys-137 to Asn) and a single mutation in GyrA (either Ser-81 to Phe or Glu-85 to Lys). Some of these changes have been demonstrated in an in vitro resistance selection study (13) to produce low-level resistant mutants, with additional mutations in GyrA at Glu-85 and ParC at Asp-83 required to result in high-level resistance (MIC, 2 to 16 μg/ml). Eight of the 11 strains were from Hong Kong. All were of the same serotype (14) and had similar mutations as those described by Ho et al. (14), suggesting the presence of a specific clone in that area. The Australian isolates also were clonal in nature; they all came from the one center, were of the same serotype (14), and exhibited similar QRDR mutations. Similar shifts in MICs of clinafloxacin, gatifloxacin, trovafloxacin, and gemifloxacin have been demonstrated with levofloxacin-resistant isolates of *S. pneumoniae* (19). These changes in MIC were shown to be due to mutations in the QRDR, where small increases in MIC were associated with one-step mutations in *parC*, and up to 32-fold increases in MIC were associated with double mutations in *parC* and *gyrA*. It is also possible that the elevation of MICs for the fluoroquinolones is due to active efflux (9), although this is unlikely to be the sole cause of high-level resistance (5). No active efflux was demonstrated in this study for the 24 isolates for which the MIC was ≥0.25 μg/ml. All of these isolates had mutations in the QRDR.

In conclusion, our study shows that in the Asia-Pacific region, where fluoroquinolone resistance is well established, garenoxacin activity has been maintained against the respiratory pathogens. There were, however, strains of *S. pneumoniae* that had mutations in both the GyrA and ParC regions of the QRDR. Mutations conferring high-level resistance were not detected, but with the addition of only one further mutation required, resistance can be expected to emerge. For *S. aureus*, in particular ORSA, resistance is already present. Other studies have shown that ciprofloxacin resistance, conferred by mutations in the QRDR, is stable in an in vitro antibiotic-free environment (17) and in clonal lineages in the clinical environment over a period of years (32). It is therefore likely that staphylococcal fluoroquinolone resistance will persist in the Asia-Pacific region. Whether an agent such as garenoxacin has less potential to select for resistant mutants and therefore maintain clinical utility is unclear, but its high potency compared to its pharmacokinetics (35) suggests that this may be the case.

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