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Detection of Resistance to Gatifloxacin and Moxifloxacin in *Streptococcus pneumoniae* with the VITEK 2 Instrument

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A group of 72 pneumococcal isolates resistant or intermediate to levofloxacin and 124 pneumococcal isolates susceptible to fluoroquinolones were tested by the VITEK 2 instrument using investigational test cards and by a broth microdilution reference method. The VITEK 2 instrument performed well, detecting 52 of 60 (86.7%) gatifloxacin-resistant isolates and 22 of 23 moxifloxacin-resistant isolates, and did not falsely classify any susceptible isolates as resistant.

The prevalence of antimicrobial resistance among both invasive and respiratory isolates of *Streptococcus pneumoniae* has increased steadily over the last 15 years. In addition to resistance to penicillin, cephalosporins, macrolides, lincosamides, trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline, there has been concern about emerging resistance to fluoroquinolones (2, 3, 5, 15). At this time, the incidence of fluoroquinolone resistance is low (i.e., <1%) in the United States (2, 3, 15). However, higher rates of resistance to ciprofloxacin or levofloxacin have been reported in some areas of Canada and in Hong Kong (2, 5). Certain newer fluoroquinolones (e.g., gatifloxacin and moxifloxacin) have greater potency (i.e., lower MICs) than the older agents. However, mutations in the genes that encode topoisomerase IV (*parC*) and gyrase A (*gyrA*) also result in increased MICs for the newer drugs (7, 8). It will be important for clinical microbiology laboratories to be able to detect emerging fluoroquinolone-resistant pneumococcal isolates by their standard susceptibility testing methods. This study has evaluated the ability of the bioMerieux VITEK 2 instrument with investigational test cards to detect fluoroquinolone resistance in a selected group of pneumococcal isolates.

A total of 196 isolates of *S. pneumoniae* were selected for inclusion in this study. Most of these isolates originated from the Centers for Disease Control and Prevention's Active Bacterial Core surveillance (ABCs) program. These isolates included 71 isolates with previously determined resistance to levofloxacin (MIC of ≥8 μg/ml) and one strain with an intermediate levofloxacin MIC of 4 μg/ml. Seventeen of these isolates had been previously examined for mutations in the quinolone resistance-determining region of the *parC* and *gyrA* loci known to influence susceptibility to fluoroquinolones (7, 8). More than half of these isolates were also resistant to one or more additional drugs of other classes. The remaining 124 isolates were recent fluoroquinolone-susceptible isolates selected to represent the current surveillance areas of the ABCs program.

All isolates were tested simultaneously by the VITEK 2 instrument with cards specially formulated for this study containing the study fluoroquinolones interpreted using developmental software and by a reference broth microdilution method. The VITEK 2 tests were performed in accordance with the manufacturer's standard protocol for testing *S. pneumoniae*. The reference method consisted of specially prepared frozen broth microdilution panels (TREK Diagnostics, West Lake, Ohio) used in accordance with the NCCLS reference MIC procedure (12). This reference procedure included the use of cation-adjusted Mueller-Hinton broth supplemented with 3% lysed horse blood as the test medium and a final inoculum density of 5×10^5 CFU/ml in the wells of the microdilution panels. Panels were incubated at 35°C in ambient air for 20 to 24 h prior to visual determination of MICs. *S. pneumoniae* ATCC 49619 was tested for quality control purposes in both the VITEK 2 and reference procedures. The most recent NCCLS MIC breakpoints were applied to determine the rates of susceptibility and resistance to the study drugs (13).

The VITEK 2 instrument detected 52 of 60 (86.7%) gatifloxacin-resistant isolates (MIC of ≥4 μg/ml) and 22 of 23

TABLE 1. Susceptibilities of 196 *S. pneumoniae* clinical isolates as determined by reference and VITEK 2 test methods

Antimicrobial agent	Reference MICs (μg/ml)			% Resistance by method:	
	MIC_{50}	MIC_{90}	Range	Reference	VITEK 2
Levofloxacin-susceptible isolates (n = 124)					
Gatifloxacin	0.25	0.5	≤0.12–0.5	0	0
Moxifloxacin	0.12	0.12	≤0.06–0.25	0	0
Levofloxacin-resistant or intermediate isolates (n = 72)					
Gatifloxacin	4	8	0.25–8	83.3	72.2[a]
Moxifloxacin	2	4	≤0.06–4	31.9	54.2[b]

[a] 86.7% of gatifloxacin-resistant strains were categorized as resistant by the VITEK 2 instrument.
[b] 100% of moxifloxacin-resistant strains were categorized as resistant by the VITEK 2 instrument.

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TABLE 2. MIC frequencies and NCCLS interpretive criteria of gatifloxacin as determined by the reference MIC method and the VITEK 2 instrument

Gatifloxacin VITEK 2 MICs	No. of isolates with reference MIC					
	0.25 μg/ml	Susceptible (1 μg/ml)	Intermediate (2 μg/ml)	Resistant		Total
				4 μg/ml	8 μg/ml	
0.5 μg/ml	2	2		1		5
Susceptible (1 μg/ml)		2	5			7
Intermediate (2 μg/ml)			1	7		8
Resistant (4 μg/ml)				24	9	33
8 μg/ml				3	16	19
Total	2	4	6	35	25	72

(95.6%) moxifloxacin-resistant isolates (MIC of ≥4 μg/ml) (Tables 1 and 2). With seven of the eight isolates not recognized as resistant to gatifloxacin by the VITEK 2 instrument, an intermediate MIC was obtained (i.e., 2 μg/ml), while the other isolate was reported as susceptible (MIC of 0.5 μg/ml) (Table 2). The one moxifloxacin-resistant isolate not correctly classified by the VITEK 2 instrument was reported as intermediate (MIC of 2 μg/ml) (Table 3). The MICs found using the VITEK 2 instrument generally agreed well with the reference values (Tables 2 and 3). The essential agreement between the VITEK 2 MICs and reference MICs was 98.6% with gatifloxacin and 97.2% with moxifloxacin (Table 4). Using the VITEK 2 instrument, there was only one very major error (1.67%) with gatifloxacin, although there were 12 (6.1%) minor errors with gatifloxacin and 26 (13.3%) minor errors with moxifloxacin. The relatively high rate of minor errors can be attributed in part to the fact that the interpretive breakpoints assigned to these more potent fluoroquinolones bisect the distribution of elevated MICs of levofloxacin-resistant strains and the fact that a one-dilution error near the breakpoints contributed to the large number of errors with both drugs (Tables 2, 3 and 4). All of the gatifloxacin minor errors and 25 of 26 moxifloxacin minor errors were within one dilution of the reference MIC (Tables 2 and 3). The VITEK 2 instrument did not falsely classify any of the fluoroquinolone-susceptible isolates as resistant, although five of six gatifloxacin-intermediate strains were classified as susceptible by the VITEK 2 instrument. With moxifloxacin, 17 of 30 -intermediate strains were classified as resistant by the VITEK 2 instrument.

TABLE 3. MIC frequencies and NCCLS interpretive criteria of moxifloxacin as determined by the reference MIC method and the VITEK 2 instrument

Moxifloxacin VITEK 2 MICs	No. of isolates with reference MIC						
	0.06 μg/ml	0.125 μg/ml	0.5 μg/ml	Susceptible (1 μg/ml)	Intermediate (2 μg/ml)	Resistant (4 μg/ml)	Total
0.25 μg/ml	1	1			1		3
0.5 μg/ml			3	3			6
Susceptible (1 μg/ml)				3	7		10
Intermediate (2 μg/ml)					13	1	14
Resistant (4 μg/ml)					17	22	39
Total	1	1	3	6	38	23	72

TABLE 4. Comparison of VITEK 2 MICs with reference MICs for the 72 levofloxacin-nonsusceptible isolates

Drug	No. of VITEK 2 MICs that differed from reference MICs[a]							% EA[b]
	−3	−2	−1	Same	+1	+2	+3	
Gatifloxacin	1	0	23	43	5	0	0	98.6
Moxifloxacin	1	0	11	41	18	1	0	97.2

[a] Dilutions indicate the number of VITEK 2 MIC dilutions as compared to reference MICs.
[b] EA, essential agreement (percent VITEK 2 MICs within one dilution of reference MICs).

Fluoroquinolones have been recommended for initial empirical therapy of community-acquired pneumonia by several American and Canadian consensus groups (1, 10, 11, 14). However, clinical failures have been encountered due to invasive infections with fluoroquinolone-resistant strains (4, 16). While fluoroquinolone resistance is uncommon among pneumococci in the general population in the United States at this time, it is much higher in some groups in the United States (e.g., long-term care facility residents [9]) and in some parts of the world (e.g., Hong Kong [6]). First step (usually *parC*) mutations often do not result in high-level levofloxacin resistance in pneumococci or resistance to the newer fluoroquinolones examined in this study. Indeed, as shown in the present study, strains that are resistant to levofloxacin (and contain mutations in *parC* and *gyrA*) may be classified as susceptible to the newer, more potent fluoroquinolones (8). Thus, it will be important for clinical laboratories to be able to quickly and accurately detect fluoroquinolone-resistant pneumococci by their standard susceptibility testing methods. This study has shown that this may be accomplished by use of the VITEK 2 instrument and the investigational cards and interpretive software examined in this study, especially with moxifloxacin. The Food and Drug Administration has approved the use of the VITEK 2 instrument for testing of isolates for moxifloxacin resistance, and cards containing moxifloxacin and levofloxacin have recently been marketed. This extends the list of drugs approved for testing in the VITEK 2 instrument, including those agents previously shown to provide reliable results (6).

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