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Surveillance

Therapeutic options among broad-spectrum β-lactams for infections caused by levofloxacin-nonsusceptible *Streptococcus pneumoniae*

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Abstract

Streptococcus pneumoniae has consistently become more resistant to primary, orally administered treatment regimens used for community-acquired respiratory tract infections (CARTI; sinusitis, bronchitis, pneumonia). As resistance rates approach 40–50% in the United States and North America for penicillin and macrolides, other agents also have exhibited coresistance rates of 10–20% (tetracycline, clindamycin, trimethoprim/sulfamethoxazole). These facts led to altered clinical treatment guidelines (IDSA) supporting the use of respiratory fluoroquinolones (levofloxacin, gatifloxacin, gemifloxacin, and moxifloxacin). This report from the SENTRY Antimicrobial Surveillance Program lists possible parenterally administered treatment alternatives for the fluoroquinolone (levofloxacin)-nonsusceptible pneumococci. The SENTRY Program isolates from CARTI (1997–2003), totaling 21 605 strains from Europe, Asia Pacific, and the Americas, were screened for fluoroquinolone-resistant *S. pneumoniae*. A total of 157 (0.7%) levofloxacin-nonsusceptible (MIC ≥ 4 μg/mL) strains were identified and tested by reference broth microdilution methods against 27 antimicrobials. Quinolone resistance-determining region (QRDR) mutations were determined by PCR amplification and gene sequencing. The entire population of *S. pneumoniae* had the following antibiogram demographics: penicillin-nonsusceptible (32%), macrolide resistance (24%), tetracycline resistance (21%), clindamycin resistance (11%), trimethoprim/sulfamethoxazole resistance (33%), and 6% of strains were resistant to all 5 drugs. Levofloxacin-resistant strains routinely had 2 or more QRDR mutations most frequently in *gyrA* at Ser81Phe or Tyr and in *parC* at Ser79Phe or Tyr and Lys137Asn. Four agents had extremely low rates of resistance when tested against the 157 levofloxacin-nonsusceptible strains (e.g., quinupristin/dalfopristin, 0% resistance; vancomycin, 0%; cefepime, 1%; ceftriaxone, 1%). Levofloxacin-nonsusceptible pneumococcal isolates remain uncommon, but are a growing problem in CARTI (1.4% in 2003), especially in previously fluoroquinolone-treated cases. Parenteral cephalosporins (cefepime or ceftriaxone) continue to be potent and safe for use in hospitalized patients with *S. pneumoniae* community-acquired pneumonia, used with or without co-drugs according to published guidelines.

Keywords: *S. pneumoniae*; Resistance; Levofloxacin; Cefepime

1. Introduction

Community-acquired pneumonia (CAP) is usually caused by *Streptococcus pneumoniae*, a pathogen associated with escalating resistances to β-lactams (penicillins, cephalosporins, carbapenems), macrolides (erythromycin, azithromycin, clarithromycin), and other antimicrobial classes (Mandell et al., 2003; Sader et al., 2003). Rates of penicillin and macrolide resistances have approached 20% and 30%, respectively, since 2000 in the SENTRY Antimicrobial Surveillance Program summarized by Sader et al. (2003). To address this increasing resistance crisis among *S. pneumoniae* isolates, treatment guidelines (Mandell et al., 2003) have embraced the use of respiratory fluoroquinolones for CAP in patients presenting in the clinic, nursing home, and hospital environments. These recommendations of respiratory fluoroquinolones have been supported by voluminous amounts of in vitro information (Jones, 2002) and supportive clinical trial results (Klugman et al., 2004; Mandell et al., 2003).

Resistance to the fluoroquinolones has been well summarized in recent reviews (Eliopoulos, 2004), and rates of resistance using levofloxacin (least active by weight) as

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an indicator compound has been increasing, first documented by Chen et al. (1999) for a series of Canadian isolates in the late 1990s. Levofloxacin resistance has been consistently evolving from 1997 through 2002 in the United States and Canada moving from 0.4% to 1.8% (Brown et al., 2004; Chen et al., 1999; Hoban et al., 2001; Powis et al., 2004; Sader et al., 2003). This rate may be even more striking if breakpoints consistent with the application of genetic and pharmacokinetic/pharmacodynamic principles were used that would reduce the levofloxacin susceptible breakpoint (750 mg dosing) to ≤1 μg/mL (Smith et al., 2004). Previous studies of clinical outcomes related to genetic resistances and MIC breakpoints for macrolides have supported the principal that breakthrough bacteremias and adverse clinical responses were noted associated with *S. pneumoniae* strains having efflux and methylase-mediated mechanisms and elevated MIC values (≥0.5 μg/mL) (Lonks et al., 2002). Other features of the macrolide azalide class must be considered including modulation (inhibition) of the protective immune response to pneumococcal infection or vaccine challenge (Fernandez et al., 2004).

Reports of levofloxacin clinical treatment failures versus *S. pneumoniae* infections have increased since the earliest cases (Davidson et al., 2002; Wortmann and Bennett, 1999). To assess the agents that could rescue hospitalized patients infected with fluoroquinolone-resistant *S. pneumoniae*, we searched the SENTRY Program collection for strains having elevated MIC values to levofloxacin. Antibiograms for selected parenteral antimicrobials were determined for the levofloxacin refractory isolates (157 strains) by reference methods and compared with the recommendations of contemporary treatment guidelines (Mandell et al., 2003).

2. Materials and methods

2.1. Organisms

All *S. pneumoniae* strains were derived from the SENTRY Program collection worldwide for the years 1997–2003. A total of 21 405 isolates were tested from Europe, the Asia Pacific region, North America, and Latin America, from which 157 strains (0.7%) were found to have levofloxacin-nonsusceptible (MIC ≥ 4 μg/mL) results; all isolates were from respiratory tract sources.

Characteristics of the entire collection antibiograms were penicillin-nonsusceptible at 32%, erythromycin/clindamycin resistance at 24/11%, tetracycline resistance at 21%, and trimethoprim/sulfamethoxazole resistance at 33%. Six percent of all tested isolates were resistant to all 5 agents listed above.

2.2. Susceptibility testing

All antimicrobial susceptibility tests were performed using reference broth microdilution methods as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2003) in the M7-A6 document. All quality control results for the 27 tested agents (11 shown here) were within published control limits (NCCLS, 2005). To judge the in vitro efficacy of those agents that would be candidates for treatment using recently published therapeutic guidelines for CAP possibly caused by *S. pneumoniae*, only parenterally administered agents were compared (Klugman et al., 2004; Mandell et al., 2003). Two agents were added to this list of possible agents, cefepime and cefdinir, as demonstrating significant activity versus contemporary *S. pneumoniae* strains (Pottumarthy et al., 2005; Sader et al., 2003).

Table 1
Distribution of QRDR mutations for *S. pneumoniae* strains having nonsusceptible (MIC ≥ 4 μg/mL) in vitro testing results for levofloxacin (SENTRY Program)

Levofloxacin MIC (μg/mL) (no. tested)	No. of strains (%) by QRDR site			
	gyrA	*gyrB*	*parC*	*parE*
>4 (40)	32(80)[a]	3(8)[b]	35(88)[c]	24(60)[d]
4 (2)[e]	1(50)	1(50)	1(50)	2(100)

[a] Mutation sites (occurrences) were Ser81Phe (27), Ser81Tyr (3), Glu85Lys (3), and Ala17Thr (1).
[b] Mutation sites (occurrences) were Asp435Ile (2) and Val432Asp (1).
[c] Mutation sites (occurrences) were Ser79Phe (25), Lys137Asn (8), Ser79Tyr (5), Asn91Asp (2), Asp83Tyr (2), and 1 occurrence each of Asp83Asn, Asp83Gly, Glu135Asp, Gly77Glu, and Ser52Gly.
[d] Mutation sites (occurrences) were Ile460Val (23), Asp435Asn (3), and Pro454Ser (1).
[e] Mutations were found for *gyrA* (Ser81Phe, 1), *gyrB* (Asp435Glu, 1), *parC* (Asp83Asn, 1), and for *parE* (Asp435Asn and Ile460Val, 1 each).

2.3. Fluoroquinolone resistance mechanisms

All isolates available for testing having a levofloxacin MIC ≥ 4 μg/mL were processed for possible mutations in the quinolone resistance-determining region (QRDR) by DNA extraction and amplification methods using procedures described before (Morrissey et al., 2003). These *gyrA* and *gyrB* or *parC* and *parE* DNA gyrase and topoisomerase IV encoding genes were assessed for 42 isolates (Table 1), 40 having a levofloxacin MIC ≥ 8 μg/mL. A total of 96 mutations in the QRDR were detected (2.3 per strain), least often for *gyrB*. An additional group of 28 *S. pneumoniae* with a levofloxacin MIC at 2 μg/mL was tested for QRDR mutations to determine the frequency of silent genetic modifications (susceptible MIC) occurring at the current NCCLS (2005) breakpoint concentration (Smith et al., 2004).

3. Results and discussion

3.1. Mutations within the QRDR

Those QRDR mutations associated with elevated levofloxacin MIC results (MIC ≥ 4 μg/mL) are listed in Table 1. Two or more mutations were common, most notable for *parC* (36 occurrences) > *gyrA* (33 occurrences) > *parE* (26 occurrences). The documented QRDR mutations were similar to those described in levofloxacin- or other fluoroquinolone-resistant strains (Brown et al., 2004; Eliopoulos, 2004; Morrissey et al., 2003; Pletz et al., 2004; Smith et al., 2004;

Weiss et al., 2001). Among the *gyrA* mutations, the modification of Ser81 to Phe (28 occurrences) or Tyr (3 occurrences) accounted for 31 of 35 (88.6%) amino acid changes (Table 1). Nearly 90% of all strains with an elevated levofloxacin MIC had a *parC* alteration, most commonly Ser79 to Phe (25 occurrences) or Tyr (5 occurrences). At *parC*, the second most common mutation site was Lys137Asn (only 8 occurrences; Table 1). The *parE* mutations were dominated by the Ile460Val change (82.8% of modifications detected). Clearly, 2 or more QRDR mutations are required for significant elevation of the levofloxacin MIC into the intermediate or resistant range.

In contrast, the following mutations in *parC* were detected among a sample of 28 *S. pneumoniae* having a levofloxacin MIC at 2 μg/mL (susceptible breakpoint by NCCLS, 2005, criteria): Lys137Asn (8), Ser79Phe (5), or Tyr (4), Asn91Asp (3), Asp83Asn (2), Asp83Tyr (1), and Glu135Asp (1). For the 8 strains having no *parC* changes, only 1 strain did not have an alteration in either *parE* or *gyrA* (data not shown).

3.2. Activity of alternative agents

Table 2 lists the cumulative percentage of strains inhibited by increasing concentrations of 11 parenterally administered agents tested against 157 levofloxacin-nonsusceptible strains. Each of these strains had a ciprofloxacin MIC ≥ 4 μg/mL (Chen et al., 1999), indicating high likelihood of QRDR mutation and poor clinical response. The lowest rates of resistance were encountered for vancomycin and quinupristin/dalfopristin (no resistant strains, 0%) and cefepime or ceftriaxone (1% resistance; 1 strain at 4 μg/mL). All other alternative parenteral agents exhibited poor susceptibility rates ranging from 13% (gatifloxacin) to 73% (chloramphenicol).

When comparing these results to the Infectious Diseases Society of America (IDSA) therapy recommendations for CAP (Mandell et al., 2003; see Table 3), options for the treatment of inpatient or nursing home patient populations becomes limited to the third (ceftriaxone or cefotaxime)- and fourth (cefepime)-generation cephalosporins, especially for patients that have received prior fluoroquinolone treatment. If *Pseudomonas aeruginosa* was suspected, then cefepime would become an option plus co-drug(s) (an active fluoroquinolone) or aminoglycoside plus a respiratory fluoroquinolone or a macrolide. Cefdinir among the orally administered cephalosporins also appears to be another option for use in the ambulatory, clinic patients with suspected CAP (Sader et al., 2003; see Table 3). The cefdinir potency and spectrum was most similar to listed cephalosporin agents such as cefpodoxime, cefprozil, and cefuroxime axetil (Mandell et al., 2003).

3.3. Comments

As fluoroquinolones become more widely used for numerous indications, selective pressure toward resistance in *S. pneumoniae* will evolve at predictable rates demonstrated by prior experience (Brown et al., 2004; Chen et al., 1999; Hoban et al., 2001; Powis et al., 2004; Sader et al., 2003; Smith et al., 2004). This can be adversely affected with increased rates by clonal occurrences as exhibited in Hong Kong (Ho et al., 1999; Morrissey et al., 2003), in the Centers for Disease Control and Prevention Active Bacterial Core Surveillance (CDC-ABC) (Pletz et al., 2004), in specific elderly age groups (Powis et al., 2004), and in nosocomial epidemics (Weiss et al., 2001). An association of fluoroquinolone resistance has already been established with 5 pneumococcal international clones, highest with the Spain^{23F}-1 (Ho et al., 1999; Morrissey et al., 2003; Pletz et al., 2004; Weiss et al., 2001), a clone commonly resistant to penicillin and macrolides (Pletz et al., 2004). Furthermore, the fluoroquinolone (levofloxacin)-resistant *S. pneumoniae* have been produced by 2 or more mutations in the QRDR (Brown et al., 2004; Pletz et al., 2004; Smith et al., 2004) with the probability (higher mutational rate) of a greater frequency of additional genetic changes (Eliopoulos, 2004).

In contrast, a favorable vaccine effect on the resistance rates for β-lactams and macrolides has emerged with the use of the heptavalent conjugate product as shown by the SENTRY Program in 2002 (Sader et al., 2003) and by the CDC-ABC Surveillance for the same year (Pletz et al., 2004).

Table 2
Activity of alternative parenterally administered antimicrobial agents tested against 157 levofloxacin-nonsusceptible (MIC ≥ 4 μg/mL) isolates from the SENTRY Antimicrobial Surveillance Program (1997–2003)

Antimicrobial agent	Cumulative % inhibited at MIC (μg/mL)									% by category	
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	Susceptible	Resistant
Cefepime	–	50	55	64	83	99	100	–	–	83	1
Ceftriaxone	45	48	56	64	85	99	100	–	–	85	1
Chloramphenicol	–	–	–	–	–	41	73	73	95	73	27
Clindamycin	–	–	59	61	62	63	–	–	–	59	39
Erythromycin	–	–	34	34	35	44	54	59	–	34	66
Gatifloxacin	0	0	1	8	13	25	79	–	–	13	75
Penicillin	44	50	54	58	65	87	98	–	–	44	35
Quinupristin/dalfopristin	–	–	19	82	98	100	–	–	–	98	0
Tetracycline	–	–	–	–	–	45	48	54	–	45	52
Trimethoprim/sulfamethoxazole	–	–	–	46	50	57	–	–	–	46	43
Vancomycin	–	10	57	98	100	–	–	–	–	100	–

Table 3
Guidelines for antimicrobial treatment of CAP (modified from Mandell et al., 2003; Klugman et al., 2004)

Patient variables	Preferred treatment options
Outpatient	
Previously healthy	
No recent antibiotic therapy	Macrolide or doxycycline
Recent antibiotic therapy	Respiratory fluoroquinolone,[a] advanced macrolide[b] plus high-dose amoxicillin or advanced macrolide plus high-dose amoxicillin/clavulanate
Comorbidities (chronic obstructive pulmonary disease, diabetes, renal, or congestive heart failure)	
No recent antibiotic therapy	Advanced macrolide or respiratory fluoroquinolone
Recent antibiotic therapy	Respiratory fluoroquinolone or advanced macrolide plus oral β-lactam[c]
Suspected aspiration	Amoxicillin/clavulanate or clindamycin
Influenza with bacterial superinfection	Oral β-lactam or respiratory fluoroquinolone
Inpatient	
Medical ward	
No recent antibiotic therapy	Respiratory fluoroquinolone or advanced macrolide plus parenteral β-lactam[d]
Recent antibiotic therapy	Advanced macrolide plus parenteral β-lactam or respiratory fluoroquinolone alone
Intensive care unit	
Pseudomonas infection not an issue	Parenteral β-lactam plus either an advanced macrolide or respiratory fluoroquinolone
Patient β-lactam allergic	Respiratory fluoroquinolone with or without clindamycin
Pseudomonas infection possible	Antipseudomonal agent plus ciprofloxacin or antipseudomonal agent plus an aminoglycoside plus respiratory fluoroquinolone or a macrolide
Patient β-lactam allergic	Aztreonam plus gatifloxacin or levofloxacin or moxifloxacin with or without an aminoglycoside
Nursing home	
Receiving treatment in nursing home	Respiratory fluoroquinolone or amoxicillin/clavulanate plus an advanced macrolide
Hospitalized	Same as for inpatient on a medical ward

[a] Gatifloxacin, gemifloxacin, levofloxacin, or moxifloxacin.
[b] Clarithromcyin or azithromcyin.
[c] High-dose amoxicillin/clavulanate, cefpodoxime, cefprozil, cefuroxime, and (cefdinir), all orally taken.
[d] Ampicillin/clavulanate, (cefepime), cefotaxime, ceftriaxone, and ertapenem, all by parenteral route.

The latter program also noted a significant reduction in levofloxacin-resistant isolates (Pletz et al., 2004). Concerns have been voiced that capsular switching will occur negating the favorable decreases in multiresistant serotypes (Klugman, 2004). Concurrently, however, the vaccine has reduced the occurrence of invasive *S. pneumoniae* disease and the resulting morbidity or mortality (Klugman, 2004; Klugman et al., 2004; Pletz et al., 2004). This continued evolution of levofloxacin resistance could be further diminished by using appropriate doses of the newer, more potent, respiratory fluoroquinolones (gatifloxacin, gemifloxacin, and moxifloxacin) to produce concurrent mutant prevention concentrations (MPCs) and high pharmacokinetic/pharmacodynamic target attainment (Smith et al., 2003, 2004).

As the fluoroquinolone-resistant and/or multidrug-resistant pneumococci emerge, alternative regimens will be required and selected from among suggested therapies (Mandell et al., 2003). Data presented here (Table 2) and elsewhere (Pottumarthy et al., 2005) suggest that combinations of potent parenteral cephalosporins (cefepime and ceftriaxone) and some newer fluoroquinolones (gatifloxacin, gemifloxacin, and moxifloxacin) offer the greatest initial empiric coverage (Mandell et al., 2003). Only 1% of levofloxacin-resistant strains tested in the SENTRY Program (1997–2003) were resistant (MIC $\geq$ 4 μg/mL) to cefepime or ceftriaxone, slightly greater than vancomycin or quinupristin/dalfopristin, but these latter agents possess narrower spectrums of overall activity and higher associated toxicity. Jones et al. (2002) showed that the increased NCCLS (2005) breakpoint for respiratory tract infection isolates of *S. pneumoniae* indicates that 96.5%, 96.5%, and 95.2% of US isolates were susceptible to cefepime, cefotaxime, and ceftriaxone, respectively. In fact, only 2.9% of penicillin-resistant *S. pneumoniae* were cefepime-resistant, a 2- to 3-fold lower rate of resistance when compared with cefotaxime or ceftriaxone (Jones et al., 2002).

As these fluoroquinolones and broad-spectrum parenteral cephalosporins become more widely used, local susceptibility test profiling must be provided. Furthermore, information concerning prior exposure histories to these parenteral agents and orally administered ambulatory practice antimicrobials (Klugman et al., 2004) should be available along with other agents listed in practice guidelines (Mandell et al., 2003).

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