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Potential Options for Parenteral Therapy of Community-Acquired Infections Caused by Levofloxacin-Resistant *Streptococcus pneumoniae*: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: *S. pneumoniae* has consistently become more resistant (R) to primary, orally administered treatment regimens used for community-acquired respiratory tract infections (CARTI; sinusitis, bronchitis, pneumonia). As R rates approach 40 - 50% in the US for penicillin (PEN) and macrolides (ER), other agents also have exhibited co-resistances of 10 - 20% (tetracycline [TC], clindamycin [CM], trimethoprim/sulfamethoxazole [T/S]). These facts led to clinical guidelines (IDSA) supporting the use of respiratory fluoroquinolones (FQs; levofloxacin [LEV], gatifloxacin [GATI] and moxifloxacin). This report from the SENTRY Program lists possible treatment alternatives for the FQ-R pneumococci.

Methods: The SENTRY Program isolates from CARTI (1997-2003); 21,605 strains) from Europe, Asia-Pacific and the Americas were screened for FQ-R *S. pneumoniae* (LEV MIC, ≥ 4 µg/ml). A total of 157 strains (0.7%) were identified and tested by NCCLS reference broth microdilution methods against 27 antimicrobials, 11 reported here. QRDR mutations were determined by PCR amplification and gene sequencing.

Results: The entire population of *S. pneumoniae* had the following antibiogram demographics: PEN-I and -R (32%), ER-R (24%), TC-R (21%), CM-R (11%), T/S-R (33%); 6% were R to all 5 drugs. The following table summarizes the results for 157 FQR isolates:

	MIC (µg/ml)		% by category:	
Antimicrobial	50%	90%	S	R
Cefepime (CPM)	≤0.12	2	83	1
Ceftriaxone (CTX)	0.25	2	85	1
Chloramphenicol	4	16	73	27
CM	≤0.25	>2	59	39
ER	4	>8	34	66
GATI	4	>4	13	75
PEN	0.12	4	44	35
Quinupristin/Dalfopristin (Q/D)	0.5	1	98	0
TC	8	>8	45	52
T/S	1	>2	46	43
Vancomycin (VAN)	0.25	0.5	100	-

FQR strains routinely had ≥ 2 QRDR mutations most frequently: *gyrA* at Ser81Phe or Tyr and *parC* at Ser79Phe or Tyr and Lys137Asn.

Conclusions: FQ-R (LEV MIC, ≥ 4 µg/ml) pneumococcal isolates are a rare, but growing problem in CARTI (1.4% in 2003) especially in previously FQ-treated cases. VAN (0% R), Q/D (0%), CPM and CTX (1%) are the best alternatives for parenteral therapy in patients requiring hospitalization. All other options tested only inhibited 13 - 73% of strains at the NCCLS S breakpoints.

INTRODUCTION

Community-acquired pneumonia (CAP) is usually caused by *Streptococcus pneumoniae*, a pathogen associated with escalating resistances to ß-lactams (penicillins, cephalosporins, carbapenems), macrolides (erythromycin, azithromycin, clarithromycin) and other antimicrobial classes. Rates of penicillin and macrolide resistances have approached 20% and 30%, respectively, since 2000 in the SENTRY Antimicrobial Surveillance Program. To address this increasing resistance crisis among *S. pneumoniae* isolates, treatment guidelines 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 and supportive clinical trial results.

Resistance to the fluoroquinolones has been well summarized in recent reviews, and rates of resistance using levofloxacin (least active by weight) as an indicator compound has been increasing, first documented by Chen et al. for a series of Canadian isolates in the late 1990s. Levofloxacin resistance has been consistently evolving from 1997 through 2002 in the United States (US) and Canada moving from 0.4 to 1.8%. This rate may be even more striking if breakpoints consistent with the application of genetic and pharmacokinetic/pharmacodynamic (PK/PD) principles were utilized that would reduce the levofloxacin susceptible breakpoint (750 mg dosing) to ≤ 1 µg/ml. 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 (*mefA*) and methylase-mediated (*erm*) mechanisms and elevated MIC values of ≥ 0.5 µg/ml. Other features of the macrolide-azalide class must be considered including modulation (inhibition) of the protective immune response to pneumococcal infection or vaccine challenge.

Reports of levofloxacin clinical treatment failures versus *S. pneumoniae* infections have increased since the earliest cited cases. 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 MIC methods and compared to the recommendations of contemporary CAP treatment guidelines.

MATERIALS AND METHODS

Organisms. All *S. pneumoniae* strains were derived from the SENTRY Program collection worldwide for the years 1997 - 2003. A total of 21,605 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-non-susceptible (MIC, ≥ 4 µg/ml) results; all isolates were from respiratory tract sources. Characteristics of the entire collection antibiogram were: penicillin-non-susceptible 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 five agents listed above.

Susceptibility testing. All antimicrobial susceptibility tests were performed using reference broth microdilution methods as described by the CLSI/NCCLS in the M7-A6 document. All quality control results for the 27 tested agents (11 presented here) were within published control limits. Only parenterally administered agents were compared, 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*. Two agents were added to this list of possible agents, cefepime and cefdinir, as demonstrating significant activity versus contemporary *S. pneumoniae* strains.

Fluoroquinolone resistance mechanisms. All isolates available for testing having a levofloxacin MIC of ≥ 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. These *gyrA* and *B* or *parC* and *E* DNA gyrase and topoisomerase IV encoding genes were assessed for 42 isolates (Table 1), 40 having a levofloxacin MIC of ≥ 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 were tested for QRDR mutations to determine the frequency of silent genetic modifications (susceptible MIC) occurring at the current CLSI/NCCLS breakpoint concentration.

RESULTS

- Two or more mutations were common, most notable for *parC* (36 occurrences) > *gyrA* (33 occurrences) > *parE* (26 occurrences). Among the *gyrA* mutations, the modification of Ser81 to Phe (28 occurrences) or Tyr (three 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 (five occurrences). Clearly two or more QRDR mutations are required for significant elevation of the levofloxacin MIC into the intermediate or resistant range (Table 1).

- 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 CLSI): Lys137Asn (eight occurrences), Ser79Phe (five) or Tyr (four), Asn91Asp (three), Asp83Asn (two), Asp83Tyr (one) and Glu135Asp (one). For the eight strains having no *parC* changes, only one strain did not have an alteration in either *parE* or *gyrA* (data not shown).

Table 1. Distribution of QRDR[a] mutations for *S. pneumoniae* strains having non-susceptible (MIC, ≥ 4 µg/ml) in vitro testing results for levofloxacin (SENTRY Program).

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

a. QRDR = quinolone resistance determining region.
b. Mutation sites (occurrences) were: Ser81Phe (27), Ser81Tyr (three), Glu85Lys (three) and Ala17Thr (one).
c. Mutation sites (occurrences) were: Asp435Ile (two) and Val432Asp (one).
d. Mutation sites (occurrences) were: Ser79Phe (25), Lys137Asn (eight), Ser79Tyr (five), Asn91Asp (two), Asp83Tyr (two) and one occurrence each of Asp83Asn, Asp83Gly, Glu135Asp, Gly77Glu and Ser52Gly.
e. Mutation sites (occurrences) were: Ile460Val (23), Asp435Asn (three) and Pro454Ser(one).
f. Mutations found: for *gyrA* (Ser81Phe [one]), for *gyrB* (Asp435Glu [one]), for *parC* (Asp83Asn [one]) and for *parE* (Asp435Asn and Ile460Val [one each]).

Table 2. Activity of alternative parenterally administered antimicrobial agents tested against 157 levofloxacin non-susceptible (MIC, ≥ 4 µg/ml) isolates from the SENTRY Antimicrobial Surveillance Program (1997-2003).

	Cum. % inhibited at MIC (µg/ml):								% by category:	
Antimicrobial agent	≤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
Trim/Sulfa[a]	-	-	-	46	50	57	-	-	-	46	43
Vancomycin	-	10	57	98	100	-	-	-	-	100	-

a. Trimethoprim/Sulfamethoxazole

- Table 2 lists the cumulative percentage of strains inhibited by increasing concentrations of 11 parenterally administered agents tested against 157 levofloxacin-non-susceptible strains. Each of these strains had a ciprofloxacin MIC at ≥ 4 µg/ml, 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; one 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 IDSA therapy recommendations for CAP, 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 (Table 3).

Table 3. Guidelines for antimicrobial treatment of community-acquired pneumonia (modified from Mandell et al., 2003 and 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
Co-morbidities (COPD, diabetes, renal or CHF)	
No recent antibiotic therapy	Advanced macrolide or respiratory fluoroquinolone
Recent antibiotic therapy	Respiratory fluoroquinolone or advanced macrolide plus oral ß-lactam[c]
Suspected aspiration	Amoxicillin/clavulanate or clindamycin
Influenza with bacterial superinfection	Oral ß-lactam or respiratory fluoroquinolone
Inpatient	
Medical ward	
No recent antibiotic therapy	Respiratory fluoroquinolone or advanced macrolide plus parenteral ß-lactam[d]
Recent antibiotic therapy	Advanced macrolide plus parenteral ß-lactam or respiratory fluoroquinolone alone
ICU	
Pseudomonas infection not an issue	Parenteral ß-lactam plus either an advanced macrolide or respiratory fluoroquinolone
Patient ß-lactam allergic	Respiratory fluoroquinolone with or without clindamycin
Pseudomonas infection possible	Anti-pseudomonal agent plus ciprofloxacin or anti-pseudomonal agent plus an aminoglycoside plus respiratory fluoroquinolone or a macrolide
Patient ß-lactam allergic	Aztreonam plus gatifloxacin or levofloxacin or moxifloxacin with or without an aminoglycoside
Nursing home	
Receiving treatment in nursing home	Respiratory fluoroquinolone or amoxicilliin/clavulanate plus an advanced macrolide
Hospitalized	Same as for inpatient on a medical ward

a. Gatifloxacin, gemifloxacin, levofloxacin or moxifloxacin.
b. Clarithromycin or azithromycin.
c. High-dose amoxicillin/clavulanate, cefpodoxime, cefprozil, cefuroxime and (cefdinir); all PO.
d. Ampicillin/clavulanate, (cefepime), cefotaxime, ceftriaxone and ertapenem; all by parenteral route.

CONCLUSIONS

- 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. This can be adversely affected with increased rates by clonal occurrences as exhibited in Hong Kong, in the Center for Disease Control and Prevention Active Bacterial Core Surveillance (CDC-ABC), in specific elderly age groups and in nosocomial epidemics. Furthermore, the fluoroquinolone (levofloxacin)-resistant *S. pneumoniae* have been produced by ≥ 2 mutations in the QRDR with the probability (higher mutational rate) of a greater frequency of additional genetic changes.

- A favorable vaccine effect on the resistance rates for ß-lactams and macrolides has emerged with the use of the heptavalent conjugate product as demonstrated by the SENTRY Program in 2002, and by the CDC-ABC Surveillance for the same year. The 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 PK/PD target attainment.

- As the fluoroquinolone-resistant and/or multidrug-resistant pneumococci emerge, alternative regimens will be required and selected from among suggested CAP therapies. Data presented here (Table 2) and elsewhere suggests that combinations of potent parenteral cephalosporins (cefepime and ceftriaxone) and some newer fluoroquinolones (gatifloxacin, gemifloxacin and moxifloxacin) offer the greatest initial empiric coverage. Only 1% of levofloxacin-resistant strains tested in the SENTRY Program (1997 - 2003) were resistant (MIC, ≥ 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.

- As these fluoroquinolones and broad-spectrum parenteral cephalosporins become more widely used, local susceptibility test profiling must be provided.

SELECTED REFERENCES

Chen D, McGeer A, de Azavedo JC, Low DE. The Canadian Bacterial Surveillance Network (1999) Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. *New England Journal of Medicine* 341:233-239.

Davidson R, Cavalcanti R, Brunton JL, Bast DJ, de Azavedo JC, Kibsey P, Faming C, Low DE (2002) Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. *New England Journal of Medicine* 346:747-750.

Ho PL, Que TL, Tsang DN, Ng TK, Chow KH, Seto WH (1999) Emergence of fluoroquinolone resistance among multiply resistant strains of *Streptococcus pneumoniae* in Hong Kong. *Antimicrobial Agents and Chemotherapy* 43:1310-1313.

Hoban DJ, Doern GV, Fluit AC, Roussel-Delvallez M, Jones RN (2001) Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* in the SENTRY Antimicrobial Surveillance Program, 1997 - 1999. *Clinical Infectious Disease* 32(Suppl 2):81-93.

Jones RN, Mutnick AH, Varnam DJ (2002) Impact of modified nonmeningeal *Streptococcus pneumoniae* interpretive criteria (NCCLS M100-S12) on the susceptibility patterns of five parenteral cephalosporins: Report from the SENTRY Antimicrobial Surveillance Program (1997 to 2001). *Journal of Clinical Microbiology* 40:4332-4333.

Klugman KP, Low DE, Mettay J, Pechere J-C, Weiss K (2004) Community-acquired pneumonia: New management strategies for evolving pathogens and antimicrobial susceptibilities. *International Journal of Antimicrobial Agents* 24:411-422.

Lonks JR, Garau J, Gomez L, Xercavins M, Ochea de Echaguen A, Gareen IF, Reiss PT, Medeiros AA (2002) Failure of macrolide antibiotic treatment in patients with bacteremia due to erythromycin-resistant *Streptococcus pneumoniae*. *Clinical Infectious Disease* 35:556-564.

Mandell LA, Bartlett JG, Dowell SF, File Jr TM, Musher DM, Whitney C (2003) Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. *Clinical Infectious Disease* 37:1405-1433.

National Committee for Clinical Laboratory Standards (2003) *Performance standards for antimicrobial Susceptibility Testing. Document M7-A6*. Wayne, PA:NCCLS.

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

Powis J, McGeer A, Green K, Vanderkooi O, Weiss K, Zhanel G, Mazzulli T, Kuhn M, Church D, Davidson R, Forward K, Hoban D, Simor A, The Canadian Bacterial Surveillance Network, Low DE (2004) In vitro antimicrobial susceptibilities of *Streptococcus pneumoniae* clinical isolates obtained in Canada in 2002. *Antimicrobial Agents and Chemotherapy* 48:3304-3311.

Sader HS, Fritsche TR, Mutnick AH, Jones RN (2003) Contemporary evaluation of the in vitro activity and spectrum of cefdinir compared to other orally administered antimicrobials tested against common respiratory tract pathogens. *Diagnostic Microbiology and Infectious Disease* 47:515-526.

Weiss K, Restieri C, Gauthier R, Laverdiere M, McGeer A, Davidson RJ, Kilburn L, Bast DJ, de Azavedo J, Low DE (2001) A nosocomial outbreak of fluoroquinolone-resistant *Streptococcus pneumoniae*. *Clinical Infectious Disease* 33:517-522.

Wortmann GW, Bennett SP (1999) Fatal meningitis due to levofloxacin-resistant *Streptococcus pneumoniae*. *Clinical Infectious Disease* 29:1599-1600.