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Emergence and Epidemic Clonality of Multi-Drug Resistant *S. pneumoniae* (1999 - 2003): Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: Emerging resistance (R) among *S. pneumoniae* (SPN) particularly to penicillin (PEN), erythromycin (ER), clindamycin (CC), tetracyclines (TC) and trimethoprim/sulfamethoxazole (T/S) continues to levels compromising orally administered therapy for community-acquired (CA) RTI. Concern has increased that multi-drug resistant (MDR)-SPN strains could also become fluoroquinolone (FQ)-R, as well as emerging R among *H. influenzae* (HI) and *M. catarrhalis* (MCAT).
Methods: SPN (2379), HI (2456) and MCAT (901) isolated in the SENTRY Program in 2003 were tested by reference MIC methods against > 30 antimicrobials. Also SPN from 1999 - 2003 (592 strains) were assessed for trends in MDR to PEN, ER, CC, TC and T/S. FQR rates were analyzed for trends or clones by ribotyping, PFGE and PCR of QRDR (2003).
Results: HI ß-lactamase (BL) production varied from 11.6 (Latin America [LA]) to 27.3% (N. America [NA]), with BL rates in MCAT stable at 94.7 - 95.6%. PEN-R in SPN was 14.7, 12.7 and 15.9% for EU, LA and NA, respectively. MDR-SPN increased from 5.7% (1999) to 6.3% (2003) in NA, but no FQR increase for the 2001 - 2003 MDR-SPN samples. An epidemic SPN FQR (levofloxacin MIC, > 32 µg/ml) cluster was detected in Italy (ribotype/PFGE = 333-3/A; same serotype; 23F), observed in 5 patients in 2002 (1) and 2003 (4). These strains were S to ß-lactams, T/S, chloramphenicol and rifampin and R to MLS_B drugs and TC. Patients were generally female (4) with ages 5 - 65 y (ave, 29 y). QRDR mutations were: *gyrA* (S81F), *parC* (S79F, K137N) and *parE* (I460V). Excluding this clone, overall FQR rates did not significantly vary from the prior year (2002) experience across regions (NA > EU > LA).
Conclusions: MDR- (variable rate) and FQR-SPN (increasing rate) continue to occur across all regions with some detectable epidemic, CARTI clones. HI- and MCAT-R rates remain stable in 2003 for all regions. Surveillance appears to be a prudent practice to follow impacts of vaccine and FQ use on R and co-R rates for clonal occurrences.

INTRODUCTION

Community-acquired respiratory tract infections (CARTI) remain the leading cause of clinical office visits and use of antimicrobial agents. *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* are the most common bacterial pathogens associated with CARTI. Therefore, empirical antimicrobial therapeutic treatment should be directed towards eradication of these organisms. Worldwide, the reports of increasing resistance among these fastidious organisms to many antimicrobial agents has made treatment management more difficult. Therefore, multicenter, global, longitudinal studies such as the SENTRY Antimicrobial Surveillance Program (established in 1997) were initiated to monitor the antimicrobial resistant trends in CARTI to provide clinicians with a valuable tool in their decision making process.

ß-lactams, macrolides and more recently, fluoroquinolones are the classes of antimicrobial agents most often prescribed for the treatment of CARTI. The main pathogens, *S. pneumoniae, H. influenzae* and *M. catarrhalis* have resistance mechanisms that can compromise the activity of these compounds. ß-lactamase production is very common among *M. catarrhalis* strains (> 95%) and *H. influenzae* isolates with ampicillin-resistant rates ranging from 12% in parts of Latin America to > 25% in North America. In contrast, *S. pneumoniae* strains primarily have target modifications (altered penicillin-binding proteins), erythromycin-resistant methylation, mutations in the quinolone resistant-determining region (QRDR), and drug efflux (macrolides and fluoroquinolones) as resistant mechanisms. The rates of penicillin-non-susceptible *S. pneumoniae* has been reported at > 50% in some parts of the world. Recent surveillance studies have detected a rapid increase in macrolide resistant strains of pneumococci while fluoroquinolone-resistance (FQR) has remained relatively low in most areas of the world. Most alarming is the increase in the rate of multi-drug resistant (MDR) isolates (resistant to ≥ three classes of compounds). Genes encoding resistance to tetracycline, trimethoprim/sulfamethoxazole and chloramphenicol are carried on the same transposon facilitating their dissemination. Studies have shown that the increase in the prevalence of pneumococcal clones has spread globally and complicated the treatment options for CARTI.

In this study, we determined the in vitro activity of more than 30 antimicrobial agents against 5,736 clinical isolates of *S. pneumoniae, H. influenzae* and *M. catarrhalis*. These organisms were acquired from patients diagnosed with CARTI as part of the SENTRY Antimicrobial Surveillance Program during 2003 from medical centers in Europe, Latin America and North America. Analysis of 592 strains of MDR *S. pneumoniae* (collected from 1999 - 2003) and the discovery of an epidemic cluster of FQR in Italy (2002 - 2003) are also presented.

MATERIALS AND METHODS

Organisms tested. The organisms tested in this study were collected by more than 60 geographically diverse medical centers in North America, Latin America and Europe as part of the SENTRY Antimicrobial Surveillance Program in 2003. The 5,736 isolates included *S. pneumoniae* (2,379 strains), *H. influenzae* (2,456 strains) and *M. catarrhalis* (901 strains) from patients diagnosed with CARTI. In addition, a subset of 592 *S. pneumoniae* strains collected from 1999 - 2003 were analyzed for trends in drug resistance. Organism identification was confirmed at the monitoring site (North Liberty, IA) using colony morphology and the bile solubility test for *S. pneumoniae*; oxidase production and butyrate disk for *M. catarrhalis*. ß-lactamase production was assessed for *M. catarrhalis* and *H. influenzae* using the nitrocefin disk test.

Antimicrobial agents. More than 30 antimicrobial compounds were tested including ß-lactams, macrolide-lincosamide-streptogramin B agents, fluoroquinolones, tetracycline, rifampin, chloramphenicol and trimethoprim/sulfamethoxazole.

Susceptibility testing. All strains were tested in validated dry-form panels (TREK Diagnostics, Cleveland, OH) containing two-fold serial dilutions of the antimicrobial agents. Testing was performed according to reference broth microdilution methods (NCCLS). After overnight growth, cultures were suspended in 5 ml of cation-adjusted Mueller-Hinton broth to a standard inoculum density (0.5 McFarland). Fifty ml (100 for *S. pneumoniae*) of this solution was transferred to either 10 ml of cation-adjusted Mueller-Hinton broth, Haemophilus Test Medium (*H. influenzae*) or Mueller-Hinton broth supplemented with 3 - 5% lysed horse blood (*S. pneumoniae*). One hundred ml was then delivered to each well to achieve a target concentration of 5 x 10^4 CFU per well. The panels were placed in an ambient air incubator for 16 - 24 hours at 35ºC. Minimum inhibitor concentrations (MIC) were determined by visual inspection and susceptible/resistant breakpoints were those defined by the NCCLS (2004) tables. The monitoring of quality control was performed by utilizing the following organisms: *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247 and 49766, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 35218 and 25922, and *Pseudomonas aeruginosa* ATCC 27853. All results were within published control limits.

Table 1. Geographic variation of the incidence of ß-lactam resistance mechanisms among *H. influenzae, M. catarrhalis* and *S. pneumoniae* during 2003 surveillance of SENTRY participant hospitals.

	Region (%)		
Organism/resistance phenotype	Europe	Latin America	North America
H. influenzae/ampicillin-resistant	16.0(0.6)[a]	11.6(0.5)[a]	27.3(0.3)[a]
M. catarrhalis/ß-lactamase positive	95.1	94.7	95.6
S. pneumoniae/penicillin-non-susceptible[b]	28.6(14.7)[c]	28.7(12.7)[c]	33.0(15.9)[c]

a. ß-lactamase-negative, ampicillin-resistant (MIC, ≥ 2 µg/ml) rate for 1999 - 2003 (0.4% overall).
b. Penicillin MIC values at ≥ 0.12 µg/ml.
c. High-level penicillin resistance (MIC, ≥ 2 µg/ml).

RESULTS

- The ampicillin resistance rates (Table 1) for *H. influenzae* were higher in North America (27.3%) compared to Europe (16.0%) and Latin America (11.6%). Among *M. catarrhalis* isolates (Table 1), ß-lactamase production rates ranged from 94.7 (Latin America) to 95.6% (Europe).
- BLNAR strains (ß-lactamase-negative, ampicillin MIC ≥ 2 µg/ml) remained stable over the five-year period (0.4% overall) with the highest rates occurring in Europe (0.6%).
- In 2003, penicillin-non-susceptible rates for *S. pneumoniae* were similar in Europe (28.6%) and Latin America (28.7%), but significantly higher in North America (33.0%). High-level resistance rates were 12.7, 14.7 and 15.9% for Latin America, Europe and North America, respectively (Table 1).
- In 2003, 49.7% of *S. pneumoniae* isolates in Latin America were resistant to trimethoprim/sulfamethoxazole; higher than other regions (31.0 - 31.3%). However, erythromycin and clindamycin (86.4 and 95.5% susceptible) in Latin America were significantly more active than in Europe (71.1 and 81.0% susceptible) and North America (71.0 and 88.4% susceptible). There were no FQR (levofloxacin MICs, ≥ 4 µg/ml) strains in Latin America. Tetracycline-resistant rates were much higher in Europe (26.0%) compared to North America (18.0%) and Latin America (14.9%; Table 2).
- During the five-year period (1999-2003), MDR *S. pneumoniae* rates were much lower in Latin America (average 1.2%) compared to North America (average 5.6%) and Europe (average 7.8%).
- Only five gatifloxacin-resistant strains were detected among the 592 MDR strains isolated between 1999 - 2003.
- In Italy, an ongoing FQR *S. pneumoniae* epidemic cluster (serotype 23F) was observed in five patients (one strain in 2002 and four strains in 2003) at one institution (085). QRDR mutations (Table 5) were identical, as were ribotypes (333.3) and PFGE patterns (A). These isolates were also resistant to MLS_B drugs and tetracycline, but susceptible to ß-lactams, trimethoprim/sulfamethoxazole and chloramphenicol.

Table 2. In vitro activity of 17 antimicrobial compounds against 2,379 *Streptococcus pneumoniae* from the SENTRY Program (2003).

	$MIC_{50/90}$ (% susceptible)		
Antimicrobial agent	Europe (n=1,053)	Latin America (n=312)	North America (n=1,014)
Penicillin	≤0.03/2(71.4)	≤0.03/2(71.3)	≤0.03/2(67.0)
Erythromycin	≤0.25/>32(71.1)	≤0.25/2(86.4)	≤0.25/32(71.0)
Clindamycin	≤0.25/>2(81.0)	≤0.25/≤0.25(95.5)	≤0.25/>2(88.4)
Tetracycline	≤2/>16(74.0)	≤2/16(85.1)	≤2/>16(82.0)
Trimethoprim/ Sulfamethoxazole	≤0.5/4(69.0)	≤0.5/>4(50.3)	≤0.5/>4(68.7)
Ciprofloxacin	(6.7)[a]	(8.0)[a]	(4.9)[a]
Levofloxacin	1/1(99.0)	1/1(100.0)	1/1(98.9)
Gatifloxacin	0.25/0.5(99.0)	0.25/0.5(100.0)	0.25/0.5(99.1)
Cefepime	≤0.06/1(98.4)	≤0.06/1(96.8)	≤0.06/1(97.4)
Ceftriaxone	0.03/1(99.1)	0.03/1(100.0)	0.03/1(98.4)
Cefdinir	0.06/4(79.0)	0.06/>4(80.9)	0.12/>4(76.8)
Cefprozil	≤0.12/8(81.3)	≤0.12/8(85.6)	≤0.12/8(81.2)
Cefuroxime	≤0.06/4(79.9)	≤0.06/4(85.9)	≤0.06/4(78.2)
Linezolid	1/1(100.0)	1/1(100.0)	1/1(100.0)
Quinupristin/ Dalfopristin	≤0.5/≤0.5(100.0)	≤0.5/≤0.5(100.0)	≤0.5/≤0.5(100.0)
Vancomycin	0.25/0.5(100.0)	0.25/0.5(100.0)	0.25/0.5(100.0)

a. % of isolates with a MIC at ≥ 4 µg/ml [Chen et al., 1999].

Table 3. Multi-drug resistant *S. pneumoniae* isolated by the SENTRY Program from 1999 - 2003.[a]

Year	Europe	Latin America	North America	Total
1999	16/213(7.5%)	1/257(0.4%)	68/1,201(5.7%)	85/1,671(5.1%)
2000	55/536(10.3%)	5/246(2.0%)	66/1,100(6.0%)	126/1,882(6.7%)
2001	84/864(9.7%)	3/201(1.5%)	59/1,001(5.9%)	146/2,066(7.1%)
2002	50/863(5.8%)	1/183(0.6%)	47/1,098(4.3%)	98/2,144(4.6%)
2003	69/1,053(6.6%)	4/312(1.3%)	64/1,014(6.3%)	137/2,379(5.8%)
1999-2003	274/3,529(7.8%)	14/1,119(1.2%)	304/5,414(5.6%)	592/10,142(5.8%)

a. Defined as isolates resistant to penicilin, erythromycin, clindamycin, tetracycline and trimethoprim/sulfamethoxazole.

Table 4. In vitro activity of ciprofloxacin and gatifloxacin against 592 multi-drug resistant *S. pneumoniae* isolates from the SENTRY Program (1999 - 2003).

	% resistant to ciprofloxacin (≥ 4 µg/ml)/gatifloxacin (≥ 2 µg/ml)		
Year (no. tested)	Europe	Latin America	North America
1999 (85)	6.3/0.0	0.0/0.0	1.5/1.5
2000 (126)	1.8/0.0	0.0/0.0	7.6/3.0
2001 (146)	7.1/1.2	0.0/0.0	3.4/0.0
2002 (98)	2.0/0.0	0.0/0.0	2.1/0.0
2003 (137)	5.8/1.4	0.0/0.0	4.7/0.0

Table 5. Analysis of fluoroquinolone-resistant *S. pneumoniae* cluster isolated from patients in an Italian hospital.[a]

		Antibiogram (MIC, µg/ml)								QRDR-mutations at:			
Isolate no.	Age/sex	Penicillin	Ceftriaxone	Erythromycin	Clindamycin	Chloramphenicol	Rifampin	Tetracycline	Trimethoprim/ Sulfamethoxazole	*gyrA*	*parC*	*parE*	Ribotype/ PFGE
3318C[b]	5/M	≤0.03	≤0.25	>8	>2	4	≤0.5	>8	≤0.5	S81F	S79F, K137N	I460V	338.3/A
3650B	65/F	≤0.03	0.03	>8	>2	≤2	≤0.5	>8	≤0.5	S81F	S79F, K137N	I460V	338.3/A
3662B	9/M	≤0.03	0.03	>8	>2	4	≤0.5	>8	≤0.5	S81F	S79F, K137N	I460V	338.3/A
3666B	55/M	≤0.03	0.03	>8	>2	4	≤0.5	>8	≤0.5	S81F	S79F, K137N	I460V	338.3/A
3689B	11/F	≤0.03	0.016	>8	>2	4	≤0.5	>8	≤0.5	S81F	S79F, K137N	I460V	338.3/A

a. All isolates were serotype 23F.
b. Isolate from 2002.

CONCLUSIONS

- The results of this SENTRY Program analysis for 1999 - 2003 showed that resistance rates for *M. catarrhalis* and *H. influenzae* on three continents remained stable.
- The prevalence of penicillin-non-susceptible and multi-drug-resistant *S. pneumoniae* was greater in Europe and North America when compared to Latin America. However, the rates of resistance increased across all regions over the five years.
- Multidrug-resistance in *S. pneumoniae* is not associated with co-resistance to fluoroquinolones (gatifloxacin).
- The identification of an epidemic FQR clones of *S. pneumoniae* demonstrates the importance of local and global surveillance systems in the monitoring of resistant trends, and their utility for guiding clinicians in empiric CARTI therapy.

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