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Changing Antimicrobial Susceptibility Patterns among *S. pneumoniae* and *H. influenzae* from Brazil: Report from the SENTRY Antimicrobial Surveillance Program (1998-2003)

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AMENDED ABSTRACT

Background: Although antimicrobial resistance (R) rates among *S. pneumoniae* (SPN) and *H. influenzae* (HI) have increased significantly in most countries in the last years, studies from Brazil report relatively low R rates among these pathogens. We analyzed the susceptibility (S) patterns of SPN and HI from Brazil (6 years) for significant trends.

Methods: 729 SPN and 566 HI collected from 1998-2003, mainly from respiratory tract and bloodstream infections, were susceptibility (S) tested by NCCLS broth microdilution methods against >30 drugs and the results analyzed by year.

Results: Results are summarized below:

	% S by year (SPN/HI)					
Antimicrobial	1998	1999	2000	2001	2002	2003
Penicillin (PEN)	84(3)/9[b]	83(6)/14[b]	75(5)/7[b]	82(8)/18[b]	78(8)/15[b]	72(10)/12[b]
Erythromycin (ERY)/Clarithromycin	85/90	88/100	91/92	90/91	94/90	91/94
Clindamycin (CLI)	94/-	95/-	96/-	95/-	100/-	98/-
Gatifloxacin/Levofloxacin	100/100	100/100	100/100	100/100	100/100	100/100
Chloramphenicol (CHL)	99/96	98/99	99/97	100/95	99/93	100/96
Tetracycline (TET)	72/96	75/84	82/95	78/95	86/98	85/96
Trimethoprim/Sulfamethoxazole	50/53	52/53	51/55	40/48	39/54	39/57

a. % R (MIC, ≥ 2 mg/L) to PEN.
b. ß-lactamase producing strains.

Conclusions: R to PEN has increased markedly among SPN over 6 years (from 3 to 10%). R to T/S also escalated from 50 to 61%, but S to ERY, CLI and TET significantly decreased among SPN strains. R to the antimicrobials tested remained very stable among HI with only some year-to-year variations. R to the newer fluoroquinolones was not detected and CHL showed an excellent spectrum (> 95% S) against both pathogens.

INTRODUCTION

Streptococcus pneumoniae and *Haemophilus influenzae* represent major bacterial pathogens which are significant causes of respiratory tract infections, bacteremia, and meningitis, especially in children and in the elderly. The emergence of antimicrobial resistance among these pathogens has been widely reported and highlights the need for alternative agents for the treatment of these infections.

Since penicillin resistance in *S. pneumoniae* was first reported in the mid-1960s, increasing resistance to this compound, and other antimicrobial agents, has been reported worldwide. Therefore, it is extremely important to understand the local epidemiology of *S. pneumoniae* in specific geographic settings, especially in developing countries, where invasive pneumococcal disease has a major impact among children. Furthermore, resistance of *H. influenzae* to ampicillin began to emerge in the early 1970's. Production of ß-lactamase (TEM-1 type) is the primary mechanism of resistance to ampicillin and other ß-lactams, and the prevalence of ß-lactamase positive *H. influenzae* varies considerably between geographic regions.

In this study we present data and assess trends on antimicrobial resistance among *S. pneumoniae* and *H. influenzae* isolates from both invasive and non invasive infections in Brazilian medical centers participating in the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Study Design: The SENTRY Antimicrobial Surveillance Program monitors antimicrobial resistance patterns of predominant pathogens causing nosocomial and community-acquired infections through sentinel medical centers worldwide. Four medical centers participate in the program in Brazil. These centers are located in four distinct cities: São Paulo, Florianópolis, Porto Alegre and Brasilia. The monitored infections for these pathogens included mainly community-acquired respiratory tract infections and bloodstream infections.

Bacterial Isolates: A total of 729 *S. pneumoniae* and 566 *H. influenzae* strains were collected during the 1998-2003 period and evaluated in the present study. Only one isolate per patient, determined to be clinically significant based on local criteria was included in the analysis. The isolates were identified at the participating institutions by the routine methodology in use at each laboratory. Upon receipt at the coordinating center (JMI Laboratories, North Liberty, IA, USA), isolates were subcultured onto blood agar to ensure viability and purity. Confirmation of species identification was performed with Vitek (bioMerieux Vitek, Hazelwood, MO, USA) or conventional methods, as required.

Antimicrobial Susceptibility: Susceptibility testing was performed by reference broth microdilution methods as described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Antimicrobial agents were obtained from the respective manufacturers or purchased from Sigma Chemical Co. (St Louis, MO, USA). Quality control was performed by testing *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247, among other control strains.

RESULTS

- In general, susceptibility rates were relatively high for *S. pneumoniae* (729 isolates tested) when compared to data from other geographic regions (Table 1). High-level resistance to penicillin (MIC ≥ 2 mg/L) was detected in only 7.0% of isolates and resistance rates to erythromycin (8.5%) and clindamycin (3.4%) were also relatively low. On the other hand, only 44.6% of isolates were susceptible to trimethoprim/sulfamethoxazole.
- All *S. pneumoniae* isolates were susceptible to levofloxacin (MIC_{90}, 1 mg/L) and gatifloxacin (MIC_{90}, 0.5 mg/L). However, 3.7% of isolates showed elevated ciprofloxacin MIC results (≥ 4 mg/L; Table 1)
- Resistance to ampicillin was detected in 12.8% of *H. influenzae* isolates (566 tested). All other antimicrobial agents tested were active against >95% of isolates; except trimethoprim/sulfamethoxazole (53.0% susceptible) and tetracycline (93.6% susceptible; Table 1).

Table 1. Antimicrobial susceptibility of *S. pneumoniae* and *H. influenzae* isolates from Brazil (SENTRY Antimicrobial Surveillance Program, 1998 - 2003).

	MIC (mg/L)				
Organism/antimicrobial agent (no. tested)	50%	90%	Range	% susceptible[a]	% resistant[a]
S. pneumoniae (729)					
Penicillin	≤0.03	0.5	≤0.03->4	78.3	7.0
Amoxicillin/Clavulanate	≤0.25	1	≤0.25-8	98.6	0.4
Cefuroxime	≤0.06	1	≤0.06->8	90.7	8.7
Ceftriaxone	0.03	0.5	≤0.008-8	99.4	0.6
Cefepime	≤0.12	0.5	≤0.12->8	99.0	0.3
Erythromycin	≤0.25	≤0.25	≤0.25->32	90.0	8.5
Clindamycin	≤0.25	≤0.25	≤0.25->16	96.4	3.4
Ciprofloxacin	1	2	≤0.25->2	-[b]	3.7[c]
Levofloxacin	1	1	≤0.5-2	100.0	0.0
Gatifloxacin	0.25	0.5	≤0.03-1	100.0	0.0
Chloramphenicol	≤2	4	≤2-16	99.2	0.8
Tetracycline	≤2	>16	≤2->16	82.0[d]	18.0
Trimethoprim/Sulfamethoxazole	1	>2	≤0.5->2	44.6	38.1
Vancomycin	0.25	0.5	≤0.12-1	100.0	0.0
H. influenzae (566)					
Ampicillin	≤1	>4	≤1->4	86.0[e]	14.0[e]
Amoxicillin/Clavulanate	0.5	1	≤0.06-4	100.0	0.0
Cefuroxime	1	2	≤0.06->8	98.1	0.2
Ceftriaxone	≤0.008	0.016	≤0.008-2	100.0	-
Cefepime	≤0.06	0.12	≤0.06-0.5	100.0	-
Azithromycin	1	2	≤0.5-8	99.8	-
Ciprofloxacin	≤0.25	≤0.25	≤0.25	100.0	-
Levofloxacin	≤0.5	≤0.5	≤0.5	100.0	-
Gatifloxacin	≤0.03	≤0.03	≤0.03-0.06	100.0	-
Chloramphenicol	≤2	≤2	≤2-16	96.2	3.5
Tetracycline	≤2	≤2	≤2-16	93.6	3.7
Trimethoprim/Sulfamethoxazole	≤0.5	>4	≤0.5->4	53.0	42.0

a. According to CLSI/NCCLS breakpoints.
b. - = no breakpoint has been established.
c. Isolates with ciprofloxacin MIC at ≥ 4 mg/L.
d. Includes susceptible and intermediate strains.
e. Based on the result of the ß-lactamase test.

- Amoxicillin/clavulanate (MIC_{90}, 1 mg/L), ceftriaxone (MIC_{90}, 0.016 mg/L), cefepime (MIC_{90}, 0.12 mg/L), ciprofloxacin (MIC_{90}, ≤ 0.25 mg/L), levofloxacin (MIC_{90}, 0.5 mg/L) and gatifloxacin (MIC_{90}, ≤ 0.03 mg/L) were active against 100% of *H. influenzae* isolates at the respective susceptible breakpoints (Table 1).

Table 2. Antimicrobial susceptibility of *S. pneumoniae* and *H. influenzae* from Brazil listed according to the year of isolation (SENTRY Antimicrobial Surveillance Program, 1998 - 2003).

	% susceptible/resistant[a] by year (no. tested):					
Antimicrobial agent	1998	1999	2000	2001	2002	2003
S. pneumoniae	(103)	(126)	(120)	(115)	(90)	(175)
Penicillin	83.5/2.9	83.3/5.6	75.0/5.0	81.7/7.8	77.8/7.8	72.0/10.3
Amoxicillin/Clavulanate	99.0/0.0	98.4/0.0	99.2/0.0	98.3/0.9	98.9/0.0	98.3/1.1
Cefuroxime	96.1/2.9	92.8/6.0	93.8/6.2	89.9/10.1	87.7/11.1	86.3/13.7
Ceftriaxone	97.1/0.0	97.7/2.3	100.0/0.0	99.1/0.9	100.0/0.0	100.0/0.0
Cefepime	99.0/0.0	98.2/0.8	100.0/0.0	99.1/0.9	100.0/0.0	97.1/0.6
Erythromycin	85.4/12.6	88.1/10/3	90.9/8.3	90.4/8.7	94.4/3.3	91.4/7.5
Clindamycin	94.2/5.8	95.2/4.0	95.8/4.2	94.8/5.2	100.0/0.0	97.7/2.3
Ciprofloxacin	-/1.9[b]	-/1.6[b]	-/1.7[b]	-/5.2[b]	-/1.9[b]	-/8.0[b]
Chloramphenicol	99.0/1.0	97.6/2.4	99.2/0.8	100.0/0.0	98.9/1.1	100.0/0.0
Tetracycline	71.8/26.2	74.6/22.8	81.7/18.3	78.3/17.4	85.6/13.3	84.9/11.6
Trimethoprim/Sulfamethoxazole	49.5/41.7	51.6/44.4	50.8/44.2	39.5/47.0	38.9/52.2	38.9/50.9
H. influenzae[c]	(90)	(80)	(120)	(129)	(41)	(106)
Ampicillin[d]	91.1/8.9	86.2/13.8	93.3/6.7	82.2/17.8	85.4/14.6	88.5/11.5
Cefuroxime	96.7/0.0	100.0/0.0	100.0/0.0	96.1/0.0	100.0/0.0	97.2/0.9
Azithromycin	100.0/-	100.0/-	100.0/-	99.2/-	100.0/-	100.0/-
Tetracycline	95.6/4.4	83.8/1.2	99.2/0.0	94.6/3.9	97.6/2.4	96.2/3.8
Chloramphenicol	95.6/4.4	98.5/1.5	96.6/2.5	96.1/3.9	92.7/4.9	96.1/3.9
Trimethoprim/Sulfamethoxazole	53.3/45.6	52.5/47.5	55.0/43.3	48.1/47.3	53.7/46.3	56.6/42.5

a. According to CLSI/NCCLS breakpoints.
b. Percentage of isolates with ciprofloxacin MIC at ≥ 4 mg/L. All isolates were susceptible to levofloxacin and gatifloxacin in all years.
c. All isolates were susceptible to amoxicillin/clavulanate, ceftriaxone, cefepime, ciprofloxacin, levofloxacin and gatifloxacin in all years.
d. Based on the ß-lactamase test result.

Table 3. Antimicrobial susceptibility of *S. pneumoniae* from Brazil according to type of infection (SENTRY Antimicrobial Surveillance Program, 1998 - 2003).

	% susceptible/resistant[a] by objective (no. tested)		
Antimicrobial agent	BSI[b] (93)	CARTI[c] (610)	Pneumonia[d] (25)
Penicillin	88.2/4.3	76.7/7.8	80.0/8.0
Amoxicillin/Clavulanate	98.9/0.0	98.4/0.5	100.0/0.0
Ceftriaxone	98.9/1.1	99.2/0.3	96.0/4.0
Cefepime	98.9/0.0	99.0/0.3	96.0/4.0
Erythromycin	94.6/4.3	89.3/9.2	88.0/8.0
Clindamycin	97.8/2.2	96.1/3.8	96.0/4.0
Ciprofloxacin	-/3.2[e]	-/3.8[e]	-/4.0[e]
Levofloxacin	100.0/0.0	100.0/0.0	100.0/0.0
Gatifloxacin	100.0/0.0	100.0/0.0	100.0/0.0
Chloramphenicol	100.0/0.0	99.0/1.0	100.0/0.0
Tetracycline	78.5/20.4	79.0/18.2	96.0/4.0
Trimethoprim/Sulfamethoxazole	58.1/34.4	42.6/48.7	40.0/52.0

a. According to CLIS/NCCLS breakpoints.
b. BSI = bloodstream infections.
c. CARTI = community-acquired respiratory tract infection.
d. Pneumonia in hospitalized patients.
e. Isolates with ciprofloxacin MIC ≥ 4 mg/L.

- Resistance to penicillin increased markedly from 2.9% in 1998 to 10.3% in 2003 among *S. pneumoniae* (Table 2). Susceptibility to trimethoprim/sulfamethoxazole decreased from 49.5 to 38.9% in the same period. Conversely, resistance rates to erythromycin, clindamycin and tetracycline decreased during the study period (Table 2).
- The prevalence ß-lactamase producing *H. influenzae* strains, as well as the susceptibility pattern of this pathogen against the antimicrobial agents evaluated remained very stable during the study period with only minor year-to-year variations (Table 2).
- *S. pneumoniae* isolates from respiratory tract infections showed higher rates of resistance to penicillin, macrolides and trimethoprim/sulfamethoxazole when compared to isolates from bloodstream infections (Table 3).
- Penicillin-susceptible *S. pneumoniae* showed high rates of susceptibility to most antimicrobial agents evaluated, except trimethoprim/sulfamethoxazole (53.4% susceptible) and tetracycline (80.9% susceptible). As expected, penicillin-resistant strains showed lower rates of susceptibility to other ß-lactams (0.0% and 92.2% susceptibility to cefuroxime and ceftriaxone respectively), macrolides (70.0% susceptibility to erythromycin), and trimethoprim/sulfamethoxazole (2.0% susceptible) than penicillin-susceptible strains (Table 4).

Table 4. Antimicrobial susceptibility of *S. pneumoniae* from Brazil according to penicillin susceptibility profile.

	% susceptible/resistant by penicillin susceptibility category (no. tested)[a]		
Antimicrobial agent	Susceptible (571)	Intermediate (107)	Resistant (51)
Amoxicillin/Clavulanate	100.0/0.0	100.0/0.0	80.4/5.9
Cefuroxime	99.8/0.0	86.6/10.3	0.0/100.0
Ceftriaxone	100.0/0.0	100.0/0.0	92.2/3.9
Cefepime	100.0/0.0	99.1/0.0	88.2/3.9
Erythromycin	92.8/5.6	84.1/15.0	70.0/28.8
Clindamycin	97.2/2.8	93.4/5.7	94.1/5.9
Ciprofloxacin	-/3.3[b]	-/3.7[b]	-/7.8[b]
Levofloxacin	100.0/0.0	100.0/0.0	100.0/0.0
Gatifloxacin	100.0/0.0	100.0/0.0	100.0/0.0
Chloramphenicol	99.3/0.7	100.0/0.0	96.1/3.9
Tetracycline	80.9'/19.1	87.5'/12.5	83.0'/17.0
Trimethoprim/Sulfamethoxazole	53.4/26.5	17.8/58.9	2.0/88.2

a. According to CLSI/NCCLS breakpoints.
b. Isolates with ciprofloxacin MIC ≥ 4 mg/L.
c. Includes susceptible and intermediate strains.

CONCLUSIONS

- Resistance to penicillin increased markedly among *S. pneumoniae* isolated in Brazil in the last few years (1998-2003), but remained relatively low when compared to other geographic areas of the world.
- The prevalence of ß-lactamase producing *H. influenzae* remained stable and moderately low (12.8%) during the study period.
- Resistance to respiratory fluoroquinolones (levofloxacin and gatifloxacin) was not detected among *S. pneumoniae* or *H. influenzae* isolated in Brazil by the SENTRY Program (1998-2003).
- Continued longitudinal surveillance is critical to follow the antimicrobial susceptibility patterns of these important respiratory pathogens in areas where resistance levels remain low and to initiate correlations with antimicrobial consumption.

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