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Characterization of Antimicrobial Susceptibility Patterns Among Community-Acquired Isolates of ß-Haemolytic Streptococci in Latin America: Results of SENTRY Antimicrobial Surveillance Program.

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AMENDED ABSTRACT

Background: Recently, ß-haemolytic streptococci (BHS) have been recognized as a cause of sepsis and soft tissue infections in adults with chronic illnesses. The main objective of this study was to determine the level of emerging resistance (R) among BHS isolates collected in the Latin America (LA) region through the SENTRY Antimicrobial Surveillance Program.

Methods: A total of 195 BHS clinical specimens were collected from patients with community-acquired infections in 10 LA sites, between January and March 2001. The susceptibility to penicillin (PEN) and other antimicrobials was evaluated by broth microdilution method [NCCLS, 2003]. Quality control was performed using recommended ATCC strains.

Results: PEN (MIC_{50}, 0.015 μg/ml) was the most potent agent. One group A streptococcal isolate from a Chilean site having reduced susceptibility (0.25 μg/ml) was detected initially. However, this result was not reproducible. Although azithromycin (MIC_{50}, 0.06 μg/ml) had shown to be the second most potent drug against GBS, only 93.3% of isolates were susceptible (S) to this agent. Interestingly, the R phenotype of MLS_B was observed in less than 3% of BHS isolates. Quinupristin/dalfopristin, linezolid, vancomycin, and gatifloxacin inhibited the growth of all GBS isolates at concentrations of ≤ 1, 2, 1, and 0.5 μg/ml, respectively.

Conclusions: Our results showed that BHS isolates were highly susceptible to ß-lactam agents. We confirm that penicillin remains the best therapeutic option for treatment of BHS infections in Latin America. However, continuing surveillance must be warranted to detect any decrease in the susceptibility to these agents, especially the emerging R for macrolides.

Table 1. Number of ß-haemolytic streptococci tested according to the Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Nation/Medical Center	No. of isolates tested (%)
Argentina	
39	22 (11.3)
Brazil	
46	30 (15.4)
48	24 (12.3)
57	23 (11.8)
101	18 (9.2)
Chile	
42	28 (14.4)
43	46 (23.6)
Venezuela	
49	4 (2.1)

Table 2. Number of ß-haemolytic streptococci tested according to the site of infection (SENTRY Antimicrobial Surveillance Program, 2001).

Site of Infection	No. of isolates tested (%)
Throat	94 (48.2)
Urine	46 (23.6)
Skin and soft tissue	33 (16.9)
Blood	14 (7.2)
Sputum	3 (1.5)
Cerebral spine fluid	1 (0.5)
Others	4 (2.1)

Table 3. Distribution of ß-haemolytic streptococci according to the Lancefield serologic tests (SENTRY Antimicrobial Surveillance Program, 2001).

ß-haemolytic Streptococci Group	No. of isolates tested (%)
Group A	120 (61.5)
Group B	64 (32.8)
Group G	5 (2.7)
Group C	4 (2.1)
Other groups	2 (1.0)

INTRODUCTION

Although antimicrobial resistance continues to increase among most clinically important bacterial species, the ß-haemolytic streptococci have remained remarkably susceptible to most antimicrobial agents. In spite of more than 50 years of extensive use of penicillin, group A streptococci remain susceptible to this ß-lactam agent. However, sporadic cases of group A streptococcal pharyngitis and tonsillitis that did not respond properly to penicillin treatment have been reported.

The main objective of this study was to determine the level of emerging resistance among ß-haemolytic streptococci isolates collected from the Latin American region through the SENTRY Antimicrobial Surveillance Program (2001).

Table 4. In vitro antimicrobial susceptibility of the 195 ß-haemolytic streptococci isolates collected from the participating Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Antimicrobial	Cumulative percentage inhibited at MIC (μg/ml)												$MIC_{50/90}$	% Susc.[a]
Agents	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	(μg/ml)	
Penicillin	65.0	90.4	99.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.015/0.06	100.0
Ceftriaxone	-	-	-	-	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	≤0.25/≤0.25	100.0
Cefepime	-	-	-	97.0	99.0	100.0	100.0	100.0	100.0	100.0	100.0	[b]	≤ 0.12/≤ 0.12	100.0
Erythromycin	-	-	91.2	92.3	92.8	92.8	93.8	95.9	96.9	99.5	[b]	-	≤ 0.06/≤ 0.06	92.8
Clindamycin	-	-	95.9	97.9	97.9	97.9	97.9	97.9	97.9	97.9	[b]	-	≤ 0.06/≤ 0.06	97.9
Ciprofloxacin	0.0	0.5	0.5	0.5	15.5	77.7	91.7	100.0	[b]	-	-	-	0.5/1.0	[c]
Levofloxacin	-	0.0	0.5	0.5	11.3	82.1	97.9	100.0	100.0	[b]	-	-	0.5/1	100.0
Gatifloxacin	-	0.5	2.0	15.4	90.2	100.0	100.0	100.0	100.0	100.0	[b]	-	0.25/0.5	100.0
Garenoxacin	-	18.5	90.2	100.0	100.0	100.0	100.0	100.0	100.0	[b]	-	-	0.06/0.12	[c]
Chloramphenicol	-	-	-	-	-	-	-	89.4	97.9	97.9	97.9	[b]	≤ 2/4	97.9
Linezolid	-	-	0.5	1.0	1.0	12.0	99.5	100.0	100.0	100.0	[b]	-	1/1	100.0
Quinupristin/ dalfopristin	-	-	2.0	50.5	80.8	99.0	100.0	100.0	100.0	100.0	[b]	-	0.12/0.5	100.0
Teicoplanin	-	-	-	96.9	99.0	100.0	100.0	100.0	100.0	100.0	100.0	[b]	≤ 0.12/≤ 0.12	[c]
Vancomycin	-	-	-	1.0	49.5	99.0	100.0	100.0	100.0	100.0	100.0	[b]	0.25/0.5	100.0

a. Percentage of susceptibility interpreted using NCCLS breakpoints. Underlined values indicate percentage inhibited at the susceptible breakpoint.
b. - = indicates untested concentration.
c. No interpretive criteria for this category has been published in the NCCLS standards.

Table 5. In vitro antimicrobial susceptibility of the 120 group A streptococci isolates collected from the participating Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Susc.[a]	% Res.[a]
Penicillin	≤ 0.015	≤ 0.015	100.0	0.0
Ceftriaxone	≤ 0.25	≤ 0.25	100.0	[c]
Cefepime	≤ 0.12	≤ 0.12	100.0	[c]
Erythromycin	≤ 0.06	≤ 0.06	95.0	5.0[b]
Azithromycin	≤ 0.06	≤ 0.06	95.0	5.0[b]
Clindamycin	≤ 0.06	≤ 0.06	100.0	0.0
Ciprofloxacin	0.5	0.5	[c]	[c]
Levofloxacin	0.5	0.5	100.0	0.0
Gatifloxacin	0.25	0.25	100.0	0.0
Garenoxacin (BMS284756)	0.06	0.06	[c]	[c]
Chloramphenicol	≤ 2	≤ 2	100.0	0.0
Linezolid	1	1	100.0	[c]
Quinupristin/Dalfopristin	0.12	0.12	100.0	0.0
Teicoplanin	≤ 0.12	≤ 0.12	[c]	[c]
Vancomycin	0.25	0.25	100.0	[c]

a. Percentage of susceptibility interpreted using NCCLS breakpoints.
b. Among the 6 macrolide-resistant isolates, 5 had the *mef*(A) genotype and 1 had the *erm*(A) subclass *erm*(TR) genotype.
c. No interpretive criteria for this category have been published in the NCCLS [2003] standards.

MATERIALS AND METHODS

Protocol Design. The SENTRY Program participating medical centers were guided by protocol to submit 25 consecutive ß-haemolytic streptococci isolated from patients with community-acquired infections. The following sites of infection were accepted: throat, high quality sputum, skin/soft tissue, urine, blood, cerebral spine fluid. No more than 20 strains from throat culture samples could be submitted. In addition, just a single isolate per patient could be referred to the monitoring center (JMI Laboratories, North Liberty, IA, USA).

Medical centers. The participants included 10 medical centers located in 9 cities (5 countries): Brasília, São Paulo, Florianópolis and Porto Alegre in Brazil; Buenos Aires and San Isidro in Argentina; Santiago in Chile (two sites); Mexico City in Mexico; and Caracas in Venezuela.

Bacterial strains. A total of 195 viable clinical isolates were processed between January and March 2001. The isolates were identified to the species level by the participant medical center and shipped to the monitoring laboratory for identification confirmation and reference susceptibility testing.

Susceptibility testing. Antimicrobial susceptibility testing was performed using the reference broth microdilution method as described by the National Committee for Clinical Laboratory Standards (NCCLS). Antimicrobial agents were obtained from the respective manufacturers and quality control was performed by testing *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, and *Enterococcus faecalis* ATCC 29212.

Characterization of macrolide resistance. Erythromycin-resistant isolates were molecularly characterized to determine the presence of *erm* and *mef* genes by multiplex rapid cycle PCR with microwell-format probe hybridization.

COMMENTS

- Groups A (61.5%) and B (32.8%) accounted for more than 90% of ß-haemolytic streptococci collected.
- Nearly one half of the ß-haemolytic streptococci were collected from patients with pharyngitis.
- Most of the isolates were collected from Brazil (48.7%), Chile (37.9%) and Argentina (11.3%).
- All ß-haemolytic streptococci remained susceptible to penicillin (MIC_{90}, 0.03 μg/ml).
- 6 group A streptococci (5.0%) were resistant to macrolides. None of these strains were resistant to other antimicrobials.
- Among group B streptococci the resistance rates to macrolides (7.8%), clindamycin (4.7%) and chloramphenicol (4.7%) were higher than those observed for group A streptococci (5.0%, 0.0%, and 0.0%, respectively).
- The novel des(F)-quinolone, garenoxacin (MIC_{90}, 0.06 μg/ml), was the most potent quinolone tested. It was four-fold more active than gatifloxacin (MIC_{90}, 0.25 μg/ml) and 16-fold more active than either levofloxacin and ciprofloxacin (MIC_{90}, 1 μg/ml).

Table 6. In vitro antimicrobial susceptibility of the 64 group B streptococci isolates collected from the participating Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Susc.[a]	% Res.[a]
Penicillin	0.03	0.06	100.0	0.0
Ceftriaxone	≤ 0.25	≤ 0.25	100.0	[c]
Cefepime	≤ 0.12	≤ 0.12	100.0	[c]
Erythromycin	≤ 0.06	0.12	92.2	7.8[b]
Azithromycin	≤ 0.06	≤ 0.06	92.2	7.8[b]
Clindamycin	≤ 0.06	≤ 0.06	93.7	4.7
Ciprofloxacin	0.5	1	[c]	[c]
Levofloxacin	0.5	1	100.0	0.0
Gatifloxacin	0.25	0.25	100.0	0.0
Garenoxacin (BMS284756)	0.06	0.06	[c]	[c]
Chloramphenicol	≤ 2	≤ 2	93.7	6.3
Linezolid	1	1	100.0	[c]
Quinupristin/Dalfopristin	0.25	0.5	100.0	0.0
Teicoplanin	≤ 0.12	≤ 0.12	[c]	[c]
Vancomycin	0.5	0.5	100.0	[c]

a. Percentage of susceptibility interpreted using NCCLS breakpoints.
b. Among the 5 macrolide-resistant isolates, 3 had the *erm*(A) subclass *erm*(TR) genotype and 2 had the *erm*(B) genotype.
c. No interpretive criteria for this category have been published in the NCCLS standards.

CONCLUSIONS

- ß-haemolytic streptococci remained highly susceptible to penicillin and other ß-lactam drugs in Latin America. However, macrolide resistance was detected among these pathogens, especially among groups A and B.
- The *mef*(A) genotype predominated among macrolide-resistant group A streptococci (5 of 6 isolates – 83.3%), while the *erm* genotype predominated among macrolide-resistant group B streptococci (all 5 isolates – 100%).
- Continue surveillance is necessary to monitor antimicrobial resistance among clinically significant ß-haemolytic streptococci in the Latin American region.

Table 7. Results of the macrolide resistance mechanism analysis by PCR.

Organism group-bank no.	Medical center	Country	Erythromycin MIC (μg/ml)	Clindamycin MIC (μg/ml)	Resistance mechanisms
Group A – 143	42	Chile	8	≤ 0.06	*mef*(A)
Group A – 146	42	Chile	8	≤ 0.06	*mef*(A)
Group A – 52	43	Chile	8	≤ 0.06	*mef*(A)
Group A – 93	43	Chile	4	≤ 0.06	*mef*(A)
Group A – 125	46	Brazil	8	≤ 0.06	*mef*(A)
Group A – 129	46	Brazil	1	≤ 0.06	*erm*(A) subclass *erm*(TR)
Group B – 16	39	Argentina	1	>8	*erm*(B)
Group B – 39	39	Argentina	8	>8	*erm*(B)
Group B – 106	43	Chile	1	≤ 0.06	erm(A) subclass *erm*(TR)
Group B – 110	43	Chile	>8	>8	*erm*(B)
Group B – 894	48	Brazil	2	≤ 0.06	*erm*(A) subclass *erm*(TR)
Group G - 387	57	Brazil	1	≤ 0.06	ND[a]

a. ND: Not done.

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