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Evaluation of the Macrolide-Lincosamide-Streptogramin (MLS_B) Susceptibility Patterns Among Community-Acquired *S. pneumoniae* Isolates: A 5-Year Report from the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Background: Patterns of MLS_B resistance (R) can vary greatly, even within a single nation. Phenotype and genotypic studies can help determine the potential clinical roles of various MLS_B agents and emerging R trends. This summary of the initial 5 years of the SENTRY Program focuses on MLS_B-R in Europe (EU), Latin (LA) and North America (NA) for 1997 - 2001.

Methods: A total of 8,837 *S. pneumoniae* (SPN) strains isolated from community-acquired RTI were tested from EU (2,229), LA (1,141) and NA (5,467). Strains were evenly distributed by year within each region, with consistent (> 85%) participation among sites. NCCLS MIC results for erythromycin (ER), clindamycin (CM), quinupristin/dalfopristin (Q/D) and penicillin (PEN) were compared. Screening for *erm B* and *mef A* genes were performed by multiplex rapid cycle PCR with probe detection and R strains negative by the screen were rRNA sequenced.

Results: ER-susceptibility (S) decreased in all regions from 1997 - 2001 as follows: EU (81.6 to 67.9%), LA (91.0 to 85.8%), NA (86.2 to 69.8%) for these SPN strains that were only 66.5 to 70.7% S to PEN overall. Rare Q/D-non-S (< 1.0%) was detected. M-phenotypes (presumed *mef A*) were found in EU (27.7%; range, 18.8 - 36.4%), LA (56.3%; range, 27.8 - 70.5%) and NA (69.6%; range, 66.9 - 71.6%). 332 ER-R strains (2001 isolates) were molecular typed and the (n tested [sites]/% *erm B*/*mef A*/both/rRNA mutant) follow: For EU (120 [18 sites]/82.5/17.5/0.0/0.0), for LA (23 [6 sites]/43.5/56.5/0.0/0.0), for USA (158 [22 sites]/17.7/70.9/7.6/3.8) and for Canada (31 [5 sites]/35.5/38.7/16.1/9.7). All double R mechanisms and rRNA mutant strains occurred in NA (nine strains; one with a unique L22 mutation coding for streptogramin-R [MIC, 4 µg/ml]).

Conclusions: MLS_B R mechanisms varied greatly between continents/nations and an increased *erm B* trend has been established. Phenotypic patterns compared favorably to molecular derived rates (within 0.5 - 6.8% by region for 2001). Continued monitoring of this evolving macrolide-R pattern seems prudent.

INTRODUCTION

Streptococcus pneumoniae is one of the most commonly isolated organisms from patients diagnosed with community-acquired respiratory tract infections (CARTI). Recent reports have chronicled the rapid increase and global spread of antimicrobial resistance in *S. pneumoniae* to several classes of agents including penicillins, cephalosporins, quinolones, and macrolides. CARTI are the leading cause of physician office visits and antimicrobial prescriptions for which standard treatment is empiric therapy with broad-spectrum antimicrobial agents. The macrolide-lincosamide-streptogramin (MLS_B) group of antimicrobials are often prescribed for CARTI because of their spectrum of activity versus key typical and atypical pathogens. However, the increasing rates of common resistance mechanisms mediated by ribosomal methylases or efflux pumps in this group of antimicrobials has raised concern among clinicians.

The initiation of the SENTRY Antimicrobial Surveillance Program in 1997 was to monitor global antimicrobial resistances in a variety of infections. CARTI is one of the infection types that has been monitored for emerging resistances including MLS_B agents used in the treatment for this indication. The results of this prospective surveillance program utilizing reference antimicrobial susceptibility test methods are summarized for 1997-2001.

MATERIALS AND METHODS

Organisms tested. A total of 8,837 *S. pneumoniae* strains isolated from patients diagnosed with CARTI were tested. The isolates were collected by more than 60 geographically diverse medical centers in NA (5,467 strains), EU (2,229 strains), and LA (1,141 strains) from 1997 to 2001 as part of the SENTRY Program. All isolates were shipped to a central international monitoring site (Iowa) on Amies charcoal swabs where identification was confirmed based on colony morphology, optochin disks, and bile solubility with 10% desoxycholate (Remel, Lenexa, KS).

Antimicrobial agents. All strains were tested by reference broth microdilution methods recommended by the NCCLS using validated dry form panels (TREK Diagnostics, Westlake, OH). Organisms from pure overnight culture plates were suspended into a Mueller-Hinton broth media equal to a 0.5 McFarland standard, and then 100 µl of this suspension was transferred to 10 ml of Mueller-Hinton broth supplemented with 3-5% lysed horse blood. Each panel well concentration was 100 µl, with a bacterial inoculum of 5 x 10^5 CFU/ml. The panels were incubated in ambient air at 35ºC for 20-24 hours. The MIC for each antimicrobial agent was determined by examining the panels for the lowest drug concentration which visually inhibited growth. Susceptibility breakpoints were those defined by the NCCLS [2002] tables. Colony counts were performed periodically to confirm inoculum concentration and quality control was monitored using ATCC strains recommended by the NCCLS [2002].

Molecular analysis. Screening for *erm B* and *mef A* genes were performed by multiplex rapid cycle PCR with probe detection and resistant strains negative by the PCR screen were rPNA sequenced [Farrell et al., 2001].

RESULTS

- Penicillin susceptibility (Table 1) decreased from 1997 - 2001 in EU (80.1 to 63.3%) and NA (73.4 to 63.8%), however, it showed no clear trend in LA.
- Erythromycin susceptibility (Table 1) decreased in all regions from 1997 through 2001 as follows: NA (86.2 to 69.8%), EU (81.6 to 67.9%) and LA (91.0 to 85.8%). Clindamycin susceptibility also decreased in EU (88.3 to 75.7%) and NA (95.8 to 90.0%).
- Quinupristin/dalfopristin non-susceptible strains were rarely detected (≤ 1.0%; Table 1).

Table 1. Resistance trends in MLS_B and penicillin among 8,837 *S. pneumoniae* isolates in Europe (EU), Latin America (LA), and North America (NA; USA and Canada) from the SENTRY Antimicrobial Surveillance Program (1997-2001).[a]

		% susceptible by year:					
Antimicrobial agent	Region (no. tested)	1997	1998	1999	2000	2001	Trend
Erythromycin	EU (2,229)	81.6	76.3	80.3	70.1	67.9	-13.7%
	LA (1,141)	91.0	84.6	86.8	85.3	85.8	-5.2%
	NA (5,467)	86.2	80.4	75.3	73.0	69.8	-16.4%
Clindamycin	EU	88.3	84.3	84.0	78.2	75.7	-12.6%
	LA	93.5	92.9	96.1	93.5	94.4	NC[b]
	NA	95.8	94.4	92.3	91.2	90.0	-5.8%
Quinupristin/dalfopristin	EU	99.0	99.3	100.0	99.6	99.8	NC
	LA	100.0	100.0	100.0	100.0	100.0	NC
	NA	99.8	100.0	100.0	100.0	99.6	NC
Penicillin	EU	80.1	73.1	68.7	66.0	63.3	-16.8
	LA	69.2	71.4	72.4	66.9	73.6	NC
	NA	73.4	69.1	67.2	66.0	63.8	-9.6

a. Community-acquired respiratory tract isolates only (Objective B).
b. NC = no significant change.

Table 2. Molecular characterization of the MLS_B resistance patterns among year 2001 *S. pneumoniae* isolates from the SENTRY Antimicrobial Surveillance Program (332 strain sample; three regions).

Region (no. sites)	No. of strains	Resistance genes	No (%) occurrence
Europe (18)	120	*erm B*	99 (82.5)
		mef A	21 (17.5)
Latin America (6)	23	*erm B*	10 (43.5)
		mef A	13 (56.5)
United States (22)	158	*erm B*	28 (17.7)
		mef A	112 (70.9)
		erm B + mef A	12 (7.6)
		negative	6 (3.8)
Canada (5)	31	*erm B*	11 (35.5)
		mef A	12 (38.7)
		erm B + mef A	5 (16.1)
		negative	3 (9.7)

- The overall percentage of M-phenotypes (presumed *mef A*) were: NA (69.6%; range 66.9 - 71.6% across 5 years), LA (65.3%; range 27.8 - 70.5%), and EU (27.7%; range 18.8 - 36.4%) (Table 1).
- Molecular characterization of MLS_B resistance for a sample of 332 isolates from 2001, showed *erm B* domination in Europe (82.5%), in contrast to *mef A* in the United States (70.9%). Latin America and Canada had a more equal distribution of these resistance genes (Table 2).
- Novel resistances or combinations of resistance genes were observed only in North America (Canada > United States).
- In nine strains, negative by screen for *mef A* or *erm B*, 23S ribosomal mutations (A2059G, A2058G, A2059C), and/or L22 and/or A2503C alterations were identified. One novel L22 mutation produced an elevated quinupristin/dalfopristin MIC (Table 3, footnote a).
- Table 4 shows the remarkable correlations between the prevalence of the M-phenotype and the documented occurrence of *mef A* in the random sample in each monitored region.

Table 3. Non-*erm B* or *mef A* mechanisms of MLS_B resistances detected in North American isolates during 2001 (nine strains).

			Resistance mechanisms				
Country of origin	Site	Strain no.	23S mutation	No. alleles	L4	L22	2505
Canada	031	2473	A2059G	3	-	-	A2503C
	031	2497	A2059G	4	-	-	-
	038	2106	A2058G	4	-	-	-
United States	001	1956	A2059G	3	-	-	-
	017	3167	A2059G	1	-	a	-
	019	2691	A2059G	2	-	P84T	-
	021	2403	A2059G	3	-	-	-
	030	0068	A2059G	3	-	-	-
	030	0302	A2058G	4	-	P84T	-

a. Unusual strain with quinupristin/dalfopristin MIC of 4 µg/ml and a documented gene insertion (5AA) in L22 (Malbruny et al., AAC 46:2200-7, 2002), unique in *S. pneumoniae*.

Table 4. Comparison of the MLS_B resistance phenotype with that rate determined by molecular methods for year 2001 SENTRY Program strains.

Region	M-phenotype	*mef A* positive[a]
Europe	24.3%	17.5%
Latin America	60.6%	56.5%
North America	66.9%	67.4%

a. *mef A* alone, without any additional mechanisms from a sample of 332 strains (approximately 61% of all erythromycin-resistant isolates observed).

CONCLUSIONS

- Marked variations in the resistance patterns of MLS_B compounds between continents/nations were noted:
  - erythromycin resistance was much higher in EU and NA than for LA.
  - M-phenotypes, supported by molecular studies, were much greater in NA and LA than observed in EU.
  - non-*erm*, non-*mef* resistance mechanisms were most frequent in NA.
- Clindamycin had wider activity than erythromycin in all regions, but much less than quinupristin/dalfopristin (> 99%).
- The increasing rates of resistances to antimicrobial agents in the MLS_B group requires continued monitoring by well structured networks such as the SENTRY Program suppported by molecular tests.

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