C2-295

Antimicrobial Surveillance of ß-Haemolytic Streptococci in Europe and North America: Report from the SENTRY Program (2001)

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ICAAC 2002

AMENDED ABSTRACT

Background: To determine the variation of emerging resistance (R) among ß-haemolytic streptococci (ßHS), SENTRY sites forwarded community-acquired isolates for testing against numerous antimicrobials. Strains included *S. pyogenes* (SGA) from patients with clinical pharyngitis as well as serogroups (SG) B, C, F and G from a variety of infections.
Methods: The collection included 985 isolates from Europe (EU) dominated by SGA (703) > SGB (150) > SGG (66) > SGC (41) > SGF (8); and 815 North America (NA) isolates SGA (397) > SGB (318) > SGG (45) > SGC (19) > SGF (8). Sites of infection were throat, lower respiratory tract, wounds, urine, blood and CSF. ß-haemolysis was confirmed on subculture, and bacitracin-susceptibility (SGA) and CAMP factor tests (SGB) were routinely performed. Isolates were tested by NCCLS microdilution methods in Mueller-Hinton broth supplemented with 3 - 5% lysed horse blood. Susceptibility (S) to ß-lactams, levofloxacin (LEVO), erythromycin (ER), quin/dalfo (Q/D), linezolid (LZD), chloramphenicol (CH), vancomycin (VAN) and tetracycline (TET) was monitored.
Results: All ßHS from NA and EU were S to penicillin (PEN), ceftriaxone, cefepime, LZD and VAN. PEN potency was greater (MIC_{90} in µg/ml) against SGA (≤ 0.015) and SGG (≤0.015-0.03) compared to the other SGs (0.03 - 0.12). ER-non-susceptible % was EU > NA overall, for SGA (15.0 > 9.1); SGC (7.3 > 5.3); SGF (12.5 > 0.0) and SGG (31.3 > 15.6). In contrast, ER-non-susceptible SGB was much higher in NA (30.8%) than EU (12.7%). M-phenotypes (*mef A*) dominated SGA (72.7 - 91.2%), SGC (67.1 - 100.0%) SGF (100.0%) in both NA and EU. M-phenotypes in NA/EU for SGB (61.4/11.0%) and SGG (85.9/32.3%) were significantly different. TET-R was common among SGB and SGG (MIC_{90}, 8 - > 8 µg/ml) compared to other SGs ($MIC_{90/90}$, ≤ 4/> 8 µg/ml). CH-R was found only in EU (2.7%), 1 isolate was R to LEVO in NA (data not shown).
Conclusions: ß-lactams, LZD, VAN and Q/D were very active against ßHS strains from NA and EU. Macrolide-R mechanisms vary greatly between NA and EU, and require continued surveillance efforts to guide alternative treatments.

INTRODUCTION

The SENTRY Antimicrobial Surveillance Program was designed to detect global antimicrobial resistance trends and is longitudinally focused primarily on blood stream and respiratory tract infections. In 2001, the program initiated an objective to determine the current antimicrobial resistance patterns of ß-haemolytic streptococci and determine if the prevalence of resistance mechanisms differs geographically.

ß-haemolytic streptococci are significant pathogens responsible for respiratory tract infections, endocarditis, sepsis, glomerulonephritis as a sequelae, and bone and joint infections. *Streptococcus* serogroup A (*S. pyogenes*) is most commonly associated with acute pharyngitis in both children and adults, although serious invasive infections including pylonephritis, necrotizing facitis and sepsis are also caused by this serogroup. Serogroup B is an important cause of post-partum and neonatal infections due to colonization of the birth canal.

Penicillins are most commonly used to treat streptococcal infections, however, macrolides are the alternative drug of choice for patients with penicillin allergy. Although susceptibility to penicillin has been maintained against ß-haemolytic streptococci, macrolide resistance has been evolving rapidly. Macrolide resistance was first observed in the 1950s and has been increasing in occurrence across the globe ever since. This has created a need for active surveillance studies and prudent antimicrobial therapy. Macrolide resistance is caused by two principal mechanisms: 1) *erm* which encodes for ribosomal modification; and 2) *mef A* which encodes for drug efflux. The resistance rates and selected molecular characterization of resistant strains are reported here.

MATERIALS AND METHODS

In 2001, the SENTRY Program launched a focused surveillance objective to monitor resistance among ß-haemolytic streptococci with a network of clinical laboratories in North and Latin America and Europe. A total of 1,800 strains of ß-haemolytic streptococci were collected from North America and Europe. The European collection included 985 isolates dominated by serogroups A (703 strains)> B (150 strains) > G (66 strains) > C (41 strains) > F (eight strains). North American isolates included serogroups A (397 strains) > B (318 strains) > G (45 strains) > C (19 strains) > F (eight strains). Sites of infection included throat, lower respiratory tract, wound, urine, blood and CSF. Isolates were sent to an international monitor for identification confirmation and susceptibility testing against numerous antimicrobial agents. Upon receipt, isolate identification was confirmed using colony morphology and haemolysis of blood agar, bacitracin disks and the CAMP test.

MICs were determined for numerous ß-lactams, fluoroquinolones, MLS_B compounds, linezolid, chloramphenicol, vancomycin, and tetracycline. Susceptibility testing was performed using validated dry-form panels manufactured by TREK Diagnostics Systems (Westlake, Ohio) and National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution methods in Mueller-Hinton agar supplemented with 3-5% lysed horse blood. All interpretations of results were performed using NCCLS M100-S12 (2002).

The molecular characterizations of *erm* and *mef* were determined by multiplex rapid cycle PCR with microwell-format probe hybridization.

RESULTS

- Strains of ß-haemolytic streptococci (1,800) from North America and Europe were susceptible to penicillin, cefepime, ceftriaxone, quinupristin/dalfopristin, linezolid, and vancomycin (Tables 1 and 2).
- Tables 1 and 2 show that the highest penicillin MICs were found among serogroups B, C and F (MIC_{90}S [MIC_{50}, serogroup F] 0.03 - 0.5 µg/ml) for isolates in North America and Europe.
- ß-haemolytic streptococci serogroups A, C, G and F from Europe were less susceptible to erythromycin than those in North America (15.0 > 9.1%), (7.3 > 5.3%), (31.3 > 15.6%), (12.5 > 0.0%), respectively (Tables 1 and 2). In contrast, serogroup B isolates were less susceptible to erythromycin in North America (30.8%) compared to Europe (12.7%).
- M-phenotypes (presumptive *mef A*) dominated amongst serogroups A (72.7 - 91.2%), C (67.1 - 100.0%), and F (100.0% when resistance occurred) in both North America and Europe (Tables 1 and 2). However, this phenotype was prevelant in North America compared to Europe for serogroups C (85.9 vs. 33.3%) and B (61.4 vs. 11.0%).
- The greatest variation in susceptibility rates was observed for tetracycline, with highest susceptibility among SGA in North America (85.6%; Table 1) and lowest susceptibility for SGB in Europe (0.0%; Table 2).
- Chloramphenicol resistance was not detected in North America (Table 1), but was consistent at 1.5 - 3.0% among the three most prevalent serogroups (A, B, G) in Europe (Table 2).
- A random sample of erythromycin-resistant isolates for molecular mechanisms analysis (NA isolates only, Table 3) revealed: occurrences of *erm (A)* subclass *erm (TR)/erm (B)/mef (A)* were 5/1/3 for serogroup A, 8/6/3 for serogroup B, and 4/0/2 for serogroup G. One isolate of serogroup B had both *erm (A)* subclass *erm (TR)* plus *mef (A)*.

Table 1. Activity and susceptibility rates[a] for 13 antimicrobial agents tested against five serogroups of ß-haemolytic streptococci isolated in North America (SENTRY Antimicrobial Surveillance Program, 2001).

	Activity by organism tested:									
	Serogroup A (397)		Serogroup B (318)		Serogroup G (45)		Serogroup C (19)		Serogroup F (8)	
Antimicrobial agent	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S
Erythromycin	≤0.06/0.12	90.9	≤0.06/8	69.2	≤0.06/2	84.4	≤0.06/≤0.06	94.7	≤0.06/-	100.0
Clindamycin	≤0.06/≤0.06	99.2	≤0.06/>8	88.1	≤0.06/≤0.06	97.8	≤0.06/≤0.06	100.0	≤0.06/-	100.0
Tetracycline	≤4/>8	85.6	>8/>8	14.8	8/>8	48.9	≤4/>8	57.9	≤4/-	75.0
Chloramphenicol	≤2/4	100.0	≤2/≤2	100.0	≤2/4	100.0	≤2/4	100.0	≤2/-	100.0
Penicillin	≤0.015/≤0.015	100.0	0.03/0.06	100.0	≤0.015/0.03	100.0	0.03/0.06	100.0	0.03/-	100.0
Cefepime	≤0.12/≤0.12	100.0	≤0.12/≤0.12	100.0	≤0.12/≤0.12	100.0	≤0.12/0.5	100.0	0.5/-	100.0
Ceftriaxone	≤0.25/≤0.25	100.0	≤0.25/≤0.25	100.0	≤0.25/≤0.25	100.0	≤0.25/≤0.25	100.0	≤0.25/-	100.0
Quinupristin/Dalfopristin	0.12/0.25	100.0	0.5/0.5	100.0	0.25/0.5	100.0	0.5/0.5	100.0	1/-	100.0
Linezolid	1/1	100.0	1/1	100.0	1/1	100.0	1/1	100.0	1/-	100.0
Vancomycin	0.25/0.5	100.0	0.5/0.5	100.0	0.25/0.5	100.0	0.5/0.5	100.0	1/-	100.0
Garenoxacin[b]	0.06/0.06	100.0	0.06/0.06	100.0	0.06/0.06	100.0	≤0.03/0.06	100.0	≤0.03/-	100.0
Gatifloxacin	0.25/0.25	100.0	0.25/0.25	100.0	0.25/0.25	100.0	0.12/0.25	100.0	0.12/-	100.0
Levofloxacin	0.5/0.5	100.0	0.5/1	100.0	0.5/0.5	100.0	0.25/0.5	100.0	0.25/-	100.0

a. Susceptibility criteria per NCCLS [2002].
b. Garenoxacin susceptibility was defined the same as gatifloxacin [NCCLS, 2002].

Table 2. Activity and susceptibility rates[a] for 13 antimicrobial agents tested against five serogroups of ß-haemolytic streptococci isolates in Europe (SENTRY Antimicrobial Surveillance Program, 2001).

	Activity by organism tested:									
	Serogroup A (703)		Serogroup B (150)		Serogroup G (67)		Serogroup C (41)		Serogroup F (8)	
Antimicrobial agent	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S	$MIC_{50/90}$	% S
Erythromycin	≤0.06/2	85.0	≤0.06/4	87.3	≤0.06/8	68.7	≤0.06/≤0.06	92.7	≤0.06/-	87.5
Clindamycin	≤0.06/≤0.06	95.9	≤0.06/0.5	88.7	≤0.06/>8	78.8	≤0.06/≤0.06	97.6	≤0.06/-	100.0
Tetracycline	≤4/>8	73.6	>8/>8	0.0	8/>8	47.8	≤4/>8	65.9	≤4/-	87.5
Chloramphenicol	≤2/4	97.0	≤2/4	96.7	4/4	97.0	≤2/4	100.0	≤2/-	87.5
Penicillin	≤0.015/≤0.015	100.0	0.03/0.06	100.0	≤0.015/≤0.015	100.0	≤0.015/0.03	100.0	0.5/-	100.0
Cefepime	≤0.12/≤0.12	100.0	≤0.12/≤0.12	100.0	≤0.12/≤0.12	100.0	≤0.12/0.5	100.0	0.5/-	100.0
Ceftriaxone	≤0.25/≤0.25	100.0	≤0.25/≤0.25	100.0	≤0.25/≤0.25	100.0	≤0.025/≤0.25	100.0	≤0.25/-	100.0
Quinupristin/Dalfopristin	0.12/0.12	100.0	0.25/0.5	100.0	0.5/0.25	100.0	0.25/0.5	100.0	0.5/-	100.0
Linezolid	1/1	100.0	1/1	100.0	1/1	100.0	1/1	100.0	1/-	100.0
Vancomycin	0.25/0.5	100.0	0.5/0.5	100.0	0.25/0.5	100.0	0.5/0.5	100.0	1/-	100.0
Garenoxacin[b]	0.06/0.12	100.0	0.06/0.06	100.0	0.03/0.06	100.0	0.06/0.06	100.0	0.06/-	100.0
Gatifloxacin	0.25/0.25	100.0	0.25/0.25	100.0	0.25/0.25	98.5	0.25/0.25	100.0	0.12/-	100.0
Levofloxacin	0.5/1	100.0	0.5/1	100.0	0.5/0.5	100.0	0.5/0.5	100.0	0.5/-	100.0

a. Susceptibility criteria per NCCLS [2002].
b. Garenoxacin susceptibility was defined the same as gatifloxacin [NCCLS, 2002].

Table 3. Molecular characterization of MLS_B resistances among 33 ß-haemolytic streptococci isolated in the SENTRY Antimicrobial Surveillance Program (2001).

		Resistance mechanism			
Organism	Region (no. tested)	*erm (A)* subclass *erm (TR)*	*erm (B)*	*mef (A)*	*erm (A)* subclass *erm (TR)+mef (A)*
Serogroup A	USA (7)	4	0	3	0
	Canada (2)	1	1	0	0
Serogroup B	USA (17)	8	5	3	1
	Canada (1)	0	1	0	0
Serogroup G	USA (4)	4	0	0	0
	Canada (2)	0	0	2	0

CONCLUSIONS

- The tested ß-lactams, linezolid, vancomycin and quinupristin/dalfopristin continue to be very active against ß-haemolytic streptococci in both North America and Europe.
- Europe had more resistant stains (chloramphenicol, tetracycline, clindamycin and erythromycin) than North America.
- Macrolide resistance continues to evolve with 15.8% and 18.5% of ß-haemolytic strains from North America and Europe, respectively, being resistant to erythromycin.
- Resistance mechanisms vary greatly among streptococci and require continued surveillance programs to monitor and guide resistance and antimicrobial chemotherapy.

SELECTED REFERENCES

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