

Detection of Clindamycin and Erythromycin Resistance in Isolates of *Streptococcus agalactiae* using Three Commercial Systems

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REVISED ABSTRACT

BACKGROUND: Macrolide resistance (MacR) for *Streptococcus agalactiae* (STRAGA) has been reported as high as 20% in the U.S. Both erythromycin (E) and clindamycin (CC) are used for penicillin-allergic patients with STRAGA infections. MacR is usually mediated by the *erm* and *mef* genes. *erm* – mediated resistance includes macrolide-lincosamide-streptogramin b – inducible (iMLS_b) and constitutive (cMLS_b) phenotypes. Both MLS_b phenotypes are E-resistant (E^R) and CC-resistant (CC^R) but iMLS_b requires induction to show CC^R. *mef* (M phenotype) are E^R and CC-susceptible (CC^S). This study evaluated three commercial systems for the detection of CC and E resistance in STRAGA.

METHODS: Sixty-four STRAGA strains characterized as E^R using the CLSI recommended standard broth microdilution were selected. These strains were further evaluated using the D Zone test (E and CC double disk diffusion method) to define resistant phenotypes. PCR was performed to verify the presence of *erm* or *mef* genes. All isolates were tested for CC and E resistance using the BD PhoenixTM Automated Microbiology System (BD Diagnostics) – PHX, Vitek (bioMerieux) – V1, and MicroScan MICroSTREP Plus (Dade Behring) – MS.

RESULTS: The 64 STRAGA strains were classified as 37 iMLS_b, 20 cMLS_b, and 7 M phenotype. For the 37 iMLS_b strains, PHX detected CC resistance for 32 as CC^R (86%) and 5 CC^I (14%). V1 and MS incorrectly reported 35 and 36 iMLS_b strains as CC^S, respectively. For the 20 cMLS_b strains, PHX detected CC resistance for all 20 strains (100%), V1 detected 9 (45%), and MS detected 16 (80%). For the 7 M phenotype strains, PHX reported 6 CC^S and 1 CC^I. Both V1 and MS correctly reported all 7 CC^S. For E resistance detection, PHX correctly reported all 64 strains as E^R but V1 and MS only reported 42 E^R (66%) and 49 E^R (77%), respectively. V1 failed to detect E resistance for 20 strains (14E^S and 6E^I) and MS did not detect E resistance for 15 strains (E^I).

CONCLUSIONS: PHX was able to accurately report E and CC resistance for iMLS_b, cMLS_b and M phenotypes. The need for the D Zone test to detect CC resistance in iMLS_b phenotype would be reduced using PHX but would remain an important part of CC resistance detection for V1 and MS.

BACKGROUND

Streptococcus agalactiae is a leading cause of infections in neonates and pregnant women. Macrolides and clindamycin remain important drug alternatives for treatment of penicillin-allergic patients with *S. agalactiae* infections. An increasing incidence of macrolide and clindamycin resistance has been reported during the past decade for this group of organisms. This has resulted in an increased importance to accurately and rapidly test and report macrolide and clindamycin resistance for these isolates. Clinical and Laboratory Standards Institute (CLSI) has recently recommended performing the D Zone test for macrolide-resistant isolates of beta-hemolytic streptococci with inducible clindamycin resistance. This supplemental testing is required due to the failure of the reference and most automated AST systems to accurately detect clindamycin resistance for beta-hemolytic streptococci with inducible resistance to clindamycin. Table 1 summarizes the different macrolide resistant beta-hemolytic streptococci phenotypes and expected D zone results with the final clindamycin interpretation.

Table 1. Description of three common macrolide resistant beta-hemolytic streptococci phenotypes

Macrolide-resistant phenotype	Resistance Mechanism	Gene Type	Expected SIR Result		D Zone Test Result	Final Clindamycin Interpretation
			E	CC		
cMLS _b – constitutive	Methylation of the 23S rRNA	<i>erm</i>	R	R	Not required	CC – Resistant
iMLS _b – inducible	Methylation of the 23S rRNA	<i>erm</i>	R	S, I, or R	Positive ^a	CC – Resistant
M phenotype	efflux	<i>mef</i>	R	S	Negative	CC – Susceptible

Abbreviations: MLS_b, macrolide, lincosamide, and type B streptogramin; SIR, susceptible, intermediate, resistant; E, erythromycin; CC, clindamycin
a. D Zone testing would be required for CC^S or CC^I results.

This study compared the BD PhoenixTM Automated Microbiology System (BD Diagnostic Systems, Sparks, MD), Vitek[®] (bioMerieux, Hazelwood, Mo.) and MicroScan[®] (Dade Behring, Sacramento, CA.) for detection of clindamycin and erythromycin resistance in isolates of *S. agalactiae*.

* Product not available in the US for susceptibility testing.

METHODS

Strains:

Table 2 summarizes the 64 erythromycin resistant *S.agalactiae* strains used in this study. Strains were obtained from various hospitals located in the United States and Canada.

Commercial and Reference AST Methods:

All commercial AST testing was performed per manufacturer recommended procedures. Reference AST testing was performed according to CLSI recommended guidelines (M7-A6). Bacterial suspensions from an overnight culture on TSAII™ were prepared and adjusted to a 0.5 McFarland standard as required for each test method.

- Phoenix** — Suspensions were prepared and adjusted to a 0.5 McFarland standard in Phoenix ID Broth. The Phoenix AST-S Broth, a proprietary non-blood containing broth specifically formulated for rapid susceptibility testing for streptococci was inoculated and used in the Phoenix Strep Panel. The inoculated Phoenix panels were then placed into the Phoenix Instrument for incubation (ambient air) and continuous reading. The Phoenix Strep Panel utilizes an enhanced clindamycin formulation to increase detection of inducible resistance and thus potentially decrease the need for the additional D Zone test.
- Vitek** — Suspensions were prepared and adjusted to a 0.5 McFarland standard in saline. The VITEKGPS-105 panels were inoculated and placed into the VITEK instrument for incubation and continuous reading.
- MicroScan** — Suspensions were prepared and adjusted to a 0.5 McFarland standard in saline. Mueller-Hinton Broth with 3% lysed horse blood was inoculated and used in the MICroSTREP Plus panels. Panels were incubated for 20-24 hours at 35°±1°C in ambient air and interpreted visually by trained technologists.
- Reference Microdilution (SBM) Method** — The SBM panels containing 3.5% lysed horse blood were prepared and inoculated according to CLSI recommended guidelines (M7-A6). Suspensions were prepared and adjusted to a 0.5 McFarland standard in Phoenix ID Broth. After 20 – 24 hours of incubation at 35°±1°C in ambient air, the SBM panels were interpreted visually by trained technologists. The CLSI (M100-S15) was used for categorical interpretation.

Antimicrobial Agent	MIC (ug/ml) Interpretative Standard		
	S	I	R
Erythromycin	≤ 0.25	0.5	1
Clindamycin	≤ 0.25	0.5	1

BD Phoenix™ Automated Microbiology System



D ZONE TEST: All testing performed per CLSI standard disk diffusion testing recommendation (CLSI M100-S15, page 68, Comment 13). Direct colony suspension prepared in Mueller-Hinton broth to an equivalent 0.5 McFarland Standard. Mueller-Hinton agar with 5% sheep blood plates were streaked using a sterile cotton tipped swab to achieve a uniform lawn of growth. The erythromycin disk (15 µg) was placed 12 mm (edge to edge) from the clindamycin disk (2 µg). After incubation at 35°C, 5% CO₂ for 20 – 24 hours, plates were examined for flattened clindamycin zone (D – shape) between the erythromycin and clindamycin disks. Results were reported as:

Clear D-Shape = D Zone Positive

No D-Shape = D Zone Negative

Refer to Figure 1 – 3 for typical D Zone results.

Figure 1.
iMLSb phenotype showing positive D Zone test



Figure 2.
M phenotype showing negative D Zone test



Figure 3.
cMLSb phenotype showing negative D Zone, typically not required since CC would appear resistant



PCR TESTING: The presence of erythromycin resistant genes was determined by PCR using DNA amplification with primers specific for *erm*(TR), *erm*(B) and *mef*(A/E). DNA preparation and PCR conditions were carried out as described in the following references:

- Seppälä, H., M. Skurnik, H. Soini, M. C. Roberts, and P. Huovinen. 1998. A novel erythromycin resistance methylase gene (*erm*TR) in *Streptococcus pyogenes*. *Antimicrob. Agents Chemother.* 42:257-262.
- PCR Protocol: Qiagen Multiplex PCR Handbook Cat # 1029475.

Specific PCR protocol conditions included initial activation at 15 min. 95°C, denaturation at 30 sec. 94°C, annealing at 90 sec. 57°C, extension at 60 sec. 72°C, 35 cycles, and final extension at 30 min. 60°C.

QUALITY CONTROL TESTING: The following QC strains were used prior to testing.

- S. galactiae* 1832 - D Zone Test Negative
- S. galactiae* 4472 - D zone Test Positive, *erm* positive
- S. pneumoniae* ATCC 49619

RESULTS

Table 2. Summary of the 64 Erythromycin Resistant *S. agalactiae* Strains

Strain No.	Reference Interpretation (SIR)		D Zone Result	Phenotype	erm/mef PCR
	Erythromycin	Clindamycin			
6941	R	R	NA	cMLSb	NT
6962	R	R	NA	cMLSb	erm
6971	R	R	NA	cMLSb	erm
6977	R	R	NA	cMLSb	erm
7012	R	R	NA	cMLSb	erm
7016	R	R	NA	cMLSb	erm
7018	R	R	NA	cMLSb	erm
7019	R	R	NA	cMLSb	erm
7021	R	R	NA	cMLSb	erm
7024	R	R	NA	cMLSb	erm
7025	R	R	NA	cMLSb	erm
7026	R	R	NA	cMLSb	erm
7027	R	R	NA	cMLSb	erm
7029	R	R	NA	cMLSb	erm
7030	R	R	NA	cMLSb	Negative
7031	R	R	NA	cMLSb	erm
7034	R	R	NA	cMLSb	erm
7035	R	R	NA	cMLSb	erm
7037	R	R	NA	cMLSb	erm
7039	R	R	NA	cMLSb	erm
3002	R	S	Positive	iMLSb	NT
4472	R	S	Positive	iMLSb	erm
4473	R	S	Positive	iMLSb	NT
4864	R	S	Positive	iMLSb	NT
5548	R	S	Positive	iMLSb	NT
5898	R	S	Positive	iMLSb	NT
6049	R	S	Positive	iMLSb	erm
6068	R	S	Positive	iMLSb	erm
6069	R	S	Positive	iMLSb	Negative
6071	R	S	Positive	iMLSb	erm
6082	R	S	Positive	iMLSb	NT
6942	R	S	Positive	iMLSb	NT
6960	R	S	Positive	iMLSb	erm
6961	R	S	Positive	iMLSb	erm
6963	R	S	Positive	iMLSb	erm
6964	R	S	Positive	iMLSb	erm
6966	R	S	Positive	iMLSb	Negative
6967	R	S	Positive	iMLSb	erm
6968	R	S	Positive	iMLSb	erm
6969	R	S	Positive	iMLSb	erm
6970	R	S	Positive	iMLSb	erm
6972	R	S	Positive	iMLSb	erm
6973	R	S	Positive	iMLSb	erm
6974	R	S	Positive	iMLSb	erm
6975	R	S	Positive	iMLSb	erm
6976	R	S	Positive	iMLSb	erm
6978	R	S	Positive	iMLSb	erm
7014	R	S	Positive	iMLSb	Negative
7020	R	S	Positive	iMLSb	Negative
7022	R	S	Positive	iMLSb	Negative
7023	R	S	Positive	iMLSb	Negative
7028	R	S	Positive	iMLSb	erm
7032	R	S	Positive	iMLSb	erm
7036	R	S	Positive	iMLSb	erm
7038	R	S	Positive	iMLSb	erm
7040	R	S	Positive	iMLSb	erm
7041	R	S	Positive	iMLSb	erm
6939	R	S	Negative	M_type	NT
6940	R	S	Negative	M_type	NT
6943	R	S	Negative	M_type	NT
6965	R	S	Negative	M_type	erm
7011	R	S	Negative	M_type	Negative
7015	R	S	Negative	M_type	mef
7017	R	S	Negative	M_type	Negative

Abbrev: NT, Not Tested; NA, Not Applicable

Table 3. Detection of erythromycin resistance with 64 Macrolide-resistant *S. agalactiae* using various commercial systems

Test System	Total Tested	Erythromycin Interpretation			%VME
		S	I	R	
Reference	64			64	NA
Phoenix	64			64	0
Vitek 1	62 ^a	14 ^b	6	42	23
MicroScan	64		15	49	0

a. Two strains failed to grow in Vitek 1.

b. 14 VME were 9-MLSb(i), 4-MLSb(c), and 1-M phenotype

Table 2 summarizes the phenotype and genotype for the 64 erythromycin resistant *S. agalactiae* strains used for this study. Susceptibility to erythromycin and clindamycin based on reference SBM was determined. Further testing to detect inducible clindamycin resistance was performed using the D Zone test. Based on E and CC reference AST results and D Zone results, each strain was phenotypically classified as cMLSb, iMLSb or M phenotype. Additional PCR for *erm* and *mef* genes was performed for 53 of the strains.

Table 3 summarizes E resistance detection for the three commercial systems. Phoenix detected resistance for all 64 E^R isolates (100%). Vitek detected 42 strains as E^R (68%) with 14 strains as E^S (23% Very Major Error -VME). MicroScan detected 49 strains as E^R (77%) with no VME, but 15 (23%) Minor Errors.

Table 4 summarizes the detection of CC resistance for MLSb phenotype strains using three commercial systems. These strains would include iMLSb and cMLSb phenotypes, both of which should be reported as CC^R. The ability to detect inducible CC resistance for these strains would reduce the need to perform the additional D Zone test. Of the 57 MLSb strains evaluated, Phoenix detected CC resistance for 52 strains (91%), Vitek detected 9 (16%), and MicroScan detected 17 (30%).

Table 5 provides the CC results for each test system, further summarized by each isolate phenotype. Phoenix correctly detected CC resistance for all 20 cMLSb. Vitek detected CC^R for 9/20 (45%) and MicroScan detected CC^R for 16/20 (80%) of the cMLSb phenotypes. For the iMLSb, Phoenix detected 32/37 (86%) as CC^R, Vitek detected 0/35 (0%) as CC^R, and MicroScan detected 1/37 (3%) as CC^R. For the M phenotype with expected CC^S, Phoenix gave 6/7 (86%) as CC^S and both Vitek and MicroScan reported all 7 M phenotype as CC^S.

Table 4. Detection of clindamycin resistance with 39 MLSb phenotype *S. agalactiae* using various commercial systems

Test System	Total Tested	Clindamycin Interpretation			No. of strains requiring D Zone test to report clindamycin result
		S	I	R	
Reference	57	36		21	36
Phoenix	57		5	52	5
Vitek 1	55 ^a	45	1	9	46
MicroScan	57	40		17	40

a. Two strains failed to grow in Vitek 1

Table 5. Clindamycin interpretative result for each *S. agalactiae* phenotype and test system

Test System	Pheno_Type	Total	S	I	R
Reference	cMLSb	20			20
	iMLSb	37	37		
	M_type	7	7		
Phoenix	cMLSb	20		20	
	iMLSb	37		5	32
	M_type	7	6		1
Vitek 1	cMLSb	20	10	1	9
	iMLSb	35	35		
	M_type	7	7		
MicroScan	cMLSb	20	4		16
	iMLSb	37	36		1
	M_type	7	7		

CONCLUSIONS

- The Phoenix System provides accurate detection of erythromycin resistance for *S. agalactiae*. Vitek failed to detect erythromycin resistance for numerous strains resulting in a high VME rate. MicroScan showed improved detection for erythromycin resistance but reported numerous strains as intermediate, which would result in Minor Errors.
- The Phoenix System was able to detect clindamycin resistance for a large number of the clindamycin inducible *S. agalactiae* isolates. This would greatly reduce the amount of D Zone testing required to correctly interpret and report clindamycin results. Both Vitek and MicroScan would require D Zone testing for all clindamycin inducible isolates.