

Detection and Interpretation of Macrolide-Lincosamide-Streptogramin Resistance among *Staphylococcus* with Phoenix Automated Microbiology System and BDXpert™ System

B. TURNG, J. SINHA, M. DEAL, J. POLLITT, D. CALLIHAN, B. BRASSO, T. WILES AND J. REUBEN

BD Diagnostics • 7 Loveton Circle • Sparks, MD 21152, USA

REVISED ABSTRACT

OBJECTIVES: The National Committee for Clinical Laboratory Standards (NCCLS) recently published recommendations for the detection of macrolide-lincosamide-streptogramin (MLSb) resistance in *Staphylococcus*. These statements provide guidelines for laboratorians to detect the MLSb resistance phenotypes in macrolide resistant staphylococci. The Societe Francaise De Microbiologie (SFM) and Deutsches Institut fur Normung (DIN) have not yet implemented similar guidelines. This study evaluated the detection and reporting of MLSb results in *Staphylococcus* using the BD Phoenix™ Automated Microbiology System and BDXpert System with NCCLS, SFM or DIN as interpretative standards.

METHODS: Comments regarding MLSb resistance as listed in the NCCLS M100-S14 were converted into expert rules. Special BDXpert rules were also constructed for the detection and reporting of MLSb resistance when applying SFM or DIN breakpoints. A total of 182 strains of *Staphylococcus* (148 *S. aureus*, 12 *S. epidermidis*, and 22 coagulase-negative staphylococci) were tested in Phoenix panels containing erythromycin (E) and clindamycin (CC). NCCLS, SFM or DIN breakpoints were used to interpret the Phoenix MIC results. The double disk diffusion D zone test (E and CC) was used as the reference method for the determination of MLSb resistance phenotypes.

RESULTS: The Phoenix E and CC MIC values were interpreted based on the standard selected. The BDXpert rules were executed and applicable expert messages were displayed. The Phoenix correctly detected 38 out of 43 constitutive MLSb (cMLSb) phenotypes as compared to the D zone test results. Four cMLSb strains were interpreted by the BDXpert as potential inducible MLSb (iMLSb)/efflux phenotypes. A total of 72 iMLSb and 22 efflux phenotype isolates were all reported by the BDXpert as iMLSb/efflux phenotype and the users were alerted to perform D-test before reporting the CC results. The CC interpretation was suppressed in these isolates. The NCCLS, SFM, or DIN showed identical detection and interpretation of MLSb resistant phenotypes by the Phoenix and BDXpert systems.

CONCLUSIONS: The Phoenix and BDXpert Systems can assist laboratories in rapid detection and accurate interpretation of MLSb results for *Staphylococcus*. Special messages can be used to communicate timely and accurate information to clinicians for proper therapy of staphylococcal infections with MLSb resistant phenotypes.

INTRODUCTION

Detection of macrolide-lincosamide-streptogramin (MLS) resistance among *Staphylococcus* has recently drawn more attention in the clinical laboratories. Macrolide resistance in *Staphylococcus* is mainly mediated by two mechanisms, Macrolide-Lincosamide-Streptogramin type B (MLSb) or efflux mechanism phenotypes. The MLSb carries the *erm* gene encoding rRNA methylase that alters the antimicrobial binding site on the 23S rRNA, resulting in macrolides, lincosamides, and streptogramin B resistance. The expression of MLSb phenotype can be further classified as inducible MLSb or constitutive MLSb. When an inducible MLSb strain is exposed to an inducer (such as a low-level of erythromycin), the level of rRNA methylase expression increases resulting in an increased resistance to antimicrobics of the MLS class (such as clindamycin). The efflux mechanism encoded by the *msrA* gene confers resistance only to macrolides and streptogramin type B.

The National Committee for Clinical Laboratory Standards (NCCLS, USA; currently CLSI) recently published recommendations for the detection of macrolide-lincosamide-streptogramin (MLSb) resistance in *Staphylococcus* using the D-test (D-zone test) in order to differentiate between inducible MLSb and efflux mechanisms. These statements provide guidelines for laboratorians to detect the MLSb resistance phenotypes in macrolide resistant staphylococci. The Societe Francaise De Microbiologie (SFM) and Deutsches Institut fur Normung (DIN) have not yet implemented similar guidelines.

The NCCLS, SFM or DIN standard documents contain information that clinical laboratorians use to assist clinicians in reporting and selection of appropriate antimicrobial agents for treating bacterial infections. Currently several automated or semi-automated antibiotic susceptibility testing (AST) systems utilize an expert system to interpret AST results and provide a descriptive message or required actions. The BDXpert System used together with the BD Phoenix™ Automated Microbiology System and BD EpiCenter™ System (BD Diagnostic Systems, Sparks, Maryland, USA) provides interpretation of AST test results as recommended by NCCLS, SFM or DIN standards.

This study evaluated the detection and reporting of MLSb results in *Staphylococcus* using the BD Phoenix™ Automated Microbiology System and BDXpert System with NCCLS, SFM or DIN as interpretative standards.

MATERIALS AND METHODS

RULE SETS.

The NCCLS document, “Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement M100-S14, Vol. 24, No. 1, January, 2004” (NCCLS M100-S14) and the “Antibiogram Committee of the French Microbiology Society, Report 2004, January, 2004” (SFM) and DIN 58940-4, January 2000 were used to construct the expert rules. Comments regarding detection of MLSb resistance as listed in the NCCLS M100-S14 were converted into expert rules. Special BDXpert rules were also constructed for the detection and reporting of MLSb resistance when applying SFM or DIN breakpoints.

TEST STRAINS.

A total of 182 strains of *Staphylococcus*, including 148 *S. aureus*, 12 *S. epidermidis*, and 22 other coagulase-negative staphylococci (Table 1) were tested in Phoenix panels containing erythromycin (E, 0.25 – 4 µg/mL) and clindamycin (CC, 0.25 – 4 µg/mL). NCCLS, SFM or DIN breakpoints were used to interpret the Phoenix MIC results.

D ZONE TEST.

The double disk diffusion (D zone test) with E (15 µg) and CC (2 µg) BBL Sensi-Discs (BDDS) was performed as the reference method for the determination of MLSb resistance phenotypes. The standard NCCLS recommended procedures and medium type were used. A visible D zone indicates an inducible MLSb phenotype.

RESISTANT MARKERS.

a) Constitutive MLSb Phenotype — BDXpert rules 1539 and 1622 report constitutive MLSb phenotype. When azithromycin, clarithromycin or erythromycin is resistant and clindamycin is also resistant, Rule 1539 reports the resistance marker. BDXpert rule 1622 is designed for the user to manually check and report the resistance marker.

b) Inducible MLSb Phenotype — BDXpert rule Rule 1618 reports inducible MLSb phenotype. When azithromycin, clarithromycin or erythromycin is resistant and clindamycin is not resistant, with a positive D zone test result, users can then manually check to report inducible MLSb phenotype.

c) Macrolide Efflux Phenotype — BDXpert rule 1621 reports macrolide efflux phenotype. When azithromycin, clarithromycin or erythromycin is resistant and clindamycin is not resistant, with a negative D zone test result, users can then manually check to report macrolide efflux phenotype. When azithromycin, clarithromycin or erythromycin is resistant, but clindamycin is not resistant, Rule 335 suppresses the interpretation of clindamycin and alerts the user to perform the D zone test. Flowchart 1 summarizes the workflow of the BDXpert System in reporting the resistance markers.

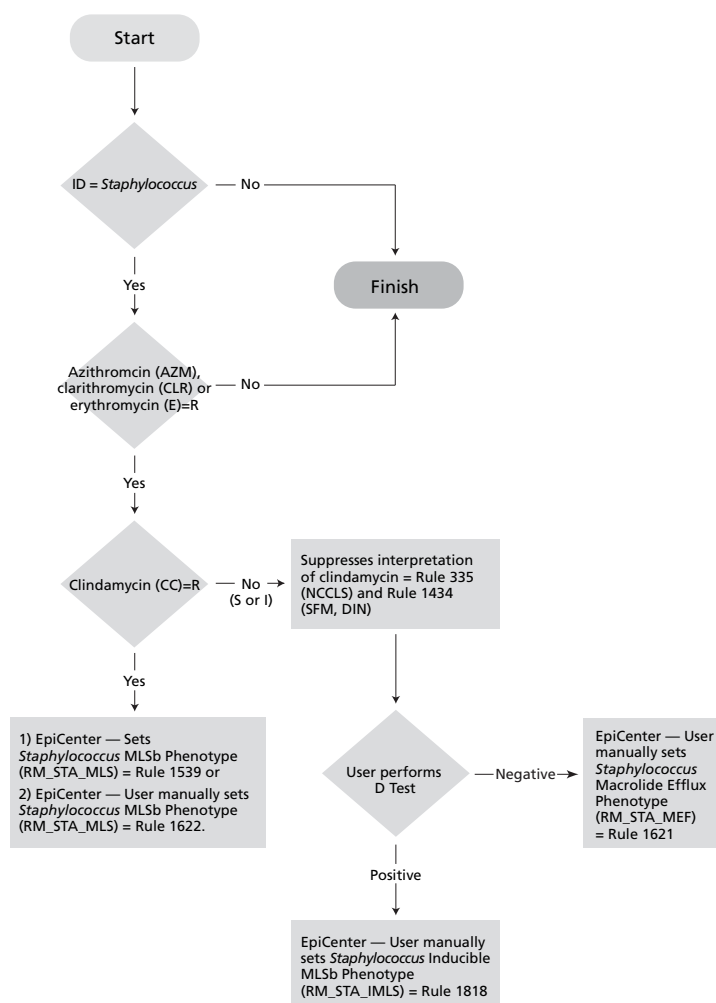
TEST PROCEDURES.

The SIR interpretation results generated by the Phoenix System were used to evaluate the BDXpert System. A printed report for each test isolate was reviewed by two experienced microbiologists. Expert rules were activated and were evaluated based on the MIC and interpretation results on the report. Messages of any expert rules triggered were noted and compared to the original recommendations in the standards documents.

Table 1. List of *Staphylococcus* species Tested

Species	Number of Strains
<i>Staphylococcus aureus</i>	148
<i>Staphylococcus epidermidis</i>	12
<i>Staphylococcus haemolyticus</i>	10
<i>Staphylococcus hyicus</i>	2
<i>Staphylococcus cohnii</i>	2
<i>Staphylococcus xylosus</i>	1
<i>Staphylococcus saprophyticus</i>	1
<i>Staphylococcus lugdunensis</i>	1
<i>Staphylococcus hominis</i>	1
<i>Staphylococcus caprae</i>	1
<i>Staphylococcus capitis</i>	1
<i>Staphylococcus species</i>	1
Coagulase-negative <i>Staphylococcus</i>	1
TOTAL	182

Flowchart 1. Reporting MLSb Phenotypes in the BDXpert System for NCCLS, SFM or DIN



RESULTS

The Phoenix E and CC MIC values were interpreted based on the NCCLS, SFM or DIN. The BDxpert rules were executed and applicable expert messages were displayed according to the SIR interpretation of E and CC.

The Phoenix correctly detected 38 out of 43 constitutive MLSb (cMLSb) phenotypes compared to the D zone test results. Four cMLSb strains were interpreted by the BDxpert as potential inducible MLSb (iMLSb)/efflux phenotypes. A total of 72 iMLSb and 22 efflux phenotype isolates were reported by the BDxpert as iMLSb/efflux phenotype. The user was alerted to perform D zone test before reporting the CC results. The CC interpretation was suppressed in these isolates. However, there was 1 cMLSb, 3 iMLSb and 1 efflux strains, by the reference method, mis-identified as wild-type by the Phoenix System. The overall accuracy of MLSb phenotypes as detected by Phoenix and BDxpert System was 94.5% (172/182).

When applying NCCLS, SFM, or DIN, identical detection and interpretation of MLSb resistant phenotypes were observed by the Phoenix and BDxpert systems.

Table 2. Phenotype of Test Strains Using D Zone Test

Species	Reference Phenotypes			
	cMLSb	iMLSb	Efflux	Wild-Type
<i>Staphylococcus aureus</i>	33	69	16	30
<i>Staphylococcus epidermidis</i>	2	3	1	6
<i>Staphylococcus haemolyticus</i>	6	1	3	
<i>Staphylococcus hyicus</i>		1		1
<i>Staphylococcus xylosum</i>		1		
<i>Staphylococcus species</i>			1	
<i>Staphylococcus saprophyticus</i>				1
<i>Staphylococcus lugdunensis</i>				1
<i>Staphylococcus hominis</i>	1			
<i>Staphylococcus cohnii</i>			2	
<i>Staphylococcus caprae</i>				1
<i>Staphylococcus capitis</i>				1
Coagulase-negative <i>Staphylococcus</i>	1			
TOTAL	43	75	23	41

Table 3. Comparison of Phoenix vs Reference Phenotypes

Species	Phoenix Phenotype	Reference Phenotypes			
		cMLSb	iMLSb	Efflux	Wild-Type
<i>Staphylococcus aureus</i>	cMLSb	28	1		
	iMLSb/Efflux	4	66	15	
	Wild-Type	1	2	1	30
<i>Staphylococcus caprae</i>	Wild-Type				1
<i>Staphylococcus capitis</i>	Wild-Type				1
<i>Staphylococcus cohnii</i>	iMLSb/Efflux			2	
	cMLSb	2	1		
<i>Staphylococcus epidermidis</i>	iMLSb/Efflux		2	1	1
	Wild-Type				5
<i>Staphylococcus haemolyticus</i>	cMLSb	6			
	iMLSb/Efflux		1	3	
<i>Staphylococcus hominis</i>	cMLSb	1			
<i>Staphylococcus hyicus</i>	iMLSb/Efflux		1		1
<i>Staphylococcus lugdunensis</i>	Wild-Type				1
<i>Staphylococcus saprophyticus</i>	Wild-Type				1
<i>Staphylococcus species</i>	iMLSb/Efflux			1	
<i>Staphylococcus xylosum</i>	Wild-Type		1		
Coagulase-negative <i>Staphylococcus</i>	cMLSb	1			
TOTAL		43	75	23	41

Table 4. BDxpert Rules for MLSb Phenotypes

BDxpert Text	
Rule #	NCCLS
335	Macrolide-resistant staphylococci with an interpretation of susceptible or intermediate for clindamycin may have inducible resistance to clindamycin (MLSb resistance). User must perform a clindamycin induction test (D test). If positive, isolate should be reported as clindamycin resistant. However, clindamycin may still be effective in some patients. If D test is negative, isolate should be reported as susceptible.
Rule #	SFM/DIN
1434	Macrolide-resistant staphylococci with an interpretation of susceptible or intermediate for clindamycin may have inducible resistance to clindamycin (MLSb resistance). User must perform a clindamycin induction test (D test). If positive, isolate should be reported as clindamycin resistant. However, clindamycin may still be effective in some patients. If D test is negative, isolate should be reported as susceptible.
Rule #	ALL Standards
1539	Isolate is a MLSb phenotype in <i>Staphylococcus</i> . Consultation with an infectious disease practitioner is recommended.
1618	Inducible clindamycin resistant phenotype in <i>Staphylococcus</i> was set manually by the user. Clindamycin may still be effective in some patients. Consultation with an infectious disease practitioner is recommended.
1621	Macrolide efflux phenotype in <i>Staphylococcus</i> was set manually by the user. Consultation with an infectious disease practitioner is recommended.
1622	MLSb phenotype in <i>Staphylococcus</i> was set manually by the user Consultation with an infectious disease practitioner is recommended.

CONCLUSIONS

- n The Phoenix and BDxpert Systems can assist laboratories in rapid detection and accurate interpretation of MLSb results for *Staphylococcus*. Special messages can be used to communicate timely and accurate information to clinicians for proper therapy of staphylococcal infections with MLSb resistant phenotypes.
- n The BDxpert system reported appropriate messages, especially to alert users to perform D zone test as necessary. Actions associated with the resistance were also activated correctly. The BDxpert System has proven to be a reliable system that can assist laboratory reporting and proper detection and interpretation of AST results for MLSb resistance in staphylococci.

