

KeyPath™ MRSA/MSSA Blood Culture Test – BT accurately identifies *S. aureus* isolates of broad phylogenetic diversity

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Abstract

Background: *Staphylococcus aureus* bacteremia leads to high morbidity and mortality, and appropriate antibiotic therapy can be delayed due to the 2-3 days needed for susceptibility testing. The KeyPath MRSA/ MSSA Blood Culture Test – BT is a phage amplification based test that identifies *S. aureus* in positive blood culture and determines its susceptibility to methicillin in about 5 hours. The test has one of three possible outcomes, namely MRSA, MSSA or NSA (non – *S. aureus*). Here we examine the KeyPath test accuracy when challenged with a genetically diverse set of *S. aureus* isolates.

Methods: Bacterial strains were grown in Trypticase soy broth and were spiked into BACTEC™ blood culture bottles charged with freshly-drawn blood. Cultures were grown in a BACTEC™ 9050 until positive. Up to 24 hours post-alarm, samples were withdrawn and diluted 10-fold to attain a bacterial input level at or near the limit of detection. KeyPath MRSA/MSSA Blood Culture Test – BT was run according to the package insert instructions. At each day of testing, three control strains (MRSA, MSSA and an NSA) were run in parallel.

Results: A total of 114 *S. aureus* strains representing geographic and phylogenetic diversity of *S. aureus* were tested. The panel included members of 46 MLS (multilocus sequence) types representing 17 clonal complexes. Each strain was tested in duplicate for a total of 228 test runs. Of the 22 incorrect calls, 20 were called NSA and 2 MSSA were called MRSA. The overall sensitivity of detection for *S. aureus* was 91.8% (208/228). Among *S. aureus* true positives, category agreement was 99% for determination of methicillin-resistance and 100% for determination of methicillin-susceptibility.

Conclusion: The KeyPath MRSA/ MSSA Blood Culture Test – BT is able to detect highly diverse *S. aureus* strains from different geographic locations and of different genetic makeup. The test was also not affected by PFGE class, PVL carriage, SCCmec type or methicillin resistance. The high sensitivity of this phage based system is indicative of strong reactivity of its phage against a wide variety of *S. aureus* strains.

Introduction

Staphylococci are frequent cause of blood stream infection in the US resulting in significant morbidity and mortality. Appropriate treatment for *S. aureus* bacteremia can be delayed due to the 2-3 days needed for microbiological testing. The KeyPath MRSA/ MSSA Blood Culture Test reports results within 6 hours of positive blood culture detection. The test is based on bacteriophage amplification, detected by lateral flow immunoassay and returns methicillin-resistant *S. aureus* (MRSA), methicillin-susceptible *S. aureus* (MSSA) or non-*S. aureus* (NSA) calls. The assay has recently been cleared for marketing by the FDA. Here we determine performance of the assay when challenged with a diverse set of strains representing the geographic and phylogenetic diversity of *S. aureus*.

Methods

A panel of *S. aureus* strains was collected that represents geographic and phylogenetic diversity. The strains are clinical isolates from North America and Western Europe. A total of 114 strains that were tested included 46 MLS (multilocus sequence) types representing 17 clonal complexes. The MRSA or MSSA category of each strain was confirmed by cefoxitin disk diffusion testing following the guidance of CLSI M100-S20.

Bacteria were spiked into blood culture bottles containing growth media and blood drawn from volunteers and grown in a BACTEC 9050 instrument. ‘Culture positive’ samples were inoculated into the test and the test carried out according to product insert.

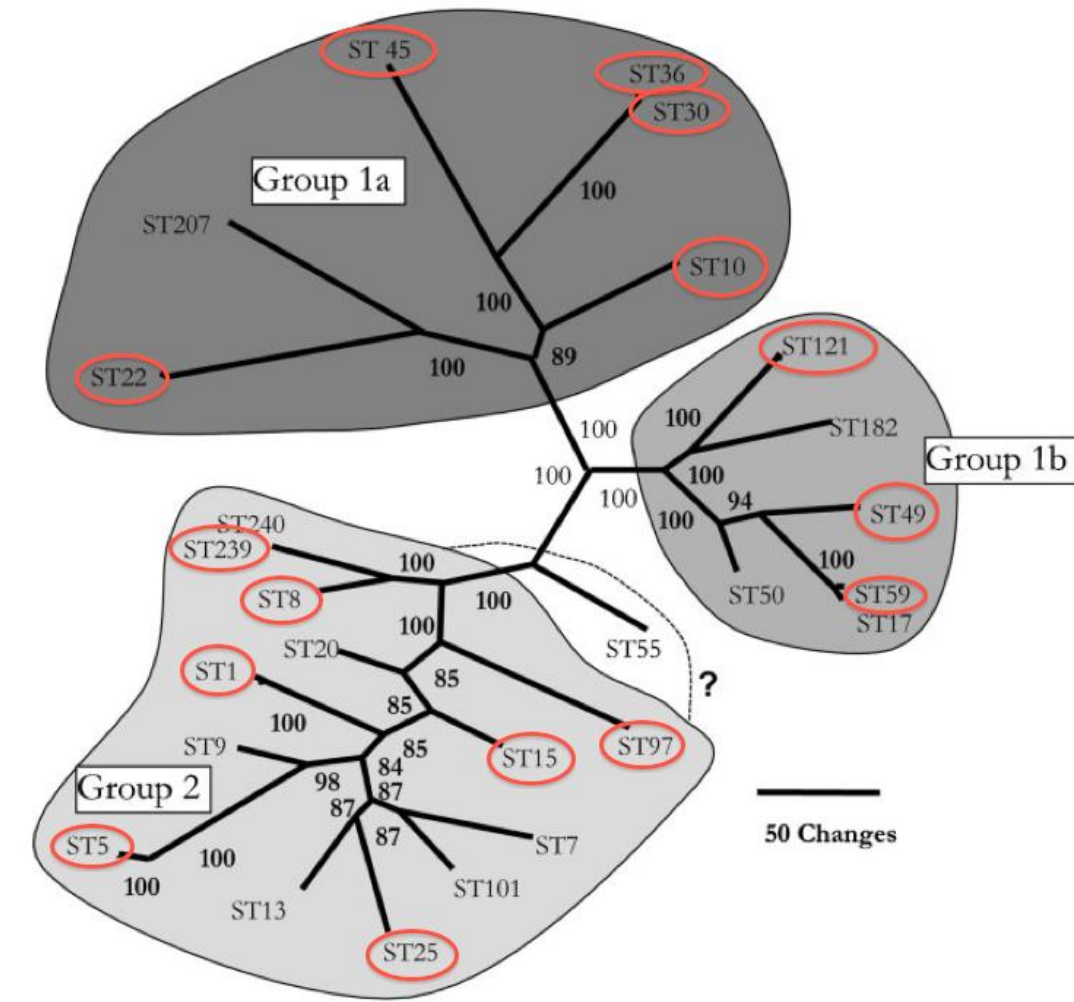


Figure 1. Phylogenetic structure of *S. aureus*. Phylogenetic structure of different MLS types is shown (Ref.) MLS types included in this study are circled in red. An additional 31 MLS types, whose phylogenetic relationships were not included in the analysis above, were also included in this study.

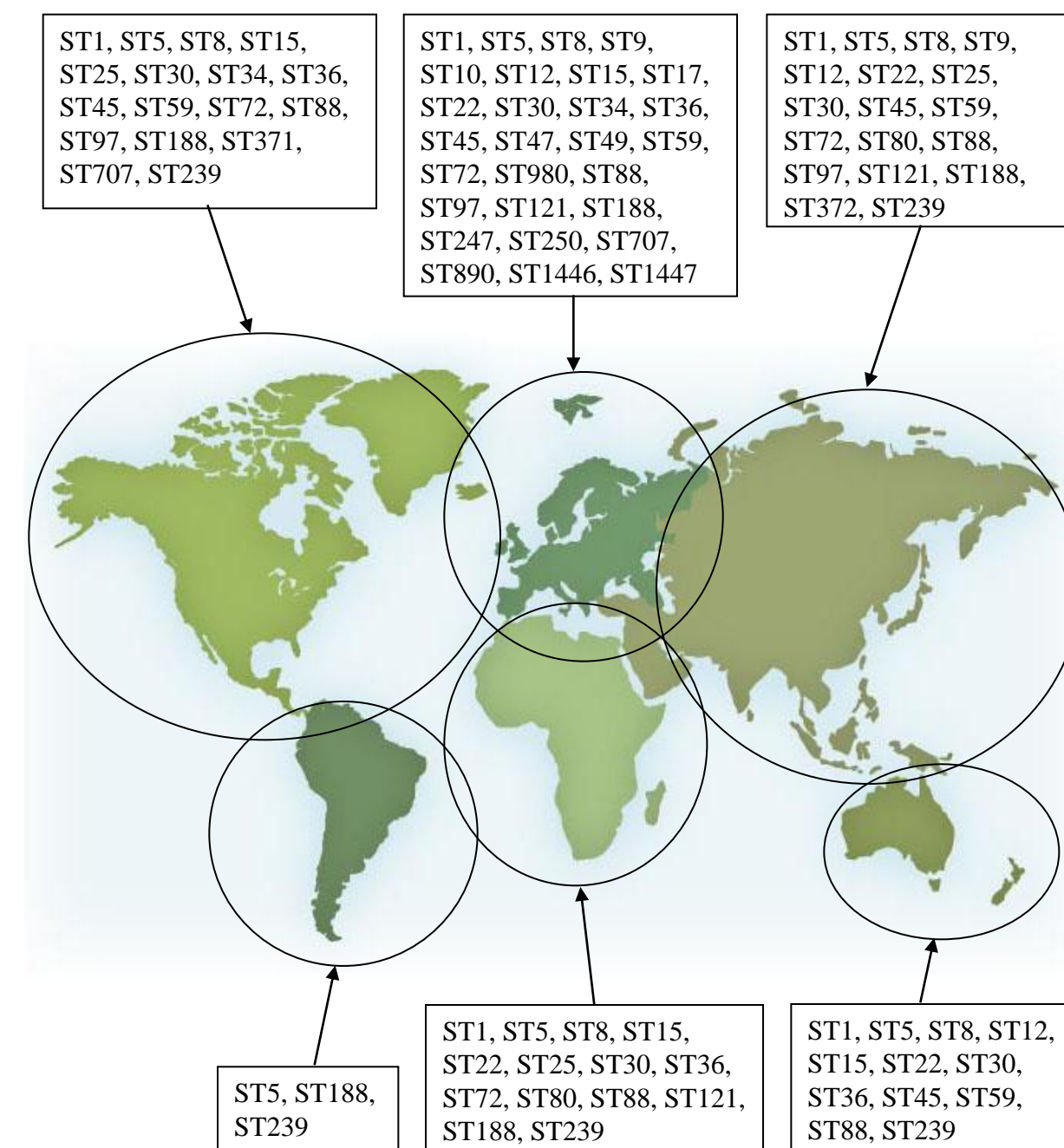


Figure 2. Global distribution of *S. aureus*. Geographic distribution of different MLS types used in this study is shown.

Results

The KeyPath MRSA/MSSA Test detects *S. aureus* of diverse MLS types. 114 *S. aureus* clinical isolates were tested in duplicate for a total of 228 test runs. Of these, 206 tests returned true calls (MRSA/ MSSA) while 208 samples were correctly identified as *S. aureus*. Of the 22 incorrect calls, 20 were ID false-negatives (NSA) and 2 were false resistant (a single strain, NRS 274, OXA MIC = 2 µg/mL). The overall sensitivity of detection for *S. aureus* is therefore 208/228 = 91.8%. Among *S. aureus* true positives, accuracy of methicillin resistance and susceptibility determination is 204/206 = 99.0%.

The only MLS Type with more than one false call was ST239, a member of CC8. These strains grew slowly on initial testing, requiring up to 36 hours. Upon repeat testing, the strains alarmed in the normal time window (12 to 16 hours), and returned true positive results.

The KeyPath MRSA/MSSA Test is not affected by PFGE type and PVL status of the *S. aureus* isolate. The major PFGE types (100, 300, 500) were represented by multiple isolates, and all yielded correct results (MRSA) upon testing. Types 700 and 1000 are represented by single isolates and gave false results (NSA) on initial testing. NRS 386 (USA 700) gave correct results on retesting, while NRS 483 (USA 1000) repeated the NSA result on retesting.

Table 1. KeyPath assay performance from various Pulsed Field Gel Electrophoresis (PFGE) Type *S. aureus* isolates (left panel) and Panton-Valentine Leukocidin (PVL) carrying isolates (right panel). Assay results are classified as True (T) if consistent with the strain category and False (F) otherwise. Inconsistent replicate results are presented as Mixed (M).

Strain ID	PFGE Type	KeyPath Result	Strain ID	PVL	KeyPath Result
NRS 382	100	T	NRS 22	-	T
NRS 741	100	T	NRS 648	-	T
NRS 383	200	T	NRS 708	-	T
NRS 71	200	T	NRS 715	-	T
NRS 722	200	M	NRS 722	-	T
NRS 384	300	T	NRS 741	-	T
NRS 643	300	T	NRS 123	+	T
NRS 739	300	T	NRS 192	+	T
NRS 123	400	T	NRS 193	+	T
NRS 192	400	T	NRS 384	+	M
NRS 193	400	T	NRS 483	+	F
NRS 385	500	T	NRS 484	+	T
NRS 708	500	T	NRS 643	+	T
NRS 22	600	F	NRS 739	+	T
NRS 648	600	T			
NRS 715	600	T			
NRS 386	700	F			
NRS 387	800	M			
NRS 483	1000	F			
NRS 484	1100	T			

Performance of KeyPath MRSA/MSSA test by SCCmec type. Performance data for all strains tested with known SCCmec types is shown in Table 2. All false calls were non-*S. aureus* (NSA) and not MSSA. Thus no false-susceptible results were reported.

Table 2. Performance of KeyPath MRSA/MSSA test by SCCmec type. All the strains tested returned either MRSA or MSSA call. Inconsistent replicate results are denoted as ‘Mixed’ where both MRSA and MSSA calls were obtained.

SCCmec Type	MRSA	MSSA	NSA	Mixed
I	2	0	1	0
II	14	0	1	1
IV	20	0	2	1
IVa	2	0	1	0
V	0	0	1	0

Conclusion

Bacterial identification and antibiotic susceptibility testing are crucial for delivering appropriate therapy and implementing control measures to prevent infection spread. The recent FDA cleared KeyPath MRSA/MSSA Blood Culture Test – BT provide MRSA/MSSA call under six hours from positive blood culture. The performance of the test was evaluated on a total of 114 MRSA and MSSA strains. Ten strains yielded incorrect results comprising 9 false-negative NSA calls, and 1 false-resistant MRSA call. Errors were distributed randomly among clonal complexes and MLS types, with the exception of ST239, which returned 3/4 NSA calls on initial testing. These strains grew anomalously slowly, and yielded correct results upon retesting. The data presented indicates that the Test is not affected by MLST, PFGE, PVL carriage or SCCmec type and is able to correctly identify a wide range of *S. aureus* strains.

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