

Rapid Screening for Methicillin-Resistant *S. aureus* (MRSA) by Bacteriophage Amplification

BP Sportmann, M Izzo, JC Rees, D Smith
MicroPhage Incorporated, Longmont, CO 80503 USA
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ABSTRACT:

Background

We describe initial feasibility studies for development of a rapid, accurate, low-cost MRSA screen based on bacteriophage amplification technology. In this scheme, nasal swabs are incubated in broth containing bacteriophage specific to *S. aureus* +/- the beta-lactam antibiotic cefoxitin. Detection of bacteriophage amplification indicates the presence of *S. aureus* in the inoculum. Detection in cefoxitin-containing broth additionally identifies the inoculate as MRSA.

Methods

Bacterial strains were acquired from volunteer nasal swabs and from expired clinical nasal swabs. The number and identity of bacteria recovered from swabs was determined by plating on Chromagar Staph, tryptic soy-broth, and blood agar plates. Species identity was confirmed by the API-Staph test procedure, or by standard culture methods. Methicillin resistance was determined by oxacillin MIC on a Microscan Walkaway-40. A panel of 42 MRSA, 72 MSSA and 102 coagulase-negative Staph (CoNS) was tested.

A spiked model was developed in which single-colony isolates were dispersed in broth to a 0.5 McFarland standard, diluted as appropriate and spotted on to sample swabs. The swabs were then placed in 0.5 ml of the appropriate broth containing bacteriophage and incubated for 10 hours at 35°. The broths used were a proprietary ID broth, which allows identification of *S. aureus*-positive samples, and an antibiotic-resistance broth containing the cefoxitin, which identifies *S. aureus*-positive samples as resistant or susceptible. Samples that tested positive for identity and resistance were called as "MRSA", a negative on either test resulted in a call of "not MRSA".

At the end of the incubation period, samples were diluted and plated in soft agar for plaque assays and were scored positive for phage amplification at $> 10^7$ pfu/ml. In dipstick tests, a clearly visible test line was scored positive.

Results

The MicroPhage prototype test proved to be highly sensitive and specific in detecting MRSA-positive nasal swab samples. 95% (40/42) MRSA swabs were identified correctly, with no false positives from MSSA (0/72) or CoNS (0/102) swabs. The test has good analytic sensitivity and specificity: MRSA were identified correctly from swabs inoculated with as few as 300 cfu, and CoNS and MSSA were rejected on swabs inoculated with as many as 7000 cfu. We found that these quantitative results could be replicated by a simple qualitative dipstick immunoassay test that uses antibody directed against our bacteriophage.

(This Abstract has been revised from the original)

INTRODUCTION:

The value of screening in MRSA control and management is widely accepted, but implementation of screening programs in hospitals and other facilities is limited by the cost and technical expertise required. Our goal is to develop a low-cost test that requires minimal equipment, hands-on time and expertise. We are developing a test that uses bacteriophage amplification technology to meet these specifications while delivering a high level of performance.

The principle of the test is outlined in Figure 1. We have developed a cocktail of bacteriophage that have broad but highly specific coverage of *S. aureus* clinical strains. The performance of the bacteriophage is enhanced by

broth formulation. We identify MRSA in two parallel tests, one for species ID and one for antibiotic resistance. The ID test distinguishes *S. aureus* - positive samples from *S. aureus* - negative samples: positives amplify bacteriophage and negatives do not. The resistance test additionally distinguishes resistant strains: MRSA strains amplify bacteriophage and MSSA strains do not.

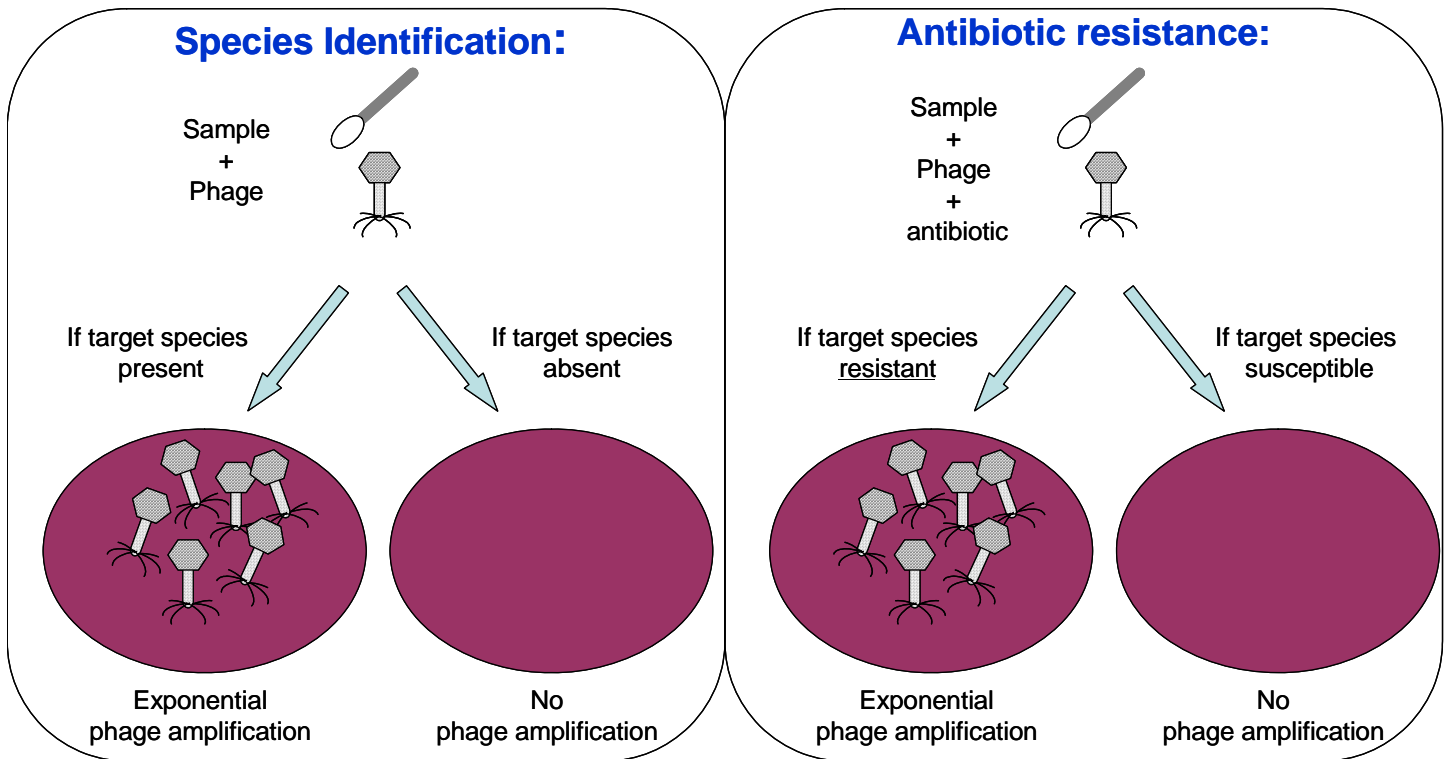


Figure 1. Using bacteriophage amplification for bacterial diagnostics.

MATERIALS and METHODS:

Bacteria

Bacterial strains were acquired from volunteer nasal swabs and from expired clinical nasal swabs. The number and identity of bacteria recovered from swabs was determined by plating on Chromagar Staph, tryptic soy-broth, and blood agar plates. Species identity was confirmed by the API-Staph test procedure, or by standard culture methods. Methicillin resistance was determined by oxacillin MIC on a Microscan Walkaway-40. The test panel had the species composition detailed in Table 1.

Species	n
<i>S. aureus</i> - MRSA	42
<i>S. aureus</i> - MSSA	72
Coagulase-negative Staph	99
<i>S. epidermidis</i>	52
<i>S. warneri</i>	12
<i>S. haemolyticus</i>	12
<i>S. capitis</i>	10
<i>S. simulans</i>	5
<i>S. hominis</i>	4
<i>S. lugdunensis</i>	3
<i>S. lentus</i>	2

Table 1. Nasal bacteria tested in this study.

Testing Model

A spiked model was developed in which single-colony isolates were dispersed in broth to a 0.5 McFarland standard, diluted as appropriate and spotted on to sample swabs. The swabs were then placed in 0.5 ml of the appropriate broth containing bacteriophage at 5×10^5 pfu/ml and incubated for 10 hours at 35°. The broths used were a proprietary ID broth, which allows identification of *S. aureus*-positive samples, and an antibiotic-resistance broth containing the beta-lactam antibiotic cefoxitin, which identifies *S. aureus*-positive samples as resistant or susceptible. Samples that tested positive for identity and resistance were called as "MRSA", a negative on either test resulted in a call of "not MRSA".

Samples were scored positive for phage amplification at $> 10^7$ pfu/ml, equivalent to a 20-fold increase in phage. In rapid-test detection devices, a clearly visible test line was scored positive.

RESULTS:

Bacteriophage Performance

The bacteriophage amplification test proved to be both highly sensitive and specific in detecting MRSA-positive samples. Samples that scored positive for bacteriophage amplification in both the ID and resistance tests were classified as "MRSA"; samples that were negative in either test were classified as "not MRSA". The results of these evaluations are summarized in Tables 2 and 3, below.

		Reference	
		MRSA	not MRSA
MicroPhage	MRSA	40	0
	not MRSA	2	174

Table 2. Two-by-two results of the experimental MRSA test.

Performance Summary	
Parameter	Result
Sensitivity	95%
Specificity	100%
PPV	100%
NPV	99%

Table 3. Performance summary of experimental MRSA test.

None of the CoNS or MSSA samples yielded a false-positive result, giving the test perfect fidelity in specificity and positive predictive value. Two of the 42 MRSA samples yielded a false-negative result. These samples were negative in both the ID and resistance tests.

Nasal swabs are an inherently heterogenous sample type. To assess the robustness of the test with regard to levels of bacterial input, we deliberately spiked samples with a wide range of input levels of bacteria. We consistently detected positives spiked with as little as 300 cfu, and rejected negatives spiked at up to 8000 cfu.

A small sample of these experiments was repeated using the spike model but detecting amplification using a rapid-test detection device. An example of these results is shown in Figure 2.

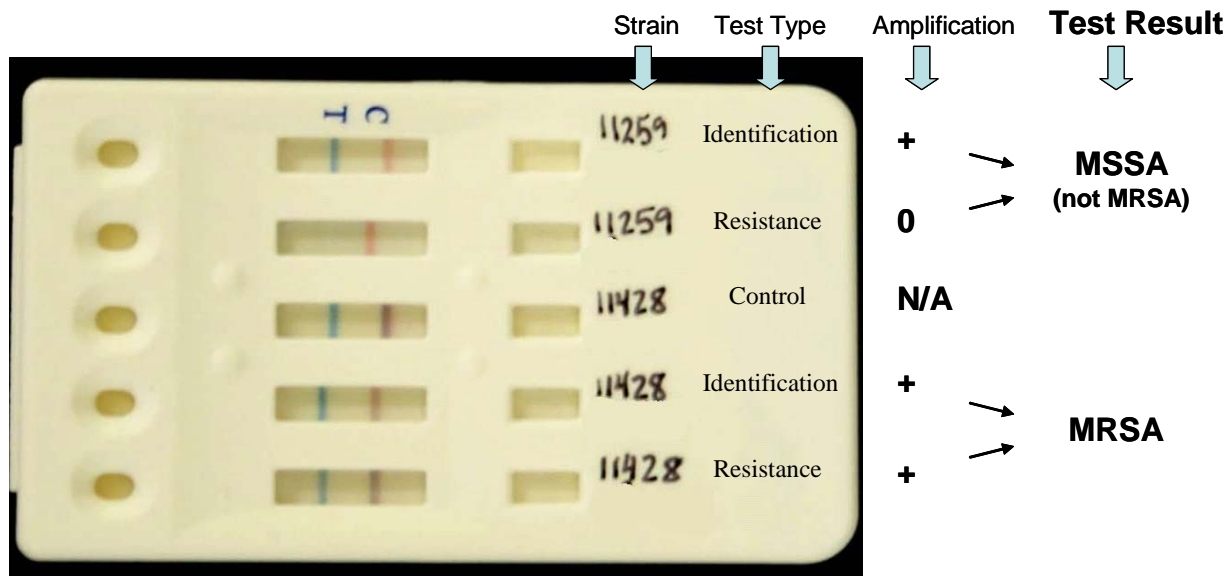


Figure 2. Rapid-test results of bacteriophage amplification in spiked nasal swabs.

CONCLUSIONS:

Our results indicate that high-sensitivity, high-accuracy screening for MRSA is feasible using a bacteriophage amplification test. The cost of the test components, consisting of a swab and incubator device, a cocktail of bacteriophage in broth and a rapid-test detector, will be low, and the hands-on time of the test will be minimal. The test protocol, with test prototypes, is shown in Figure 3, below.

The only equipment required is a simple heating block or incubator. The test requires only minimal skills and training to perform and interpret, and thus should be suitable for use outside the clinical microbiology laboratory.

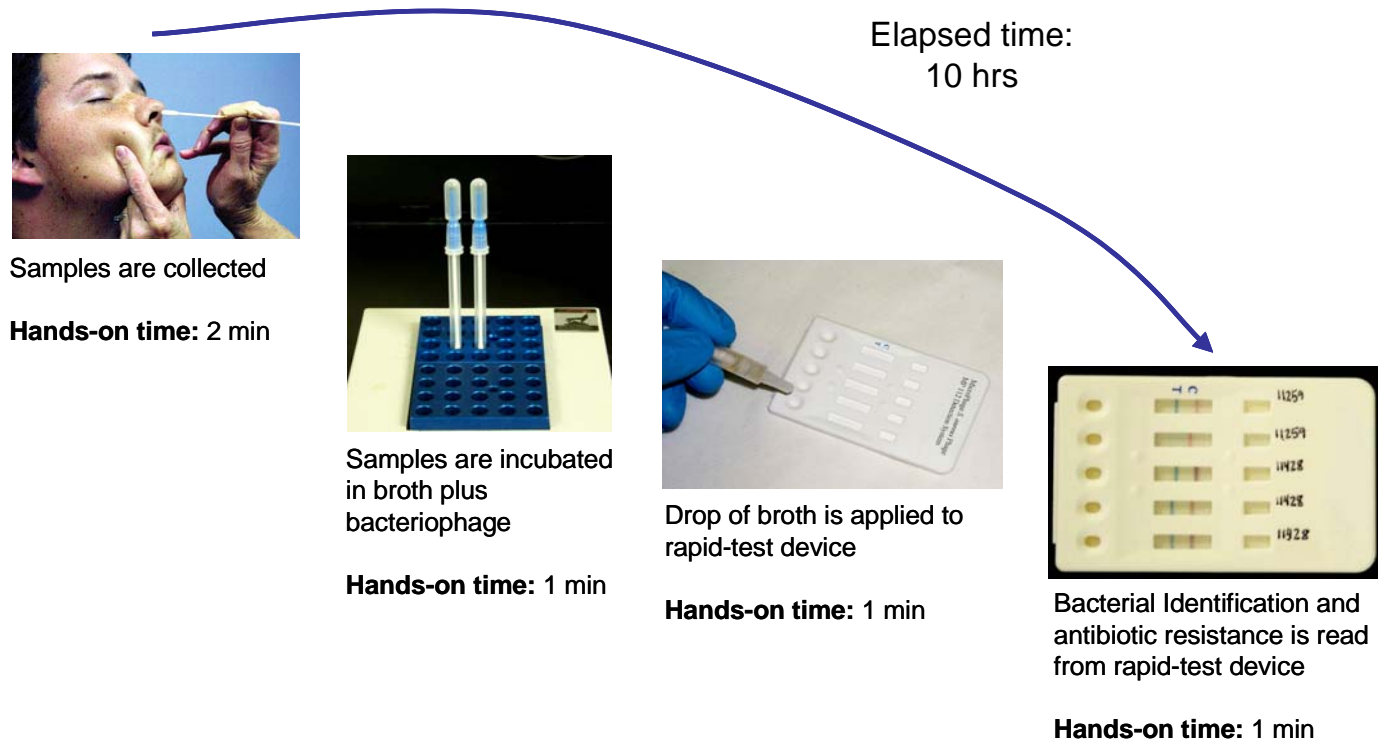


Figure 3. Envisioned rapid-test protocol with test prototypes for rapid MRSA Screening.