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## ABSTRACT:

**Background**  
Because bacteriophage amplification relies upon a metabolically active host bacterium, antibiotic susceptibility may be established by incubating phage and bacteria in a medium with an antibiotic, and comparing these results to a control without the antibiotic. If phage amplification can be detected in the presence of an antibiotic, then the bacterium is deemed resistant. Conversely, if no phage amplification occurs, then the bacterium is deemed susceptible.

This study establishes the efficacy of phage amplification to determine *S. aureus* susceptibility to cefoxitin. Samples were modeled as blood culture, a clinically significant sample type.

**Methods**  
96 clinical *S. aureus* strains were inoculated into Bactec culture medium containing 20% human blood and grown to 1-5 x 10<sup>8</sup> cfu/ml at 35C to model a positive blood culture. These samples were diluted into broth containing 2 bacteriophages in the presence and absence of cefoxitin and incubated at 35°C for 4 hours. Phage amplification was quantified by counting plaques on host lawns. Phage amplification of >10-fold over input was scored as positive. Strains were validated as MRSA or MSSA per CLSI standard M07-A7.

**Results**  
42/48 MSSA and 42/48 MRSA strains amplified phage in the absence of cefoxitin, and thus could be scored for susceptibility or resistance in the presence of cefoxitin. 41/42 phage-sensitive MSSA strains scored negative for phage amplification in 2ug/ml cefoxitin. 41/42 phage-sensitive MRSA scored positive for phage amplification. Assay performance parameters were determined to be: 88% sensitivity, 98% positive predictive value for susceptibility, and 98% positive predictive value for resistance.

**Conclusions**  
Because bacteriophage amplification requires a viable host, this method can provide a means of determining host bacterial drug resistance or sensitivity. Convenient, inexpensive methods of bacteriophage detection, such as antibody-based assays, can thus be used to determine bacterial drug resistance and susceptibility rapidly and reliably.

(This Abstract has been revised from the original)

## INTRODUCTION:

The value of rapidly and correctly identifying causative bacteria and determining their antibiotic susceptibility is critical to achieving positive patient outcomes in many types of infections. With the rate of methicillin-resistant *Staphylococcus aureus* (MRSA) increasing to epidemic levels, clinicians now often empirically treat patients with suspected bacteremia with broad spectrum antibiotic such as the glycopeptide vancomycin as a precautionary measure until laboratory results are available. Although generally accepted as the standard of care, deescalation of therapy to more narrow spectrum antibiotics for certain organisms such as methicillin-susceptible *S. aureus* (MSSA) is relatively new. In order to confidently deescalate therapy, the clinician must be given more rapid and reliable identification and susceptibility result than the current laboratory standard of 48 to 72 hours for bacteremia. Our goal is to develop a test that can eliminate organism subculturing and directly identify bacteria and their antibiotic susceptibility from blood culture on a platform that could be used 24 hours a day by a laboratory technician. 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 below in Figure 1, below. 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 susceptibility test additionally distinguishes resistant strains: MRSA strains amplify bacteriophage and MSSA strains do not.

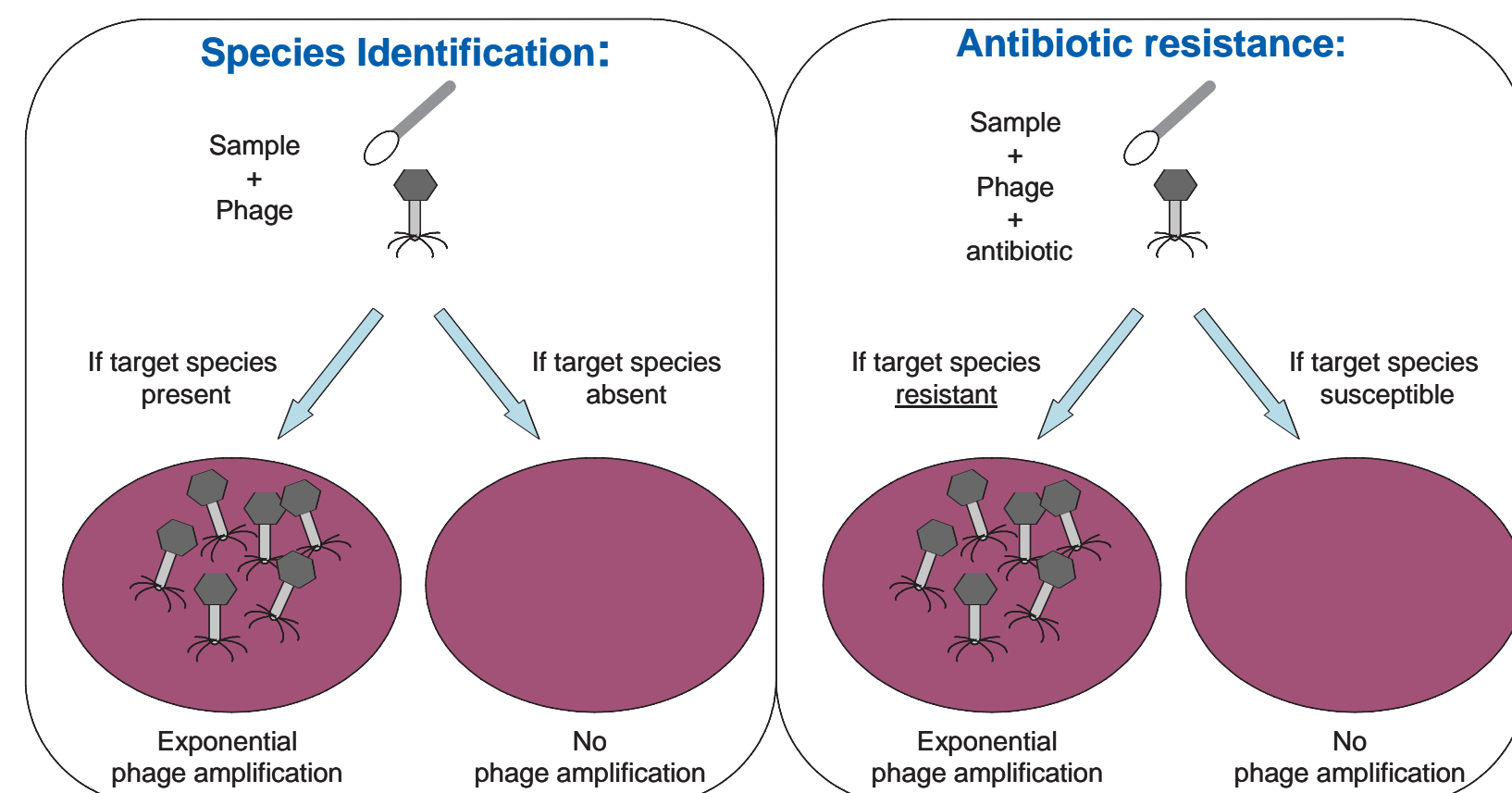


Figure 1. Using bacteriophage amplification for bacterial identification and breakpoint antibiotic susceptibility testing.

## MATERIALS and METHODS:

### Bacteria

202 clinical strains of *S. aureus* and 154 clinical coagulase negative Staphylococci were sourced from JMI laboratories (North Liberty, IA). The breakdown of the strain identification is listed to the right in Table 1..

### Blood Culture Testing Model

Each strain was inoculated into Bactec culture medium (Becton Dickinson, Franklin Lakes, NJ) containing 20% human blood and grown at 35 C until a concentration of 1-5 x 10<sup>8</sup> cfu/ml was achieved. Subsequently, each sample was diluted into a tryptic soy based broth for identification (ID), or diluted into a tryptic soy-based broth containing cefoxitin for the purpose of determining antibiotic susceptibility (AbS). To both the ID and AbS samples, a phage cocktail of 3 Staphylococci phages MP115, MP131 and MP112 (developed in-house) were added and samples were incubated at 35°C for 4 hours.

### Amplification Determination Method

Subsequent to incubation, samples were quantified for bacteriophage using the traditional plaque assay methodology. If a final phage concentration exceeded initial phage input greater than 10 fold, then phage amplification was said to have occurred. Conversely, if the final phage concentration was determined to be below 10 fold, then phage amplification was deemed insufficient. An insufficient phage amplification for the ID test means that the sample was identified as an organism other than the *S. aureus*, and an insufficient amplification in the ABs broth combined with a greater than 10 fold increase in the ID broth means that the sample is identified as MSSA. Figure 2 below shows a schematic of interpreting results. **ADD REFERENCE TO HOW RELATED TO LFI THIS IS (a PREVIOUS MicroPhage POSTER).**

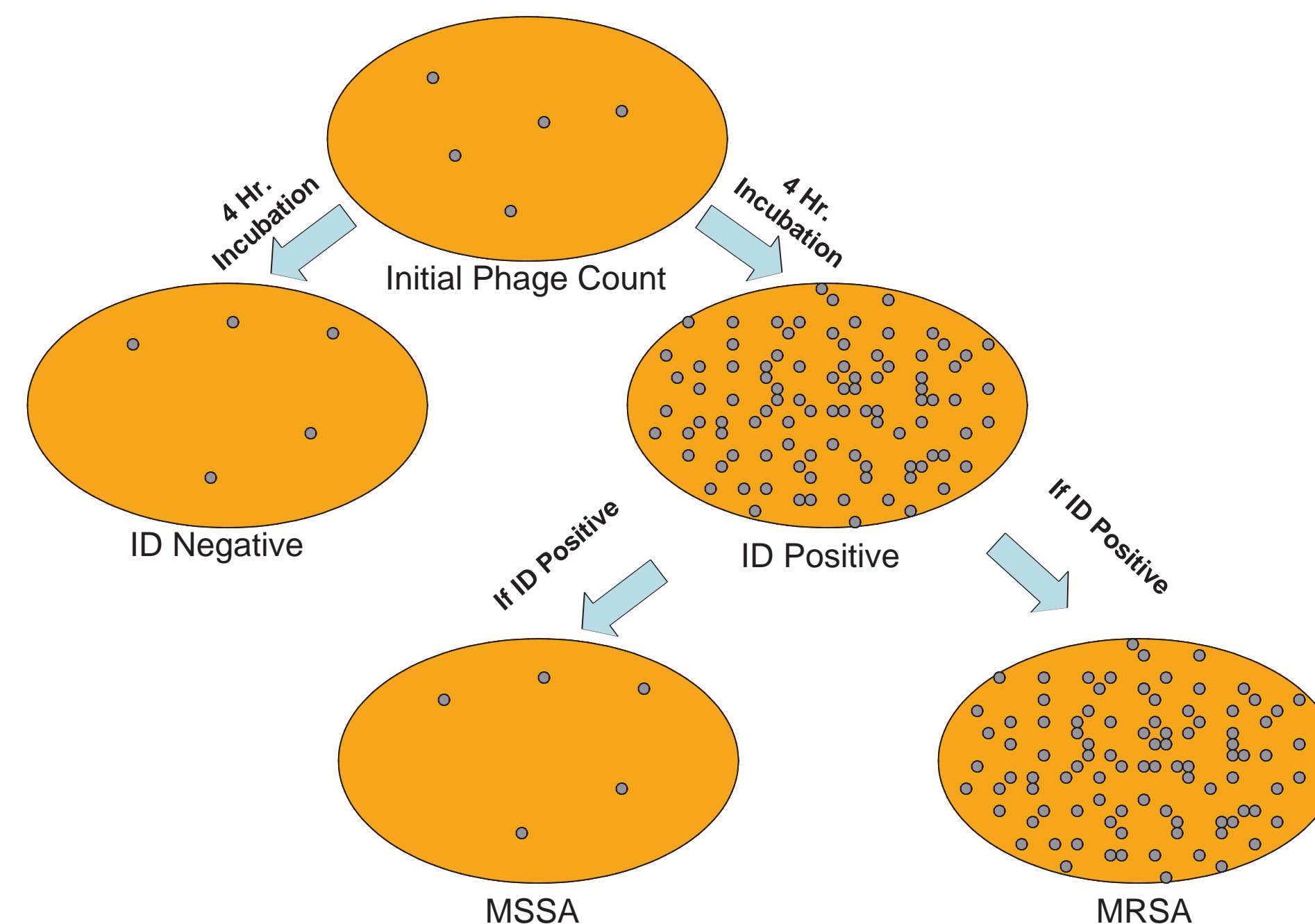


Figure 2. Bacteriophage amplification determination by traditional plaque assay for identification and antibiotic susceptibility testing.

Species	n
<b><i>S. aureus</i> - MRSA</b>	<b>98</b>
<b><i>S. aureus</i> - MSSA</b>	<b>104</b>
<b>Coagulase-negative Staph</b>	<b>154</b>
<i>S. epidermidis</i>	100
<i>S. hominis</i>	16
<i>S. haemolyticus</i>	13
<i>S. capitis</i>	9
<i>S. warneri</i>	8
<i>S. lugdenensis</i>	3
<i>S. saprophyticus</i>	2
<i>S. simulans</i>	2

Table 1. Nasal bacteria tested in this study.

## RESULTS:

### Bacterial Identification Performance

Of the 203 *S. aureus* strains tested in this study, 187 were correctly identified as *S. aureus*, as shown in Table 2. Importantly, no CoNS strain showed phage amplification greater than 10X. Because of the high specificity of the test, the positive predictive value of the ID test is 100%, a notable achievement for this type of assay. Undoubtedly, as the sensitivity of the phage cocktail is honed to amplify in the refractory *S. aureus* strains - an intrinsic advantage of this technology - the utility of this assay becomes evermore attractive.

### Antibiotic Susceptibility Performance

The results of the AbS testing are shown below right in Table 3. Of the 97 strains of MRSA that were phage amplification positive without cefoxitin, all 97 showed phage amplification in the presence of cefoxitin. Furthermore, of the 100 MSSA strains that were phage amplification positive in the absence of cefoxitin, only 3 showed any evidence of phage amplification above the 10X cutoff threshold. Seven strains of *S. aureus* were refractory to the phage cocktail as currently constituted.

Table 2. Results of the bacterial identification test with *Staphylococci*.

Table 3. Results of the antibiotic susceptibility test of *S. aureus* strains.

### Combined Test Performance

The combination of the ID and AbS test is shown to the right in Table 4, which includes results for all *S. aureus* and CoNS strains tested in this study. Because the overwhelming majority of *S. aureus* strains refractory to the phage cocktail were MSSAs, these were classified as false negatives, which causes the sensitivity of the test to suffer. However, because the combined ID/AbS test shows only one MSSA giving a false positive, the inherent PPV hovers at 99%.

## CONCLUSIONS:

Our results indicate that an AbS test using phage amplification detection can generate a remarkable PPV. Because phage need a viable host to generate progeny, the effects of cefoxitin (or for that matter almost any antibiotic) to suppress phage amplification when compared to a positive ID test indicates the presence of MSSA. As the bacteriophage cocktail continues to be refined, performance of the assay, particularly in the area of sensitivity, is expected to improve.

While the standard plaque assay is not being considered as an actual detection method, other rapid detection devices can seamlessly be integrated as a detector. As shown in previous work by MicroPhage researchers (REF), immunoassays are a prime platform for which to detect phage amplification, as high concentrations of phage-derived protein are generated in an amplification process. Prototype lateral flow immunochromatography strips developed to detect one component of the phage cocktail is shown in Figure 3, and antibodies to the other two phage components are currently being analyzed. Continued refinement of the phage cocktail along with the development of a rapid detector already in progress add to the momentum of a product being introduced shortly to detect antibiotic susceptibility in *S. aureus*.

## REFERENCES:

Add here (Duane paper on plaque assay to LFI, others).

Table 4. Identification, antibiotic susceptibility and combined interpretation performance results.