Supporting Information

Simultaneous identification and susceptibility determination to multiple antibiotics of *Staphylococcus aureus* by bacteriophage amplification detection combined with mass spectrometry.

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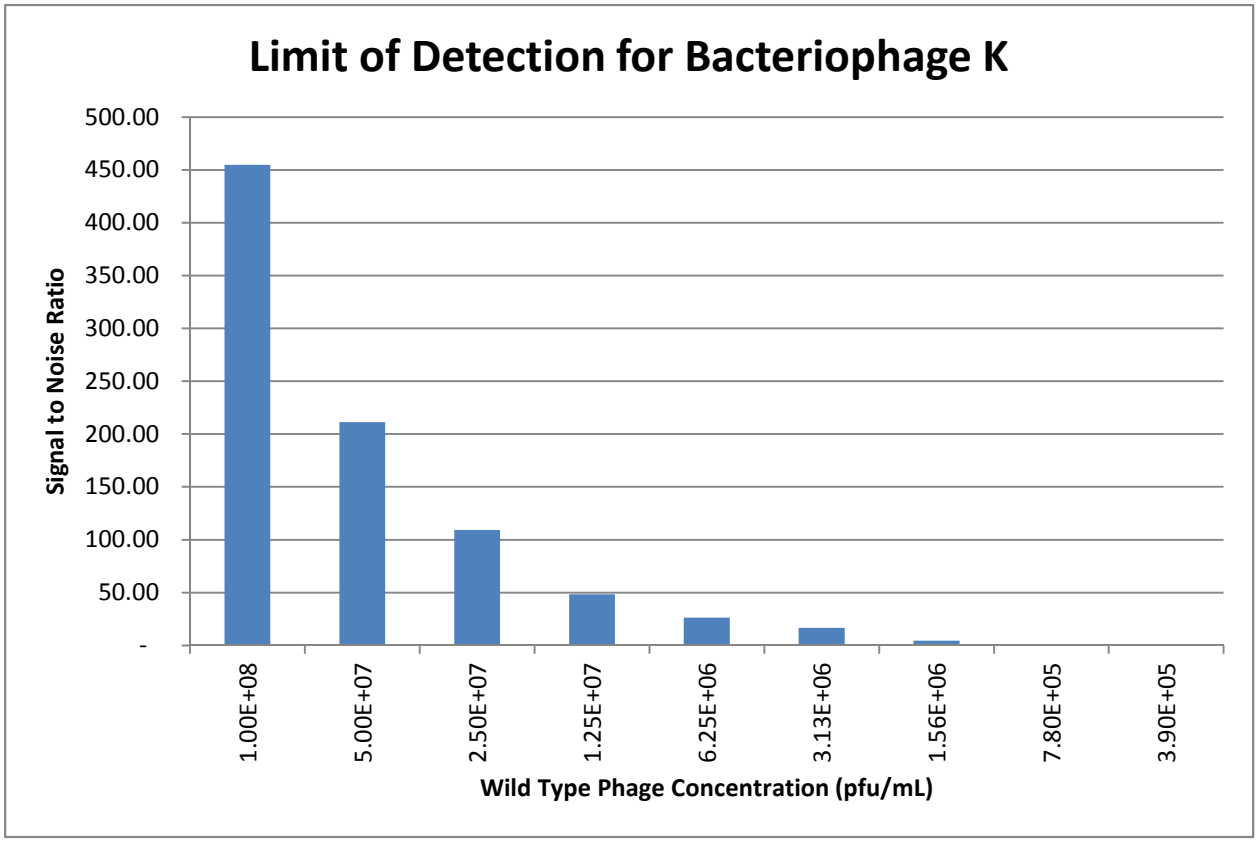
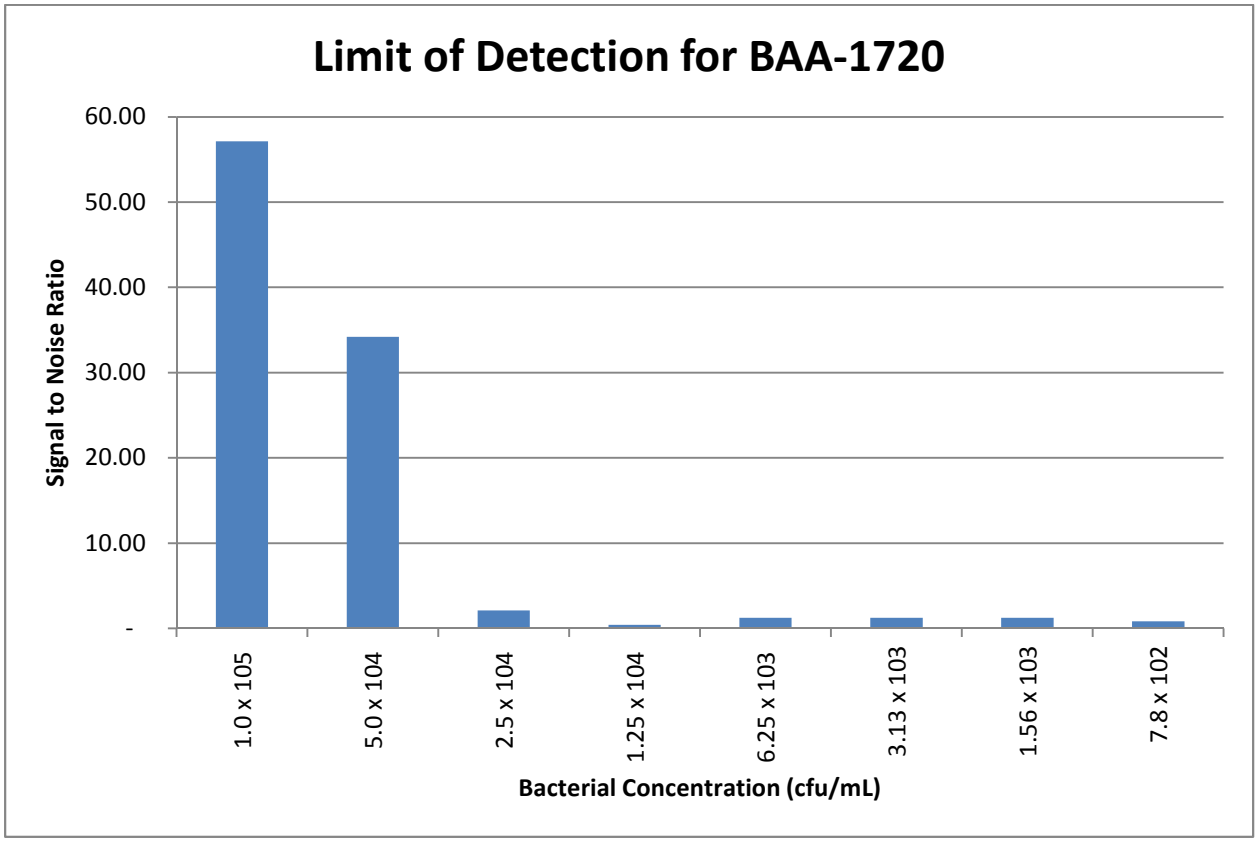
ABSTRACT: The continued advance of antibiotic resistance in clinically relevant bacterial strains necessitates the development and refinement of assays that can rapidly and cost-effectively identify bacteria and determine their susceptibility to a panel of antibiotics. A methodology is described herein that exploits the specificity and physiology of the Staphylococci bacteriophage K to identify Staphylococcus aureus (S. aureus) and determine its susceptibility to clindamycin and cefoxitin. The method uses liquid chromatography-mass spectrometry to monitor the replication of bacteriophage after it is used to infect samples thought to contain S. aureus. Amplification of bacteriophage K indicates the sample contains S. aureus, for it is only in the presence of a suitable host that bacteriophage K can amplify. If bacteriophage amplification is detected in samples containing the antibiotics clindamycin or cefoxitin, the sample is deemed to be resistant to these antibiotics, respectively, for bacteriophage can only amplify in a viable host. Thus, with a single work flow, S. aureus can be detected in an unknown sample and susceptibility to clindamycin and cefoxitin can be ascertained. This paper discusses implications for the use of bacteriophage amplification in the clinical laboratory.

Supplement Table 1 - List of proteins identified from bacteriophage K preparations by LC-MS/MS followed by database searching.

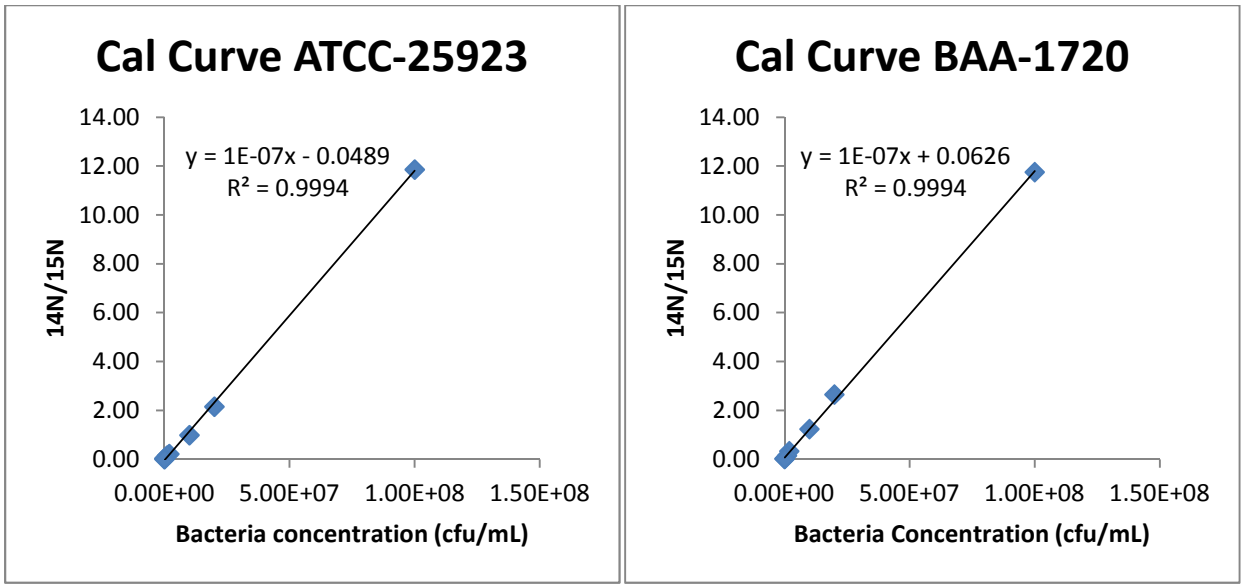
Protein	Accession #	Normalized Spectral Count	M_w
Phage K Capsid Protein	gi|66394965	71	51 kDa
Phage K ORF 65	gi|66394951	28	129 kDa
Phage K ORF 95	gi|66395008	25	23 kDa
Phage K Major Tail Protein	gi|46394852	13	13 kDa
Phage K ORF 68	gi|37729150	9	50 kDa
Phage K ORF 43	gi|37729123	9	36 kDa
Phage K ORF 66	gi|46394848	8	73 kDa
Phage K ORF 64	gi|66395027	5	19 kDa
Phage K ORF 42	gi|66394997	5	27 kDa



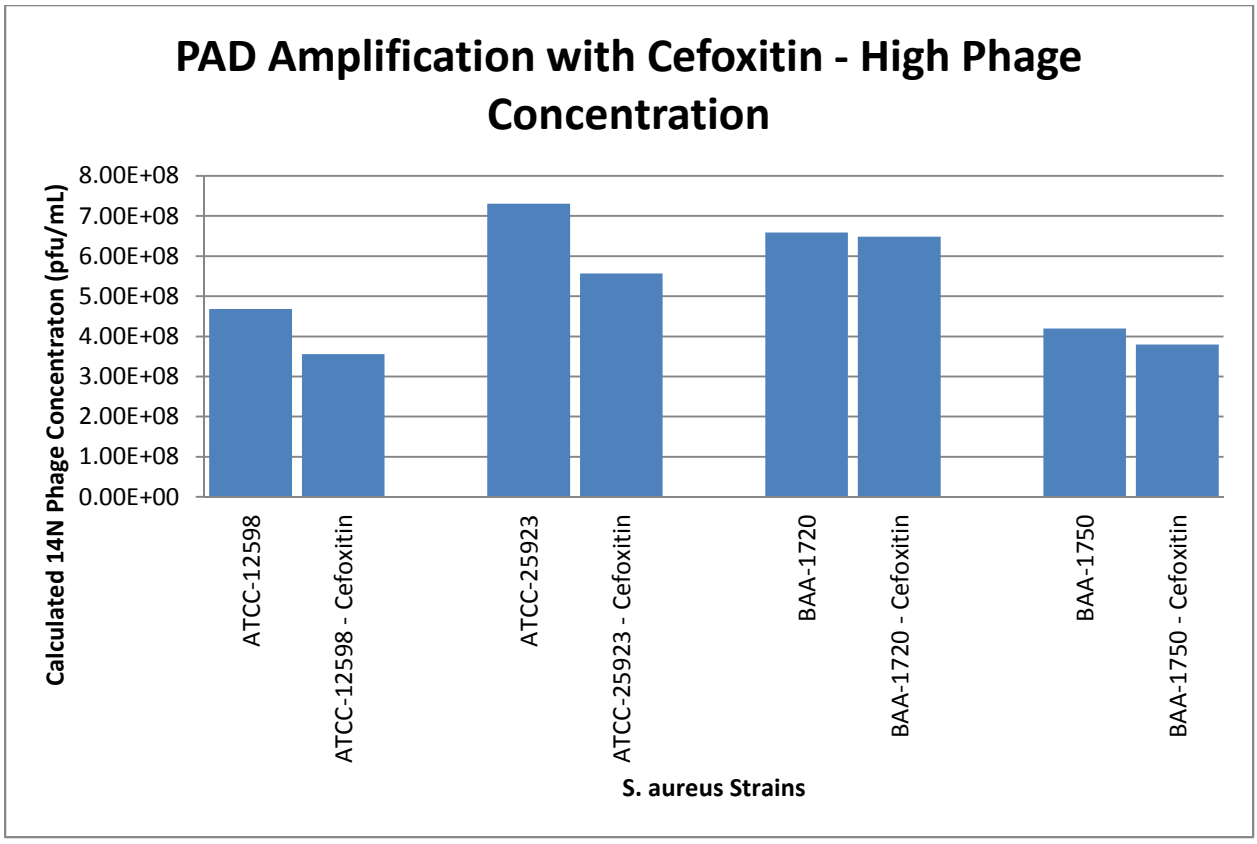
Supplemental Figure 1 – Signal to noise ratios of serial dilutions of bacteriophage K. The limit of detection is approximately 1 x 10^6 pfu/mL, where the S/N ratio drops below 3.



Supplemental Figure 2 – PAD signal to noise ratios for serial dilution of BAA-1720. Limit of detection for bacteria is approximately 5 x 10^4 cfu/mL.



Supplemental Figure 3 – Calibration curves for *S. aureus* ATCC-25923 and *S. aureus* BAA-1720.



Supplemental Figure 4 – PAD in the presence of cefoxitin using high initial (T = 0 h) concentrations of bacteria and bacteriophage.