1. Introduction

Hospital-acquired (HA) infections of antibiotic-resistant bacteria are a growing problem worldwide, costing billions of dollars in treatment and resulting in tens of thousands of deaths in North America alone [1].

HA infections are typically diagnosed by PCR or agar plating with antibiotics. While these methods are sensitive and accurate, they are laborious, have limited throughput and may require expensive equipment. Screening for HA infections is therefore priority-based and doesn’t focus on reducing the spread of infection.

To address this urgent need, we have developed a rapid sandwich assay based on silver enhancement to detect pathogenic bacteria with low-cost imaging equipment. This approach is well suited for a biosensor that can be used at the point-of-care to quickly screen patients and reduce the spread of HA infections.

“Superbugs”

The three most prevalent HA infections in North America are MRSA, C. difficile and VRE.

2. Methodology

A sandwich assay based on surface expressed antigens has been developed to rapidly detect bacteria. The assay will be integrated into an autonomous point-of-care diagnostic.

- The proof-of-concept assay is developed using E. coli K12 and O157:H7 (a common pathogenic strain).
- Antibodies against surface expressed antigens provide specificity for the target strain.
- Bacteria captured from the sample are detected by the development of a silver coating, making them easily visible with low-cost imaging equipment (Figure 1).
- Through this approach, individual cells can be counted, allowing for improved sensitivity and theoretical single cell detection.

3. Bacteria Capture

- Bacteriophage (3.1) and antibodies (3.2) were investigated as potential binders for the capture of E. coli from samples.
- Immobilization conditions (pH, buffer, conc.) were optimized using a microarray format (Figure 2) on 4 different surfaces (Epoxy, Poly-L-lysine coated-glass, Xenobind and (3-Aminopropyl)trimethoxysilane (APTES)/glutaraldehyde functionalized glass).

3.1 Bacteriophage Capture

- Optimal T4 phage immobilization was found on reactive aldehyde surfaces, i.e. Xenobind and APTES/glut. (Figure 3).
- High pH (9.0) with 50% glycerol buffer yielded a maximum capture density of 2.5 x 10^4 cfu/mm² (Figure 4).

3.2 Antibody Capture

- Immobilization of antibodies against E. coli O157:H7 were optimized using a microarray on different surfaces (Figure 5).
- Optimal conditions were found to be 200 µg/ml on Xenobind at pH 9.0 with a betaine/butanediol buffer.
- E. coli O157:H7 were captured in high density with reduced background compared to T4 phage (Figure 6).

4. Bacteria Detection by Silver Enhancement

- Following capture of E. coli from the sample, the cell surface is coated with detection antibodies and streptavidin nanogold.
- Silver enhancement reagents are incubated for 5 min; nanogold catalyzes the local reduction of silver on the cell surface.
- Silver amplified bacteria become easily visible as bright particles (Figure 7) that can be enumerated by particle counting algorithms.

5. Conclusions and Future Work

A novel assay for pathogenic bacteria detection has been developed using silver enhancement to detect whole cells.

- Bacteriophage and antibodies can be used as binders for capturing target bacteria strains based on their surface antigens.
- Detection can be achieved with low-cost imaging equipment (no fluorescence or high magnification necessary).
- Demonstrated here with E. coli K12 and O157:H7, this approach can be applied to other pathogens by choosing strain-specific binders.
- Future work will apply this approach to MRSA detection, and use an autonomous microfluidic circuit [4] to complete the assay at the point-of-care.

References