

Nanoparticle Gene Array: The Future of Bedside Diagnostics?

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Introduction

The emergence of drug resistant bacteria occurs frequently in the Intensive Care Unit (ICU). Both gram-negative and gram-positive bacteria are involved. This issue is problematic for critical care clinicians because there are now several pathogens that can only be effectively treated with a limited number of antimicrobial agents. Examples include methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecium*, and Gram-negative bacteria producing extended spectrum β -lactamases.

Multi-drug resistant bacterial infections are associated with increased mortality, length of hospital stay, and cost of care. For instance, in a study of patients from a large tertiary-care teaching hospital in Boston, Massachusetts, median length of stay and hospital charges were significantly greater for individuals with MRSA compared to methicillin-sensitive *S. aureus* (MSSA).

Successful treatment of patients admitted to the ICU with nosocomial and community-acquired infections depends on adequate initial antibiotic treatment. A commonly used strategy is to begin with broad spectrum antibiotic therapy, later de-escalating antibiotic administration based upon culture and sensitivity data. Initial broad-spectrum antibiotic therapy is necessary until culture data is available to guide focused antimicrobial administration. However, broad spectrum antibiotics are a leading cause of drug-resistant bacteria emergence.

Several strategies have been investigated as a means of reducing the emergence of multi-drug resistant bacteria in ICUs. One such strategy is to employ only narrow spectrum antibiotics directed at culprit pathogens identified using rapid bedside detection devices. This strategy requires technology capable of identifying pathogens within minutes of sample collection, technology capable of pathogen identification and analysis of antibiotic resistance patterns, and technology capable of identifying organisms that may reside in intracellular compartments. Our critical care research group has developed a nanoparticle gene array, which preliminary studies suggest is more sensitive than conventional gene array technology.

Hypothesis

We hypothesized that our critical care research group could:

1. Develop a nanoparticle gene array that would be sensitive enough to detect infectious pathogens at concentrations as low as 500 femtomolar.
2. Design nanoparticle array probe sets capable of distinguishing common ICU pathogens from other organisms.

Methods

Probe Selection

Ten published genomes from the 6 known *Pseudomonas* groups were analyzed for common nucleic acid sequences. Probes were identified using a selection algorithm finding unique midmers of the pattern $8n+9n+8n$. Probe candidates were compared to other bacterial and human genomes using BLAST software. Finally ClustalX Alignment Analysis software was utilized to elucidate the specific nucleic acid sequences.

Nanoparticle Gene Array

Oligonucleotide probes (IDT Technologies) covalently linked to the polyallylamine-coated chip surface were inoculated with a 10 picomolar solution of biotinylated target ssDNA. (Figure 2). PBS solution was then applied to the chip surface briefly to remove all free nucleotides. Our magnetic label (Miltenyi Biotech), functionalized with streptavidin molecules, was then allowed to incubate on the chip until the signal saturated. Finally the PBS solution containing the magnetic nanoparticles was removed from the chip by rinsing with deionized water and dried again with a vacuum pump.

Results

We have identified 7 nucleic acid sequences, all found within the same region of the genome, that we predict will be capable of identifying the specific strain of *Pseudomonas* organism present. All seven sequences (except *P. stutzeri*) are located in the intron following the gene PA5438 which encodes a transcriptional regulatory protein from the RpiR family. Within these sequences lies a homological midmer, 9 nucleotides long, that is conserved among all human pathogenic strains of *Pseudomonas*. Additionally, in our preliminary study, the data would suggest that when using a 10 picomolar sample of human papillomavirus, the device is capable of detecting pathogens at concentrations as low as 500 femtomolar.

Conclusions

Our critical care research group has developed a gene array that may obviate the need for amplification of genomic material by polymerase chain reaction prior to labeling and hybridization. This advancement could facilitate development of a bedside ICU device capable of identifying pathogens in sputum, blood, urine, and cerebrospinal fluid within minutes. Our initial findings suggest that enough data is available in the public domain to design nanoparticle array probe sets capable of identifying common ICU pathogens.

References

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