Development of a PCR Assay for Pathogens from the Middle East

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Abstract

Acinetobacter baumannii or "Iraqibacter" is a pathogen found to infect soft tissue. It was identified by American doctors in soldiers returning from Iraq and Afghanistan as it was blown into their shrapnel wounds. Acinetobacter baumannii is a major heath care problem because it forms biofilms on living tissue, medical devices, and surfaces. The purpose of this study is to test designed primers (*A. baumannii* CsuAB forward and reverse and other) purchased from IDT by using PCR high resolution melt and multiplex them with previous Bacillus primers developed in the lab and optimize the reaction. Preliminary analysis NCBI Blast show the primers are specific. By using agarose gel electrophoresis, we will determine if the amplicon is the correct size. The csuAB gene encodes the Csu pili organelle. We hypothesize that the CsuAB primers will amplify the Acinetobacter baumannii DNA and no other species of bacteria. The goal of this experiment is to develop and test the comparison of a wound sample to see if the infection is caused by Acinetobacter baumannii by comparing the melt curves.

Introduction

Acinetobacter baumannii or "Iraqibacter" is commonly found in soil, water, and hospitals. It is one of the most successful pathogens responsible for hospital-acquired infections in the modern healthcare system (Chang-Ro Lee, 2017). This successful pathogen has been identified in soldiers who served in Iraq and Afghanistan by American doctors. A. baumanni is reported to cause infections of the skin, bloodstream, urinary tract, and other soft tissues. According to the CDC, it can also “colonize” or live in a patient without causing infections or symptoms, especially in respiratory secretions (sputum) or open wounds (Prevention, 2019).

Acinetobacter baumannii is constantly figuring out ways to avoid the affects of antibiotics when trying to be treated, making them antibiotic and multidrug-resistant. Acinetobacter can live for long periods of time on environmental surfaces and people who are at high risk typically include patients in hospitals. According to the CDC’s Antibiotic Resistance Threats in the United States, 2019, in 2017, Acinetobacter caused an estimated 8,500 infections in hospitalized patients and 700 estimated deaths in the United States (Prevention C. f., 2012). Researchers in Beijing, China who conducted a study for A. baumannii, developed a loop-mediated isothermal amplification (LAMP) assay for the rapid detection of A. baumannii in clinical samples by using high-specificity primers of the blaOXA-51 gene (Puyuan Li, 2015). The results showed that the LAMP assay could detect target DNA within 60 min at 65°C. The detection limit was 50 pg/µl, which was about 10-fold greater than that of PCR (Puyuan Li, 2015). The goal of this study is to make a more sensitive and faster assay.

Methods

- Specific pathogenic organism DNA were obtained from ATCC. Both forward and reverse primers were designed using the NCBI Blastn and evaluated using the IDT OligoAnalyzer 3.1.
- Primers were optimized purchased from IDT.
- Each similar aneling temperatures closest to 60 and 4 or less complimented base pairs.
- Primers annealing melt temperature was estimated in OligoCalc. The PCR was conducted on the Rotor-Gene Q using the LightScanner master mix.
- Primers were quantitated using the NanoDrop then diluted to 5 micromolar.
- The DNA was also quantitated to 1 nanogram.
- A 3% agarose gel electrophoresis was conducted using the Ultra Low Range DNA Ladder
- A multiplex PCR was run with multiple primer sets and species DNA.

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Results

Target Organism	Primer Sequence (5'-3')	Target gene	Amplicon length (bp)	Melt (LightScanner, °C)
B. cereus	F-GAAAAGTACAGTGGCAAAAGTTGTTGCG R-CGCTAACTCTTGCTGACGACGT	*cmk*	103	83.90±0.15
A. baumannii	F-TGGTGAACGTACAGACCGCACT R-GGTGTACCTGTGTTTGGAGCAA	*csuA/B*	186	85.37±0.16
A. baumannii	F-CTGTGCAGCATTGTATGGTATCACTTC R-GTTAGCATTCCTCGGGTCCC	*abaR*	163	85.61
A. baumannii	F- CAGTCCCAGTCTATCAGGAACTTGCG R - TATCAACCTGCTGTCCAATTTCAG	*oxa*	108	81.05
A. baumannii	F- AACTGGAGCAACTTCTACAGGAGCA R- GGTAACGCTGGTGTTGGTGCTTTC	*ompA*	180	86
P. aeruginosa	F- AGA AGA TGG CGA GCG ACC TT R - CTCGTAGTCCTGGCTGTCCTTAG	*lasR*	74	
B. thuringiensis	F- ATGGATAACAATCCGAACATCAATGAATG R- TCCAGCACCGGGAACAAATTC	cry	165	77.27±0.03

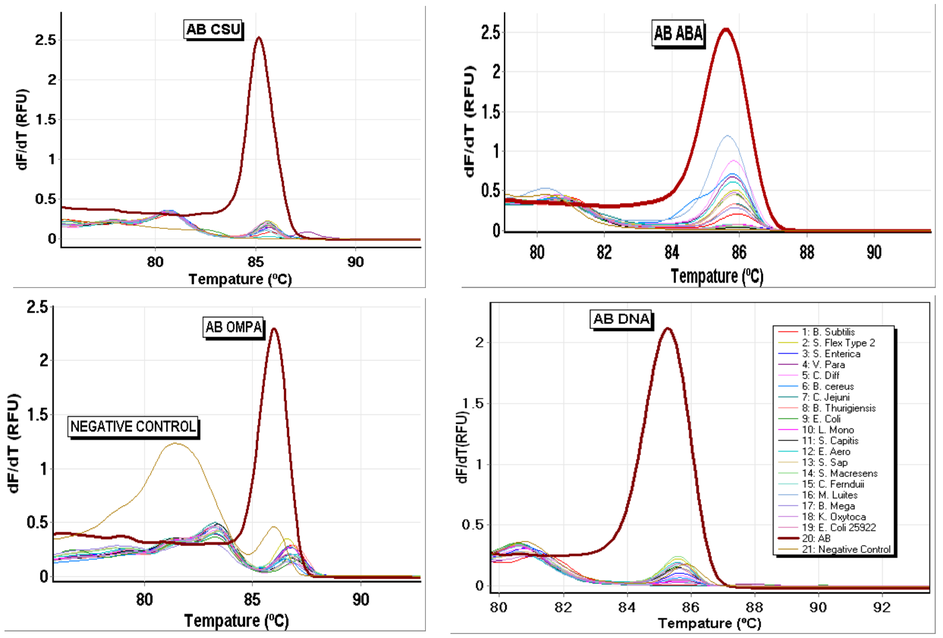


Fig. 1. Specificity of *A. baumannii* primers tested against the other bacterial DNA.

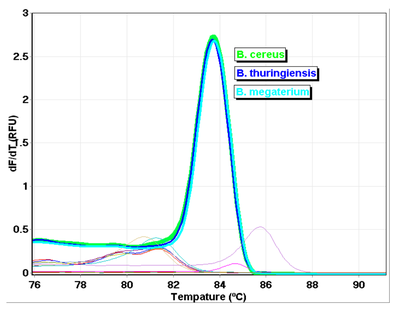


Fig. 2. Specificity of *B. cereus, B. thuringiensis,* and *B. megaterium* all peak at about the same RFU.

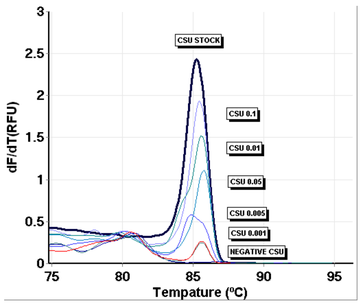


Fig. 3. The sensitivity *A. Baumannii* CSU dilutions.

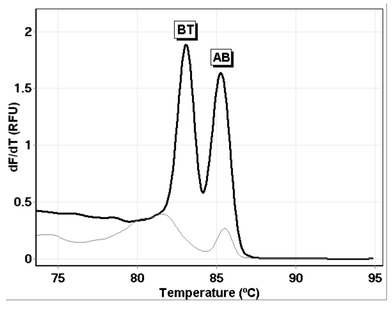


Fig. 4. Multiplex of *B. thuringiensis* and *A. baumannii*

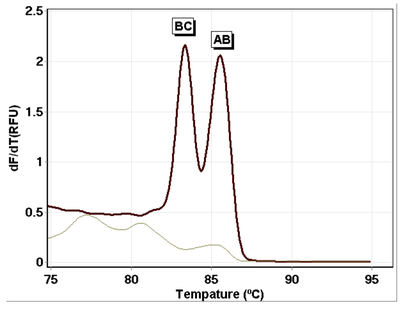


Fig. 5. Multiplex of *B. cereus* and *A. Baumannii*

Gel Electrophoresis

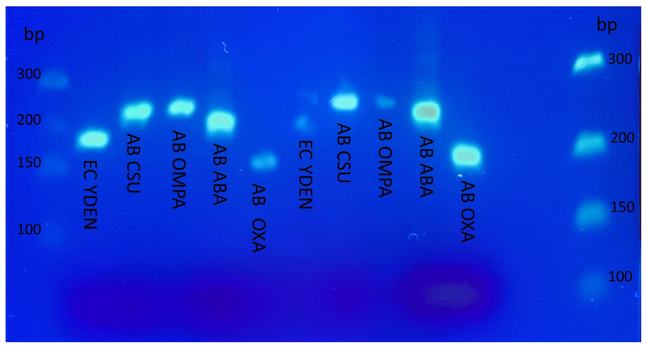
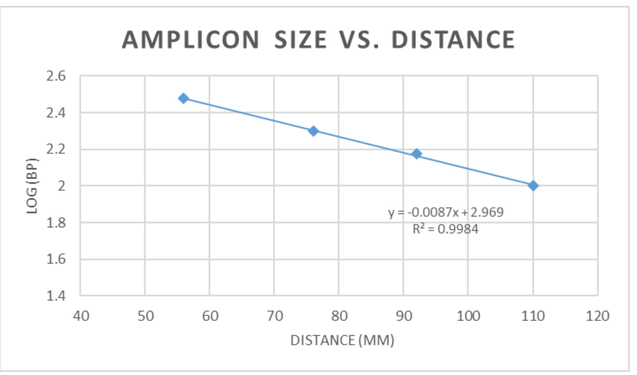


Fig. 6. Agarose gel electrophoresis to estimate amplicon size.



Conclusions

- All four of the *A. baumannii* primer sets amplified the target.
- *A. baumannii* CSU and *A. baumannii* OMPA were the most specific out of all the various primers.
- Multiplex assays were developed for *A. baumannii* CSU and *B. thuringiensis.*
- Multiplex assays were developed for *A. baumannii* CSU and *B. cereus.*
- The assays were sensitive and reproducible.

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Acknowledgements

The authors acknowledge the NIH Bridges to Baccalaureate Program for their funding and thank Dr. Michelle Snyder, Dr. Cindy Zeller for their support.