# Development of a PCR Assay for the Soil Pathogen *Acinetobacter baumannii*

**Larissa Speaks[1], Shannon Carr[1], and Kelly M. Elkins[1]**

Forensic Science Program, Chemistry Department, Towson University, 8000 York Road,

Towson, MD 21252-0001, USA;

* Correspondence: kmelkins@towson.edu; Tel.: +1-410-704-6217

**Abstract:** *Acinetobacter baumannii*, *Bacillus cereus*, and *Escherichia coli* are three well-documented human pathogens found in soil. They can be transmitted by contaminated food, water, or infiltration of open wounds. Herein we describe the design and testing of a new PCR assays to detect and identify these serious threat pathogens. PCR primers were designed to be specific to each bacterium. Primers for *A. baumannii* were designed and tested targeting the *csu*A/B, *omp*A, *aba*R, and *oxa* genes. Multiplexes with previously published primers for *E. coli* and *B. cereus* targeted the *yed*N and *cmk* genes, respectively. The target DNA for each species was obtained from ATCC and the primers were purchased from IDT. The primers were tested using real-time PCR paired with the LightScanner master mix and detected using the LC Green Plus fluorescent intercalating dye. The *csu*A/B and *omp*A primers were specific for *A. baumannii* and the sensitivity of each primer set was tested. The *csu*A/B primers proved to have higher specificity and strong amplification; all further testing for *A. baumannii* was conducted using this primer set. Gel electrophoresis was used to confirm the sizes of the target amplicons. The PCR high-resolution melt assays were specific, sensitive, reproducible, and rapid in experiments. The unique melting temperatures for the PCR assays are: 82.65 ± 0.83 °C for *E. coli*, 84.31 ± 0.24°C for *B. cereus,* and 85.70 ± 0.44°C for *A. baumannii* using the *csu*A/B primers. A duplex PCR assay is demonstrated for *B. cereus* and *A. baumannii* and a triplex assay is reported for *E. coli, B. cereus, and A. baumannii*.

**Keywords:** soil; pathogen; *Acinetobacter baumannii*; *Bacillus cereus*; *Escherichia coli*; real-time PCR melt assay; rapid screening

## 1. Introduction

The soil beneath our feet has a greater impact on human health than some may realize. A teaspoon of soil can contain between 100 million and 1 billion bacteria which are invisible to the human eye [1]. Bacteria can survive in varying oxygen concentrations, temperature environments, pH levels and moisture contents. The bacteria in soils can have positive influences on the environment including breaking down decaying matter, recycling nutrients,

promoting plant diversity, purifying water, and capturing water run-off [1, 2]. Soil bacteria have also been found to be a source of medicines, especially antibiotics [2]. However, soil bacteria also provide negative influences if land management practices are poor; the accumulation of pathogenic soil bacteria can cause illness or disease to plants, animals, and humans [2]. There are three common ways humans can be exposed to soil bacteria: ingestion, spore inhalation, and penetration into wounds [2].

This study focused on the rapid detection and identification of an urgent and serious threat multi-antibiotic-resistant soil pathogen, as listed by the United States' (US) Centers for Disease Control and Prevention (CDC), *Acinetobacter baumannii* (*A. baumannii*) with creating a multiplex identification panel with one or more of the following: *Bacillus cereus* (*B. cereus*) or *Escherichia coli* (*E. coli*).

*A. baumannii* or "Iraqibacter" is new to the US CDC "urgent threats" and among the Antibiotic Resistance Threats in the United States (2019) [3]. This species is a gram-negative, aerobic bacterium which is commonly found in soil, water, manure, and hospitals [4, 5]. In 2017, it was estimated to cause 700 deaths and 8500 infection cases in hospitalized, and especially immunocompromised, patients [3]. *A. baumannii* can exist for long periods of time on environmental surfaces and equipment including catheters and drainage tubes [4, 6] which increases the risk of transmission. *A. baumanni* causes symptomatic and asymptomatic infections of the skin, bloodstream, urinary tract, lungs, and other soft tissues [7]. It has caused infections in U.S. soldiers who have been hit by improvised explosive device (IED) bomb shrapnel while serving in the Middle East, including in Iraq and Afghanistan which has earned it the moniker "Iraqibacter" [5]. The pathogen also causes foodborne illness and can be transmitted through raw fruits and vegetables and contaminated dairy products [8]. Many strains of *A. baumannii* are antibiotic and multidrug-resistant [5, 9, 10]. Additionally, the bacterium is difficult to isolate and culture, which lengthens the identification time [8]. *A. baumannii* is difficult to de-stain, leading to the bacteria being misidentified as Gram-positive, and the metabolic tests currently in use struggle to differentiate *Acinetobacter* species from other non-fermenting Gram-negative bacteria [5]. Additionally, *A. baumannii* has acquired a wide array of antibiotic resistances, which means effective treatment requires performing antimicrobial susceptibility testing [5]. Rather than letting the patient go unmedicated while antimicrobial susceptibility testing is underway carbapenems are often prescribed which, while effective for now, is giving *A. baumannii* opportunity to develop drug resistance to that line of antibiotics as well [5]. A more rapid identification of *A. baumannii* would leave more time for proper susceptibility testing which, in turn, should result in more effective and targeted treatment and better outcomes for the patients with lower risk of the bacterium gaining widespread carbapenem resistance.

*E. coli* is a Gram-negative, facultatively anaerobic bacteria widely found in the environment, in the intestines of healthy humans, and in the GI tract of some animals [11]. While most types of *E. coli* are harmless in the intestines

and aid in a healthy digestive track, some strains may cause unwanted symptoms if ingested. Ingestion often occurs through contaminated foods or handling food with unwashed hands. There are six pathogenic *E. coli* strains that are associated with diarrhea; these are referred to as diarrheagenic *E. coli* [11, 12]. The six phenotypes are Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) [11, 12]. The STEC form can cause severe diarrhea and foodborne disease as it is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables. While widely associated with food poisoning, pneumonia and urinary tract infections can also be caused by *E. coli* bacteria [11]. In fact, around 80% of urinary tract infections are caused by *E. coli* [13]. The CDC reports STEC to be the 4$^{th}$ most common cause of foodborne outbreaks, resulting in around 17% of the total hospitalizations resulting from foodborne bacteria in 2017 [14].

*Bacillus cereus* is classified in the same genus as anthrax (*B. anthracis*) and is found in soil, vegetation, and contaminated cooked and uncooked food products [15]. It is a facultatively anaerobic, Gram-positive rod-shaped bacterium that produces emetic toxins. Like *B. anthracis*, *B. cereus* is spore-forming which allows it to survive longer in extreme temperatures, and when at room temperature this bacterium can quickly multiply. Ingestion of *B. cereus* can cause nausea, vomiting, and diarrhea [15]. Although there are confirmed outbreaks of *B. cereus* in China, Belgium, and the US, deaths due to this gastrointestinal illness are rare [14, 15]. The CDC reported that *B. cereus* was the 7$^{th}$ most common cause of foodborne outbreaks in 2017, accounting for around 4% of bacterial foodborne outbreaks and 6% of the illnesses relating to bacterial pathogens [14].

The direct method of bacterial identification is to culture and microscopically evaluate the shape, color, growth, and staining on a medium. While this method is reliable and inexpensive, it may take more than a week to identify the pathogen [16]. Some bacteria grow slowly in culture, and it may take several tries on different media to coax the organism to grow sufficiently for examination. Waiting a week or more is not ideal when an infection or outbreak is ravaging cruise ships, hospitals, or communities. Thus, much effort is ongoing to develop additional and more sensitive and rapid detection and identification methods. Certain enzyme-linked immunosorbent (ELISA) antibody assays are highly sensitive but often require 1-2 days to perform with intensive analyst time and rely on well selected antibodies. Several groups have focused on developing isothermal and polymerase chain reaction (PCR) methods [12, 16-23]. Real-time or post-PCR identification can be performed using fluorescence detection or gel electrophoresis, respectively. PCR assays have been developed for *E. coli* and *B. cereus* among other bacterial pathogens [19, 22, 23]. Post-PCR amplicon melt analysis can enable fast *in situ* identification without gel electrophoresis analysis. Targeted and deep sequencing using Sanger and next generation

sequencing (NGS) methods have also been developed, but samples often need to be cultured and amplified prior to sequencing. Furthermore, NGS methods are relatively expensive, standardized analysis procedures are still under development, and samples can be identified only if sequences are reported in databases. Real-time PCR methods have been widely used for testing in the COVID-19 pandemic because they are rapid, sensitive, inexpensive, and high-throughput and many labs had existing thermocyclers on site. Some procedures recommend DNA extraction prior to PCR while others employ direct PCR in which no prior DNA extraction is necessary. PCR assays are extremely sensitive with detection limits of a picogram or even femtograms.

Researchers in Beijing, China recently developed a loop-mediated isothermal amplification (LAMP) assay for the rapid detection of *A. baumannii* in clinical samples by using high-specificity primers targeting the blaOXA-51 gene [6]. The results showed that the LAMP assay detected the target DNA within 60 minutes at 65°C. The detection limit was 50 pg/µl, which was about 10-fold greater than that of existing PCR methods for *A. baumannii* [6].

The goal of this study was to develop and test new more sensitive and faster real-time PCR assays with *in situ* detection using post-PCR high resolution melt to identify and differentiate the *A. baumannii* urgent and serious threat to human health and create multiplex assays for the pathogen for simultaneous detection and identification with other soil pathogens.

## 2. Materials and Methods

### *2.1. Bacterial Strains*

Fifteen bacterial strains from numerous species were obtained from American Type Culture Collection. These standards included *Acinetobacter baumannii*, *Bacillus cereus*, str. NRRL B-568, and *Escherichia coli*, str. MG1655. These were obtained to test the developed assays and perform specificity testing (Table 1). Additional bacterial strains were obtained for specificity testing from Carolina Biological, Ward's Natural Science, Midwest Culture Service, and the TU Biology Department (Table 2). The material was received as extracted and lyophilized DNA which was reconstituted in nuclease-free water. The DNA was diluted to 1 ng/µL and serially to 0.001 ng/µL or 1 pg/µL for PCR experiments.

**Table 1.** Standards obtained from ATCC for assay development and specificity tests.

| Bacterial Strain | Source |
|---|---|
| *Acinetobacter baumanii* | ATCC |
| *Bacillus cereus*, str. NRRL B-568 | ATCC (10876D-5) |
| *Bacillus subtilis*, str. 168 | ATCC (23857D-5) |
| *Bacillus thuringiensis*, str. USDA H522 | ATCC (35646D-5) |
| *Campylobacter jejuni*, subsp. jejuni | ATCC (33560D-5) |
| *Clostridium difficile*, str. 90556-M6S | ATCC (9689D-5) |
| *Escherichia coli*, str. MG1655 | ATCC (700926D-5) |
| *Escherichia coli,* str. FDA strain Seattle 1946 | ATCC (25922) |
| *Klebsiella oxytoca*, str. MsA1 | ATCC |
| *Listeria monocytogenes*, str. EGDe | ATCC (BAA-679D-5) |
| *Pseudomonas aeruginosa,* str. *PAO1-LAC* | ATCC (47085D-5) |
| *Salmonella enterica*, subsp. enterica | ATCC (700720) |
| *Shigella flexneri* type 2, str. 24570 | ATCC (29903D-5) |
| *Staphylococcus capitis* subsp. *capitis* Kloos and Schleifer | ATCC (35661) |
| *Vibrio parahaemolyticus*, str. EB101 | ATCC (17802D-5) |

**Table 2.** Additional standards obtained for additional specificity tests.

| Bacterial Strain | Source |
|---|---|
| *Bacillus megaterium* | TU Biology |
| *Citrobacter freundii* | Carolina Biological |
| *Enterobacter aerogenes* | TU Biology |
| *Micrococcus luteus* | Midwest Culture Service |
| *Serratia marcescens* | Carolina Biological |
| *Staphylococcus saprophyticus* | Ward's Natural Science |

*2.2. PCR Primers*

Oligonucleotide primer locations were selected based on previously determined and published gene sequences available in the National Center for Biotechnology Information (NCBI) database. The genes selected as targets in *A. baumannii* are: *csu*A/B, *aba*R, *oxa*, and *omp*A [24-27] (Table 3). Published PCR primers for *B. cereus* [22] and *E. coli* [19] targeting the *yed*N and *cmk* genes were used in designing multiplex assays (Table 4). The forward and reverse primers were designed using the Integrated DNA Technologies (IDT) OligoAnalyzer Tool v.3.1 and checked for specificity in NCBI Basic and Local Alignment Search Tool nucleotide (BLASTn) using the site's default settings. Each was designed to yield different sized amplicons. The primers were purchased from IDT. Upon receipt, the primers were quantitated using a NanoDrop 2000 (Thermo Fisher) and diluted to 5 μM stocks.

**Table 3.** Gene target and PCR primers designed and tested for *A. baumannii* (AB) assay.

| Gene Target | Forward Primer (5′ to 3′) | Reverse Primer (5′ to 3′) | Amplicon Size (bp) |
|---|---|---|---|
| *aba*R | CTGTGCAGCATTGTATGGTATCACTTC | GTTAGCATTCCTCGGGTCCC | 163 |
| *csu*A/B | TGGTGAACGTACAGACCGCACT | GGTGTACCTGTGTTTGGAGCAA | 186 |
| *omp*A | AACTGGAGCAACTTCTACAGGAGCA | GGTAACGCTGGTGTTGGTGCTTTC | 180 |
| *oxa* | CAGTCCCAGTCTATCAGGAACTTGCG | TATCAACCTGCTGTCCAATTTCAG | 108 |

**Table 4.** Gene target and PCR primers designed and tested for *B. cereus* (BC) and *E. coli* (EC) in multiplex assays [20].

| Specie | Gene Target | Forward Primer (5′ to 3′) | Reverse Primer (5′ to 3′) | Amplicon Size (bp) |
|---|---|---|---|---|
| BC | *cmk* | GAAAAGTACAGTGGCAAAAGTTGTTGCG | CGCTAACTCTTGCTGACGACGT | 103 |
| EC | *yed*N | TCCTGGATTGAGGTGCTTTATC | CTACGGAGACCTGGGTAATTCC | 142 |

*2.3. PCR Reaction Conditions and HRM Analysis*

PCR was conducted using a Rotor-Gene Q real-time PCR instrument (Qiagen) with a pre-amplification touchdown and the detection set at HRM with the following cycling parameters: an initial 10 minute hold at 95°C, 40 cycles of 15 second denaturation at 95°C, 15 seconds for primer annealing at 60°C, and 15 seconds of extension at 72°C, a five minute hold at 72°C, and a final 1 minute hold at 45°C before beginning the melt. The melt analysis was performed using the settings of 65 to 95°C, increasing by 0.3°C in 3 second intervals.

An assay consisted of 8 μL of 2.5X LightScanner master mix (BioFire Defense), 1 μL forward primer (5 μM), 1 μL reverse primer (5 μM), 9 μL of nuclease-free sterile water, and 1 μL of 1 ng/μL target DNA in a 20 μL PCR tube. Each assay was tested in triplicate; a no template control was tested concurrently substituting water for template DNA.

For specificity testing, the DNA target included each of the species in Tables 1 and 2 in separate reactions as described above.

For sensitivity testing, the standard DNA was serially diluted to 0.001 ng/μL and PCR reactions were prepared with 1 μL of each of the DNA dilutions as described above.

A duplex assay consisting of the *A. baumannii csu*A/B and *B. cereus* primer sets was created. The primer concentrations were varied until even amplification was observed. The final primer ratio was 0.5 μL of each of the

*A. baumannii csu*A/B primers and 1 μL of each of the *B. cereus* primers. The water was reduced appropriately to continue to have a final volume of 20 μL.

For the triplex assay, the primer concentrations were varied until even amplification was observed. The final primer ratio was 0.75 μL of each of the *A. baumannii csu*A/B primers, 1.5 μL of each of the *E. coli* primers, and 0.5 μL of each of the *B. cereus* primers.

*2.4. Sizing using Agarose Gel Electrophoresis*

A 3% agarose gel was prepared and electrophoresis was conducted with 1x Tris-acetate-EDTA (TAE) using a mini gel apparatus with the Ultra Low Range DNA Ladder. The bands were carefully measured, and the log of the base pair amplicon length was plotted against the distance travelled. A best fit line and line equation was used to compute the size of the amplicons.

**3. Results**

The amplification and melt temperatures were tested for four *A. baumanii* primer sets targeting four different genes. The melt temperatures were similar for the four targeted genes: 85.70 ± 0.44°C (n=12), 86.24 ± 0.20°C (n=4), 85.61°C (n=1), and 81.50°C (n=1) for *csu*A/B, *omp*A, *aba*R, and *oxa*, respectively. The specificity of the *A. baumannii* primers was tested against DNA from the bacterial species listed in Tables 1 and 2. In specificity testing, only the *A. baumannii* DNA target amplified with the *csu*A/B and *omp*A primer sets (Figures 1a and 1b). Several other species also amplified with the *oxa* and *aba*R primer sets (Figures 1c and 1d). The primers for *A. baumannii csu*A/B were chosen for additional development due to their higher specificity and strong fluorescence detection signal. The amplicons were estimated using 3% agarose gel electrophoresis and the expected amplicon sizes were confirmed (data not shown).

A dilution series was prepared from each of the *A. baumannii* standard DNA stocks from 1 ng to 0.001 ng. The *A. baumannii csu*A/B primer set was evaluated for the sensitivity to low input DNA; the *A. baumannii csu*A/B primers produced reliable data down to 0.01 ng of DNA (Figure 2).

The average observed melt temperature for the *B. cereus* and *E. coli* primer set was 84.31 ± 0.24°C (n=3) and 82.63± 0.83°C (n=2), respectively, using the PCR setting described in the methods. The *E. coli* and *B. cereus* assays were found to be specific in prior studies [19, 22]. The observed melt temperatures are consistent with the previously reported temperature of 82.15 ± 0.37°C for *E. coli* and 84.43 ± 0.50°C for *B. cereus* collected using slightly different PCR parameters [17, 20]. Non-template controls were tested with each primer set and none produced amplicons with melt temperatures similar to that of the desired targets. Duplex tests were performed using the *A. baumannii csu*A/B and *B. cereus* primer sets (Figure 3). A triplex assay was tested for *E. coli*, *B. cereus*, and *A. baumannii* using the *csu*A/B primers (Figure 3). In each of the multiplexes the bacteria amplified and melted (labeled on graphs) at the expected temperature. The species are differentiable visually or using the peak picking tool in the Rotor-gene software.

(**a**) (**b**)

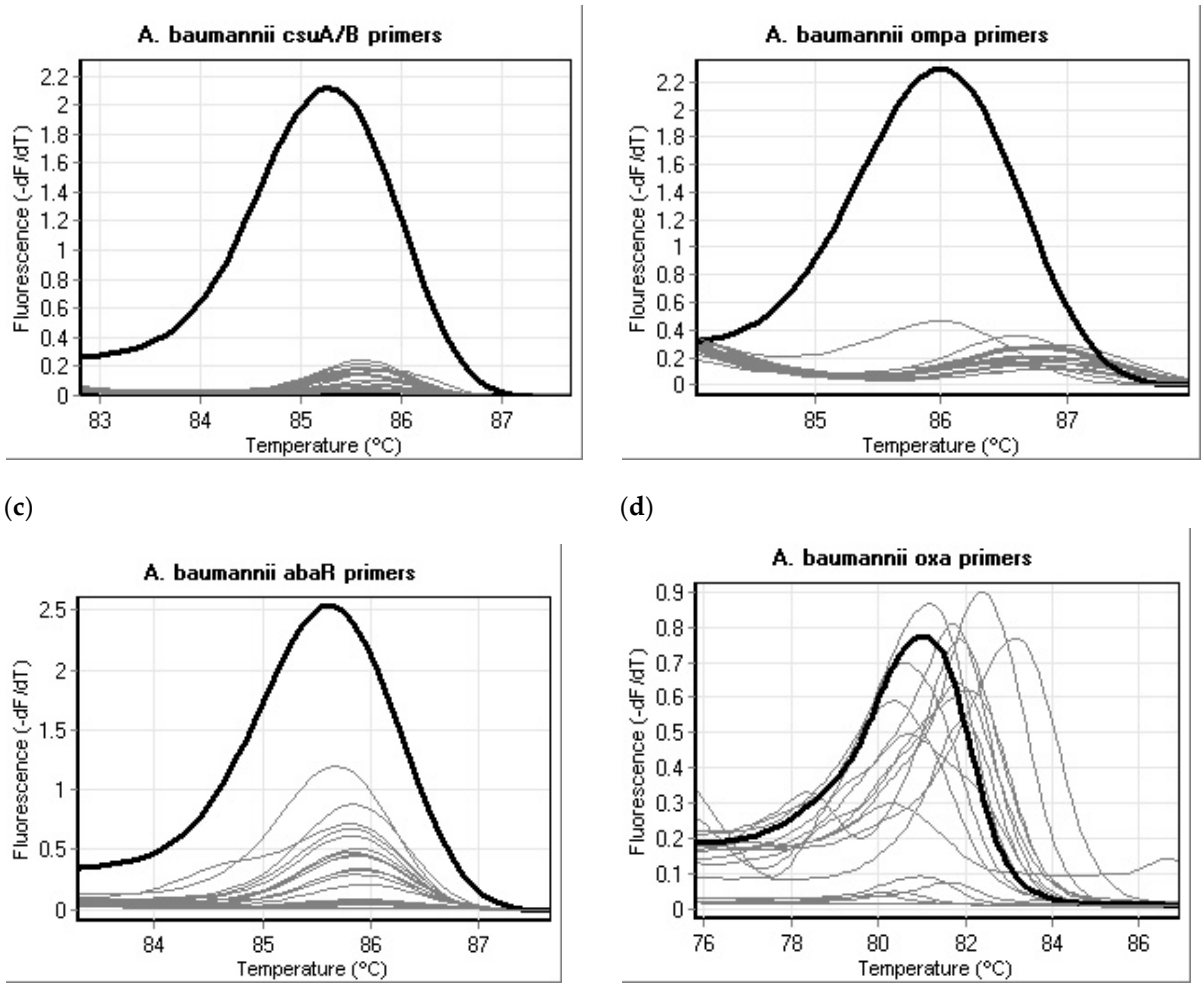


**Figure 1.** Specificity tests of *A. baumannii* (a) *csu*A/B, (b) *omp*A, (c) *aba*R, and (d) *oxa* primers (dark black line) tested against the other bacterial DNA . The non-*A. baumannii* DNA are shown in light grey. Only the *csu*A/B and *omp*A primers were specific to *A. baumannii*.

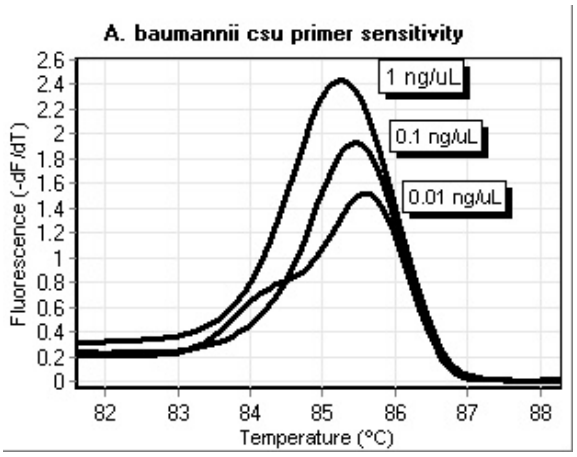


**Figure 2.** Sensitivity of *A. baumannii csu*A/B primers tested against a dilution of the *A. baumannii* DNA standard down to 0.001 ng of DNA. The assay remained reliable to 0.01 ng/uL of target DNA.

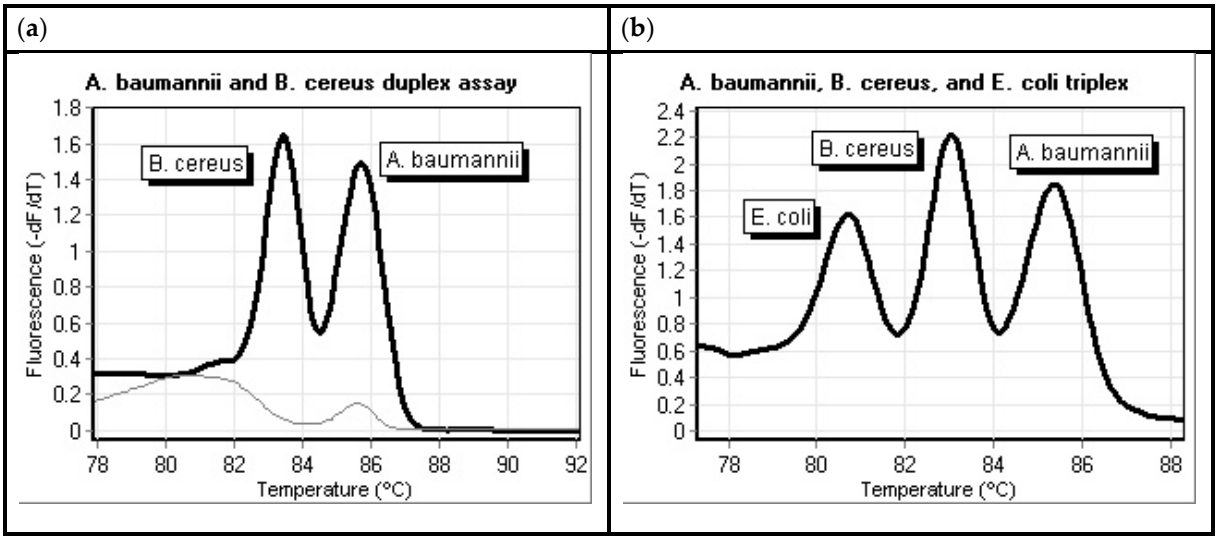


**Figure 3.** Duplex assay (a) detecting *B. cereus* (BC) and *A. baumannii* (AB) DNA as labeled. The no template control trace is shown in gray. Triplex assay (b) detecting *B. cereus* (BC), *E. coli* (EC), and *A. baumannii* (AB) DNA as labeled. The *A. baumannii* assay employed the designed *csu*A/B primers.

## 4. Discussion

We designed PCR primers for a new *A. baumannii* singleplex, duplex and triplex assay using genes unique to each species as reported in the literature. We initially prepared and tested four sets of PCR primers to detect and identify *A. baumannii*. The PCR primers for *A. baumannii* targeted the *csu*A/B gene encoding the csuA/B subunit of the Csu pili, a binding protein which plays a key role in biofilm formation [24], the *omp*A gene encoding an outer membrane protein which plays a role in biofilm formation [25], the *aba*R gene encoding an AI synthase receptor, which plays a role in antimicrobial

resistance [26], and the *oxa* gene encoding a beta-lactamase enzyme, which exhibit carbapenem-hydrolysis activity to convey increased antimicrobial resistance [27]. After obtaining the DNA sequence for the gene from the public NCBI GenBank repository, we designed PCR primer sets to have an annealing temperature near 60 °C, no more than four homo- or hetero-dimer hydrogen bonding regions, and no hairpin formation approaching the annealing temperature when tested using the IDT OligoAnalyzer Tool. The BLASTn tool with the default settings was used to evaluate if the primers were specific for the desired target bacteria. The final primer sets were ordered from IDT.

The average melt temperatures for the *A. baumannii* primer sets were similar: 85.70 °C, 86.24 °C, 85.61 °C, and 81.50 °C for *csu*A/B, *omp*A, *aba*R, and *oxa*, respectively. In evaluating the four primer sets, we aimed to identify one or more primer sets that were specific for the target organism, capable of detecting low quantities of DNA, and that could be multiplexed with the published *E. coli* and *B. cereus* assays to simultaneously detect and identify the three soil pathogens. The *oxa* primer set produced a shorter amplicon with a lower melting temperature. The melt temperature is generally higher with a longer or more CG-rich amplicon [19]. The *csu*A/B, *omp*A, and *aba*R primer set melt temperatures were higher than the other target species and would be suitable for multiplexing. However, specificity testing determined that the *aba*R and *oxa* primer sets were not specific to *A. baumannii* while the *A. baumannii csu*A/B and *omp*A primer sets were specific. The *csu*A/B primers were selected for further testing based on the lower rate of non-specific amplification and strong fluorescence signal.

The *A. baumannii csu*A/B primer set was shown to be sensitive, detecting and identifying 0.01 ng/µL of input DNA. Multiplex testing using the *A. baumannii* and *B. cereus* demonstrated that the two primer sets can simultaneously detect and identify both species in a single assay. A triplex assay was tested for *E. coli*, *B. cereus*, and *A. baumannii* using the *csu*A/B primers and was also successful. It is an advantage that one assay can be used to simultaneously detect and identify multiple species all found in soil that can make people ill by eye or computationally within the software.

PCR methods are used for routine clinical testing including COVID-19 tests. The tests are rapid and specific. Previously reported PCR detection methods for *A. baumannii* were less sensitive than a published LAMP assay with sensitivity to 50 pg of the target [6]. The sensitivity of *A. baumannii csu*A/B assay is 10 pg of DNA or 5x more sensitive than the recently published LAMP assay [6].

## 5. Conclusions

Herein we have described the development and testing of new PCR assays for detecting and identifying soil pathogens. All four of the *A. baumannii* primer sets amplified the target DNA. The *A. baumannii csu*A/B and *omp*A primers were found to be specific to the target organism, with the csuA/B primers exhibiting a lower amount of non-specific amplification. The *A. baumannii* PCR assay was 5x more sensitive than a recently published assay. Furthermore, the PCR assays were easy to set-up and rapid to conduct.

Testing of primers to detect and identify *E. coli* and *B. cereus* also identified specific gene targets and led to amplified products with discrete melting temperatures. Following specificity, sensitivity, and reproducibility testing by new testers, the primers were multiplexed. The multiplex assays that were developed for *E. coli, B. cereus,* and *A. baumannii* can be used to simultaneously detect the species alone or in duplex or triplex. Future work will involve the testing of soil from various locations to assess their pathogen composition using the developed assays. Many bacteria are present in low concentrations of soil and only detectable with prior culture and deep sequencing, but we are interested in what is detected without prior culturing for rapid assay detection.

**Author Contributions:** All authors have read and agreed to the published version of the manuscript. Conceptualization, K.M.E.; methodology, K.M.E.; validation, L.S.; formal analysis, L.S., S.M.C. and K.M.E.; investigation, L.S. and K.M.E.; resources, K.M.E.; data curation, L.S. and K.M.E.; writing, L.S., S.M.C., and K.M.E..; editing and approval of the final version, L.S., S.M.C., and K.M.E..; visualization, S.M.C.; supervision, K.M.E.; project administration, K.M.E.; funding acquisition, K.M.E.

**Funding:** This research was funded by a National Institutes of Health Bridges to the Baccalaureate Grant (Award #2R25GM058264-15) to M. Snyder, E.S. Ehrlich, J.E. Weldon, B.J. Margulies, P.A. Tsuji, K.M. Elkins, R. Dickie, J.M. Doyle, and D.J. Hearn and a FCSM Undergraduate Research Grant to L. Speaks.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors thank the Towson University Chemistry Department and Biology Department for support, Ilya Grabylnikov for suggesting the *omp*A gene, Noelle Neff for preliminary primer designs, and Jen Streb for ordering supplies.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Wagg C, Bender SF, Widmer F, Van Der Heijden MG. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci USA. 2014;111(14): 5266-5270.
2. Steffan JJ, Brevik EC, Burgess LC, Cerdà A. The effect of soil on human health: an overview. Eur J Soil Sci. **2018;**69: 159-171. https://doi.org/10.1111/ejss.12451.
3. CDC. Antibiotic resistance threats in the United States, 2019. Centers for Disease Control and Prevention. 2019. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf
4. Visca P, Seifert H, Towner KJ. Acinetobacter infection–an emerging threat to human health. IUBMB life. 2011;63(12): 1048-1054.
5. Howard A, O'Donoghue M, Feeney A, Sleator RD. Acinetobacter baumannii. Virulence. 2012;3(3): 243–50. https://doi.org/10.4161/viru.19700
6. Li P, Niu W, Li H, Lei H, Liu W, Zhao X, Guo L, Zou D, Yuan X, Liu H, Yuan J, Bai C. Rapid detection of Acinetobacter baumannii and molecular epidemiology of carbapenem-resistant A. baumannii in two comprehensive hospitals of Beijing, China. Front Microbiol. 2015;6: 997. https://doi.org/10.3389/fmicb.2015.00997.

7. CDC. Acinetobacter in healthcare settings. Centers for Disease Control and Prevention. 2019. https://www.cdc.gov/hai/organisms/acinetobacter.html
8. de Amorim, AMB, dos Santos Nascimento J. Acinetobacter: an underrated foodborne pathogen?. J Infect Dev Ctries. 2017;11(02): 111-114.
9. Fernando DM, Khan IU, Patidar R, Lapen DR, Talbot G, Topp E, Kumar A. Isolation and Characterization of Acinetobacter baumannii Recovered from Campylobacter Selective Medium. Front Microbiol. 2016;7:1871. https://doi.org/10.3389/fmicb.2016.01871.
10. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, Cha CJ, Jeong BC, Lee SH. Biology of Acinetobacter baumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. Front Cell Infect Microbiol. 2017;13;7:55. https://doi.org/10.3389/fcimb.2017.00055.
11. CDC. E. coli (Escherichia coli). Centers for Disease Control and Prevention. 2024. https://www.cdc.gov/ecoli/index.html
12. Toma C, Lu Y, Higa N, Nakasone N, Chinen I, Baschkier A, Rivas M, et al. Multiplex PCR assay for identification of human diarrheagenic Escherichia coli. J. Clin. Microbiol. 2003;41(6): 2669-2671.
13. Lee DS, Lee SJ, Choe HS. Community-Acquired Urinary Tract Infection by Escherichia coli in the Era of Antibiotic Resistance. BioMed Res Int. 2018; 7656752. https://doi.org/10.1155/2018/7656752
14. CDC. Surveillance for foodborne disease outbreaks, United States, 2017. Centers for Disease Control and Prevention. 2019. https://www.cdc.gov/fdoss/pdf/2017_FoodBorneOutbreaks_508.pdf
15. Schneider KR, Schneider RG, Silverberg R. Preventing Foodborne Illness: Bacillus cereus: FSHN15-06/FS269, 8/2015. EDIS. 2015;(7): 5-5.
16. Law JWF, Mutalib NSA, Chan KG, Lee LH. Rapid methods for detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. Front. Microbiol. 2015;5: 770.
17. Priyanka B, Patil RK, Dwarakanath S. A review on detection methods used for foodborne pathogens. Indian J Med Res. 2016;144: 327–338. https://doi.org/10.4103/0971-5916.198677.
18. Druml B, Cichna-Markl M. High resolution melting (HRM) analysis of DNA—Its role and potential in food analysis. Food Chem. 2014;158: 245–254. https://doi.org/10.1016/j.foodchem.2014.02.111.
19. Elkins KM, Perez ACU, Sweetin KC. Rapid and inexpensive species differentiation using a multiplex real-time polymerase chain reaction high-resolution melt assay. Anal. Biochem. 2016;500: 15–17. https://doi.org/10.1016/j.ab.2016.01.013.
20. Tamburro M, Ripabelli G. High Resolution Melting as a rapid, reliable, accurate and cost-effective emerging tool for genotyping pathogenic bacteria and enhancing molecular epidemiological surveillance: A comprehensive review of the literature. Ann. Ig. 2017;29: 293–316. https://doi.org/10.7416/ai.2017.2153.
21. Li B, Liu H, Wang W. Multiplex real-time PCR assay for detection of Escherichia coli O157:H7 and screening for non-O157 Shiga toxin-producing E. coli. BMC Microbiol. 2017;17: 215. https://doi.org/10.1186/s12866-017-1123-2.
22. Bender AC, Faulkner JA, Tulimieri K, Boise TH, Elkins KM. High Resolution Melt Assays to Detect and Identify Vibrio parahaemolyticus, Bacillus cereus, Escherichia coli, and Clostridioides difficile Bacteria. Microorganisms. 2020;8: 561. https://doi.org/10.3390/microorganisms8040561.
23. Reese KR, Elkins KM. Simultaneous Detection of Foodborne Pathogens Using a Real-Time PCR Triplex High-Resolution Melt Assay. Appl. Microbiol. 2022;2: 453-459. https://doi.org/10.3390/applmicrobiol2030034.
24. Ahmad I, Nadeem A, Mushtaq F, Zlatkov N, Shahzad M, Zavialov AV, Wai SN, Uhlin BE. Csu pili dependent biofilm formation and virulence of Acinetobacter baumannii. NPJ Biofilms Microbiomes. 2023;9(1):101. https://doi.org/10.1038/s41522-023-00465-6

25. Nie, D., Hu, Y., Chen, Z. et al. Outer membrane protein A (OmpA) as a potential therapeutic target for Acinetobacter baumannii infection. J Biomed Sci. 2020;27:26. https://doi.org/10.1186/s12929-020-0617-7
26. Sun X, Ni Z, Tang J, Ding Y, Wang X, Li F. The abaI/abaR Quorum Sensing System Effects on Pathogenicity in Acinetobacter baumannii. Front Microbiol. 2021;12:679241. https://doi.org/10.3389/fmicb.2021.679241
27. Ibrahim ME. Prevalence of Acinetobacter baumannii in Saudi Arabia: risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance. Ann Clin Microbiol Antimicrob. 2019 Jan 3;18(1):1. https://doi.org/10.1186/s12941-018-0301-x