**RESEARCH NOTE**

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# Impact of template denaturation prior to whole genome amplification on gene detection in high GC-content species, *Burkholderia mallei* and *B. pseudomallei*

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## Abstract

**Objective** In this study, we sought to determine the types and prevalence of antimicrobial resistance determinants (ARDs) in *Burkholderia* spp. strains using the Antimicrobial Resistance Determinant Microarray (ARDM).

**Results** Whole genome amplicons from 22 *B. mallei* (BM) and 37 *B. pseudomallei* (BP) isolates were tested for > 500 ARDs using ARDM v.3.1. ARDM detected the following *Burkholderia* spp.-derived genes, *aac(6)*, $bla_{BP/MBL-3}$, $blaA_{BPS}$, *penA-BP*, and *qacE*, in both BM and BP while $bla_{BP/MBL-1}$, *macB*, $bla_{OXA-42/43}$ and *penA-BC* were observed in BP only. The method of denaturing template for whole genome amplification greatly affected the numbers and types of genes detected by the ARDM. $Bla_{TEM}$ was detected in nearly a third of BM and BP amplicons derived from thermally, but not chemically denatured templates. $Bla_{TEM}$ results were confirmed by PCR, with 81% concordance between methods. Sequences from 414-nt PCR amplicons (13 preparations) were 100% identical to the *Klebsiella pneumoniae* reference gene. Although $bla_{TEM}$ sequences have been observed in *B. glumae*, *B. cepacia*, and other undefined *Burkholderia* strains, this is the first report of such sequences in BM/BP/*B. thailandensis* (BT) clade. These results highlight the importance of sample preparation in achieving adequate genome coverage in methods requiring untargeted amplification before analysis.

**Keywords** Antimicrobial resistance, Resistome, Extended-spectrum β-lactamase, ESBL, Select agents

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## Introduction

Antimicrobial resistance (AMR) is among the World Health Organization's most important global public health threats and is attributed with 1.27 million deaths in 2019 [1]. While significant efforts are being made to address and mitigate AMR in the public health sector [2–4], the mechanisms and prevalence of AMR in Tier 1 Select Agents and their near neighbors are not as comprehensively defined. Notably, infections with *B. pseudomallei* (BP, melioidosis) and *B. mallei* (BM, glanders) are estimated to affect up to 165,000 humans and hundreds to thousands of equids each year, respectively [5–7]. While both diseases have mortality rates of 90–95% in untreated humans, even the current two-phase therapeutic guidelines (2–8 weeks intravenous antimicrobials + 3–6 months of oral antimicrobials) may fail in up to 40% of cases [5, 8, 9]. Myriad intrinsic antimicrobial resistance (AMR) mechanisms including penicillin-binding proteins, PenA β-lactamases, drug efflux pumps, unusual lipopolysaccharide structure, altered target sites, target overproduction, and intracellular pathogen localization contribute to these therapeutic challenges [10, 11]. Furthermore, genome complexity/plasticity and reports of engineered resistance [12, 13] increase the potential for emergence and spread of resistant strains with therapeutic options more limited than those in current use [8, 9, 14].

This work is an extension to a previously published survey of Category A Select Agents and exempt strains for horizontally and vertically transferred AMR determinants [15]. Here, we use the Antimicrobial Resistance Determinant Microarray (ARDM) v.3.1 for broad spectrum screening of > 500 AMR determinants in 22 BM and 37 BP strains, with PCR as an orthogonal detection method.

## Methods

Purified DNA preparations from 22 BM and 37 BP Unified Culture Collection (UCC) strains were obtained through the Genomic Repository Program at DEVCOM CBC of the US Defense Biological Product Assurance Office (DBPAO), Frederick, MD, USA. Whole genome amplification (WGA) was performed on each sample using Illustra GenomiPhi HY kits (GE Healthcare, USA) essentially as described by the manufacturer's instructions, using 10–25 ng of starting material and amplifying for 2 h at 30 ℃. Because of *Burkholderia*'s high GC content, two approaches for WGA template denaturation were compared. Thermal denaturation involved template incubation for 5 min at 95 ℃ and 3 min on ice before WGA ("thermal amplicons"). Chemical denaturation involved template treatment for 3 min with Buffer D1 (REPLI-g Mini kit; Qiagen, USA, 1:1 volume:volume ratio), followed by two volumes of Buffer N1 (same kit) before WGA ("chemical amplicons"). Amplification time was set to 2 h to allow sufficient amplicon formation while preventing non-specific background amplification.

Equivalent amounts (3.2 μg) of thermal or chemical amplicons were fragmented and labeled using Bionick DNA-Labeling System (ThermoFisher). Fragmented, biotinylated amplicons were then applied without purification to the ARDM v.3.1 (Customarray, USA), and hybridized overnight at 60 ℃ as previously described [15, 16]. Hybridized microarrays were processed, labeled, and interrogated using the Electrasense Reader (Customarray) and previously established positive/negative thresholds [15]. Burkholderiales-specific ARDM content is found in Additional File 1.

PCR assays targeting $bla_{TEM}$ and five BM/BP-derived genes were used to confirm their presence/absence in a subset of thermal and chemical amplicons (Additional File 2). PCR amplification was assessed via electrophoresis (FlashGel, Lonza, USA). Published NCBI sequences with ≥ 95% sequence identity were used as the gold standard for sensitivity/specificity.

## Results and discussion

Samples used in the ARDM and PCR analyses were generated via phi29-based WGA, a robust and reliable method to obtain large quantities of high-fidelity amplicons with near-complete genome representation. However, several groups have observed GC content-based biases using this method [17–20] and, in a previous study, we postulated that minor differences in microarray results between thermal and chemical amplicons may have been due to differences in GC content between the specific genes and the host genome [16]. Based on *Burkholderia's* high GC content (61–68%), its genome plasticity, and the potential for genomic islands arising from other species by horizontal gene transfer [21–23], we performed WGA using templates denatured thermally or chemically to assess ARDM performance.

In general, chemical (alkali) denaturation of BM/BP templates provided higher yields after WGA than thermal denaturation (Mann–Whitney, $p < 0.001$; Additional File 3). To prevent over- or under-fragmentation and labeling in subsequent steps, both chemical and thermal amplicons were normalized to 3.2 μg before processing with the Bionick kit; labeled, fragmented amplicons were not re-quantified prior to ARDM analysis, however.

### Detection of BM/BP-derived genes (sensitivity/specificity)

ARDM and PCR results for each sample, as well as accession numbers and presence/absence of each BM/BP-derived gene, are found in Additional File 4.

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**Table 1** Overview of *Burkholderia*-derived genes and $bla_{TEM}$ detected in BM/BP thermal and chemical amplicons

| | Presence of sequences with ≥95% identity in NCBI | | Thermal amplicons | | | | Chemical amplicons | | | | ARDM/PCR concordance | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | BM | BP | BM | | BP | | BM | | BP | | Thermal | Chemical |
| | n=22 | n=37 | ARDM | PCR | ARDM | PCR | ARDM | PCR | ARDM | PCR | | |
| aac(6) | 100% | 100% | 50% (10/20) | 0% (0/8) | 14% (5/37) | | 74% (14/19) | 100% (12/12) | 59% (20/34) | 100% (35/35) | 13% | 66% |
| $blaA_{BPS}$ | 100% | 100% | 35% (7/20) | 0% (0/8) | 0% (0/37) | | 47% (9/19) | 100% (12/12) | 47% (16/34) | 57% (20/35) | 33% | 52% |
| $bla_{BP/MBL-1}$ | 0 | 100% | 0% (0/20) | 0% (0/8) | 5% (2/37) | 0% (0/1) | 0% (0/19) | 0% (0/12) | 71% (24/34) | 97% (34/35) | 92% | 80% |
| $bla_{BP/MBL-3}$ | 100% | 100% | 100% (20/20) | 100% (11/11) | 68% (25/37) | 100% (1/1) | 95% (18/19) | 100% (12/12) | 100% (34/34) | 100% (35/35) | 100% | 100% |
| $bla_{OXA-42/43}$ | 0% | 100% | 0% (0/20) | 0% (0/8) | 62% (23) | 100% (1/1) | 0% (0/19) | 0% (0/12) | 94% (32/34) | 100% (35/35) | 100% | 95% |
| $bla_{TMB}$[a] | 0% | 0% | 0% (0/20) | | 0% (0/37) | | 0% (0/19) | | 0% (0/34) | | | |
| $bla_{VEB}$[a] | 0% | 0% | 0% (0/20) | | 0% (0/37) | | 0% (0/19) | | 0% (0/34) | | | |
| macB | 0% | 0% | 0% (0/20) | | 11% (4/37) | | 0% (0/19) | | 85% (29/34) | | | |
| penA-BP | 100% | 100% | 65% (13/20) | 73% (8/11) | 3% (1/37) | 0% (0/1) | 89% (17/19) | 75% (9/12) | 76% (26/34) | 57% (20/35) | 67% | 68% |
| penA-BC[b] | 0% | 0% | 0% (0/20) | | 0% (0/37) | | 0% (0/19) | | 12% (4/34) | | | |
| qacE | 100% | 100% | 100% (20/20) | | 84% (31/37) | | 95% (18/19) | | 100% (34/34) | | | |
| $bla_{TEM}$ | 0% | 100% | 35% (7/20) | 45% (10/22) | 35% (13/37) | 14% (5/37) | 0% (0/19) | 0% (0/7) | 0% (0/34) | 0% (0/5) | 77% | 100% |

[a] Sequence derived from *Achromobacter* sp. (Burkholderiales)

[b] Sequence, derived from *B. cepacia*, has 78% sequencing identity to *penA-BP* over 82% of the entire gene

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Using thermal amplicons, ARDM analysis detected only four BM/BP-derived genes ($bla_{BP/MBL-3}$, $bla_{OXA-42/43}$, *qacE*, and *penA-BP*) in significant sample numbers, indicating this method's overall poor sensitivity with this sample set (Table 1). Sensitivities ranged from 0 to 100%, depending on the species and specific gene. In general, a higher proportion of ARDM-positives were observed in BM than in BP ($\chi^2$, $p < 0.05$); for example, sequences for $blaA_{BPS}$ and *penA-BP* are found in both species but were detected at minimal levels in BP, yielding only 12% and 25% overall sensitivities, respectively. On the other hand, $bla_{BP/MBL-1}$, $bla_{OXA-42/43}$, and *macB*—found only in BP— were not observed in any BM strains tested, indicating 100% specificity for these genes.

Confirmatory PCRs detected only $bla_{BP/MBL-3}$ and *penA-BP* in significant proportions of the limited number of the thermal amplicons tested; as expected, $bla_{OXA-42/43}$ was not detected in BM strains (100% specificity). PCR assays for both *aac(6)* and $blaA_{BPS}$ provided no positive results amongst thermal amplicons; high concordances between ARDM and PCR were observed for $bla_{BP/MBL-1}$, $bla_{BP/MBL-3}$, and $bla_{OXA-42/43}$. However, for the thermal amplicon set, comparisons between ARDM and PCR may not be significant, given the small number of samples tested by PCR.

When chemical amplicons were used as samples, ARDM detected all BM/BP-derived genes except *qac* at significantly higher proportions when compared with matched thermal amplicons ($n = 51$ pairs; McNemar, $p < 0.01$, Table 2). ARDM sensitivities were therefore higher for chemical amplicons, ranging from 59 to 100% (Table 1). As with thermal amplicons, $bla_{BP/MBL-1}$, $bla_{OXA-42/43}$, and *macB* were detected only in BP (100% specificity for each). PCR performed better than ARDM at detecting *aac(6)*, $blaA_{BPS}$, and $bla_{BP/MBL-1}$ in chemical amplicons ($n = 44$ matched samples for *aac(6)* and $blaA_{BPS}$, $n = 32$ BP only for $bla_{BP/MBL-1}$; McNemar's test, $p < 0.02$) but the opposite was true for *penA-BP* ($n = 44$, McNemar's test, $p = 0.016$).

Insufficient numbers of assays for BM/BP-derived genes were performed with both sample populations for a statistically robust comparison of PCR performance between thermal and chemical amplicons. However, PCR assays tended to detect BM/BP-derived genes in a higher proportion of chemical amplicons than in thermal amplicons (better sensitivity) and with improved ARDM concordance. PCR specificities for $bla_{BP/MBL-1}$, $bla_{OXA-42/43}$, and *macB* (found only in BP) were 100% in both thermal and chemical amplicons.

## Detection of genes not derived from BM/BP

ARDM detected 19 non-BM/BP-derived genes in thermal (7 genes) and chemical (13 genes) amplicons, all potentially representing false positives. None were detected in both chemical and thermal amplicons from the same strain. Excluding $bla_{TEM}$ (discussed below), these genes were detected more frequently in chemical versus thermal amplicons when comparing matched samples (McNemar, $p = 0.004$) but differences were not significant when comparing populations as a whole ($\chi^2$, $p = 0.902$). Interestingly, the GC contents of false-positive identifications from thermal amplicons were lower than those from chemical amplicons (t-test, $p < 0.001$; Mann–Whitney, $bla_{TEM}$ included; $p < 0.001$; Additional File 5).

Detection of $bla_{TEM}$ in nearly one-third of thermal amplicons via two orthogonal methods—but not in chemical amplicons by either—represents a unique observation, though ARDM-PCR concordance was relatively poor. PCR amplicons from thirteen $bla_{TEM}$-positive (thermal amplicon) samples were purified and sequenced, and yielded

**Table 2** Detection of BM/BP-derived genes, $bla_{TEM}$, and others in matched pairs of thermal/chemical samples (n = 51)

| Denaturation | Thermal (−)/chemical (−) | Thermal (+)/chemical (+) | Thermal (−)/chemical (+) | Thermal (+)/chemical (−) | McNemar's P |
|---|---|---|---|---|---|
| *aac(6)* | 15 | 8 | 24 | 4 | < 0.001 |
| $bla_{BP/MBL-1}$ | 27 | 1 | 23 | – | < 0.001 |
| $bla_{BP/MBL-3}$ | – | 38 | 12 | 1 | 0.006 |
| $blaA_{BPS}$ | 23 | 1 | 23 | 4 | < 0.001 |
| $bla_{OXA-42/43}$ | 19 | 20 | 12 | – | 0.001 |
| *macB* | 22 | 1 | 28 | – | < 0.001 |
| *penA-BP* | 9 | 9 | 32 | 1 | < 0.001 |
| *qacE* | – | 43 | 6 | 1 | 0.131 |
| $bla_{TEM}$ | 35 | – | – | 16 | < 0.001 |
| Other non-BM/BP genes (18 genes) | 874 | – | 32 | 12 | 0.004 |
| TOTAL | 1024 (74%) | 121 (9%) | 192 (14%) | 39 (3%) | |

**Table 3** *Bla*$_{TEM}$ PCR amplicon sequences from UCC strains (top) and other *Burkholderia* clades (bottom)

| Sequence alignment of UCC PCR amplicons and the reference sequence for *bla*$_{TEM}$ (AF309824, nucleotide position 119-979) |
|---|
| UCC BURK* 1    TCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA  120 <br>         \|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\| <br> AF309824 404   TCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA  523 <br><br> UCC BURK 121    CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  240 <br>         \|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\| <br> AF309824 524   CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  643 <br><br> UCC BURK 241    CGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  360 <br>         \|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\| <br> AF309824 644   CGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  763 <br><br> UCC BURK 361    GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCT  414 <br>         \|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\|\| <br> AF309824 764   GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCT  817 |

| Published sequences from *Burkholderia* spp. with >99% sequence identity to the 414-nt *bla*$_{TEM}$ PCR amplicon(s) purified from the UCC strains | | | |
|---|---|---|---|
| Description | Accession number | Nucleotide positions | Sequence identity |
| *B. glumae* strain PW30RS chromosome 2, complete sequence | CP100204 | 319731-320144 | 414/414 (100%) |
| *B. cepacia* (*Burkholderia* sp. COPS) contig 105 | WIXR01000105 | 1838-2698 | 413/414 (99.8%) |
| *Burkholderia* sp. LLH-Slr-24 TEM β-lactamase (*bla*$_{TEM}$) gene, partial cds | KM287448 | 262-675 | 413/414 (99.8%) |
| *Burkholderia* sp. LLH-Slr-7 TEM β-lactamase (*bla*$_{TEM}$) gene, partial cds | KM287444 | 221-634 | 413/414 (99.8%) |
| *Burkholderia* sp. LLH-Slr-1 TEM β-lactamase (*bla*$_{TEM}$) gene, partial cds | KM287442 | 227-640 | 413/414 (99.8%) |
| *Burkholderia cenocepacia* strain 11806 β-lactamase (*bla*$_{TEM-116}$) gene, partial cds | GU169410 | 283-696 | 413/414 (99.8%) |
| *Burkholderia* sp. II-02 BLA-PCR signal genomic sequence | DQ977654 | 190-603 | 413/414 (99.8%) |
| *Burkholderia* sp. LLH-Slr-6 TEM β-lactamase (*bla*$_{TEM}$) gene, partial cds | KM287443 | 236-649 | 412/414 (99.5%) |
| *Burkholderia* sp. LLH-Slr-13 TEM β-lactamase (*bla*$_{TEM}$) gene, partial cds | KM287445 | 256-669 | 410/414 (99.0%) |

[*] UCC BURK represents (identical) amplicon sequences from the following UCC *Burkholderia* spp. strains: BM: BURK007, BURK010, BURK065, BURK066, BURK073, BURK077, BURK082, BURK119, BURK131; BP: BURK132, BURK133, BURK134, and BURK135. These sequences are available in NCBI as accession numbers OR815368 through OR815380. The reference sequence for *bla*$_{TEM}$ (AF309824) is shown in red

sequences 100% identical to the ARDM *bla*$_{TEM}$ reference gene (AF309824: 119..979; Table 3, top). To our knowledge, these observations represent the first time that *bla*$_{TEM}$ has been detected in the BM/BP/BT group, though analogous sequences have been documented in strains from other *Burkholderia* clades ([24–29] and Table 3). A BLAST search of NCBI yielded nine records from *Burkholderia* spp. with > 99% identity to the purified amplicons over the

entire 414-nt length (Table 3, bottom). While eight are partial sequences, the *B. glumae* and *B. cepacia* sequences are > 99% identical to the entire 861-nt *bla*$_{TEM-1}$ reference gene.

This study's differential detection of *bla*$_{TEM}$ in thermal amplicons only may be due to template denaturation, the gene's GC content, and/or its context within the genome(s). Here, *bla*$_{TEM}$ and other genes with lower

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GC content were more frequently detected in thermal amplicons, whereas *Burkholderia* sp.-derived and other genes with GC > 50% tended to be detected more frequently in chemical amplicons. These results support observations that GC-rich regions are underrepresented in WGA amplicons from thermally denatured templates [18, 30, 31], though conflicting reports have also been published [17, 32]. While detection of $bla_{TEM}$ in our thermal amplicons may be artifacts from enrichment of lower GC regions, we note that the genome context of the *B. glumae* $bla_{TEM}$ gene (Table 3; CP100204) is within a chromosomal region with lower GC content, where the upstream six genes are > 95% identical in sequence to *Escherichia coli* analogs. It is possible that genome plasticity—documented to occur in *Burkholderia* spp. [33–38]—is responsible for horizontal transfer of this gene from a Gammaproteobacterium, *Neisseriaceae* spp., or one of the few other Betaproteobacteria species in which $bla_{TEM}$ has been documented. We have not attempted to identify the full $bla_{TEM}$ gene or its genome context within the strains tested here. It further remains to be seen whether the detected sequences are part of a complete $bla_{TEM}$ gene that is actively transcribed and is capable of conferring a clinically relevant phenotype (e.g., resistance to ceftazidime and/or amoxicillin/clavulanate, used in acute and eradication phase therapies for melioidosis and glanders).

## Limitations

While useful to track movement of ARDs in epidemiological studies, the ARDM technology used here is unable to predict AMR phenotypes based on differences in transcriptional regulation or gene duplication. Furthermore, as ARDM probes are designed to detect ARD sequences conserved amongst multiple species, ARDM chips cannot detect small sequence differences affecting phenotype (e.g., *penA* mutations conferring ceftazidime resistance; [39]). However, these limitations are shared by any DNA-based technologies using relatively long sequences for hybridization-based detection. Newer technologies such as gene expression profiling—especially when combined with next generation sequencing approaches—can provide valuable information about both transcriptional levels and the presence of mutations, increasing the potential for phenotypic predictions. Untargeted sequencing technologies also have the potential to detect all known and suspected determinants—well above the current content (~ 500 genes) of the ARDM chips—provided the full genome is sufficiently represented.

Here, we observed significant differences in the numbers and types of genes detected based on the method of pre-WGA template denaturation. These and other WGA-induced artifacts can also affect other analytical methods requiring untargeted amplification before analysis, i.e., where sample size is limited, including sequencing [40–43]. While other efforts to sequence the same UCC strains have failed to document $bla_{TEM}$ in the BM/BP/BT clade, the current study presents evidence to support its natural occurrence within the tested strains, and potentially in a wider context. Specifically, detection of the low-GC-content $bla_{TEM}$ gene in nearly a third of thermal amplicons due to a fortuitous artifact of template denaturation before WGA (i.e., enrichment of AT-rich regions) may enable detection of other genes not easily identified, although the relatively poor ARDM/PCR concordance (77% in the thermal amplicons) should be explored further. Detection of $bla_{TEM}$ in both BM and BP amplicons prepared on multiple days, in multiple preparations, and in multiple facilities suggest that positives were not due to contamination; other samples processed at the same time were all $bla_{TEM}$-negative except where expected [15]. Finally, sequences from thirteen purified PCR amplicons were 100% identical to the *E. coli* reference gene over the full 414-nt amplicon length.

More research is needed to characterize the generally poor detection of BM/BP ARDs and low ARDM/PCR concordance in thermal versus chemical amplicons. Specifically, differences in representation of various regions of the genome between thermal and chemical amplicon populations may shed light on unidentified genes and regions of the genome previously missed due to requirements for large quantities of DNA obtainable only through amplification (e.g., sequencing and hybridization applications). Overall, this study suggests that use of both thermal and chemical template denaturation may enable detection of $bla_{TEM}$ and other previously undetected genes in high or low GC backgrounds. Confirmation of $bla_{TEM}$ presence and clinical importance will require full sequence and context determinations, while application of these observations to other high GC species will require a larger and more complex sample set.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-06717-8.

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**Additional file 1.** Content of ARDM v.3.1 derived from Burkholeriales; Description—Antimicrobial resistance determinants represented on ARDM v.3.1 that are derived from species in Burkholderiales.

**Additional file 2.** PCR primers and conditions; Description—Primers (and cycling conditions) used for PCR confirmation of select BM/BP-derived determinants and $bla_{TEM.}$

**Additional file 3.** Distribution of yields from WGA; Description—Distribution curves of yields from WGA amplicons from thermally and chemically denatured templates.

**Additional file 4.** Results from ARDM and PCR; Format: Excel spreadsheet; Description—Strain descriptions, presence/absence of BM/BP-derived

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genes and *bla*<sub>TEM</sub> within published genomes, detection of BM/BP-derived genes and *bla*<sub>TEM</sub> via ARDM analysis and PCR.

**Additional file 5.** Putative false-positive (non-BM/BP-derived) genes detected by ARDM; Description—Prevalence of non-BM/BP-derived genes detected by ARDM analysis amongst thermal and chemical samples (Panel **A**) and distribution of GC contents of non-BM/BP-derived genes detected (Panel **B**).

## Author contributions
Conceptualization, CRT, TAL, SS, DLD, GJV; methodology, CRT, TAL; validation, CRT, TAL, SS; investigation, CRT, TAL, KLB, JRC, AC; resources, KLB, RWD, SS, DLD; data curation, CRT, SS; writing—original draft preparation, CRT; writing—review and editing, CRT, TAL, JRC, AC, KLB, RWD, SS, DLD, GJV; supervision, CRT, DLD, GJV; project administration, CRT, DLD, GJV; funding acquisition, CRT, SS, DLD, GJV All authors have read and agreed to the published version of the manuscript.
This manuscript is approved for public release: distribution is unlimited.

## Availability of data and materials
Supplemental data are found in the attached files; full ARDM v.3.1 content can be obtained from the US Defense Biological Product Assurance Office (DBPAO), Frederick, MD, USA. Nucleic acid materials can be obtained from the Unified Culture Collection (UCC) of DBPAO. Amplicon sequences are available in NCBI.

## Declarations

### Ethics approval and consent to participate
Not applicable.

### Consent for publication
All authors consent to coauthorship and have reviewed/approved the manuscript.

### Competing interests
Chris R. Taitt is employed by Nova Research Inc. DTRA was active in both the research design and project management, and in preparation of the manuscript. All authors declare no conflict of interest.

### Non-endorsement
References to non-federal entities or commercial products do not constitute or imply Department of Defense or U.S. Army endorsement of any company, product, or organization.

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