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# Genome exploration and predicted antimicrobial resistance profiles of *Bartonella* spp. in Thailand

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## ABSTRACT

**Background:** Bartonellosis, caused by *Bartonella species*, is a group of zoonotic infectious diseases affecting both humans and animals. Rodents serve as important reservoirs, particularly in Southeast Asia, where pathogen diversity remains underexplored.

**Objectives:** This study aimed to characterize the genomic features and antimicrobial resistance profiles of *Bartonella* isolates obtained from rodent and deer hosts in Thailand.

**Materials and methods:** Six *Bartonella* isolates recovered from rodent and deer blood samples in Thailand were confirmed by *gltA* and *rpoB* PCR and subjected to whole-genome sequencing using the Illumina MiSeq platform. Genome quality and annotation were assessed using QUAST and Prokka, respectively. Taxonomic assignment and prediction of antimicrobial resistance-associated determinants were performed using CZ ID, while orthologous gene clusters were compared using OrthoVenn3.

**Results:** Whole-genome sequencing and metagenomic analyses were performed on six *Bartonella* isolates. Sequence identity analysis revealed that all isolates were closely related to *Bartonella vinsonii*, suggesting potential zoonotic transmission. Additional species identified included *B. schoenbuchensis*, *B. kosoyi*, and *B. tribocorum*, which are primarily associated with animal infections, as well as *B. grahamii*, a species with known zoonotic potential. Draft genome sizes ranged from 1.52 to 2.57 Mb, with predicted gene counts between 1,258 and 2,115. The largest genome was observed in isolate RT1 *(B. grahamii)*. Analysis of predicted antimicrobial resistance-associated genes revealed a diverse distribution of putative resistance determinants across the isolates. Most of the identified determinants were annotated as protein homologs and associated with antibiotic inactivation mechanisms.

**Conclusion:** The genomic data generated in this study provide valuable resources for developing molecular markers for *Bartonella* detection and surveillance. These genomic findings provide preliminary information on putative antimicrobial resistance-associated determinants in *Bartonella* spp.; however, phenotypic antimicrobial susceptibility testing is required to confirm their functional relevance.


## Introduction

*Bartonella* is a genetically diverse genus of fastidious, gram-negative, facultative intracellular bacteria belonging to the class Alphaproteobacteria. These bacteria circulate among mammalian reservoir hosts and blood-feeding arthropods, and their transmission is often sustained by persistent or relapsing intraerythrocytic bacteremia in reservoir hosts, thereby facilitating acquisition by competent arthropod vectors during blood feeding.[1-3] *Bartonella* spp. occur worldwide in diverse mammalian hosts and hematophagous arthropods; however, their prevalence and genetic diversity vary substantially with geographic location, host-community composition, vector ecology, and study design.[4-6] Rodents and shrews serve as important reservoir hosts for numerous *Bartonella* lineages and may contribute to zoonotic maintenance and spillover at human-animal-vector interfaces.[5-7]

In Thailand, early investigations into Bartonella ecology in companion animals and small mammals revealed a diverse reservoir community. *B. henselae*, the causative agent of cat scratch disease, was highly prevalent among cats in northern border provinces, with high seropositivity in Chiang Rai and Mae Hong Son, whereas no antibodies were detected in dogs.[8] These findings identify cats as key reservoirs in specific ecological settings and suggest that environmental and vector-related factors contribute to spatial heterogeneity in transmission. Subsequent work in well-cared, client-owned cats in the Bangkok metropolitan area further demonstrated that *B. henselae* and *B. clarridgeiae* are the predominant feline *Bartonella* species, even in animals without clinical signs or visible flea infestation, reinforcing the role of apparently healthy urban cats as reservoirs for zoonotic Bartonella. In Southeast Asia, *B. henselae* and *B. clarridgeiae* are considered the principal feline *Bartonella* species of zoonotic and veterinary importance and are predominantly transmitted by the cat flea *Ctenocephalides felis*, which is ubiquitous on cats and dogs worldwide.[9-13]

Concurrently, *Bartonella* spp. was widely detected in rodents and shrews, with species such as *B. elizabethae, B. tribocorum, B. rattimassiliensis*, and *B. queenslandensis* reported in *Rattus* spp., *Bandicota indica*, and *Suncus murinus* at prevalences exceeding 40% in some regions.[13-17] Subsequent studies have expanded the known diversity of rodent- and shrew-associated *Bartonella* in Thailand. Investigations along the borders with Myanmar, Laos, and Cambodia identified multiple species, including *B. coopersplainsensis, B. phoceensis, B. queenslandensis, B. rochalimae, Bartonella sp.* RN24BJ, and a novel lineage proposed as "*Candidatus Bartonella* thailandensis".[18] A nationwide survey conducted between 2013 and 2016 reported an overall prevalence of 11.5% and nine species: *B. tribocorum, B. rattimassiliensis, B. queenslandensis, B. elizabethae, B. chanthaburi* sp. *nov., B. satun* sp. nov., *B. coopersplainsensis, B. ranong* sp. nov., and *B. henselae,* highlighting substantial geographic variation and marked genetic heterogeneity in *Bartonella* populations in small mammals.[7, 16]

*Bartonella* infections have also been documented in febrile patients with clinical manifestations ranging from mild regional lymphadenitis to severe endocarditis.[19] However, links between specific animal reservoirs and human disease remain poorly defined. Previous studies in Thailand have identified *Bartonella* strains in several rodent species that were phylogenetically closely related to strains *B. grahamii* and *B. elizabethae*, both of which have previously been associated with human illness.[14-17] Kosoy et al. (2010) reported that *Bartonella* genotypes identified in febrile human patients were either identical or highly similar to homologous sequences detected in rodents from Asia, and were phylogenetically closely related to *B. elizabethae*, *B. rattimassiliensis*, and *B. tribocorum*.[19]

Recent regional genomic studies have demonstrated that certain *Bartonella* clades differ substantially in genome size, G+C content, and metabolic capacity, and have even proposed reclassifying some lineages into new genera, underscoring the value of whole-genome approaches for elucidating *Bartonella* evolution, host adaptation, and pathogenic potential.[20-22] Despite extensive genotyping based on partial housekeeping genes (for example, *gltA* and *rpoB*), whole-genome data for Thai *Bartonella* isolates-particularly those recovered from rodents and ruminants-remain limited, and the distribution of antimicrobial resistance determinants within these lineages is largely unknown, even as antimicrobial use and resistance pose major public health and veterinary challenges in Thailand.

Addressing these knowledge gaps through comprehensive genomic characterization of *Bartonella* circulating in key reservoir hosts is therefore essential to assess zoonotic risk and inform One Health-aligned surveillance and control strategies.

## Materials and methods

### *Sample Isolation*

Samples were collected from several rodent hosts, including *B. indica, R. rattus,* and *R. norvegicus,* as well as from deer (*Rusa timorensis*). Rodent and cervid species were selected according to their frequent occurrence at the study sites, ecological characteristics that may affect their exposure to *Bartonella*, and their inclusion in a previous study designed to achieve geographic representation across northern, eastern, western, and southern Thailand (Table 1). Blood samples were used as the primary source for isolation. The frozen blood samples were thawed at room temperature and centrifuged at 1,800 x g for 60 minutes. After centrifugation, the pellets were plated onto brain heart infusion agar (BHIA) plates containing 5% defibrinated rabbit blood. The plates were incubated at 25°C under 5% $CO_2$ for 4 weeks. We selected two or three small, rough, gray colonies from each plate, streaked them onto fresh plates, and further cultured them under the same conditions, presumptively identifying them by colony morphology. Colonies with characteristic *Bartonella*-like morphology were subsequently confirmed as *Bartonella* spp. by PCR amplification of the *gltA*: BhCS.781p(5'-GGGGACCAGCTCATGGTGG-3'), BhCS.1137n (5'-AATGCAAAAAGAACAGTAAACA-3') and *rpoB*: 1400F(5'-CGCATTGGCTTACTTCGTATG-3'), 2300R (5'-GTAGACTGATTAGAACGCTG-3') genes before whole-genome sequencing.[7, 16]

**Table 1**. Characteristics of isolates by host species, sample type, and geographic location.

| Sample | Host | Host group | Isolation source | Location | Method of host identification |
|---|---|---|---|---|---|
| CP1 | *Rattus rattus* | Rodent | Blood | Eastern | Morphology |
| DR1 | *Rusa timorensis* | Cervid | Blood | Western | - |
| EZ1 | *Rattus rattus* | Rodent | Blood | Northern | Morphology |
| QS1 | *Rattus norvegicus* | Rodent | Blood | Eastern | Morphology |
| RT1 | *Bandicota indica* | Rodent | Blood | Southern | Morphology |
| TB1 | *Rattus rattus* | Rodent | Blood | Eastern | Morphology |

### *Genomic DNA extraction and sequencing*

Genomic DNA was extracted from *Bartonella spp*. isolates using the DNeasy® Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA concentrations of the samples were measured using fluorescence-based Qubit™ quantitation assays (Qubit® 2.0 Fluorometer, Invitrogen, USA). Paired-end libraries were prepared using the Miseq Reagent Micro Kit V2 (Illumina®, USA) and subsequently sequenced on the Illumina MiSeq platform (Illumina®, USA).

### *Bioinformatic analyses*

Paired-end reads were quality-filtered using Fastp 1.0.[23] Assembly quality was assessed using QUAST version 5.3.1. The resulting assembled contigs were subsequently annotated using Prokka version 1.14.6.[24] Sequence-based taxonomic assignment, coverage assessment, and antimicrobial resistance profiles were performed using the CZ ID pipeline (https://czid.org/) with the default pipeline settings. The orthologous gene clusters were compared using OrthoVenn3.[25]

## Results

### *Genome analyses*

Metagenomic analysis of the six samples revealed varying sequence identities and sequencing depths for *Bartonella* species. CP1 and TB1 were both assigned to *B. vinsonii*, with moderate sequence identities of 85.1% and 84.3% and depths of 20× and 32.8×, respectively; the closest reference match has been reported in animal and/or human hosts (CP1) or animals (TB1). DR1 was identified as *B. schoenbuchensis*, with 94.5% sequence identity and a depth of 74.3× in an animal-derived sample. EZ1 showed the highest sequence identity (98.3%) to *B. kosoyi*, with a depth of 57.4× (animal). QS1 was assigned to *B. tribocorum* (91.4% identity, 40.1× depth; animal), whereas RT1 was assigned to *B. grahamii* (88.8% identity, 33.1× depth); the closest reference match has been associated with human infection. (Table 2).

**Table 2**. CZ ID sequence-alignment results and preliminary taxonomic assignments of *Bartonella* spp. detected by metagenomic sequencing.

| Sample | Closest CZ ID reference hit | Sequence identity (%) | Coverage depth (x) | Preliminary taxonomic interpretation | Pathogenicity report |
|---|---|---|---|---|---|
| CP1 | *Bartonella vinsonii* (LR134529) | 85.1 | 20 | *Bartonella* sp.; species-level assignment not established. | Animal/ human |
| DR1 | *Bartonella schoenbuchensis* (CP019789) | 94.5 | 74.3 | *Bartonella* sp.; species-level assignment not established. | Animal |
| EZ1 | *Bartonella kosoyi* (CP031843) | 98.3 | 57.4 | *Bartonella* sp.; putative species-level match, requiring whole-genome validation. | Animal |
| QS1 | *Bartonella tribocorum* (HG969192) | 91.4 | 40.1 | *Bartonella* sp.; species-level assignment not established. | Animal |
| RT1 | *Bartonella grahamii* (CP001562) | 88.8 | 33.1 | *Bartonella* sp.; species-level assignment not established. | Human |
| TB1 | *Bartonella vinsonii* (LR134529) | 84.3 | 32.8 | *Bartonella* sp.; species-level assignment not established. | Animal/ human |

The assembled genomes from the six samples varied in size, contig number, GC content, and coding capacity. Genome sizes ranged from 1,517,004 bp in DR1 to 2,570,754 bp in RT1. CP1, DR1, EZ1, QS1, RT1, and TB1 contained 72, 69, 80, 80, 150, and 65 contigs, respectively (Table 3). GC content was relatively consistent, ranging from 36.97% in RT1 to 38.79% in TB1. The number of protein-coding sequences (CDSs) ranged from 1,258 in DR1 to 2,115 in RT1, with CP1, EZ1, QS1, and TB1 encoding 1,483, 1,869, 1,705, and 1,608 CDSs, respectively (Figure 1).

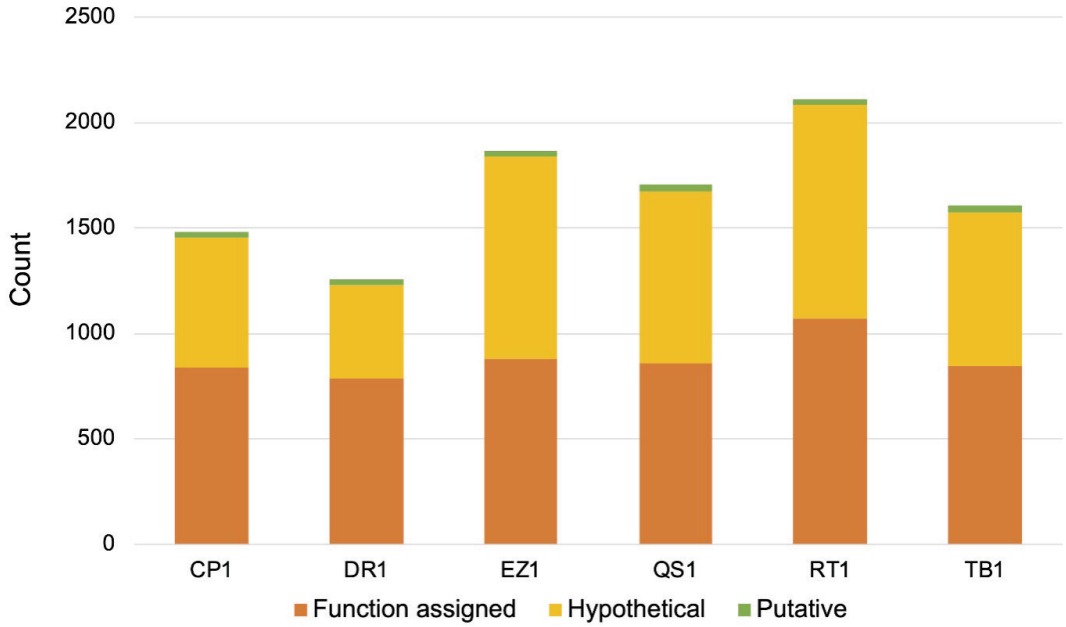



**Figure 1.** Predicted protein-coding sequences (CDSs) in draft genomes of six *Bartonella* isolates from Thailand.

Genome quality assessment indicated 100% estimated completeness for all six assemblies. Contamination estimates were low for CP1, DR1, EZ1, QS1, and TB1 (0.06%, 0.01%, 0.26%, 0.06%, and 0.03%, respectively), whereas RT1 showed substantially higher estimated contamination (22.5%) (Table 3). Although RT1 had the largest assembly size and the highest predicted CDS count, these values should be interpreted with caution because the relatively high contamination may have introduced additional genomic sequences and coding regions.

**Table 3**. Genome assembly statistics and protein-coding sequences of six *Bartonella* isolates.

| Parameter | CP1 | DR1 | EZ1 | QS1 | RT1 | TB1 |
|---|---|---|---|---|---|---|
| Genome size (bp) | 1,747,449 | 1,517,004 | 2,172,653 | 2,071,848 | 2,570,754 | 1,980,309 |
| Number of contigs | 72 | 69 | 80 | 80 | 150 | 65 |
| Total length of contigs ≥1,000 bp (bp) | 1,731,094 | 1,495,862 | 2,163,497 | 2,069,463 | 2,540,349 | 1,972,953 |
| N50 of contigs (bp) | 148,121 | 147,950 | 118,202 | 151,799 | 68,171 | 154,417 |
| Largest contig (bp) | 368,783 | 442,939 | 360,862 | 321,918 | 267,959 | 259,289 |
| GC content (%) | 37.95 | 37.46 | 38.19 | 38.19 | 36.97 | 38.79 |
| Completeness (%) | 100 | 100 | 100 | 100 | 100 | 100 |
| Contamination (%) | 0.06 | 0.01 | 0.26 | 0.06 | 22.5 | 0.03 |
| Protein-coding sequences (CDS) | 1,483 | 1,258 | 1,869 | 1,705 | 2,115 | 1,608 |

All six strains contained 10,038 predicted gene clusters. A total of 8,244 proteins were assigned to orthologous clusters, including 731 single-copy gene clusters, whereas 1,182 proteins were singletons (Figure 2). The Venn diagram showed 1,014 shared gene families among the six genomes (Figure 3). Together, these findings indicate that RT1 represents the largest and most gene-rich genome, whereas DR1 is the smallest and least gene-dense, with the other genomes exhibiting intermediate values.

Analysis of enzyme-coding genes across the six strains (CP1, DR1, EZ1, QS1, RT1, and TB1) showed that CDS counts varied by enzyme class (Figure 4). For EC1 (dehydrogenases, oxidases), counts ranged from 61 in DR1 to 80 in RT1, indicating a relatively consistent representation across strains. EC2 (transaminases, kinases) was the most abundant class, with TB1 exhibiting the highest number (211) and DR1 the lowest (158), suggesting a prominent role for these enzymes in core metabolism. For EC3 (lipases, amylases, peptidases), RT1 showed the highest number of CDSs (163), whereas DR1 had the lowest (110), reflecting variability in hydrolytic capacity among strains. EC4 (decarboxylases) ranged from 29 in DR1 to 42 in RT1, indicating moderate differences between genomes. EC5 (isomerases, mutases) had the lowest overall

counts, from 25 in EZ1 to 31 in RT1, consistent with more specialized functions. For EC6 (synthetases), RT1 again had the highest count (74), and DR1 the lowest (58), suggesting variation in biosynthetic potential. EC7 (translocases) showed comparatively low and uniform counts across all strains (24-31). Overall, RT1 carried the highest number of CDSs across most enzyme classes, consistent with a broader metabolic potential, whereas DR1 generally exhibited the lowest counts. EC2 and EC3 were the most abundant enzyme classes in all strains, underscoring their central role in metabolism, while EC5 and EC7 were less frequent, likely reflecting more specialized or restricted functions.

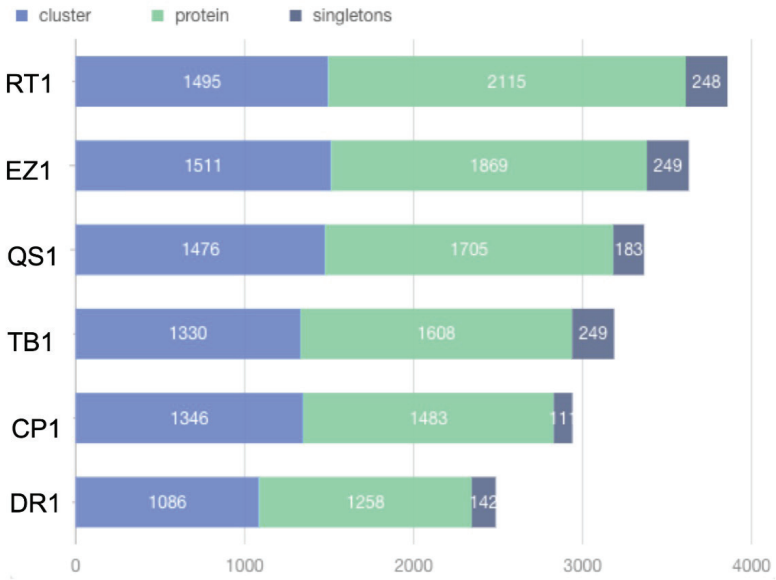



**Figure 2.** Orthologous-cluster composition of predicted proteins across six *Bartonella* isolates from Thailand.

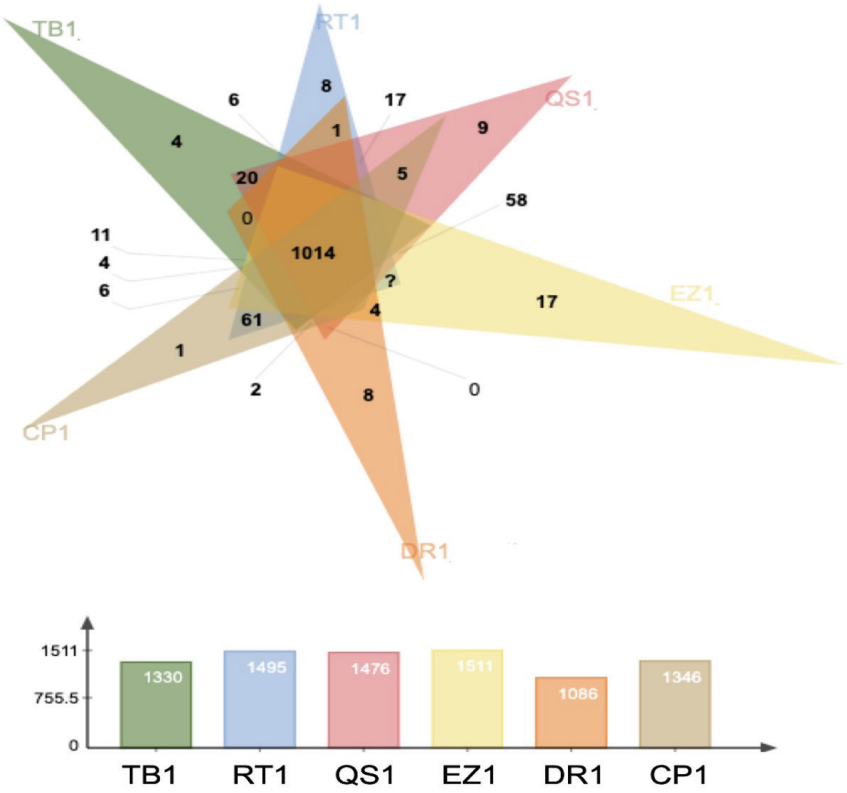



**Figure 3.** Shared and isolate-specific gene families among six *Bartonella* draft genomes from Thailand. A: Venn diagram showing the distribution of shared gene families among *CP1, DR1, EZ1, QS1, RT1*, and *TB1*, B: bar graph summarizing the numbers of shared and isolate-specific gene families. A total of 1,014 gene families were shared by all six isolates.

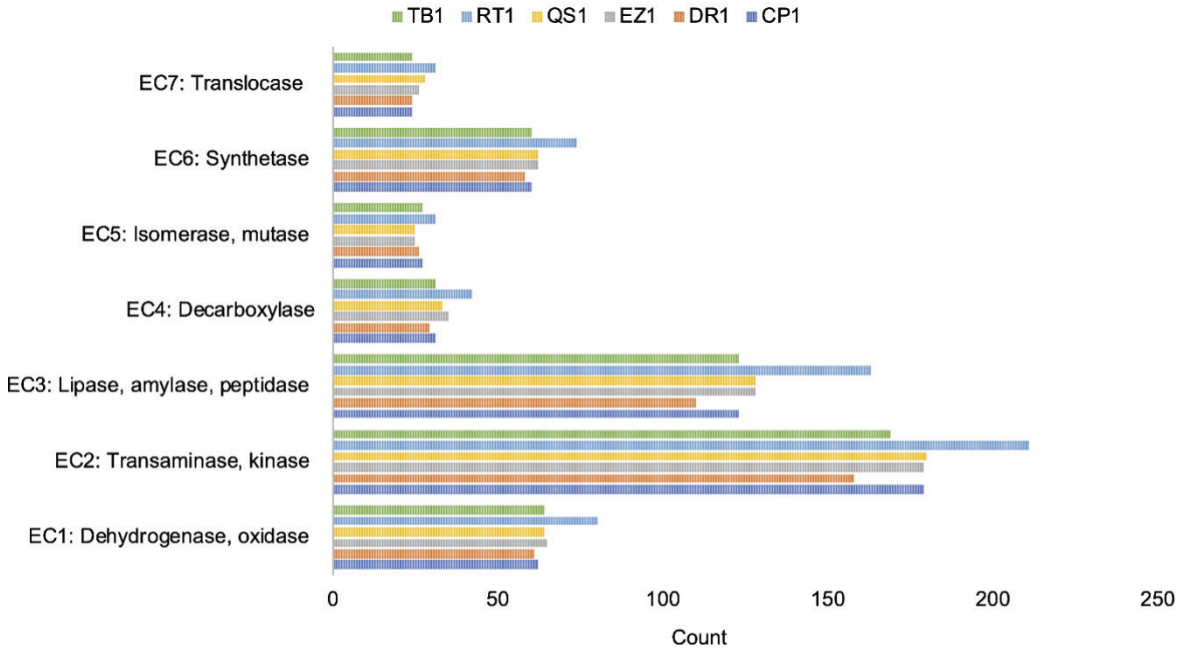



**Figure 4.** Distribution of predicted enzyme-coding genes by enzyme commission (EC) class across six *Bartonella* isolates from Thailand.

***Predicted antimicrobial resistance-associated genes***

Putative antimicrobial resistance-associated genes were predicted in the examined *Bartonella* genomes based on whole-genome sequencing data, with a range of resistance determinants and mechanisms observed across *QS1, CP1, EZ1, RT1, TB1*, and *DR1*. These genes were predominantly associated with antibiotic inactivation and were generally detected as protein homologs predicted from whole-genome sequencing data (Table 4).

In QS1, the aminoglycoside resistance gene *ANT(6)-Ia* was detected, consistent with enzymatic antibiotic inactivation as the underlying mechanism. In CP1, multiple beta-lactam resistance genes, including *OXA-290* and *SGM-6*, were identified and are predicted to confer resistance to carbapenems, cephalosporins, and penams via antibiotic inactivation.

In EZ1, the *catII* gene, associated with resistance to phenicol antibiotics, was detected together with *mecI,* which is linked to resistance to penams within the beta-lactam class; both genes are implicated in antibiotic inactivation mechanisms. Sample RT1 harbored the greatest diversity of resistance genes, including *BRO-1*, *ESP-1*, *OXA-447*, *SGM-7*, *BSU-1*, and *CSP-1*, all associated with beta-lactam resistance against carbapenems and cephalosporins, as well as dfrK, which mediates resistance to diaminopyrimidines through antibiotic target replacement.

In TB1, the OXA-308 gene, associated with beta-lactam resistance, and tet(Q), associated with tetracycline resistance, were identified; both are linked to antibiotic target replacement mechanisms. In DR1, OXA-900 was detected and is associated with beta-lactam resistance mediated by antibiotic inactivation.

**Table 4.** Predicted antimicrobial resistance-associated determinants in *Bartonella* spp. identified from whole-genome sequencing data.

| Sample | Gene | **Drug class** (specific) | High-level drug class | Mechanism | Model |
|---|---|---|---|---|---|
| QS1 | *ANT(6)-Ia* | Aminoglycoside antibiotic | Aminoglycoside antibiotic | Antibiotic inactivation | Protein homolog |
| CP1 | *OXA-290* | Carbapenem; cephalosporin; penam | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | *SGM-6* | Carbapenem | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| EZ1 | *catII* | Phenicol antibiotic | Phenicol antibiotic | Antibiotic inactivation | Protein homolog |
| | *mecI* | Penam | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |

**Table 4.** Predicted antimicrobial resistance-associated determinants in *Bartonella* spp. identified from whole-genome sequencing data. (continue)

| Sample | Gene | Drug class (specific) | High-level drug class | Mechanism | Model |
|---|---|---|---|---|---|
| RT1 | *BRO-1* | Penam | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | *ESP-1* | Carbapenem | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | *OXA-447* | Carbapenem; cephalosporin; penam | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | *SGM-7* | Carbapenem | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | *BSU-1* | Carbapenem | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | *CSP-1* | Cephalosporin | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | *dfrK* | Diaminopyrimidine antibiotic | Diaminopyrimidine antibiotic | Antibiotic target replacement | Protein homolog |
| TB1 | OXA-308 | Carbapenem; cephalosporin; penam | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |
| | Tet(Q) | Tetracycline antibiotic | Tetracycline antibiotic | Antibiotic target replacement | Protein homolog |
| DR1 | OXA-900 | Carbapenem; cephalosporin; penam | Beta-lactam antibiotic | Antibiotic inactivation | Protein homolog |

## Discussion

In this study, the draft genome sizes of six *Bartonella* isolates ranged from 1.52 to 2.57 Mb, with GC contents between 36.97% and 38.79%. The largest assembly was observed in RT1 (*B. grahamii*; 2.57 Mb), followed by EZ1 (*B. kosoyi*; 2.17 Mb), QS1 (*B. tribocorum*; 2.07 Mb), TB1 (*B. vinsonii*; 1.98 Mb), CP1 (*B. vinsonii*; 1.75 Mb), and DR1 (*B. schoenbuchensis*; 1.52 Mb). Compared with previous studies, this genome size range is broadly consistent with those reported for other *Bartonella* species, although some variation is evident. Wang et al. (2025) reported genome sizes of bat-borne *Bartonella* spp. ranging from 1.39 to 1.98 Mb, with GC contents of 35.3%-38.92%, [26] which overlaps with the lower end of our isolates, such as CP1 and DR1. In contrast, our RT1 isolate *(B. grahamii)* had a larger assembled genome (2.57 Mb) than the *B. grahamii* from pikas described by Liu et al. (2025), which had a genome size of 2.19 Mb and a GC content of 38.03%, suggesting potential strain-specific genome expansion. [27]

The genome sizes of *B. henselae* strains from shelter cats in Osaka, Japan, ranged narrowly from 1.83 to 1.85 Mb, with GC contents of 37.9%-38.0%, placing them in the mid-range of our isolates, such as QS1 and TB1. [28] Similarly, *B. quintana* isolated from a Japanese macaque had a genome size of 1.58 Mb and a GC content of 38.8%, comparable to DR1 in our study. [29] Taken together, these comparisons indicate that, although GC content among *Bartonella* species tends to remain relatively stable (approximately 36%-39%), genome sizes can vary considerably among species and strains.

The assembled genome observed in some of our isolates, particularly RT1 (*B. grahamii*), has a relatively high estimated contamination of RT1 (22.5%), which represents a limitation when interpreting its genome size, CDS number, and AMR gene diversity. Although RT1 showed 100% estimated genome completeness, contamination may have added sequences and genes to the assembly. Therefore, the higher genome size, CDS count, and diversity of putative antimicrobial resistance-associated genes observed in RT1 should be interpreted cautiously and may not necessarily reflect the characteristics of the target organism alone.

To the best of current knowledge, this study is the first report from Thailand to describe antimicrobial resistance-associated genes in *Bartonella spp.* isolates. This finding expands the current understanding of *Bartonella* epidemiology in Thailand, which has largely focused on reservoir hosts, zoonotic transmission, and molecular detection, with very limited information on the resistome of circulating strains. The identification of ANT(6)-Ia in QS1 is of particular interest because aminoglycosides, especially gentamicin in combination with doxycycline, remain important components of

treatment regimens for severe *Bartonella* infections such as bacteremia and infective endocarditis.[30,31] Likewise, the detection of Tet(Q) in TB1 is potentially relevant because doxycycline and other tetracyclines are among the most frequently recommended agents for Bartonellosis. In contrast, catII and dfrK suggest additional potential for resistance to phenicols and diaminopyrimidines.[30,31]

Several isolates carried predicted beta-lactam resistance-associated determinants, including OXA-290 and SGM-6 in CP1, OXA-308 in TB1, OXA-900 in DR1, and multiple beta-lactamase-related determinants in RT1. Although beta-lactams are not first-line therapy for Bartonella infection, these determinants suggest that *Bartonella* spp. may contribute to a broader antimicrobial resistance gene pool at the human-animal-environment interface. This observation should be interpreted with caution, as previous in vitro studies have generally reported low MICs for several agents active against Bartonella, particularly macrolides, doxycycline, rifampicin, and selected aminoglycosides. In contrast, more recent studies have shown that antimicrobial activity can vary substantially across culture conditions and may not fully predict intracellular persistence or treatment response.[30,32,33] In addition, molecular studies in *B. bacilliformis* have demonstrated that specific target-site mutations and other genetic changes can mediate resistance to several drug classes, highlighting that the presence and evolution of resistance determinants in *Bartonella spp.* is biologically plausible.[34]

Although the six genomes are insufficient for definitive marker development, they provide a preliminary reference dataset for identifying candidate conserved and variable loci through comparison with publicly available *Bartonella* genomes. These candidate targets should be validated using a larger collection of isolates and field samples before implementation in molecular detection and surveillance.

Therefore, the present findings should be regarded as genomic evidence of putative resistance determinants rather than confirmed phenotypic resistance. Although phenotypic antimicrobial susceptibility testing was not performed, these findings provide an important early signal that *Bartonella* spp. circulating in Thailand may harbor diverse resistance-associated genes. Further susceptibility testing, expression analysis, comparative genomics, and functional studies are needed to determine whether these genomic determinants contribute to antimicrobial resistance in *Bartonella* spp.[30-31, 33-34]

### *Nucleotide sequence accession numbers*

Genome sequences were uploaded as BioProject PRJNA1469401. The raw data were deposited in the NCBI/SRA database under the BioSample accession numbers SAMN60356737 (CP1), SAMN60356738 (DR1), SAMN60356739 (EZ1), SAMN60356740 (QS1), SAMN60356741 (RT1), and SAMN60356742 (TB1).

## Limitations

This study included only six *Bartonella* isolates from selected rodent and deer hosts, limiting the generalizability of the findings and comparisons across host species and geographic regions. Species-level assignments remained preliminary because they were based on sequence similarity to reference genomes rather than definitive whole-genome taxonomic validation. In addition, the high estimated contamination in the RT1 assembly (22.5%) may have inflated its genome size, coding sequence count, and diversity of predicted antimicrobial resistance-associated determinants. Resistance-associated annotations were based on computational predictions of protein homologs and were not supported by phenotypic susceptibility testing or functional validation. Therefore, these findings should not be interpreted as evidence of confirmed antimicrobial resistance.

## Future study

Future studies should include larger collections of Bartonella isolates from diverse hosts and geographic regions to better characterize genomic diversity and the distribution of putative resistance determinants in Thailand. Further purification and resequencing of RT1 are needed to resolve potential contamination. Whole-genome taxonomic analyses should also be performed to establish species-level assignments. Phenotypic antimicrobial susceptibility testing, together with gene expression and functional analyses, is required to determine whether the predicted determinants contribute to antimicrobial resistance.

## Practical implications

The draft genomes generated in this study provide a preliminary resource for comparative genomic analyses and for identifying candidate molecular markers for Bartonella detection and surveillance in Thailand. Following validation in larger isolate collections and field samples, these markers may support surveillance at the human-animal interface within a One Health framework. The predicted resistance-associated determinants also identify priorities for confirmatory laboratory investigations; however, they should not be used alone to guide antimicrobial treatment decisions.

## Conclusion

This study provides preliminary genomic characterization of six Bartonella isolates recovered from rodents and a deer in Thailand, revealing variation in genome features, gene content, and putative antimicrobial resistance-associated determinants. Interpretation is constrained by unresolved species-level assignments, substantial estimated contamination in RT1, and the absence of phenotypic and functional validation. Nevertheless, these genomic data provide a foundation for further comparative studies and for identifying candidate targets for molecular surveillance. Additional genomic validation and antimicrobial

susceptibility testing are required to establish the taxonomic and biological significance of the findings.

## Ethical approval

The study was approved by the Research Ethics Committee, Department of Medical Sciences, Ministry of Public Health (Protocol No. DMSc-EC014; approved 20 April 2024).

## Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used Perplexity (Perplexity AI) to assist with drafting and English-language editing, and Grammarly to assist with grammar, spelling, and punctuation checks. The authors subsequently reviewed and revised all AI-assisted content, verified its accuracy against the study data and cited sources, and take full responsibility for the final content of the manuscript.

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## Conflict of interests

The authors declare no conflicts of interest relevant to the content of this article.

## CRediT authorship contribution statement

**Decha Pangjai:** conceptualization, methodology, investigation, formal analysis, writing-original draft, review and edit; **Dararat Bangdee**: investigation, resources; **Ratana Tacharoenmuang**: resources, supervision, writing-review and edit; **Sittiporn Parnmen**: formal analysis, writing-original draft, review and edit; **Archawin Rojanawiwat**: supervision, writing-review and edit.

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