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First complete genome sequence of *Bordetella bronchiseptica* from a canine respiratory infection in Vietnam

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Abstract

Bordetella bronchiseptica is an important bacterial agent associated with canine infectious respiratory disease complex (CIRDC). Beyond its role in canine respiratory disease, *B. bronchiseptica* is an opportunistic pathogen capable of causing respiratory infections and, in severe cases, systemic disease in susceptible humans. However, genomic information on *B. bronchiseptica* from Vietnam remains unavailable, with no complete or draft genome sequence have been previously reported. In this study, a hybrid genome assembly strategy integrating short-read and long-read sequencing was used to generate the complete genome of *B. bronchiseptica* strain SG2023, isolated from a dog showing clinical signs of respiratory disease at a veterinary clinic in Ho Chi Minh City, Vietnam. The assembled genome consisted of a single circular chromosome of approximately 5.3 Mbp and contained two antimicrobial resistance-associated genes, including the acquired *sul2* gene and the intrinsic bla_{BOR-1} β-lactamase gene, and the conserved *bvgAS* virulence regulatory system. A distinctive chromosomal locus comprising three tandemly arranged homologous phage-related regions was also identified. Comparative genomic analysis showed that this arrangement was not observed among the examined *B. bronchiseptica* reference genomes. In addition, whole-genome phylogenetic analysis of 30 genomes, comprising 29 *B. bronchiseptica* strains and one *B. pertussis* outgroup, placed SG2023 within the mammalian-associated lineage and placed it closest to strain 253. This study provides the first complete genome reference of *B. bronchiseptica* from Vietnam and expands the genomic resources available for comparative, epidemiological, and evolutionary studies of this pathogen.

Introduction

Canine infectious respiratory disease complex (CIRDC) in dogs is a contagious respiratory disease associated with a range of bacterial and viral pathogens. *Bordetella bronchiseptica* is a respiratory pathogen capable of

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colonizing a wide range of mammalian hosts, including dogs, cats, pigs, rabbits, and humans, and is one of the bacterial agents classically implicated in CIRDC, also known as kennel cough, which is characterized by cough, nasal discharge, and, in severe cases, pneumonia (1–3). In other companion and domestic animal populations, *B. bronchiseptica* has similarly been associated with respiratory tract infections ranging from bronchitis to severe pneumonia, including cases reported in intensive care settings. Although human infections are relatively uncommon, several case reports have suggested zoonotic transmission, with human infections occurring in individuals with a history of close contact with dogs or other companion animals (4–6).

B. bronchiseptica's capacity to cause infection is related to a variety of adhesins, toxins, and secretion systems that play roles in host colonization, immune modulation, and tissue destruction. Many of these virulence factors are expressed under the control of the *bvgAS* two-component regulatory system, which controls the switch between the virulent and avirulent phenotypes (7). In addition, the classical *Bordetella* species (*B. bronchiseptica*, *B. pertussis*, and *B. parapertussis*) form a closely related evolutionary lineage, with *B. pertussis* and *B. parapertussis* believed to have emerged independently from *B. bronchiseptica*-like ancestors through host adaptation and genome reduction (8). High-quality complete genome sequences therefore provide important references for comparative genomic analyses and for resolving evolutionary relationships within the *Bordetella* genus.

Although *B. bronchiseptica* has been reported in animals from many regions worldwide, genomic data from Vietnam are currently unavailable. The absence of local genome sequences limits comparisons between Vietnamese isolates and the broader global population of the species. To address this gap, the present study generated a complete genome sequence of a *B. bronchiseptica* isolate recovered from a dog with respiratory disease in Vietnam. The assembled genome was annotated and subsequently analyzed using comparative genomic and phylogenomic approaches to place the isolate within the genomic diversity of *B. bronchiseptica*.

Materials and Methods

Isolation

A nasal mucus swab sample was collected from a dog showing clinical signs consistent with CIRDC (coughing and nasal discharge) by a veterinarian in January 2023. The sample was streaked onto MacConkey agar and incubated for 24 hours at 37°C, yielding colonies with morphology characteristic of *B. bronchiseptica*. A single such colony, designated strain SG2023, was selected and subjected to PCR using primers specific for *B. bronchiseptica* (9).

Sequencing

The single colony of *B. bronchiseptica* strain SG2023 on MacConkey agar was sub-cultured in Brain Heart Infusion medium overnight at 37°C to obtain sufficient biomass for DNA extraction, and genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA). Subsequently, genomic DNA was subjected to short-read sequencing using the DNBSEQ-G400RS system (MGI, China) and to long-read sequencing using a MinION device with an R9 flow cell (Oxford Nanopore Technologies Ltd, UK). Long-read base calling was performed using Guppy v3.1 to convert the raw signals into FASTQ format.

Genome assembly

Raw short- and long-read sequencing data were processed following the Trycycler assembly workflow (10). Short reads were first quality-filtered using fastp v0.24 (11), whereas long reads were subsampled with Trycycler v0.5.5 using the parameters --genome-size 5.3m and --min_read_depth 15.7×. Independent long-read assemblies were generated using Flye v2.9.5 with the --nano-hq option (12), Raven v1.8.3 with --disable-checkpoints (13), and miniasm_and_minipolish.sh v0.3 with default settings. The resulting assemblies were subsequently processed through the Trycycler workflow, including clustering, reconciliation, multiple sequence alignment, partitioning, and consensus generation under default parameters. Consensus polishing was performed using Medaka v1.12.1 with the model r1041_e82_400bps_fast_g632 (14), followed by short-read polishing using Polypolish v0.6.0 in combination with BWA v0.7.8-r1243 under default settings (15).

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In silico taxonomic validation

Following quality trimming, short reads were subjected to taxonomic classification using Kraken2 v2.1.3 to assess potential contamination and perform preliminary taxonomic identification (16). A sample was considered potentially contaminated if any taxon outside the *Bordetella* lineage accounted for more than 1% of the classified reads. The complete genome sequence was submitted to PubMLST for multilocus sequence typing (MLST) analysis. Average nucleotide identity (ANI) analysis was subsequently performed using PyOrthoANI (17) to compare the genome against *B. bronchiseptica* strain 253, *Bordetella pertussis* strain H640, *B. parapertussis* strain FDAARGOS_1541, and *B. holmesii* strain FDAARGOS_1539 (a negative control).

Genome annotation and visualization

The complete genome sequence was annotated using Bakta v1.9.4 with database v5.1 (18). Antimicrobial resistance genes were identified using ABRicate with the CARD database (19) using minimum thresholds of 90% sequence identity and 60% sequence coverage (20) and AMRFinderPlus with database v3.12 (21). Virulence-associated genes were screened using the VFAnalyzer tool (22) available through the Virulence Factor Database (VFDB) web interface (accessed in May 2026). Circular chromosome visualization was performed using Proksee (23), and selected genomic features, including antimicrobial resistance genes, the *bvgAS* virulence regulatory system, and phage-related regions, were manually annotated for display.

The most closely related reference sequence was identified by BLASTn search against the NCBI nucleotide database, and protein sequences extracted from its GenBank annotation file were subsequently supplied to Bakta using the --proteins option to improve gene annotation of the phage-related regions. Comparative visualization was performed using clinker v0.0.32, and genes were grouped into homologous clusters using a minimum sequence identity threshold of 80% (24).

Phylogeny

A phylogenetic tree based on whole-genome data of 29 *B. bronchiseptica* strains (Table S1) was constructed using kSNP4.1 (25). The resulting SNP matrix was subsequently analyzed with IQ-TREE v2.4.0 to build a maximum-likelihood phylogenetic tree (26) with 1000 ultrafast bootstraps replicates (27) and TVM+F+ASC+R5 model as identified by ModelFinder (28). The resulting tree was visualized and annotated using iTOL v7.6 (https://itol.embl.de/).

Results

Clinical presentation and pathogen confirmation

The dog from which strain SG2023 was isolated was a 7 kg male French Pug that presented with a 3-day history of coughing and clear nasal discharge. Auscultation revealed wheezing (rhonchi), with paroxysmal coughing and expectoration of mucous secretions. Rectal temperature ranged from 38–39°C. The dog had previously been vaccinated with a heptavalent vaccine (Recombitek® C7, which does not include *Bordetella* antigen). No prior antimicrobial or corticosteroid therapy had been administered before presentation. Thoracic radiography and a complete blood count/serum biochemistry analysis were not performed as part of the clinical workup. The dog was treated with cefotaxime (20 mg/kg), prednisone (0.5 mg/kg), furosemide (1 mg/kg), and bromhexine (1 mg/kg), and recovered fully after 10 days of treatment. In addition to bacterial culture, PCR testing of the clinical sample was positive for a target band specific to *B. bronchiseptica* (data not shown), supporting the presence of this organism in the clinical specimen.

The complete genome of Bordetella bronchiseptica strain SG2023

Kraken2 analysis identified *B. bronchiseptica* as the predominant species-level classification, accounting for 23.9% of the total reads, whereas *B. parapertussis* and *B. pertussis* each represented less than 1%. As no taxon

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outside the *Bordetella* lineage accounted for more than 1% of the classified reads, no read filtering was performed prior to genome assembly. ANI analysis further demonstrated the highest nucleotide identity between SG2023 and *B. bronchiseptica* strain 253 (99.976%), supporting the assignment of SG2023 to *B. bronchiseptica*.

The complete genome of the isolate was 5,335,397 bp in length, consisting of a single circular chromosome, with a GC content of 68.03 %. The final assembled genome was supported by a sequencing depth of 104× for short reads and 77× for long reads. This strain was identified as sequence type 27 (ST27) and annotated to contain 5,023 coding sequences, 9 rRNA genes, 67 tRNA genes, and 1 tmRNA. In addition, key genes commonly identified in the genome of *B. bronchiseptica* SG2023, such as the *bvgAS* regulatory system and antibiotic resistance genes, along with a three-copy tandem repeat of an incomplete phage-related region were identified in the assembled genome (Figure 1).

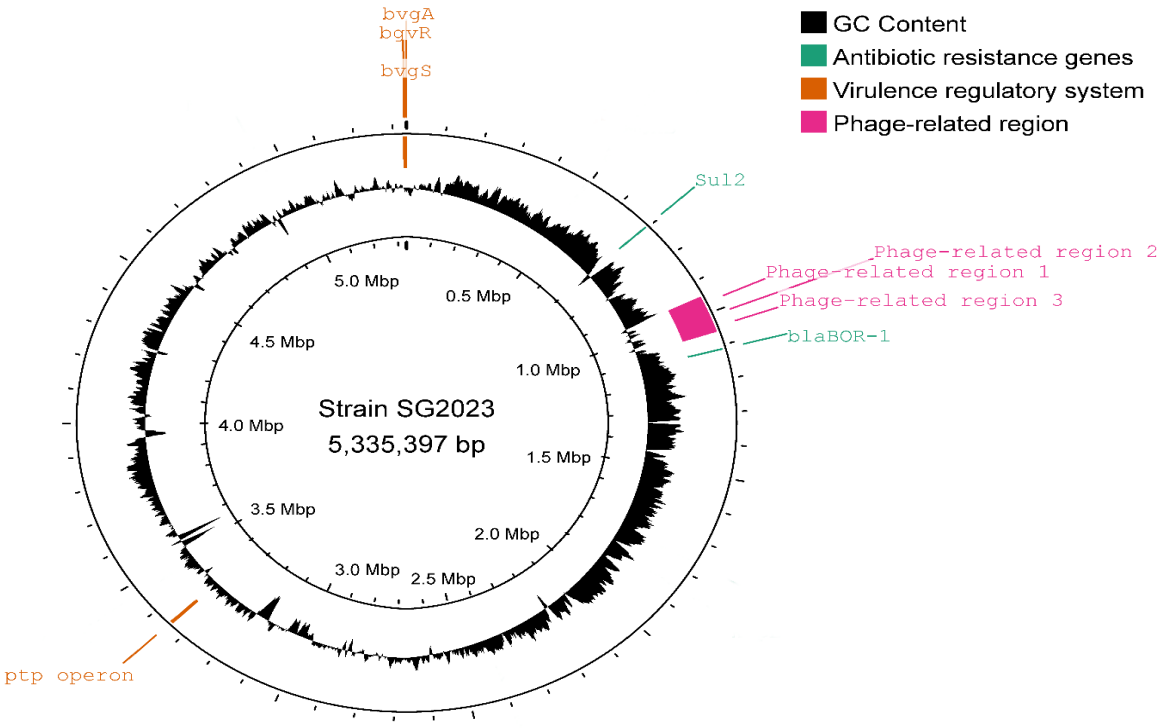


Fig. 1. Genomic features of *Bordetella bronchiseptica* strain SG2023. The figure was generated using Proksee (23).

Unique tandem homologous phage-related regions

Genome analysis of strain SG2023 identified a unique locus comprising three tandemly arranged homologous phage-related regions with a total length of 111,901 bp. The three regions were located at positions 947,031-989,600; 989,601- 1,029,160; and 1,029,161-1,058,930 on the chromosome, respectively. Comparative analysis revealed extensive sequence similarity among the three regions, with most homologous ORFs sharing nucleotide identities ranging from approximately 80% to <100%. However, the regions were not identical and differed in both gene content and organization. Regions 1 and 2 (42,569 bp and 39,559 bp, respectively) contained a relatively complete set of genes considered essential components of a prophage, including phage lysozyme and holin (lysis-associated genes), capsid structural genes, and enzymes involved in DNA replication and packaging, and were therefore predicted to represent candidate prophages. Region 3 (29,769 bp) was

approximately 10 kb shorter than Regions 1 and 2 and lacked genes encoding DNA replication and packaging enzymes; therefore, it was not predicted to represent a complete prophage. Several non-homologous ORFs were present between conserved phage-associated genes, and minor variations in ORF arrangement were observed among the three copies (Fig. 2A). No known *Bordetella*-associated virulence factor or toxin-encoding gene was identified within these regions.

BLAST analysis of the entire locus against the NCBI database did not identify any bacterial genome containing an equivalent structure composed of three adjacent homologous phage-related regions. The closest bacterial match was *B. bronchiseptica* strain 253, which contained several scattered regions homologous to individual ORFs within the SG2023 phage-related locus. Although the identities of these ORF-level matches exceeded 90%, no single region in strain 253 showed full-length coverage of an entire SG2023 phage-related region, and the homologous ORFs were located at distant, non-adjacent positions across the chromosome, in a rearranged order, rather than forming a triplicated tandem arrangement. Among viral sequences, the highest-scoring match was *Bordetella* phage vB_BbrP_003, with multiple ORFs sharing more than 80% sequence identity with SG2023; however, the homologous sequences spanned only approximately 10 kb of the more than 42 kb total length of the phage-related region (Fig. 2B). Given this high identity but limited coverage relative to the full length of the element, this locus is considered to represent a phage-related element distinct from, but related to, vB_BbrP_003. These results indicate that the tandem organization of three homologous phage-related regions observed in SG2023 is distinct from that observed in currently available *Bordetella* genomes and phage sequences in public databases.

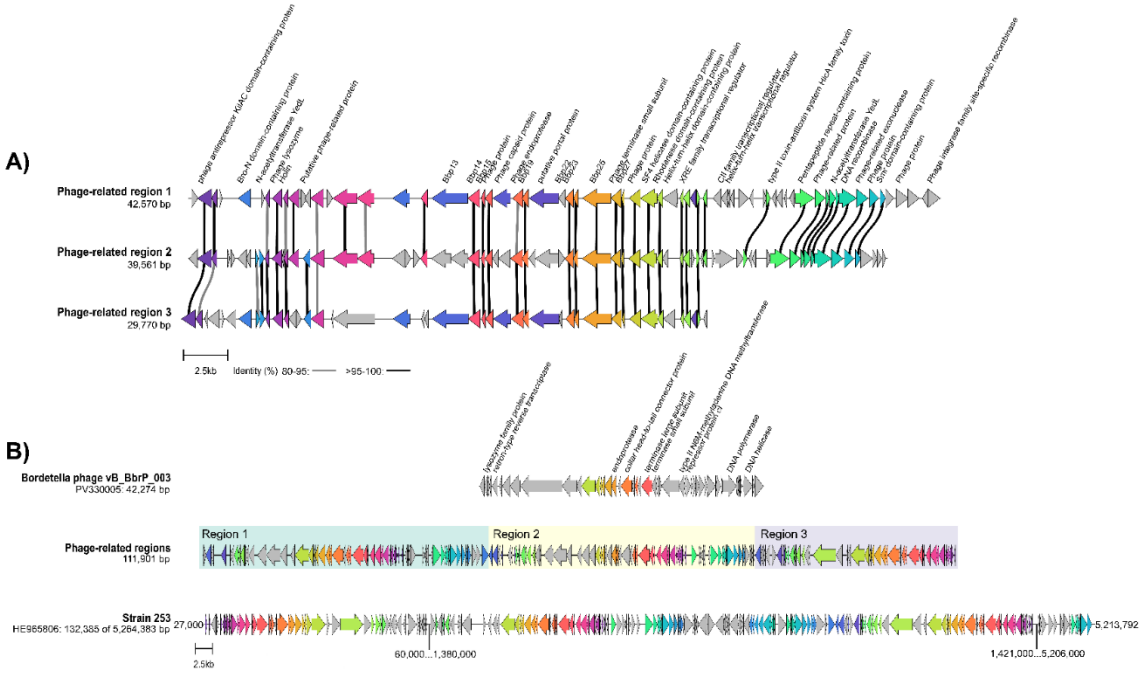


Fig. 2. Organization and comparative analysis of the tandem homologous phage-related regions identified in strain SG2023. A) Gene organization and sequence comparison among the three tandemly arranged homologous phage-related regions. B) Comparison of the entire tandem phage-related locus with the closest matches identified by NCBI BLAST analysis. Homologous ORFs sharing ≥80% sequence identity are grouped and displayed using the same color. The figure was generated using clinker v0.0.32 (24).

Antibiotic resistance genes and virulence factor identification

A total of two antimicrobial resistance genes were identified in the SG2023 genome (Table 1). The *sul2* gene, associated with resistance to sulfonamides, was identified with 100% sequence identity and 100% query coverage. The intrinsic β-lactamase gene bla_{BOR-1}, commonly reported in *B. bronchiseptica*, was also detected with 99.89% sequence identity and complete query coverage. No plasmids were identified in the SG2023 genome, suggesting that the detected resistance genes were chromosomally encoded.

Table 1. List of antimicrobial resistance genes.

Gene	Start	End strand	%Coverage	%Identity	Class
sul2	694960	695775	100.00	100.00	Sulfonamide
bla_{BOR-1}	1111623	1112540	100.00	99.89	Beta-lactamase

Screening against the VFDB revealed that strain SG2023 possessed a virulence gene repertoire largely consistent with that reported for classical *Bordetella* species, including *B. pertussis* and *B. parapertussis*. Most major virulence-associated loci involved in host colonization, immune modulation, and regulatory functions were conserved in the SG2023 genome, including the *bvgAS* regulatory system and multiple adhesin- and secretion-associated genes identified based on sequence homology (Table S2).

Despite this overall conservation, several virulence-associated genes present in other *Bordetella* genomes were not detected in SG2023. These included *fhaS* and *fhaL*, encoding FHA-like proteins; *fimA*, associated with fimbrial structures; *wbmP*, involved in lipopolysaccharide biosynthesis; *fliK*, associated with flagellar assembly; *ptlI*, of the Ptl type IV secretion system; and *cyaA*, encoding adenylate cyclase toxin (Table S2).

Previous studies have suggested that the *ptp* locus may be associated with alternative toxin secretion mechanisms in *Bordetella*. The absence of *cyaA* was consistent with observations reported for *B. bronchiseptica* strain 253 (7). Manual BLAST analysis identified the presence of the *ptp* operon in SG2023, with a genomic organization similar to that of strain 253 (Fig. 1).

Whole-genome phylogenetic analysis of Bordetella bronchiseptica

The phylogenetic tree was constructed based on the complete genomes of 30 *Bordetella* strains, including 29 *B. bronchiseptica* and the *B. pertussis* strain H640 as outgroup strain. The phylogeny revealed two major clades: Group I, consisting of isolates from humans and chickens, and Group II, consisting of isolates from mammals such as humans, dogs, pigs, cats, and rabbits. Strain SG2023 clustered within Group II and formed a subclade with *B. bronchiseptica* strain 253, representing its closest phylogenetic neighbor among the genomes included in this study (Fig. 3). This relationship was supported by a short branch length separating the two genomes, indicating a high degree of genomic similarity.

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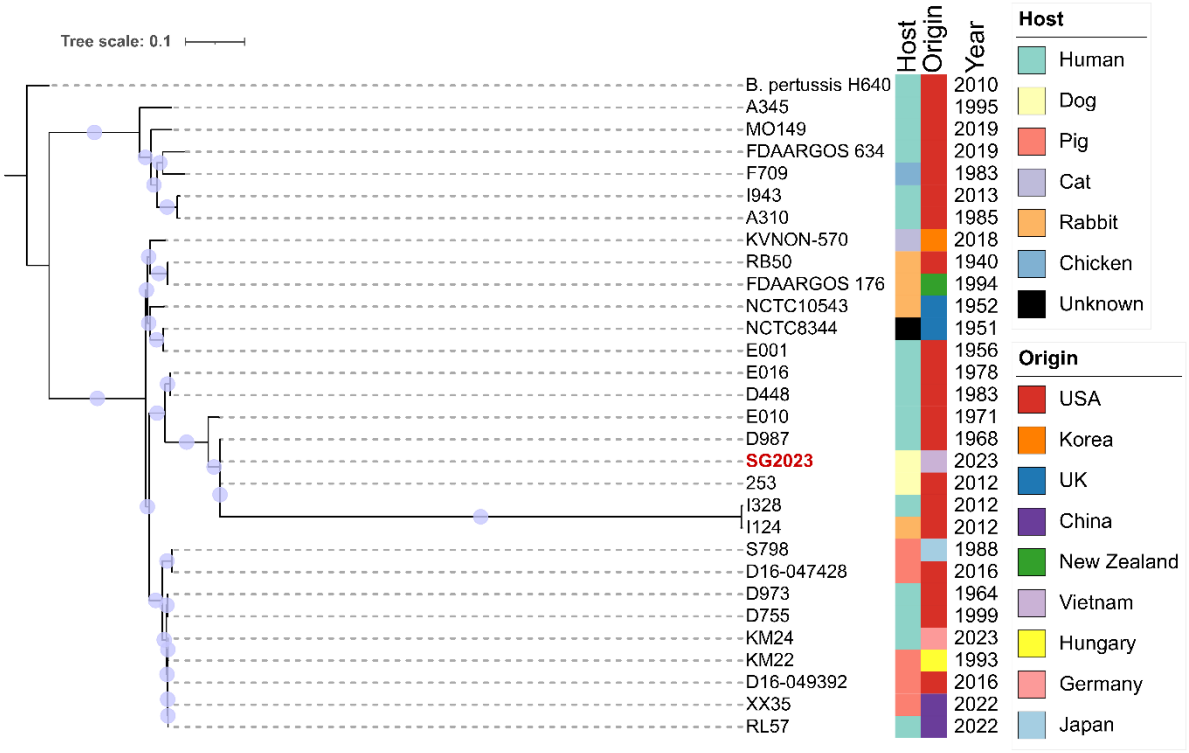


Fig. 3. Maximum-likelihood phylogeny of 29 *Bordetella bronchiseptica* genomes based on whole-genome alignment. *B. pertussis* strain H640 was included as the outgroup. Circles on branches indicate ultrafast bootstrap support values >70. Strain SG2023 identified in this study is highlighted with a red. The figure was generated using the iTOL online tool v7.6 (https://itol.embl.de).

Discussion

It should be noted that, as no additional diagnostic testing (e.g., thoracic radiography, complete blood count, or screening for other canine respiratory pathogens) was performed at the clinic, this case cannot be conclusively attributed to *B. bronchiseptica* alone, and a multifactorial etiology cannot be excluded. The detection of *B. bronchiseptica* by both culture and PCR supports its presence in the respiratory tract of this dog; however, whether SG2023 represents the primary causative agent or a co-colonizing organism, and whether it exhibits a typical or atypical virulence phenotype relative to other circulating strains, remains to be established through further clinical and experimental investigation.

MLST analysis assigned SG2023 to ST27, a sequence type that has previously been reported among *B. bronchiseptica* strains isolated from both animals and humans. Isolates sharing the same sequence type are expected to possess a closely related genetic background. Therefore, SG2023 may share genomic features with ST27 strains isolated from human infections (29).

Among the antimicrobial resistance genes identified in SG2023, *sul2* and $bla_{\text{BOR-1}}$ were the two genes meeting the applied identity/coverage threshold (Table 1). These two genes differ in their likely origin: $bla_{\text{BOR-1}}$ is an intrinsic, chromosomally encoded β-lactamase gene characteristic of *B. bronchiseptica*, whereas *sul2* is more consistent with an acquired resistance gene (30). Both genes have previously been reported in a genomic survey of *B. bronchiseptica* isolates from swine in the United States, suggesting that they are not restricted to a single host population (31). The presence of *sul2* is of particular interest because sulfonamides, often administered in combination with trimethoprim, are commonly used in the treatment of canine respiratory infections, including kennel cough (32); therefore, the detection of *sul2* may be associated with reduced susceptibility to this

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antimicrobial class. It should be noted that antimicrobial susceptibility testing was not performed for SG2023, and both genes reported here were identified based on genotypic prediction only; phenotypic confirmation (e.g., MIC testing) is required to establish clinically relevant resistance.

The genome of SG2023 did not contain the *cyaA* gene encoding adenylate cyclase toxin (Table S2), a virulence factor that inhibits host phagocytic cells. It is known that *B. bronchiseptica* strains that lack the *cya*A gene, including strain 253, are less virulent than strains that contain the gene, such as RB50 (33, 34). Thus, the absence of *cyaA* in SG2023 would be consistent with a potentially reduced virulence phenotype. Several other virulence-associated genes present in other *Bordetella* genomes, including *fhaS*, *fhaL*, *fimA*, *wbmP*, *fliK*, and *ptlI*, were also not detected in SG2023 based on VFDB homology screening. These non-detections should be interpreted with caution, as several of these loci are known to exhibit high sequence polymorphism among *Bordetella* strains, which may limit their detection by homology-based approaches rather than reflecting a true absence or loss of function (35). Notably, *fhaB*, encoding the principal filamentous hemagglutinin adhesin, and other core flagellar genes were conserved in SG2023, suggesting that the adhesion and motility machinery is largely intact despite the absence of the accessory paralogs *fhaS*, *fhaL*, and *fliK* (36, 37). Similarly, the absence of *ptlI*, a component of the Ptl type IV secretion system responsible for pertussis toxin secretion (38), is consistent with the general lack of a functional pertussis toxin in *B. bronchiseptica*, distinguishing it from *B. pertussis*, and is therefore unlikely to represent a meaningful reduction in the species' virulence potential. It should be noted that the virulence gene repertoire identified in this study, including the absence of *cyaA* and the other loci discussed above, was based on sequence homology to reference genes in the VFDB and does not confirm that these genes are actively expressed or functionally active in SG2023. Experimental validation, such as gene-expression analyses or phenotypic assays, would be required to establish the functional relevance of these findings to the clinical phenotype of SG2023.

One of the remarkable features of the SG2023 genome was the presence of a tandemly repeated phage-related region consisting of three consecutive copies (Fig. 2), two of which (Regions 1 and 2) are predicted to represent candidate prophages based on their relatively complete complement of phage-associated genes, whereas the third (Region 3) is truncated and lacks a comparable gene set. We retain the general term “phage-related region” for all three copies, including Regions 1 and 2, as confirming that these regions are bona fide prophages would require demonstrating their capacity to exist independently of the host chromosome (e.g., through induction or excision assays), which was not performed in this study. Phages and phage-derived elements are important in the diversification of bacterial pathogen genomes through horizontal gene transfer and genomic rearrangements. The presence of three copies of a similar phage-related sequence adjacent to each other suggests that this region might have been locally duplicated following integration into the chromosome. Alternatively, the repeated structure may be a remnant of historical recombination events involving phage-derived DNA (39).

The biological significance of this repeated element remains unclear. Repeated phage-derived sequences have been proposed to influence genome plasticity by providing homologous substrates for recombination, potentially contributing to structural variation during evolution (40). However, this interpretation remains speculative in the absence of experimental or comparative evidence (e.g., recombination assays or structural variation analysis) in the present study. Similar mechanisms have been reported in several bacterial pathogens in which phage-associated repeats facilitate chromosomal rearrangements and gene flux (41–43).

Because the element could not be confidently assigned to a known phage (Fig. 2B) and its activity was not experimentally evaluated, its functional role in SG2023 remains unresolved. Additional comparative genomic analyses involving a larger collection of *B. bronchiseptica* genomes will be required to determine whether this

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structure represents a lineage-specific feature or a more widespread but previously overlooked component of the species pangenome.

A phylogenomic analysis showed that SG2023 was clustered with strain 253, indicating a close evolutionary relationship between the two canine-associated isolates. This clustering pattern is in agreement with previous studies that have reported the division of *B. bronchiseptica* populations into host-associated groups, including one lineage mainly containing mammalian isolates and another lineage with isolates from birds and humans (8, 44, 45). SG2023 was grouped within the mammalian-associated lineage and was most closely related to strain 253 (Fig. 3). The results indicate that SG2023 belongs to a globally distributed canine-associated lineage of *B. bronchiseptica*. However, the current dataset does not provide sufficient evidence to infer the geographic origin of the Vietnamese isolate or to conclude that it directly descended from strain 253.

Conclusion

This study reports the first complete genome of *B. bronchiseptica* from Vietnam. Strain SG2023 harbored bla_{BOR-1} and *sul2*, a conserved virulence gene repertoire, and a unique locus containing three tandem homologous phage-related regions. Phylogenomic analysis placed SG2023 close to strain 253 within the mammalian-associated lineage. This genome provides a reference for future studies of *B. bronchiseptica* epidemiology, antimicrobial resistance, and genome evolution.

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Ethics approval

Not applicable.

Conflict of interest

The authors declare that they have neither financial nor non-financial competing interests.

Artificial Intelligence Statement

The authors confirm that no generative artificial intelligence (AI) tools were used for the writing, or data analysis of this manuscript. AI was utilized for language editing and grammar correction.

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