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Clinical long-read metagenomic sequencing of culture-negative infective endocarditis reveals genomic features and antimicrobial resistance

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Abstract

Background Infective endocarditis (IE) poses significant diagnostic challenges, particularly in blood culture-negative cases where fastidious bacteria evade detection. Metagenomic-based nanopore sequencing enables rapid pathogen detection and provides a new approach for the diagnosis of IE.

Method Two cases of blood culture-negative infective endocarditis (IE) were analyzed using nanopore sequencing with an *in silico* host-depletion approach. Complete genome reconstruction and antimicrobial resistance gene annotation were successfully performed.

Results Within an hour of sequencing, EPI2ME classified nanopore reads, identifying *Corynebacterium striatum* in IE patient 1 and *Granulicatella adiacens* in IE patient 2. After 18 h, long-read sequencing successfully reconstructed a single circular genome of *C. striatum* in IE patient 1, whereas short-read sequencing was used to compare but produced fragmented assemblies. Based on these results, long-read sequencing was exclusively used for IE patient 2, allowing for the complete and accurate assembly of *G. adiacens*, confirming the presence of these bacteria in the clinical samples. In addition to pathogen identification, antimicrobial resistance (AMR) genes were detected in both genomes. Notably, in *C. striatum*, regions containing a class 1 integron and multiple novel mobile genetic elements (IS*Cost1*, IS*Cost2*, Tn*7838* and Tn*7839*) were identified, collectively harbouring six AMR genes. This is the first report

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of such elements in *C. striatum*, highlighting the potential of nanopore long-read sequencing for comprehensive pathogen characterization in IE cases.

Conclusions This study highlights the effectiveness of host-depleted, long-read nanopore metagenomics for direct pathogen identification and accurate genome reconstruction, including antimicrobial resistance gene detection. The approach enables same-day diagnostic reporting within a matter of hours.

Keywords Adaptive sampling, Infective endocarditis, Nanopore, Short-read sequencing, Valve replacement

Background

Infective endocarditis (IE) is a life-threatening disease that has evolved considerably in both host susceptibility and pathogen spectrum, with morbidity and mortality incidence of approximately 6.78 cases per 100,000 person-years [1, 2]. A 10-year retrospective study conducted at Siriraj Hospital, Bangkok, Thailand, from January 2005 to May 2015, reported 5.67 cases per 1,000 admissions with 81.3% involving native valves [3]. Methicillin-susceptible *Staphylococcus aureus* (MSSA) and *Enterococcus* spp. were commonly associated with prosthetic valve endocarditis, whereas viridans group streptococci (VGS) predominated in native valve IE cases at both Siriraj Hospital and Srinagarind Hospital in Khon Kaen, Thailand [3, 4].

The current gold standard for diagnosing IE combines blood cultures with clinical examination and echocardiographic findings, as outlined by the 2023 Duke–International Society for Cardiovascular Infectious Diseases (ISCVID) criteria [5]. However, blood culture-negative endocarditis (BCNE) poses a diagnostic challenge, often caused by fastidious organisms that require specific growth conditions or exhibit slow replication in standard microbiological cultures. Notable pathogens such as *Abiotrophia defectiva*, *Bartonella* spp., *Coxiella burnetii*, *Granulicatella* spp., and *Tropheryma whipplei* frequently escape detection by routine blood cultures. This limitation impedes antimicrobial susceptibility testing and contributes to delayed treatment and increased mortality [6, 7]. Therefore, rapid and accurate detection of pathogens and antimicrobial resistance (AMR) genes from clinical specimens is essential for effective IE management.

Sequencing technologies have emerged as transformative tools for culture-independent microbial identification. Both short-read and long-read metagenomic sequencing have demonstrated utility in rapidly identifying pathogens and predicting AMR, thereby overcoming the limitations of traditional culture-based diagnostics [8–12]. Among these, nanopore long-read metagenomic sequencing offers additional advantages, such as real-time data generation, host DNA depletion via adaptive sampling, and the ability to recover complete bacterial genomes and AMR profiles. This technique has shown promise in other infection models, such as intraamniotic infection [13].

To date, however, there are no reports on the application of nanopore long-read metagenomic sequencing with adaptive sampling for pathogen detection in BCNE. The diagnostic performance and clinical relevance of this method in IE cases remain to be fully explored. Here, we present two BCNE cases in which long-read nanopore sequencing with adaptive sampling enable rapid pathogen identification, genome reconstruction, and resistance gene profiling directly from native valve tissues – highlight the potential of this approach for same-day diagnostics in culture-negative endocarditis.

Methods

Medical records and samples collection

This study investigates two cases of culture-negative infective endocarditis (IE) diagnosed at Siriraj Hospital, Bangkok, Thailand, in November 2023 and June 2024. Patient medical records were obtained during hospitalization. Following aortic valve replacement, the excised valves were subjected to Gram staining, microbiological culture, and metagenomic sequencing. The study was approved by the Institutional Review Board of the Faculty of Medicine at Siriraj Hospital (certificate of approval number Si 768/2025).

Microbiological culture

The native valve samples were processed using standard microbiological culture procedures. The valve tissues were firstly dissected and directly inoculated onto blood agar, chocolate agar, and MacConkey agar. Additionally, the dissected tissue was incubated in tryptic soy broth to monitor turbidity. The culture media (Biomedia, Thailand) were prepared and incubated at 37 °C for 48 h under both aerobic and anaerobic conditions.

Total DNA isolation and metagenomic sequencing

The native aortic valve tissues were collected and frozen at – 80 °C for one hour prior to processing. Samples were then homogenized using a micro tissue homogenizer for DNA extraction. Total DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300, USA) following a previously described protocol (~2 h) [14]. The DNA quality was assessed using a Nanodrop Spectrophotometer (Thermo Fisher Scientific, USA) and concentration was measured by a Qubit®

4.0 Fluorometer (Invitrogen, USA). Nanopore long-read sequencing was performed using 1 µg with the Ligation sequencing gDNA–Native Barcoding Kit 24 V14 (SQK-NBD114.24, Oxford Nanopore Technologies, ONT, UK) to prepare DNA library (~2 h). Approximately 600 ng of the DNA library was then loaded into an R10.4.1 nanopore flow cell (FLO-MIN114, ONT, UK) on a GridION platform (ONT, UK) and sequenced for 18 h. Adaptive sampling in depletion mode was used to selectively deplete human DNA based on the reference genome (T2T CHM13v2.0/hs1, GCF_009914755.1). For short-read sequencing, DNA from IE patient 1 was submitted to Bangkok Genomics Innovation Public Company Limited (BGI, Hong Kong) for sequencing using the DNBSEQ-G400 sequencing with 150-bp paired-end (PE) mode (Fig. 1).

EPI2ME real-time data visualization

For ONT real-time data analysis, sequencing reads were analyzed using the Antimicrobial Resistance v2023.04.26-1808834 workflow via the EPI2ME™ cloud platform (~40 min) [15]. Reads were initially classified using the standard Centrifuge database [16]. Then, AMR profiling was determined by aligning the taxonomically classified reads against all reference sequences available in the Comprehensive Antibiotic Resistance Database (CARD) v11.3 [17] using the Minimap2 program (Fig. 1).

Data processing and whole genome sequence reconstruction

Raw ONT sequencing reads were basecalled using Dorado v0.7.2 (https://github.com/nanoporetech/dorado) with the SUP model v4.3.0. Only reads with a quality score (Q) ≥ 10 were retained. Sequencing adapters were trimmed using Porechop v0.2.4–py38hed8969a_1 (https://github.com/rrwick/Porechop). Host depletion was performed using MinKNOW's adaptive sampling mode, which mapped reads against the human genome (hs1). The system retained or rejected reads in real-time, with decisions logged in the 'adaptive_sampling.csv'

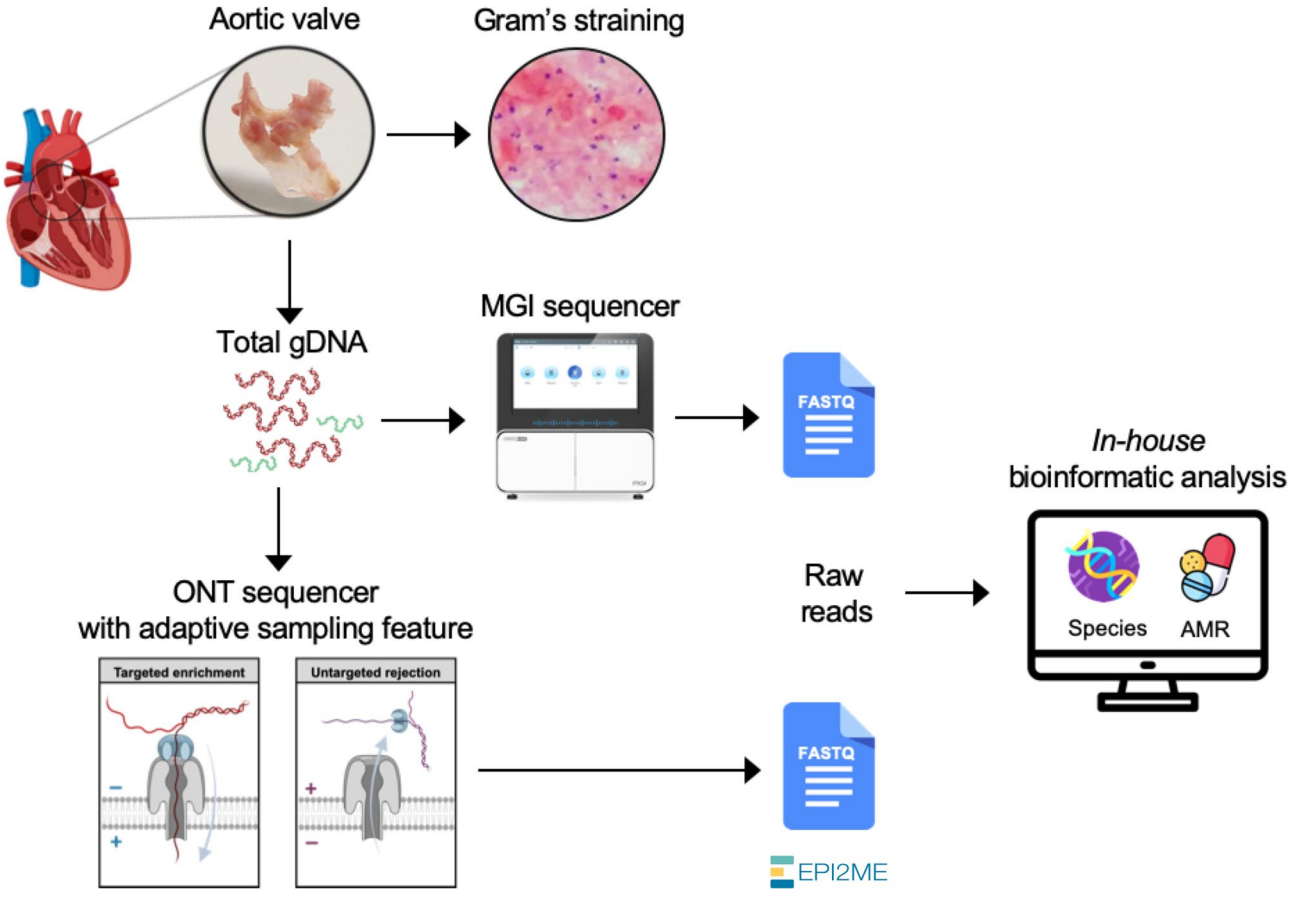

Fig. 1 Schematic representation of the experimental design for pathogen characterization in infective endocarditis cases, from sample collection to species identification and prediction of antimicrobial resistance (AMR) genes: A heart valve was collected from the patient and subjected to Gram's staining under a light microscope (100×), followed by DNA isolation. Nanopore long-read sequencing with host depletion and MGI short-read sequencing were employed for bacterial identification. EPI2ME was solely used for visualizing long-read data, while raw read sequences were assembled and analyzed for the presence of AMR genes.

file. Retained reads listed in this file were extracted and further aligned to the human genome using Minimap2 v2.21–h5bf99c6_0 [18] to remove residual host DNA. Only unmapped reads were retained for downstream analysis. These unmapped reads were *de novo* assembled using Flye v2.9.2–py39h6935b12_0 [19] with parameters '--meta -i 2', and the assemblies were polished using Medaka (https://github.com/nanoporetech/medaka) to improve accuracy.

For short-read sequencing, raw paired-end sequencing reads were quality-filtered and adapter-trimmed using Fastp v0.23.4 [20]. Host sequences were removed by aligning the reads to the human genome (T2T-CHM13v2.0/hs1, GCF_009914755.1) using BWA v0.7.18 [21]. Samtools v1.16.1 was used to extract reads from the sorted BAM, which were then converted into paired FASTQ files [22]. Genome assemblies, including either short-read or hybrid approaches, were conducted using Unicycler v0.5.0 [23].

Assembly quality was assessed using QUAST v5.0.2 [24], genome completeness and contamination were evaluated with CheckM v1.2.1 [25]. Plasmid typing was performed using MOB-suite v3.0.3 and PLSDB database (https://ccb-microbe.cs.uni-saarland.de/plsdb2025) [26]. Species identification was performed by aligning the assembled genome to the GTDB-Tk database (R207_v2), and average nucleotide identity (ANI) and alignment fraction (AF) were calculated using GTDB-Tk v2.1.1 [27]. Antimicrobial resistance (AMR) genes were annotated by aligning the assembled genomes to the CARD v3.2.8 using the nucleotide-based Resistance Gene Identifier (RGI) v6.0.2 [17], with thresholds of >95% identity and >90% coverage. Integrons and insertion sequences in the genome were annotated using IntegronFinder 2.0 and ISFinder, respectively [28, 29]. The novel transposons and insertion sequences were assigned Tn and IS numbers by The Transposon Registry and ISFinder, respectively [28, 30].

Results

Medical record of infective endocarditis cases

In this study, patient demographic and clinical data for the two IE cases are summarized in Table 1. IE patient 1 was a 56-year-old male admitted in June 2024 due to acute altered consciousness and myoclonus. Cerebrospinal fluid analysis via lumbar puncture demonstrated elevated protein and leukocytosis without hypoglycorrhachia or identifiable pathogens, raising suspicion of acute bacterial meningoencephalitis. Empirical intravenous ceftriaxone and ampicillin were initiated, later escalated to meropenem. Approximately one week later, the patient developed melena and anemia, though both esophagogastroduodenoscopy and colonoscopy were unremarkable. Video capsule endoscopy identified ileitis. Upon referral, bacterial meningoencephalitis was deemed unlikely, and antibiotics were discontinued.

Table 1 Patient identification and medical records of two infective endocarditis patients

Characteristics	IE Patient 1	IE Patient 2
Hospitalization	June 2024	November 2023
Age (year)	56	68
Gender	Male	Male
Medical conditions	Hypertension, dyslipidemia, old cerebrovascular disease, and end-stage renal disease	Type 2 diabetes, hypertension, and dyslipidemia

Ten days after discharge, the patient returned with exertional presyncope and severe anemia (Hb 4.2 g/dL, Hct 12.8%). A positive direct Coombs test led to a diagnosis of warm autoimmune hemolytic anemia (AIHA). During this admission, he developed a febrile illness and a to-and-fro cardiac murmur, prompting transthoracic echocardiography, which revealed perforation of the right coronary cusp of the aortic valve, severe aortic regurgitation, a pseudoaneurysm, and a fistulous tract to the left ventricle. Clinical deterioration ensued, including congestive heart failure and acute respiratory failure, necessitating emergency aortic valve replacement (AVR) and pseudoaneurysm repair with a bovine pericardial patch. Surgery was completed without complications. Empirical treatment with meropenem and vancomycin was initiated. Following identification of the pathogen and preliminary AMR profiling, meropenem was de-escalated to ampicillin/sulbactam, while vancomycin was continued to complete a 6-week course.

IE patient 2 was a 68-year-old male admitted in November 2023 with progressive dyspnea and generalized edema over two weeks. His medical history included hypertension, type 2 diabetes mellitus, dyslipidemia, and recent tooth extraction one month prior. He was afebrile on admission. On day 3 of hospitalization, the patient developed atrial fibrillation with rapid ventricular response, acute kidney injury, hemolytic anemia, and acute respiratory failure. Physical examination revealed a grade IV pansystolic murmur at the apex and bilateral pulmonary crepitations. Transthoracic echocardiography demonstrated severe aortic regurgitation with a 2.15×1.24 cm vegetation on the aortic valve, and severe mitral regurgitation with a 0.95×1.92 cm vegetation. Infective endocarditis involving both valves was diagnosed, with acute decompensated heart failure. Empirical ceftriaxone and cloxacillin were administered, and cardiothoracic surgery was consulted for aortic and mitral valve replacement. After pathogen identification and concurrent AMR profiling, antibiotic therapy was de-escalated to ceftriaxone for a 6-week treatment course.

Microbiology diagnostics

Gram staining of the aortic valve tissue from IE patient 1 revealed numerous Gram-negative bacilli, non-spore-forming Gram-positive bacilli, and a few Gram-positive cocci in pairs, with no polymorphonuclear cells observed. Gram staining of the valve tissues from IE patient 2 showed only Gram-positive cocci (Fig. S1).

Blood cultures from both patients were collected during admission and again prior to surgery. All aerobic and anaerobic bottles remained negative after 5 days of incubation. Similarly, the culture of the native valve tissues obtained during surgery showed no microbial growth under both aerobic and anaerobic conditions, thus precluding antimicrobial susceptibility testing for these cases.

EPI2ME real-time species identification

ONT sequencing reads were initially classified using the cloud-based Antimicrobial Resistance workflow on the EPI2ME platform. In IE patient 1, 88.7% of classified reads matched *Corynebacterium striatum*, while in IE patient 2, 93% matched *Granulicatella adiacens.* Other bacterial genera were detected at significantly lower relative abundance, as presented in Table S1. Notably, species-level identification (≥ 90% identity) was achieved within the first hour of sequencing. Given the clinical context of culture-negative infective endocarditis, these dominant species were selected for downstream genome reconstruction and resistance profiling.

Metagenomic assembly and genome characteristics

To obtain a precise diagnosis of infectious disease in the patients, we utilized either long-read or short-read metagenomic data for genome reconstruction using metagenomic-assembled genome (MAG) technique. Following the human-depletion step, a total of 134,856 out of 6,966,644 reads (1.9%) and 45,269 out of 230,687 reads (19.6%) remained in the long-read datasets of IE patient 1 and 2, respectively. These host-depleted reads exhibited improved *N50* values, increasing from 714 bp to 2,718 bp in patient 1 and from 2,859 bp to 7,880 bp in patient 2, indicating higher read contiguity suitable for genome assembly (Fig. S2 and Table S2). Additionally, a total of 6 Gb of short-read metagenomic data, comprising 385,148 host-depleted reads (1.6%) as employed in the genome assembly process (Table S2).

The long-read metagenomic data were successfully assembled into two contigs, representing the circular genome of *C. striatum*, with a total length of 2,991,631 bp and an average genome coverage of 202×. This assembled genome exhibited an average nucleotide identity (ANI) of 98.13% when compared to *C. striatum* ATCC 6940 (GCF_000159135.1) (Tables S3 and S4). In contrast, the short-read sequencing did not reconstruct the genome as a single chromosome, yielding a total of 61 contigs with a genome size of 2,858,108 bp and an average genome coverage of 61×. The hybrid assembly, combining short- and long-read sequences, produced a genome assembly comparable to that generated by long-read sequencing alone (Table 2 and Fig. S3).

Given the fragmented genome assembly from short-read sequencing and the comparable performance of long-read and hybrid assemblies in IE patient 1, DNA extracted from the valve of IE patient 2 was then sequenced solely using nanopore long-read sequencing. Similar to IE patient 1, this approach facilitated the assembly of a single chromosomal contig of *Granulicatella* sp. (95.86% ANI, GCA_ GCA_001058355.1), with a length of 1,782,318 bp, a genome coverage of 105×, and a G + C content of 39.42%. Notably, the NCBI database

Table 2 Characteristics of the reconstructed genomes from two IE patients obtained using ONT long-read, MGI short-read, or hybrid assembly approaches

Genome features	IE patient 1			IE patient 2
	Long-read	Short-read	Hybrid	Long-read
No. of contigs	2	61	2	1
Total length (bp)	2,991,631	2,858,108	2,991,463	1,782,318
Largest contig (bp)	2,952,876	296,954	2,952,875	1,782,318
Contig N50 (bp)	2,952,876	129,247	2,952,875	1,782,318
Contig L50	1	8	1	1
G + C content (%)	58.67	57.66	59.16	39.42
Completeness (%)	99.78	99.78	99.78	99.45
Contamination (%)	0.23	0.23	0.23	0.27
Plasmid	Not detected	Detected	Not detected	Not detected
Species identification	*C. striatum*	*C. striatum*	*C. striatum*	*G. adiacens*
ANI (%)	98.13	98.13	97.97	95.86
GTDB taxonomy	*C. striatum* ATCC 6940	*C. striatum* ATCC 6940	*C. striatum* ATCC 6940	*Granulicatella* sp. 916,049,935
Accession number	GCF_000159135.1	GCF_000159135.1	GCF_000159135.1	GCA_001058355.1

identified this strain as *G. adiacens* strain KHUD_009 (CP106751). The characteristics of the reconstructed genome from both IE patients are detailed in Fig. 2A; Table 2 and S3-S4.

Annotation of antimicrobial resistance genes in the reconstructed genome

AMR genes identified within the reconstructed genomes from IE cases were annotated and detailed in Fig. 2A and Table S5. All AMR identified in both genomes demonstrated over > 90% identity and coverage when aligned to reference sequences in the CARD database. The genome of *C. striatum* revealed a wide range of resistance features distributed across two distinct regions. The first region consists of a class 1 integron flanked by two IS*6* family insertion sequences, IS*6100* and IS*1628*. It features the typical architecture, with the integrase gene *intI1* located at the 5′ conserved segment, and the 3′ conserved segment comprising the *sul1* gene (conferring sulfonamide resistance) and the truncated *qacEΔ1* gene (associated with resistance to quaternary ammonium compounds). Within the integron, three gene cassettes are present: two encoding resistance to aminoglycosides (*aac(6')-Ia* and *aadA*), and one containing a novel IS*3* family insertion sequence, IS*Cost1*. Adjacent to the integron is a novel composite transposon Tn*7838*, flanked by two novel IS*3* family insertion sequences, IS*Cost2*. This transposon harbors *tetW* (tetracycline resistance) and *ermX* (macrolide-lincosamide resistance).

A second mobile region consists of a novel composite transposon, Tn*7839*, flanked by two IS*Cre1* elements. This transposon encodes a trypsin-like serine protease, a sensor histidine kinase, a DNA-binding response regulator, and the aminoglycoside resistance gene *aac(3)-XI*. This arrangement suggests coordinated horizontal transfer of resistance and regulatory elements, possibly enhancing environmental adaptability and antimicrobial resilience. In contrast, the genome of *G. adiacens* solely harbored *tetA*(60) and *tetB*(60), an ABC transporter that confers resistance to tetracycline and tigecycline. (Fig. 2B and Table S5).

Discussion

In this study, metagenomic sequencing was employed to identify pathogens in two cases of native valve infective endocarditis with negative blood cultures. The complete workflow, from sample receipt to report generation, required approximately 24 h, comprising DNA extraction (~ 2 h), library preparation (~ 2 h), sequencing (~ 18 h), and real-time data analysis using EPI2ME (~ 40 min). Utilizing nanopore long-read metagenomic sequencing with adaptive sampling, we were able to detect bacterial DNA within the first hour of sequencing. Notably, 18 h of sequencing yielded 73× bacterial sequence coverage in IE patient 1, markedly higher than the 19× coverage achieved using short-read sequencing (Table S2). These results emphasize the potential of both sequencing approaches for accurate detection, while highlighting the advantage of nanopore sequencing in reducing diagnostic turnaround time.

Although adaptive sampling has been previously applied to pus samples, its success is known to depend on the initial abundance of the target microbe and DNA fragment reads [13, 31]. This study is the first to demonstrate the feasibility of adaptive sampling in native valve tissue from IE cases. The read *N50* obtained – 2,718 bp for IE patient 1 and 7,880 bp for IE patient 2 (Table S2) - indicates that native valve tissue appears to be compatible with this technique. In addition, long-read sequencing alone yielded a complete genome assembly in IE patient 1, with comparable quality to the hybrid assembly. Consequently, for IE patient 2, only nanopore sequencing was performed.

While standard diagnostic protocols for suspected IE recommend blood cultures, clinical evaluation, and echocardiographic imaging [5], these approaches often fail in cases involving fastidious organisms, such as members of the HACEK group (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, *Kingella*), *Bartonella*, *Coxiella*, and *Granulicatella* species [6, 7, 32]. Our real-time sequencing data revealed that most sequencing reads from IE patient 1 matched *C. striatum* (Table S1). Although typically considered a skin commensal, *C. striatum* has been increasingly recognized as an opportunistic pathogen in hospital settings. Of the 22 reported IE cases through an English-language literature search in Medline, the majority (82%) involved native valve endocarditis, and over half were hospital-acquired [33, 34]. Therefore, the presence of this bacterium in the patient may pose a significant concern due to its potential antibiotic resistance.

C. striatum is known for its multidrug resistance, especially to penicillin, ceftriaxone, meropenem, clindamycin, and tetracycline [35, 36]. The genome reconstructed in this study revealed two distinct mobile genetic regions, Tn*7838* and Tn*7839*, exhibiting sequencing depths of 195× and 38×, respectively, with complete coverage (100%) for both regions (Fig. 2A). Multiple novel mobile genetic elements were identified among these regions, including IS*Cost1*, IS*Cost2*, Tn*7838*, and Tn*7839*. A class 1 integron was also identified, flanked by IS*6100* and IS*1628*, carrying resistance genes to aminoglycosides (*aac(6')-Ia*, *aadA*). While the *ermX* and *tetW* genes, carried by Tn*7838*, are prevalent in *C. striatum* globally [37], the *aadA* gene was uniquely detected in our strain. Moreover, most of the insertion sequences found in these regions belong to the IS*6*- and IS*3*-family elements, which are known to mediate the mobility of AMR genes.

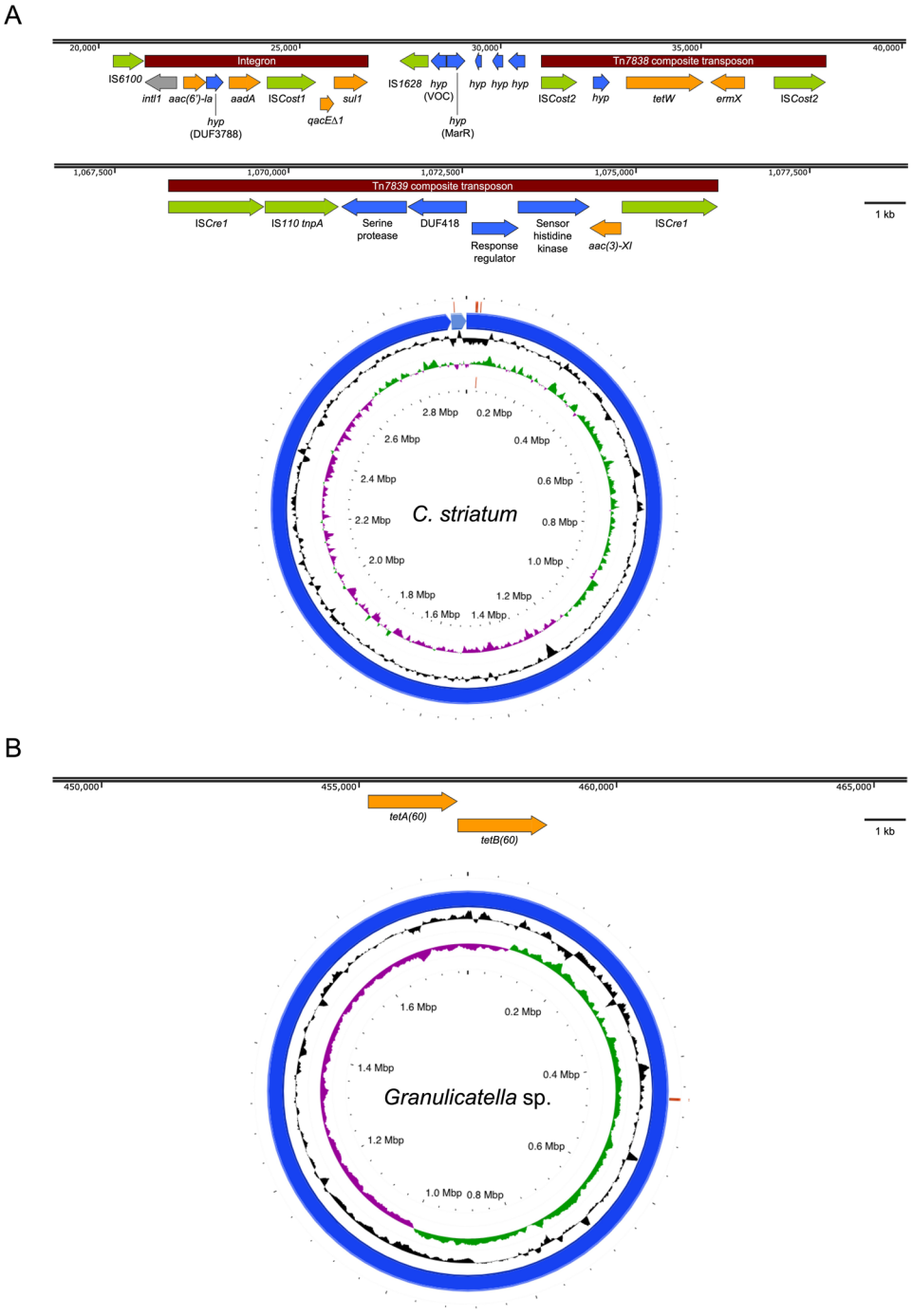

Fig. 2 Genome features and AMR genes of *Corynebacterium striatum* (**A**) and *Granulicatella adiacens* (**B**). Images were initially created using either Proksee or SnapGene. The upper part represents a map of the mobile genetic regions. In the lower part is a circular map of reconstructed genome. The contents are arranged in feature rings (starting with the outermost ring): ring 1: AMR gene positions showed in red; ring 2: coding region sequences (CDS); ring 3: G + C content; ring 4: G/C skew information in the + strand (green color) and − strand (purple color)

The IS*6* family was exclusively associated with aminoglycoside resistance genes in *C. striatum* [38]. To our knowledge, this is the first report describing multiple distinct mobile regions harboring six resistance genes, including a class 1 integron and two novel composite transposons, in *C. striatum*, particularly in a Thai clinical isolate (Fig. 2). These findings highlight the role mobile genetic elements in shaping the multidrug resistance landscape of *C. striatum*, underscoring the clinical importance of monitoring its genomic evolution.

In IE patient 2, *G. adiacens* was identified as the causative agent. *G. adiacens*, a fastidious Gram-positive

coccus within the nutritionally variant streptococci (NVS) group, requires enriched media for growth and is rarely implicated in human infection [39, 40]. Despite its rarity, *G. adiacens* is a known cause of severe endocarditis, often resulting in valve destruction and heart failure [41]. Detection is challenging due to its complex growth requirements and atypical colony morphology [42]. While *Streptococcus* species dominate IE cases in Thailand [43], *G. adiacens* infections are likely underreported due to diagnostic limitations. Using nanopore sequencing with *in silico* host depletion, we successfully reconstructed the complete genome of *G. adiacens* from a valve sample from a patient with type 2 diabetes and dyslipidemia (Fig. 2B). Infections caused by *Granulicatella* are typically treated with penicillin or ceftriaxone [44], though treatment failure and mortality rates remain high [45, 46]. Our findings showed that the *G. adiacens* genome contained only tetracycline resistance genes, *tetA*(60) and *tetB*(60), without nearby insertion sequences, suggesting limited potential for horizontal gene transfer (Fig. 2B and Table S5).

Importantly, the combined results of species identification and AMR profiling were available in real time and informed treatment decisions in both patients. No β-lactamase genes were detected, which reinforced the safety of de-escalation to β-lactam-based regimens. In IE Patient 1 (*C. striatum*), this supported the shift from meropenem to ampicillin/sulbactam, with continued vancomycin, while in IE Patient 2 (*G. adiacens*), narrowing therapy to ceftriaxone monotherapy was consistent with the AMR profile, which showed only tetracycline resistance genes. These findings demonstrate that real-time AMR profiling, when provided alongside pathogen identification, can serve as an adjunct to conventional diagnostics and support antimicrobial stewardship.

Although our study is limited to two IE cases, the successful application of long-read metagenomic sequencing with adaptive sampling for direct pathogen detection and genomic analysis demonstrates the clinical utility of this approach. Further investigations are warranted to determine the optimal sequencing depth for complete genome assembly and to validate reproducibility across broader patient cohorts.

Conclusions

Blood culture-negative infective endocarditis presents a significant diagnostic challenge, particularly when caused by fastidious or slow-growing organisms. This study demonstrates the utility of nanopore long-read metagenomic sequencing combined with adaptive sampling for direct pathogen identification and comprehensive genome reconstruction from clinical heart valve specimens. Using this approach, we successfully identified *C. striatum* and *G. adiacens* and retrieved their complete genomes, including antimicrobial resistance determinants.

Notably, we report, for the first time, the presence of multiple mobile genetic regions harbouring six AMR genes within a class 1 integron and novel composite transposons in the genome of *C. striatum*, underscoring the value of long-read sequencing in uncovering complex resistance elements. The ability to perform species-level identification, genome assembly, and resistance profiling within hours highlights the potential of this method for same-day diagnostics and precision-guided antimicrobial therapy. While our findings are based on two cases, they provide a strong proof-of-concept for integrating long-read metagenomics into the diagnostic workflow for infective endocarditis. Future studies involving larger patient cohorts are needed to validate the robustness, scalability, and clinical impact of this technique across diverse clinical settings.

Abbreviations

AF	Alignment fraction
AIHA	Autoimmune hemolytic anemia
AMR	Antimicrobial resistance
ANI	Average nucleotide identity
AVR	Aortic valve replacement
BCNE	Blood culture-negative endocarditis
CARD	Comprehensive antibiotic resistance database
C. striatum	*Corynebacterium striatum*
EGD	Esophagogastroduodenoscopy
G. adiacens	*Granulicatella adiacens*
IE	Infective endocarditis
IS	Insertion sequences
ISCVID	The international society for cardiovascular infectious diseases
NVS	Nutritionally variant streptococci
ONT	Oxford Nanopore Technologies
Q	Quality score
RGI	Resistance gene identifier
Tn	Transposon
VGS	Viridans group streptococci

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11741-5.

Supplementary Material 1. Table S1 EPI2ME real-time species identification of the predicted top five of IE patients sequnced by nanopore sequencing. Table S2 Filtered read sequencing statistics obtained from either nanopore long-read or MGI short-read sequencing. Table S3 Assembly metrics and genomic characteristics identified from metagenome-assembled genomes in two cases of IE. Table S4 Comparison of closest species identified across long-read, short-read, and hybrid assemblies in two IE cases. Table S5 Antimicrobial resistance genes annotation from metagenome-assembled genomes in two cases of IE.

Supplementary Material 2. Fig. S1 Gram staining of the valve showing Gram-negative bacilli, Gram-positive bacilli (non-spore-forming), and a few Gram-positive cocci in pairs in the aortic valve obtained from IE patient 1 (A and C), and Gram-positive cocci in the aortic valve obtained from IE patient 2 (B and D). Fig. S2 Distribution of sequencing read lengths for host-associated and host-depleted reads from IE patient 1 (A) and IE patient 2 (B), along with the number (C) and percentage (D) of raw, host-depleted, and host-associated reads obtained by nanopore sequencing. Fig. S3 Bandage visualization of reconstructed genomes from either long-read sequences (A) or short-read sequences (B) of IE patients.

Authors' contributions
WKr and TW designed the study. WKr, WKa, and ST drafted the primary manuscript. DP, WT, YS, and NC were involved in clinical management and patient follow-up. PNit, ST and WKa conducted the microbiological testing. WKr, PNim, and SA performed metagenome sequencing and data analysis. NW, PJ, TA, RD and ST analyzed genome reconstruction and annotation. All authors approved the final version for publication.

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Data availability
Sequence data was made publicly available in NCBI databases (BioProject no. PRJNA1051280).

Declarations

Ethical approval and consent to participate
The study was conducted in accordance with the principles of the Declaration of Helsinki. The study was reviewed and approved under the expedited review process by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si 768/2025, SIRB Protocol No. 778/2568(IRB3)). The IRB granted a waiver of informed consent, as the study involved leftover clinical specimens and was determined to pose minimal risk to participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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