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Bayesian reconstruction of a vancomycin-resistant *Enterococcus* transmission route using epidemiologic data and genomic variants from whole genome sequencing

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SUMMARY

Background: Outbreaks of vancomycin-resistant enterococcus (VRE) are a serious problem in hospitals. Inferring the transmission route is an important factor to institute appropriate infection control measures; however, the methodology has not been fully established.
Aim: To reconstruct and evaluate the transmission model using sequence variants extracted from whole genome sequencing (WGS) data and epidemiological information from patients involved in a VRE outbreak.
Methods: During a VRE outbreak in our hospital, 23 samples were collected from patients and environmental surfaces and analysed using WGS. By combining genome alignment information with patient epidemiological data, the VRE transmission route was reconstructed using a Bayesian approach. With the transmission model, evaluation and further analyses were performed to identify risk factors that contributed to the outbreak.
Findings: All VREs were identified as *Enterococcus faecium* belonging to sequence type 17, which consisted of two VRE genotypes: *vanA* ($N = 8$, including one environmental sample) and *vanB* ($N = 15$). The reconstruction model using the Bayesian approach showed the transmission direction with posterior probability and revealed transmission through an environmental surface. In addition, some cases acting as VRE spreaders were identified, which can interfere with appropriate infection control. Vancomycin administration was identified as a significant risk factor for spreaders.

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Conclusion: A Bayesian approach for transmission route reconstruction using epidemiologic data and genomic variants from WGS can be applied in actual VRE outbreaks. This may contribute to the design and implementation of effective infection control measures.

Introduction

Since vancomycin-resistant enterococcus (VRE) was first reported in the 1980s, VRE has rapidly emerged worldwide [1,2]. Although the vancomycin resistance rate in *Enterococcus* spp. varies among countries, VRE isolation rates higher than 30% have been reported [3]. VRE infections and outbreaks are a significant and urgent problem in many countries. An outbreak of such drug-resistant organisms has a major impact on the healthcare setting and is a burden on infection control departments.

When managing and controlling a VRE outbreak, several measures — including contact precautions, patient cohorting, surveillance, and sometimes ward closure — should be implemented simultaneously [2,4–6]. Among these measures, an epidemiological study is an important strategy, and understanding the transmission route of VREs is particularly meaningful for implementing and evaluating appropriate infection control measures and preventing the spread of VREs. Currently, molecular methods such as pulsed-field gel electrophoresis or phylogenetic analysis can be employed to evaluate strain homology [7,8]. In addition to molecular analysis, contact history between patients is often investigated from the medical records. However, even by using these personnel-intensive methods, it is usually difficult to infer the transmission route; namely, 'who infected whom'.

In recent years, whole genome sequencing (WGS) has enabled analysis of bacteria with the specific aim of detecting an outbreak and monitoring the evolution and dynamics of drug-resistant organisms. In epidemiological research in particular, a number of approaches have been developed to reconstruct the transmission route based on WGS data [8–18]. Indeed, some of these approaches are useful for ruling out or identifying transmission. However, the nature of within-host variation among infecting organisms can be misleading between genetic analysis and the epidemiological relationship [15,16]. Recently, a new reconstruction model of pathogen transmission has been developed from genetic alignment accounting for within-host variation and patient epidemiological data by using a Bayesian and Monte Carlo Markov chain (MCMC) approach [19]. The basic principle for inferring the transmission route is the Bayesian approach, which is widely used for the creation of phylogenetic trees in which many parameters assumed for model construction are processed by the MCMC method to create an optimal pathway [20].

Here, we aimed to reconstruct the transmission model using sequence variants extracted from WGS data and epidemiological information of patients involved in a VRE outbreak. In addition, we aimed to evaluate the applicability of this model through the identification of risk factors for VRE transmission.

Methods

Study site

We analysed a total of 23 VRE isolates that were collected from patients and environmental surfaces during an outbreak that occurred between January and May 2018 at the National Defense Medical College Hospital, an 800-bed tertiary hospital in Japan. This study conformed to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board (approval no. 2951).

Case detection

In January 2018, a VRE strain was first isolated from a swab specimen of an oral enanthema that occurred in a patient who had undergone chemotherapy for diffuse large B-cell lymphoma. A few days later, VRE was cultured from the autopsied lung of a patient in the same ward who had received immunosuppressive therapy. In response to the accumulation of VRE cases in a short period and in the same ward, the Department of Infection Control declared a VRE outbreak and initiated an active surveillance approach using faecal samples from the patients in that ward, which identified three new carriers. The VRE carriers were isolated immediately and contact precautions were taken. In addition, active surveillance using faecal samples and anal swabs was expanded to all patients in the hospital on a monthly basis. Finally, a total of 22 VRE carriers were identified by May 2018. By continuing active surveillance, contact precaution measures, staff education, environmental cleaning, and promotion of appropriate vancomycin use, we confirmed that no new VRE carriers were detected for three consecutive months. We finally declared cessation of the VRE outbreak in September 2018.

We also performed environmental surveillance including the VRE-positive patient room, dirty utility room, toilet and bathroom in the ward where the VRE carriers were isolated and found VRE in only one sample from 92 surfaces. Microscan Walkaway 96 Plus with a Pos Combo 3.1J panel (Beckman Coulter, Tokyo, Japan) was used for bacterial identification and the determination of antimicrobial susceptibility.

DNA extraction and whole genome sequencing

DNA was extracted from samples according to the protocol supplied with the Mora-Extract kit (Kyokuto, Tokyo, Japan). All VREs were derived from an initial culture of a freshly isolated sample from each patient. Sequencing libraries were prepared using a Nextera XT DNA Library Prep kit (Illumina, San Diego, CA, USA) with indexed adapters according to the manufacturer's instruction. A pre-processing library quality check

was performed using an Agilent 2100 Bioanalyzer with a high-sensitivity DNA assay (Agilent, Santa Clara, CA, USA). WGS was conducted with an Illumina MiSeq platform with appropriate library configuration.

Bacterial analysis

All raw sequencing data provided in FASTQ format were checked using FastQC. Assembly was performed with the SPAdes Genome Assembler or Velvet *de novo* Assembly. For species identification, multi-locus sequence type (MLST) and antimicrobial resistance genes were determined using the Bacterial Analysis Pipeline program. All bacterial analysis was performed through the BaseSpace Sequence Hub provided on the Illumina website (https://www.illumina.com/products/by-type/informatics-products/basespace-sequence-hub.html).

Integration of alignment data and epidemiological data for Bayesian reconstruction

In this study, we used a Bayesian approach to infer the direction of VRE transmission. We applied BadTrIP, an open source program package that enabled the reconstruction of the transmission inference model to the BEAST2 (version 2.5.2) open source cross-platform program for Bayesian phylogenetic analysis of molecular sequences. BadTrIP infers transmission from three information sources: pathogen alignment data, host exposure duration, and sampling time [19,21]. The pathogen alignment data were composed of the number of bases of A, T, G, and C observed at a specific position in each sample. After sequence mapping to the *Enterococcus faecium* reference sequence, single nucleotide polymorphisms/variation (SNPs/SNV) and insertions/deletions (INDELs) were acquired through SAMtools version 1.9. Sample-specific SNPs/SNV and INDELs were determined from the obtained variant call format files by using VCFtools version 0.1.17 [22,23]. Variants were filtered using the cut-offs of mapping quality ≥35 and depth ≥40.

Of these filtered variants, the representative sample-specific position of SNPs/SNV or INDELs was extracted in each sample, and the four positive natural values, being the respective number of A, T, G, and C bases observed at that position, were used as alignment information [19]. Furthermore, the duration from the first hospitalization date to the final discharge date within the outbreak period of each patient was defined as the period of host exposure. The outbreak period was defined from the date that the first infected patient was identified (September 2017) to the discharge date of the last infected patient (July 2018). The total day count was based on the day that the first patient was admitted. Similarly, sample collection dates were calculated from the patients' medical charts.

We created a BEAST-subject file (xml format) with BadTrIP, and, using this subject file as input, a correlation diagram was generated by the BEAST2 application.

Patient information

Patient information was collected from medical records. Patient activity status (ambulant, assist transfer, or bedridden) at the time of VRE sample collection, patient treatment, and

Table I
Genomic characteristics of the *vanA* isolates ($N = 8$, including environment sample) and patient information

Sample/patient no.	Accession no.	VCM resistance gene	Age (years), sex	Other drug-resistance genes	Ward	Activity	Detection site	Tracheal suction	Oral care assist	Diaper	Urinary catheter	Tube feeding	VCM PPI
1	DRX171149	*vanA*	81, M	*erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*	W8	Ambulant	Oral cavity	−	+	+	+	−	+ −
2	DRX171150	*vanA*	78, M	*aadE*, *erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*, *tet(M)*	W8	Bedridden	Lung	+	+	+	+	−	+ −
3	DRX171151	*vanA*	67, M	*aadE*, *erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*, *tet(M)*	W8	Ambulant	Faeces	−	−	−	−	−	+ −
4	DRX171152	*vanA*	26, M	*aadE*, *erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*, *tet(M)*	W8	Ambulant	Faeces	−	−	−	−	−	+ −
5	DRX171153	*vanA*	71, F	*aadE*, *erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*	W8	Ambulant	Faeces	−	−	−	−	−	− +
12	DRX171160	*vanA*	65, M	*aadE*, *erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*	W8	Bedridden	Faeces	−	+	+	+	−	− +
16	DRX171164	*vanA*	86, M	*aph(3′)-III*, *aadE*, *erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*, *tet(M)*	W6	Transfer assist	Faeces	−	−	−	+	−	− −
23	DRX171171	*vanA*	—	*aadE*, *erm(B)*, *msr(C)*, *erm(T)*, *dfrG*, *tet(L)*	W8								

VCM, vancomycin; PPI, proton pump inhibitor. Sample no. 23 was obtained from a chair in the bathroom of ward W8 through environmental culture.

Table II
Genomic characteristics of the *vanB* isolates ($N = 15$) and patient information

Sample/ patient no.	Accession no.	VCM-resistance gene	Age (years), sex	Other drug-resistance genes	Ward	Activity	Detection site	Tracheal suction	Oral care assist	Diaper	Urinary catheter	Tube feeding	VCM	PPI
6	DRX171154	*vanB*	72, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5	Bedridden	Faeces	+	+	+	+	+	+	+
7	DRX171155	*vanB*	82, F	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5	Transfer assist	Faeces	+	+	+	−	+	+	−
8	DRX171156	*vanB*	68, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	E10	Transfer assist	Faeces	−	−	+	−	+	−	+
9	DRX171157	*vanB*	67, F	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5	Transfer assist	Faeces	+	−	+	+	+	−	−
10	DRX171158	*vanB*	87, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5	Transfer assist	Faeces	−	−	−	+	−	−	−
11	DRX171159	*vanB*	80, F	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5	Ambulant	Faeces	−	−	−	−	−	−	−
13	DRX171161	*vanB*	49, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	E10→W5	Bedridden	Faeces	−	+	+	+	−	+	+
14	DRX171162	*vanB*	52, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	E8	Transfer assist	Faeces	−	−	+	+	−	−	−
15	DRX171163	*vanB*	76, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	E8	Ambulant	Faeces	−	−	−	+	−	−	+
17	DRX171165	*vanB*	75, F	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5	Transfer assist	Faeces	+	+	−	−	+	−	−
18	DRX171166	*vanB*	80, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	E8	Bedridden	Faeces	+	+	+	+	+	−	−
19	DRX171167	*vanB*	82, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W8	Transfer assist	Faeces	−	+	+	+	−	−	−
20	DRX171168	*vanB*	55, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5	Ambulant	Faeces	−	+	−	−	+	−	+
21	DRX171169	*vanB*	76, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W8	Transfer assist	Faeces	−	−	−	−	−	−	+
22	DRX171170	*vanB*	71, M	*aph(3′)-III*, *erm(B)*, *msr(C)*, *dfrG*, *tet(L)*	W5→W8	Ambulant	Faeces	−	−	−	+	−	+	+

VCM, vancomycin; PPI, proton pump inhibitor.

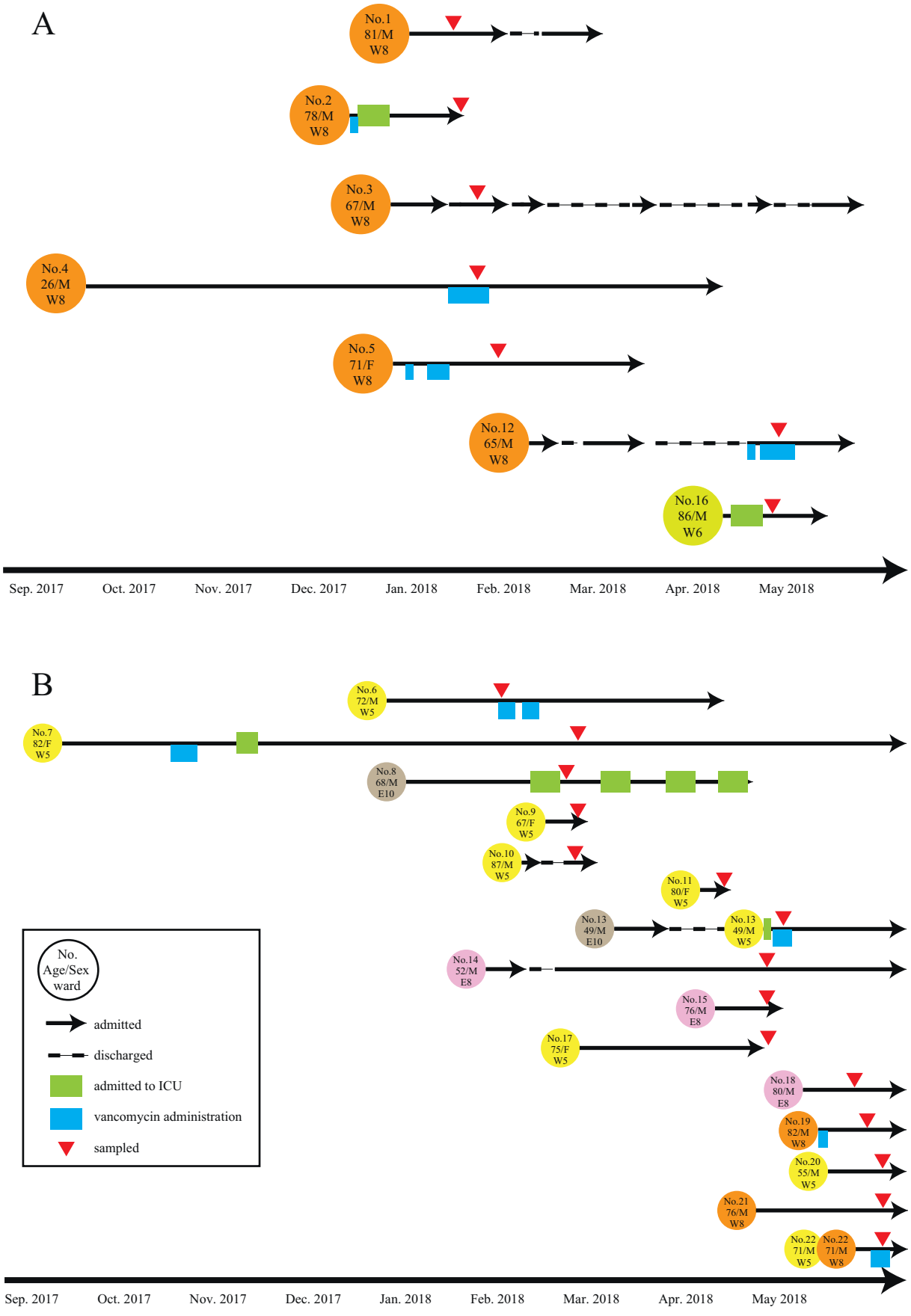

Figure 1. Outbreak timeline for (A) *vanA*-type and (B) *vanB*-type.

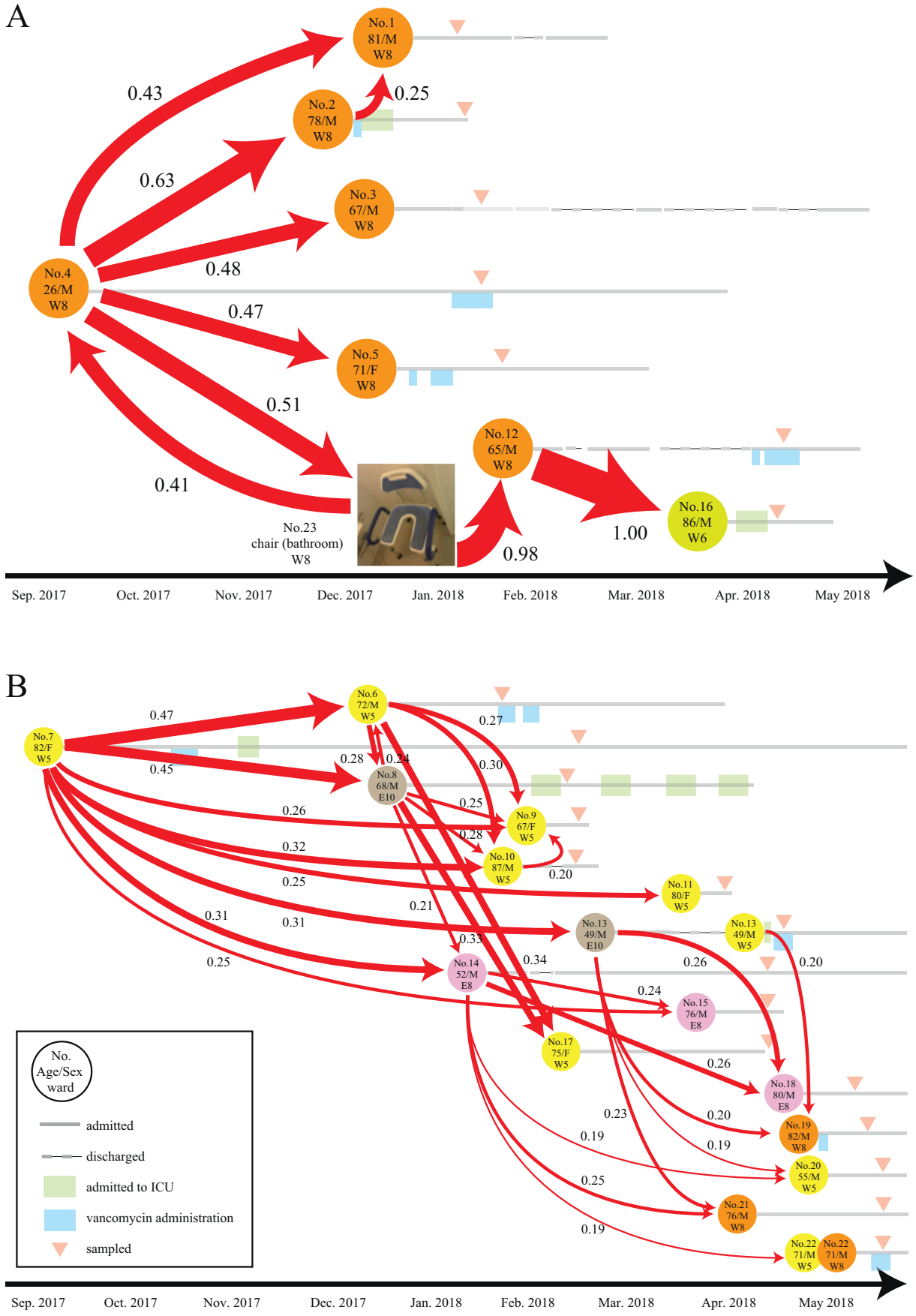

Figure 2. Bayesian reconstruction for (A) *vanA*-type and (B) *vanB*-type vancomycin-resistant enterococcus (VRE) transmission route applied to the outbreak timeline with posterior probability.

Table III
Risk factors for vancomycin-resistant enterococcus transmission

Variable	Spreader ($N = 9$)	Non-spreader ($N = 13$)	*P*-value	Multivariate analysis	OR (95% CI)
Tracheal suction	3	3	0.655		
Oral-care assist	5	5	0.666		
Diaper	7	4	0.081	0.575	1.15 (0.71–1.88)
Urinary catheter	6	7	0.674		
Tube feeding	3	4	1.000		
Vancomycin	6	2	0.026	0.011	1.71 (1.18–2.48)
Proton pump inhibitor	4	6	1.000		
Non-ambulant	8	6	0.165	0.215	1.40 (0.84–2.35)

OR, odds ratio; CI, confidence interval.

the administration history of vancomycin and a proton pump inhibitor were also investigated.

Statistical analysis

The χ^2-test was used to analyse non-parametric variables, and Fisher's exact test was used when the sample number was small. For multivariate analysis, multiple logistic regression was used to estimate risk. $P < 0.05$ was considered significant. All statistical analyses were performed using R version 3.5.3 software (R Foundation for Statistical Computing, Vienna, Austria).

Results

The epidemiological and clinical characteristics of the patients with VRE ($N = 22$) and an environmental sample with VRE ($N = 1$) in this outbreak are listed in Tables I and II. After assembling the de-novo sequencing data from the obtained whole genome sequences, MLST revealed that the causative pathogen of this outbreak was *E. faecium* belonging to sequence type 17 (ST17). In addition, two VRE genotypes, *vanA* ($N = 8$, including one environmental sample) and *vanB* ($N = 15$), were identified. Several other antimicrobial resistance genes were also identified.

Most of the patients with VRE colonization were identified by active surveillance culture; only three patients (14%; nos. 1, 2, and 12) were detected by routine clinical specimens.

Figure 1 shows the outbreak timelines for *vanA* and *vanB*. It is clear that *vanA* and *vanB* were spread concurrently. The index case for *vanA* was patient no. 1, while the index case for *vanB* was patient no. 6. Although *vanA* was mostly confined to ward W8, *vanB* spread through several wards, which complicated the identification of the transmission route. In some VRE-positive patients, vancomycin was administered before or at the time of sample collection.

Figure 2 was made by applying the inferred transmission route to Figure 1. The arrows indicate the direction of propagation, and the numerical values and thickness of the arrows show the posterior probability calculated by the Bayesian approach. Those with posterior probabilities <0.19 were excluded. These results indicated that the patients considered to be index cases were different from those inferred in Figure 1, and some patients played the role of spreaders by transmitting VRE to multiple patients, suggesting that the infection control measures were insufficient. From this approach, we could identify spreaders beyond the ward. Furthermore, it was shown that there was transmission via a bathroom chair in ward W8.

Table III summarizes the characteristics of the patients who were the sources of transmission (spreaders) and others (non-spreaders) identified from the Bayesian approach. Multivariate logistic regression analysis for the variables was performed, which assumed the risk factors for transmission with low *P*-values, and only vancomycin administration history was identified as a risk factor with an odds ratio of 1.71 (95% confidence interval: 1.18–2.48; $P = 0.011$).

Discussion

Estimating pathogen transmission during an outbreak is one of the most important steps for intervention. The initial evaluation step is the identification of a hospital infection. A number of methods for evaluating the homology of strains and determining the association among isolated samples have been developed and are widely used in clinical practice [24]. Although such molecular epidemiological analysis can provide important insights, a crucial and practical method to directly estimate who infected whom has not been established.

Recent advances in genomic analysis have made it feasible to examine the relationship between micro-organisms isolated from patients more deeply and accurately. By taking epidemiological data into account, various methods for reconstructing a pathogen transmission model from a phylogenetic tree have been devised [8–14,17]. However, such phylogenetic trees can be influenced by within-host variation rather than the actual epidemiologic relationship [15,16,25]. If within-host dynamics are not considered, the accurate reconstruction of pathogen transmission could be hindered. Furthermore, the sequence of a pure-cultured pathogen may be different from the direct sequencing data of samples taken from a host without culturing. If an unsampled host is included in the transmission chain, it can affect the inferred reconstruction of the route. However, it is impractical to perform direct sequencing of all infected individuals in an ongoing outbreak. Considering these issues and within-host variation, we determined that using the BadTrIP program package was the most feasible reconstruction model available at that time that could be applied to an actual outbreak.

In this study, MLST revealed that the VRE responsible for the hospital outbreak belonged to ST17. ST17 is now renamed and classified as clonal complex 17 (CC17), which is recognized as

the ancestral clone of the hospital-associated clade A1 [26]. CC17 has been detected in contaminated water, has been isolated from hospital wastewater, and contributed to a previous outbreak [27]. Although VRE is responsible for a significant proportion of hospital infections, its prevalence in Japan is still low; the detection rate of VRE in a national survey conducted in Japan was only 0.35% of all *Enterococcus* spp. [28]. Although we routinely conduct screening for VRE while referring to the susceptibility of an *Enterococcus* sp. that was isolated in our hospital laboratory, we have never detected VRE before. Therefore, we consider that one *vanA*-type VRE and one *vanB*-type VRE caused an outbreak independently and concurrently. In addition, this facilitated the reconstruction of the transmission model.

It should be noted that a general reconstruction model is designed to be applicable to cases in which all hosts have been observed. In an actual outbreak, it is reasonable to assume that there will be some missing data, namely non-observed or non-sampled cases. Although BadTrIP takes these missing data into account, we carried out active surveillance by screening faecal samples and anal swabs during the outbreak to obtain more accurate epidemiological data, which helped to maximize case detection. With this model, the exposure time in the epidemiological data can be set arbitrarily, which may produce a somewhat intentional result in the reconstructed infection tree and is also enhanced by insufficient alignment data. During an actual outbreak, because of the difficulties in identifying the time of infection, it is unavoidable that the exposure time will be set to the longest possible period. Instead of this, to reduce the instability of the reconstruction model, we selected an area period for which the alignment data were sufficiently prepared.

The limitations of this approach should be mentioned. First, not all hospitals have access to WGS technology and/or staff with the required computational skills. However, WGS data can be created relatively easily and the software package we used is a widely distributed programme. It is possible to reconstruct the transmission route without advanced programming knowledge and it may be feasible even in general hospitals. Second, this approach requires considerable time to calculate the posterior probability and the presence of complicated transmission routes, especially in a large dataset. Our identification of the *vanA* transmission route was clear, whereas *vanB* transmission was complicated because of the increased number of cases. In the Bayesian approach, the posterior probability tends to decrease and become complicated over time. To optimize reconstruction, collecting multiple samples from each patient and setting the exposure time more strictly can be considered. However, even in our actual outbreak, BadTrIP was able to estimate the transmission route.

In this study, we tried to identify spreaders where conduct-appropriate infection control measures had failed, and to estimate risk factors for VRE transmission based on the reconstruction model. The increased intensity of medical care due to restricted activities of daily living, excretion care, and vancomycin administration are known risk factors of VRE transmission [2]; however, in this study, only vancomycin administration was identified. Vancomycin administration to VRE carriers increases bacterial load and may result in them becoming spreaders.

The present results may be extrapolated to the outbreak response of departments of infection control. During a VRE outbreak, vancomycin administration should be carefully determined, and the promotion of antimicrobial stewardship is crucial to control risk factors [29]. Furthermore, *vanA*-VRE transmission via hospital environmental surfaces occurs with a high probability, indicating the importance of regular environmental maintenance [30]. If further environmental surveillance had been conducted and VRE had been detected in other locations, we may have been able to demonstrate other transmissions through the environment.

From our experience, active screening is significantly helpful for case detection, which suggests that routine scheduled screening should be considered during an outbreak period [31,32].

In conclusion, a Bayesian approach for transmission route reconstruction using epidemiologic data and genomic variants from WGS was applied successfully to an actual VRE outbreak. This method has the potential to be used for the analysis of various outbreaks and can contribute to our understanding of how outbreaks occur and for reviewing the efficacy of infection control measures.

Conflict of interest statement
None declared.

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