Genomic characterisation of multidrug-resistant *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* in two intensive care units in Hanoi, Viet Nam: a prospective observational cohort study

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Summary  
Background Viet Nam has high rates of antimicrobial resistance (AMR) but little capacity for genomic surveillance. This study used whole genome sequencing to examine the prevalence and transmission of key AMR pathogens in two intensive care units (ICUs) in Hanoi, Viet Nam.  
Methods A prospective surveillance study of all adults admitted to ICUs at the National Hospital for Tropical Diseases and Bach Mai Hospital was done between June 19, 2017, and Jan 16, 2018. Clinical and environmental samples were cultured on selective media, characterised with MALDI TOF mass spectrometry, and sequenced with Illumina. Phylogenies based on the de-novo assemblies (SPAdes) were constructed with MAFFT (PARsnp), Gubbins, and RAxML. Resistance genes were detected with Abricate against the US National Center for Biotechnology Information database.  
Findings Of the 369 patients, 167 clusters involving 251 (68%) of patients were detected. Phylogenetic analysis revealed predominant lineages within *A baumannii* (global clone 2, sequence types ST2 and ST571) and *K pneumoniae* (ST15, ST16, ST656, ST11, and ST147) isolates. Transmission analysis with single nucleotide polymorphisms identified 167 clusters involving 251 (68%) of patients, in some cases involving patients from both ICUs. There were no clear differences between the lineages or AMR genes recovered between the two ICUs.  
Interpretation This study represents the largest prospective surveillance study of key AMR pathogens in Vietnamese ICUs. Clusters of closely related isolates in patients across both ICUs suggests recent transmission before ICU admission in other health-care settings or in the community.  
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Introduction  
Low-income and middle-income countries (LMICs) have reported widespread antimicrobial resistance (AMR) in health-care, community, and agricultural settings. In southeast Asia, dense human populations, intensive animal farming, unrestricted access to antibiotics, and scarce laboratory infrastructure have all contributed to the rapid expansion of AMR. Much of this burden arises from excessive use of antimicrobials in human and animal populations.  
In Viet Nam, antimicrobial usage has been estimated to be two times higher in people, and one and a half times higher in animals, compared with the EU. Despite legal restrictions in Viet Nam, antibiotics are often dispensed without prescriptions in the community. Broad-spectrum antibiotics are also commonly administered in health-care settings to mitigate the effects of the scarce capacity for microbiological testing and infection control. Detection of both AMR bacteria and antimicrobials have been recorded in the environment, hospital waste, and food sources. Extensive AMR has led to increased pressure on hospitals and is particularly problematic in critical care settings. Although AMR surveillance based on phenotypic testing in Viet Nam has improved since 2015, the infrastructure required for systematic genomic surveillance remains to be established. Genomic analysis is important to identify circulating lineages; however,
LMICs remain relatively understudied, with few studies done in Vietnamese hospitals. To address this knowledge gap, we did a prospective genomic surveillance study of key AMR pathogens in two hospitals in Viet Nam. We focussed our analysis on the three most commonly isolated species (Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii) that were extended-spectrum β-lactamase (ESBL) producers or carbapenem resistant. We describe the genomic diversity of these isolates and broadly gauge the level of isolate relatedness to establish a conservative underestimate of transmission events during and immediately before intensive care unit (ICU) admission.

Methods

Study design, setting, and participants

This prospective observational cohort study was done in two hospitals, the National Hospital for Tropical Diseases (NHTD) and Bach Mai Hospital (BMH), in Hanoi, Viet Nam, between June 19, 2017, to Jan 16, 2018. All adult patients admitted to the ICUs of the two hospitals during the study period were eligible for inclusion in the study; there were no exclusion criteria. We targeted patients who were admitted to ICUs because we hypothesised that they would be the most likely to have been treated with antibiotics and to harbour AMR pathogens. Screening specimens (stool or rectal swabs, urine, skin or wound swabs or pus, and sputum or tracheal aspirates) were collected from ICU patients on admission, on discharge, and weekly during their ICU stay. Environmental samples were collected using flocked swabs (from door handles, bed rails, medical equipment, and patient tables) once per month.

The study protocol was approved by the Scientific and Ethical Committees of the National Hospital for Tropical Diseases and Bach Mai Hospital and by the University of Cambridge Human Biology and Research Ethics Committee (reference HBREC 2017.09). Written informed consent was obtained from the patient or from their relative before enrolment in the study.

Procedures

Specimens were cultured on selective media to identify ESBL producers and carbapenem-resistant organisms (appendix 1 pp 2–3). Target organisms (E coli, A baumannii, and K pneumoniae) were identified with MALDI-TOF mass spectrometry (Bruker Diagnostics, Bremen, Germany) and stored at −80°C before being shipped to the University of Cambridge, UK, for library preparation and sequencing;
DNA was sequenced in two batches on an Illumina HiSeq X10 machine (Illumina, San Diego, CA, USA). Further details of laboratory methods including read quality control, genome assembly, phylogenetic analysis, antibiotic resistance gene detection, multilocus sequence typing, and transmission cluster analysis are available in appendix 1 (pp 2–5).

Briefly, phylogenetic trees were built from core multi-alignments (PARsnp v1.2), filtered using Gubbins (v2.3.5) and constructed with RAxML (GTR-GAMMA model; v8.2.12).

Outcomes and analyses
We determined the number of E coli, K pneumoniae, and A baumannii isolates cultured from clinical and environmental samples collected during the study period. We did whole-genome sequencing of these isolates followed by phylogenetic analyses to examine genomic diversity and relatedness. We also determined the presence of antibiotic resistance genes. We did descriptive statistical analyses of numerical data.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results
A total of 3367 isolates were cultured, comprising E coli (n=765), K pneumoniae (n=1372), and A baumannii (n=1230). 31 isolates were excluded from the analysis because of poor assembly quality. A further 150 isolates were excluded because of suspected interspecies contamination, and 33 isolates were excluded because of suspected intraspecies (strain-level) contamination (appendix 1 p 14). Thus 3153 isolates (721 E coli, 1316 K pneumoniae, 1116 A baumannii), comprising 2891 isolates from 369 patients and 262 environmental isolates, passed quality filtering and were included in the final analyses.

Overall, a total of 1042 isolates (993 clinical and 49 environmental) were collected from BMH and 2111 isolates (1898 clinical and 213 environmental) from NHTD. The participant baseline characteristics and outcomes for each hospital are summarised in table 1 and appendix 1 (p 15). The number of samples collected was higher, and the median length of ICU stay was longer, at NHTD than BMH.

146 (40%) of 369 patients (55 at BMH and 91 at NHTD) were colonised or infected with all three bacterial species; 133 (36%) patients (66 at BMH and 67 at NHTD) with two of the three species; and 90 (24%) patients (61 at BMH and 29 at NHTD) had only one species detected.

Both E coli (627 [87·0%] of 721) and K pneumoniae (822 [62·5%] of 1316) were isolated primarily from stool or rectal swabs. K pneumoniae was also isolated from other sites including sputum (325 [24·7%] of 1316), urine samples (63 [4·8%] of 1316), and skin swabs (17 [1·3%] of 1316). By contrast, A baumannii isolates were predominantly isolated from sputum (621 [55·6%] of 1116), followed by stool and rectal swabs (247 [22·1%] of 1116), urine (49 [4·4%] of 1116), and skin swabs (36 [3·2%] of 1116). A baumannii also accounted for the highest number of environmental isolates (161 [14·4%] of 1116), compared with 85 (6·5%) of 1316 for K pneumoniae and 16 (2·2%) of 721 for E coli.

Phylogenetic trees for each species were constructed to explore lineage diversity. The E coli isolates were highly
diverse, with isolates spread over eight phylogroups and 80 sequence types (figure 1). The most prevalent sequence type was ST648 (phylogroup A; 11.8%), followed by ST410 (phylogroup C; 9.7%), ST617 (phylogroup A; 9.2%), ST131 (phylogroup B2; 7.9%), and ST1193 (phylogroup B2; 7.4%). Overall, 33 of 80 sequence types had only one representative isolate in this dataset.

Figure 1: Whole genome phylogenies
(A) Escherichia coli. (B) Acinetobacter baumannii. (C) Klebsiella pneumoniae. Recombination-filtered core-single nucleotide polymorphism trees with midpoint root. Tree metadata includes (from left to right column beside trees): multilocus sequence typing, source, and hospital. Outermost purple bars indicate environmental isolates. Branches corresponding to E coli phylogroups are coloured accordingly. Main sequence types are highlighted in the image with yellow boxes. Tree scale is number of substitutions.
By contrast, the *K pneumoniae* and *A baumannii* isolates had a small number of dominant lineages. 1094 (83%) of the 1316 *K pneumoniae* isolates were from one of five sequence types, including ST15 (445 [34%]), ST16 (259 [20%]), ST656 (154 [12%]), ST11 (148 [11%]), and ST147 (88 [7%]). The majority of the 1116 *A baumannii* isolates were global clone 2 (832 [75%]) and mainly belonged to ST2 (536 [48%]) and ST571 (269 [24%]; based on the Pasteur scheme). We did not identify any specific relationship between sequence types and hospitals, with all of the major sequence type lineages represented in both ICUs.

To gain broader insight into the lineages, we selected globally representative strains to contextualise our dataset. Addition of these global representatives into the *E coli* phylogeny showed that most isolates belonged to a globally diverse set of sequence types that were not unique to Viet Nam, but found across parts of North America, Europe, and Asia (appendix 1 p 16). Similarly, several of the major *K pneumoniae* lineages were represented globally, particularly ST147, ST11 (mainly from China and the USA), and ST15 (mainly other Asian countries; appendix 1 p 17). However, it was also clear that local expansion was prominent, particularly among the lineages, suggesting clonal expansion rather than diverse sampling of the species (appendix 1 p 18). There was very little representation of global strains within our dataset, and those that were available consisted mainly of strains from other parts of Asia. Closer inspection of all global representatives found several strains in each species that were closely related (less than five core single nucleotide polymorphisms [SNPs]) to isolates in our dataset (appendix 1 pp 6, 8).

Almost all isolates carried acquired resistance genes to at least three antibiotic classes, with 649 (90%) of *E coli*, 1134 (86%) of *K pneumoniae*, and 452 (41%) of *A baumannii* isolates carrying genes across five antibiotic classes (appendix 1 p 19). There were very little representation of global strains within our dataset, and those that were available consisted mainly of strains from other parts of Asia. Closer inspection of all global representatives found several strains in each species that were closely related (less than five core single nucleotide polymorphisms [SNPs]) to isolates in our dataset (appendix 1 p 19).

Resistance to antibiotics classes varied across the *E coli* phylogeny, reflective of the diversity of strains within the dataset (appendix 1 p 20). *Bla*<sub>CTX-M</sub> genes were found in 613 of the 721 *E coli* isolates (table 2), with *bla*<sub>CTX-M-15</sub> (259 [36%]), *bla*<sub>CTX-M-21</sub> (213 [30%]), and *bla*<sub>CTX-M-35</sub> (119 [17%]) being the most prevalent (appendix 1 p 9). *Bla*<sub>TEM</sub> (94 [13%]) and *bla*<sub>SHV</sub> (579 [80%]) were present in 579 (80%) of 1116 isolates, with *bla*<sub>OXA-60</sub> (642 [58%]) and *bla*<sub>OXA-23</sub> (3 [<1%]) present at much lower frequencies (phylogroup A; n=11) involving three patients from NHTD. Conversely, multidrug-resistant gene presence across the *K pneumoniae* isolates were consistent with the main lineages, suggesting clonal expansion rather than diverse sampling of the species (appendix 1 p 21). Similar to the *E coli*, incidence of *bla*<sub>CTX-M-15</sub> (493 [37·5%] of 1316) was high, but less so than *bla*<sub>KPC-2</sub> (593 [45·1%]) and *bla*<sub>NDM</sub> (716 [54·4%]; table 2).

Acquired AMR genes were overall less prevalent among the *A baumannii* isolates. Similar to the *K pneumoniae*, resistance to specific classes tended to be a feature of each distinct lineage, suggesting clonal expansion (appendix 1 p 22). The carbapenemase gene *bla*<sub>OXA-23</sub> was present in 927 (83%) of 1116 isolates, with *bla*<sub>NDM</sub> (642 [58%]) and *bla*<sub>OXA-23</sub> (3 [<1%]) present at much lower frequencies.

### Table 2: Summary of resistance genes found in the three bacterial species

| Antibiotic Class | E. coli isolates (n=721) | Acinetobacter baumannii isolates (n=1316) | Klebsiella pneumoniae isolates (n=1316) |
|------------------|-------------------------|------------------------------------------|----------------------------------------|
| Tetracycline     | 563 (78.1%)             | 701 (62.8%)                              | 753 (57.2%)                            |
| Sulphonamide     | 649 (90.0%)             | 746 (66.8%)                              | 969 (73.6%)                            |
| Fluoroquinolone  | 161 (22.3%)             | 19 (1.7%)                                | 1187* (90.2%)                          |
| Colistin         | 28 (3.9%)               | 0                                        | 10 (0.8%)                              |
| Fosfomycin       | 48 (6.7%)               | 5 (0.4%)                                 | 1316* (100%)                           |
| Macrolide        | 579 (80.3%)             | 816 (73.1%)                              | 623 (47.3%)                            |
| β-lactamase      | 718 (99.6%)             | 1018 (91.2%)                             | 1286 (97.7%)                           |
| Class C          |                         |                                          |                                        |
| EC               | 721 (100%)              | 2 (0.2%)                                 | 0                                      |
| ACT              | 0                       | 1 (0.1%)                                 | 0                                      |
| CMY              | 209 (29.0%)             | 1 (0.1%)                                 | 2 (0.2%)                               |
| DHA              | 26 (3.6%)               | 2 (0.2%)                                 | 24 (1.8%)                              |
| Class A          |                         |                                          |                                        |
| LAP              | 17 (2.4%)               | 0                                        | 142 (10.8%)                            |
| CARB             | 0                       | 63 (5.6%)                                | 0                                      |
| TEM              | 347 (48.1%)             | 607 (52.5%)                              | 686 (52.1%)                            |
| SHV              | 7 (1.0%)                | 9 (0.8%)                                 | 1292* (98.2%)                          |
| VEB              | 0                       | 12 (1.3%)                                | 3 (0.2%)                               |
| CTX              | 653 (85.0%)             | 4 (0.4%)                                 | 681 (51.8%)                            |
| KPC              | 94 (13.0%)              | 3 (0.3%)                                 | 593 (45.1%)                            |
| Class D          |                         |                                          |                                        |
| OXA              | 251 (34.8%)             | 9961 (89.2%)                             | 611 (46.4%)                            |
| Class B          |                         |                                          |                                        |
| IMP              | 0                       | 6 (0.5%)                                 | 1 (0.1%)                               |
| NDM              | 173 (24.0%)             | 35 (3.1%)                                | 716 (54.4%)                            |
| Rifampicin       | 114 (15.8%)             | 63 (5.6%)                                | 810 (61.6%)                            |
| Aminoglycoside   | 680 (94.3%)             | 1115 (99.9%)                             | 1290 (98.0%)                           |
| Streptothricin   | 11 (1.5%)               | 8 (0.7%)                                 | 0                                      |

* *Fosfomycin*, *oxa*<sub>ADR</sub> (fluoroquinolone), and *bla*<sub>NDM</sub> intrinsic in *K pneumoniae*, *bla*<sub>IMP</sub>, *bla*<sub>PROM</sub>, and *bla*<sub>NDM</sub> intrinsic in *E coli*.

†*bla*<sub>EC</sub> intrinsic in *E. coli*.

‡*bla* ADC and *bla*<sub>OXA-1</sub> intrinsic in *A baumannii*.

§*bla*<sub>ADC-1</sub> and *bla*<sub>OXA-23</sub> intrinsic in *A baumannii*.

*Numbers are greatest number present, except for *OXA-23* and *NDM* genes which are calculated from individual genes.

**Table 2: Summary of resistance genes found in the three bacterial species.**
We finally looked at how many patients had isolates from one site (urine, swab, stool, or sputum) or mixed sites. For multiple isolates, we identified whether it was collected on admission to the intensive care unit or after. For multiple isolates, species, we evaluated whether patients had only a single isolate for that species, or multiple isolates. If only a single isolate relatedness and determine a conservative estimate of transmission. Clusters were defined as sets of isolates from the same patient and environmental samples when they involved more than one patient. Clusters involving a single patient and environmental samples were not included.

Most patients with *E coli* and *K pneumoniae* had isolates only from stool (218 [81%] of 270 patients with *E coli*, and 158 [54%] of 294 patients with *K pneumoniae*). Conversely, most patients with *A baumannii* (n=230) were detected only in sputum (86 [37%]), or in sputum and stool (65 [28%]). Repeat isolation of the same bacterial species on at least two separate occasions was common (*E coli* 138 [51%] of 270, *K pneumoniae* 169 [57%] of 294, and *A baumannii* 132 [58%] of 230). Of these patients, 60–70% had different sequence types (92 [67%] of 138 *E coli*, 115 [68%] of 169 *K pneumoniae*, 83 [63%] of 132 *A baumannii*; figure 2). A large proportion also had the same sequence type isolated at two or more timepoints (103 [75%] of 138 *E coli*, 149 [88%] of 169 *K pneumoniae*, and 123 [93%] of 132 *A baumannii*). For the 103 patients with the same *E coli* sequence type isolated over multiple timepoints, the majority (73 [70%]) were detected only from stool, with fewer cases found in both stool and sputum (14 [14%]) or stool and urine (10 [10%]). 45% of patients (n=67) with the same *K pneumoniae* sequence type were largely isolated from stool (53 [36%]). Similarly, 46% of patients (n=56) with the same *A baumannii* sequence type were isolated only from sputum (42 [34%]; appendix 1 p 12).

Temporal analysis of the isolates found no obvious association of any timepoint with any sequence type to suggest an outbreak of a specific lineage. To identify closely related strains that could indicate recent transmission, we evaluated clusters on the basis of SNP distances across the core genome of each species for this dataset. Given the short sampling period, none of the three major species was likely to acquire more than one SNP while in the hospital. As such, we looked at samples with genomic evidence of most recent transmission: zero SNP clusters (followed up with higher thresholds). We chose this SNP threshold to broadly gauge the level of isolate relatedness and determine a conservative underestimate of transmission. Clusters were defined when they involved more than one patient. Clusters involving a single patient and environmental samples were not included.

Despite our conservative threshold, we identified several clusters. Most clusters were detected in *K pneumoniae* isolates (71 clusters, representing 38% of total isolates) and *A baumannii* isolates (74 clusters, representing 52% of total isolates; figure 3). *K pneumoniae* had some of the largest clusters, ranging in size from two to 79 isolates, whereas *A baumannii* clusters were smaller, from two to 33 isolates. Only 22 clusters were detected in *E coli* and were generally small (median three isolates [IQR 2–5·75]), representing only 13% of the *E coli* dataset. The sequence types with the greatest number of clusters in each species were ST410 (*E coli*), ST2 (A *baumannii*), and ST15 (*K pneumoniae*; appendix 1 p 11).

For all three species, the majority of clusters (119 [71%] of 167) were detected in patients within a single ICU. Evaluating admission and discharge dates further confirmed patient overlap in these clusters (figure 3, appendix 1 pp 25–30). Patients involved in zero SNP

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**Figure 2: Overview of strain diversity, recurrence, and source among study patients**

Patients refers to the total number of patients in this study that had at least one isolate of that species. Within each species, we evaluated whether patients had only a single isolate for that species, or multiple isolates. If only a single isolate, we identified whether it was collected on admission to the intensive care unit or after. For multiple isolates, we identified whether the patient’s isolates were the same sequence type (recurrent) or a different sequence type. We finally looked at how many patients had isolates from one site (urine, swab, stool, or sputum) or mixed sites (any combination of sites).
clusters overlapped with more patients during their time in the ICU (mean 29 patients [SD 15]) than patients not involved in clusters (20 patients [SD 12]). Compared with *E coli* and *K pneumoniae*, *A baumannii* clusters were more often associated with environmental isolates (24 [32%] of 74 clusters; figure 3). 15 (21%) of 71 *K pneumoniae* clusters and only two (9%) of 22 *E coli* clusters were associated with environmental isolates. *K pneumoniae* environmental isolates were more often found in within-hospital (ie, ICU) clusters (n=11) compared with between-hospital clusters (n=4).

In addition to suspected within-ICU transmission, we also detected several clusters involving patients from both hospital ICUs (figure 3). The most pronounced example of this was a large ST15 *K pneumoniae* cluster involving 79 isolates from 38 patients and six environmental samples (appendix 1 p 31).

The identification of closely related isolates between independently operating ICUs suggested that there might have been a common source located outside the ICU—eg, admission to the same shared location (such as a ward in the same hospital as the current ICU, or the same ward in a different prior hospital) before admission to ICU. To find out whether particular lineages were associated with acquisition within the ICU, we assessed diversity on arrival (ie, admission samples) versus diversity within the ICU (all other samples). 275 (75%) of 369 patients had a positive culture on admission. Based on sequence type alone, we found a slight increase in diversity in ICUs versus on arrival (appendix 1 p 32). However, the unique sequence types recovered in either setting represented only a small portion of the isolates overall. All the main lineages for each species were found on both admission and within ICUs (appendix 1 pp 32–33).

In relation to our zero SNP clusters, we found that clusters involving both ICUs more often had a patient with an admission positive sample (*A baumannii*: 24 [89%] of 27 clusters; *K pneumoniae*: 13 [76%] of 17) than clusters within a single ICU (*A baumannii*: 24 [53%] of 45; *K pneumoniae*: 25 [47%] of 53; appendix 1 p 12). For *E coli*, there was no bias of admission-positive isolates in either cluster group. Overall, at least half of the clusters

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**Figure 3:** Summary of zero SNP clusters in all species

(A) Clusters were defined as multiple patients (samples were derived from at least two different patients) or same patient (isolates were derived from the same patient, or only a single patient and the environment). Epidemiological evidence to support clusters was defined as confirmed patient overlap (all patient ICU stays overlap with another in the same cluster), some patient overlap (at least two patient ICU stays overlap), and zero patient overlap between all patients in cluster. (B) Environmental isolates in clusters were counted if an environmental isolate was found in that cluster. SNP=single nucleotide polymorphism. ICU=intensive care unit.
for all species involved an isolate collected from an admission sample (E coli: 11 [50%] of 22; A baumannii: 48 [67%] of 72; K pneumoniae: 38 [54%] of 70).

Over the course of this study, 251 patients (representing 68% of the cohort) were involved in 167 clusters across the three species. 112 (45%) patients were involved in only a single cluster during their time in the ICUs (figure 4). However, the remaining 139 (55%) patients were involved in at least two clusters, with one (<1%) patient involved in 12 clusters. For the 139 patients with at least two clusters, 20 (14%) had clusters from all three species, 94 (68%) had clusters from two species, and 25 (18%) had only one species. Overall, we saw a general trend towards more clusters in a single patient as they spent more time in the ICU ward.

To find out if any of our (zero SNP) clusters were potentially derived from a single original cluster predating their time in the ICU, we looked at SNP distances between clusters of the same sequence type (appendix 1 p 34). At a threshold of five SNPs, several of the prominent sequence types within each species formed large clusters, including ST804 in A baumannii and ST16 in K pneumoniae. At this threshold, we found 29 clusters in the A baumannii dataset (originally 74), 23 clusters in the K pneumoniae (originally 71) and 19 clusters in E coli (originally 22). Using these larger five SNP clusters, we found that slightly more patients (123 vs 112 previously) might have been involved in only a single cluster during their stay (appendix 1 p 35). 128 patients had two clusters, with the maximum number of clusters in a single patient being seven (n=3 patients).

**Discussion**

Here we present a large prospective genomic surveillance study of ESBL-producing or carbapenemase-producing organisms from three key AMR pathogens from two hospital ICUs in Viet Nam. Despite limiting our study samples to ESBL-producing or carbapenem-resistant isolates belonging to three species, we identified many isolates (an average of 17 isolates per day from patients in BMH and ten per day in NHTD).

Similar settings in England, UK, have found a small incidence of ESBL-producing or carbapenemase-producing Enterobacterales.15,16 However, across Europe and the USA, the spread of endemic ESBL-producing and carbapenemase-producing Enterobacterales has increased,17 particularly E coli and K pneumoniae,18 with extensive spread of high-risk lineages, such as K pneumoniae ST258 and ST512 in the USA,19 Israel, Italy,20 and Greece.21 Meanwhile, the burden of carbapenem-resistant A baumannii remains higher in south Asia than in high-income regions.22

Several of the dominant circulating lineages described here have been identified previously. All the predominant E coli sequence types (ST617, ST648, ST410, ST131, and ST1193) are well known global lineages.23 K pneumoniae ST15 is widespread in Asia and parts of Europe, but mostly absent in the Americas where ST258 and ST512 are more common.24 ST11 is prevalent in China but has been reported previously in Viet Nam.25 A baumannii global clone 2 has caused multiple outbreaks of carbapenem-resistant A baumannii globally,26 including in Viet Nam,27 and has displaced Pseudomonas aeruginosa as the main causative agent of ventilator-associated pneumonia in this region.28
In this study, we aimed to measure the breadth and volume of circulating ESBL-producing and carbapenem-resistant isolates from three species of bacteria in two ICUs. Rather than finding and investigating specific outbreaks, we set out to broadly estimate the number of transmissions occurring across the dataset (encompassing both within the ICU and the few months before admission), and show how they were distributed across the captured species diversity. Even when we used exact pairwise identity within the core genome, we found that 68% of patients were involved in recent transmission of several sequence types with no dominant spreading lineage. Just looking at these very closely related pairs alone provided a picture of high levels of circulating AMR in a wide range of lineages.

Our observation of clusters involving patients from both hospitals could be explained by a source outside ICUs, including other wards or hospitals that might have referred patients to intensive care,29 or AMR strains that have been acquired in the community,28 reflecting high rates of antibiotic use. Based on the similarity between lineages and AMR across both ICUs, we suggest that transmission is mainly circulating outside ICUs, where it is then further propagated. In this context, we should potentially reconsider AMR surveillance and control. Knowing whether hospitals are the primary source of AMR bacteria (and subsequent transmission into the community),28 or whether the high rates are part of a more general, endemic pattern of circulating resistant strains is important for our understanding of local transmission dynamics. If the latter is true, this leads to very different national and indeed global management plans.

We acknowledge that whole genome sequencing of bacteria gives little temporal resolution on transmission, particularly with patients in an ICU for just 3 months. Whole genome sequencing to identify transmission events is more feasible in species with high mutation rates (eg, some viruses) and in low-AMR settings where community transmission is scarce and epidemiological surveillance can focus on specific lineages with detailed metadata. In our setting, the level of AMR circulation was too high to provide detailed transmission analyses (even with epidemiological support) over a large and diverse sample set. We were also limited by the single colony pick method; some transmission isolates were likely to be missed, and we could not estimate the true level of within-patient diversity. Similarly, we expect a patient’s flora to contain some level of natural diversity, which is missed with our conservative SNP threshold and will inflate the number of within-patient clusters.

We also acknowledge other limitations to our study. First, we did not explore plasmid profiles among the samples because of the limitations of short read sequence data. This restricted our ability to detect interspecies and intraspecies transmission of AMR genes via plasmids. Second, we looked only at acquired AMR genes, and did not investigate resistance caused by point mutations. As such, we may have underestimated the resistance profiles of some isolates that were resistant via point mutations. Finally, this study focused on patient and environmental samples only. Therefore, we were unable to investigate potential transmission events involving hospital staff or visitors.

Nevertheless, we present the largest prospective surveillance study to date of multidrug-resistant *E coli*, *A baumannii*, and *K pneumoniae* in patients in critical care in Viet Nam, revealing frequent transmission of highly resistant bacteria within and between two ICU settings. Further work is required to expand genomic surveillance in hospital and community settings to inform AMR control strategies in Viet Nam.

### Declaration of interests

IWR reports travel fees from European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), and is on the Microbial Genomics Early Career Microbiologists board of reviewers, outside of the submitted work. JP and ZI contributed to the conceptualisation of the manuscript. FAK, NTH, ITH, NGB, DXC, NTH, TVG, CB, THN, BN, HRvD, and NVT contributed to the data collection. NTH, FAK, JEB, AH, and TF contributed to the sample processing. IWR and ZI contributed to the methodology and formal analysis. IWR contributed to the writing (original draft), ZI and MET contributed to the writing (review and editing), NVT, HRvD, NVK, ZI, and MET contributed to the supervision of the study. ITH, NVT, HRvD, and MET contributed to the project administration. MET, NVK, and JP contributed to the funding acquisition. IWR and FAK accessed and verified all the data in this study. All authors had access to the data presented in this study and had final responsibility for the decision to submit for publication.

### Data sharing

Genome sequence data have been deposited in the European Nucleotide Archive under the Bioproject PRJEB29424. A list of the sample accession numbers is available in appendix 2. Isolate genome assemblies (heterogenous sites masked and unmasked) are available on Figshare under the following DOI: 10.6084/m9.figshare.13303253 and 10.6084/m9.figshare.13302728.

### Contributions

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