Project Title
Prospective surveillance to determine the prevalence and transmission of antimicrobial resistance (AMR) determinants in Vietnamese intensive care units using whole-genome sequencing (WGS)

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Background
Antimicrobial resistance (AMR) is estimated to cause 700,000 deaths worldwide per year, and is forecast to cause 100 million deaths, and result in the loss of over US$ 100 trillion in world GDP by 2050 [1]. Multidrug-resistant organisms (MDRO) are more commonly found in healthcare settings where they contribute to the burden of healthcare-associated infections (HCAI). In the UK the estimated prevalence of HCAI was 6.4%, with the highest prevalence (24.7%) in intensive care units (ICUs) [2]. ICU patients are particularly vulnerable to HCAI as they are critically ill, have multiple invasive medical devices, and are frequently treated with broad-spectrum antibiotics. Several outbreaks of infection caused by MDROs in intensive care units have been reported [3]. They are often recognised late and are associated with high mortality.

Microbial whole-genome sequencing (WGS) is an emerging technology that will transform the practice of clinical microbiology [4]. It is now possible to sequence a microbial genome in one day, and to obtain information about organism identity, antimicrobial resistance, genotype, genetic relatedness and virulence determinants [5]. One of its major uses is likely to be in the investigation of suspected outbreaks of infection. Over the past few years our group and others have demonstrated the potential utility of this approach for a range of pathogens [6-9]. Most of these studies were conducted retrospectively, in high-income settings with low levels of AMR, and did not influence clinical management.

Vietnam has a high burden of infectious diseases with rising mortality rates. In 2010, 30% of deaths in state healthcare facilities were estimated to be infectious diseases-related. Vietnam also has high levels of AMR, particularly among Gram-negative bacteria (GNB). In a cross-sectional study in 36 hospitals in the north of Vietnam 7.8% of patients developed HCAIs and the most commonly isolated organisms were Pseudomonas aeruginosa (31.5%) and Acinetobacter baumanii (23.3%). High rates of resistance were found to 3rd and 4th generation cephalosporins, aminoglycosides, fluoroquinolones and carbapenems (considered “last line” antibiotics). There is currently no systematic surveillance for MDRO in Vietnam, and no national policy or guidance on how to detect and manage these organisms.

Experimental approach

Aims
1. To determine the prevalence and transmission of MDRO in the ICU at the National Hospital of Tropical Diseases (NHTD) hospital 1 and Bach Mai Hospital (BMH) Hanoi, Vietnam, using bacterial whole-genome sequencing (WGS)
2. To use rapid WGS for the investigation of a putative outbreak of CRE in the ICU at NHTD hospital 2 in Vietnam

Study setting and participants
Clinical studies will be based in the ICUs at the NHTD and BMH in Hanoi, Vietnam.

Study 1: A prospective observational cohort study of MDRO in patients admitted to ICU
All patients admitted to the ICUs at NHTD hospital 1 and BMH between June 2017 and January 2018 (N=400) were screened for MDRO. Clinical data and specimens (swabs, sputum or tracheal aspirates, urine, stool or faecal swabs) were collected from all patients on admission to ICU, on discharge from ICU, and weekly from patients whose duration of admission was 7 days or more. We also collected environmental samples at monthly intervals during the study. Samples were processed in the microbiology laboratory at NHTD to identify target organisms (e.g. carbapenem-resistant Enterobacteriaceae (CRE) extended-spectrum beta-lactamase producers (ESBLs) and vancomycin-resistant enterococci (VRE). 4,151
clinical and environmental samples have been collected and transferred to Cambridge for re-identification (by MALDITOF MS), antimicrobial susceptibility testing (Vitek2) and DNA extraction before being submitted to the Wellcome Trust Sanger Institute for high-throughput sequencing (HiSeq). Clinical and laboratory data have been collected and will be analysed using a standard descriptive statistical methods. The post-doctoral fellow will be responsible for bioinformatic analysis of the genomic data.

**Study 2: Investigation of putative outbreaks of MDRO in ICU patients**

All patients admitted to the ICU at NHTD hospital 2 during a 3-month study period (estimated start Autumn 2018) will be screened for CRE If a suspected outbreak of infection occurs (more than one case on the ward at the same time), intensified surveillance (e.g. twice weekly screening) will be performed. Samples will also undergo DNA extraction, library preparation, and WGS on a rapid sequencing platform (e.g. illumina MiSeq) in the microbiology laboratory at NHTD. Bacterial sequence data will be analysed in real-time to provide information to the clinicians and the infection control team.

**Data analysis**

Bacterial sequence data generated in Study 1 will be analysed by the post-doctoral fellow. He/she will also help to train a Vietnamese bioinformatician assigned to the project. Bacterial sequence data generated in study 2 will be analysed by the post-doctoral fellow and/or Vietnamese bioinformatician This will enable rapid analysis of the data in order to inform clinical decision-making and infection control procedures locally, in real-time. During the course of the project we will aim to discuss with the relevant national bodies (e.g. Ministry of Health) regarding the setting up of national databases with epidemiological data linked to pathogen genomes to enhance surveillance and management of infectious diseases in Vietnam.

**Relevance to biology and/or health and disease**

1. Study 1 is the largest prospective surveillance study of AMR conducted to date. We will not only be able to determine the true prevalence of AMR but also determine spread between patients, between patients and the environment and of AMR determinants between bacterial species.
2. Study 2, by contrast, could have real translational impact as it will inform immediate patient management and infection control procedures, provide training in sequencing and bioinformatics analysis for Vietnamese scientist and inform public health policy on surveillance and management of AMR in Vietnam.

**References**