Clinical Study Protocol

Study Title:	Whole-genome sequencing to investigate colonisation and transmission of multidrug-resistant organisms in the adult intensive care unit at Addenbrooke's Hospital
Protocol Number:	ICU001
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Study Sponsors:	Cambridge University Hospitals NHS Foundation Trust University of Cambridge

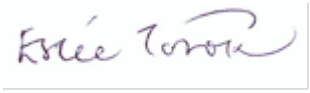
1 Protocol Signatures:

I give my approval for the attached protocol entitled "Whole-genome sequencing to investigate colonisation and transmission of multidrug-resistant organisms on the adult intensive care unit at Addenbrooke's Hospital" dated 4 January 2016

Chief Investigator

Name: Dr Estee Torok

Signature:



Date: 4 January 2016

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4 Abbreviations

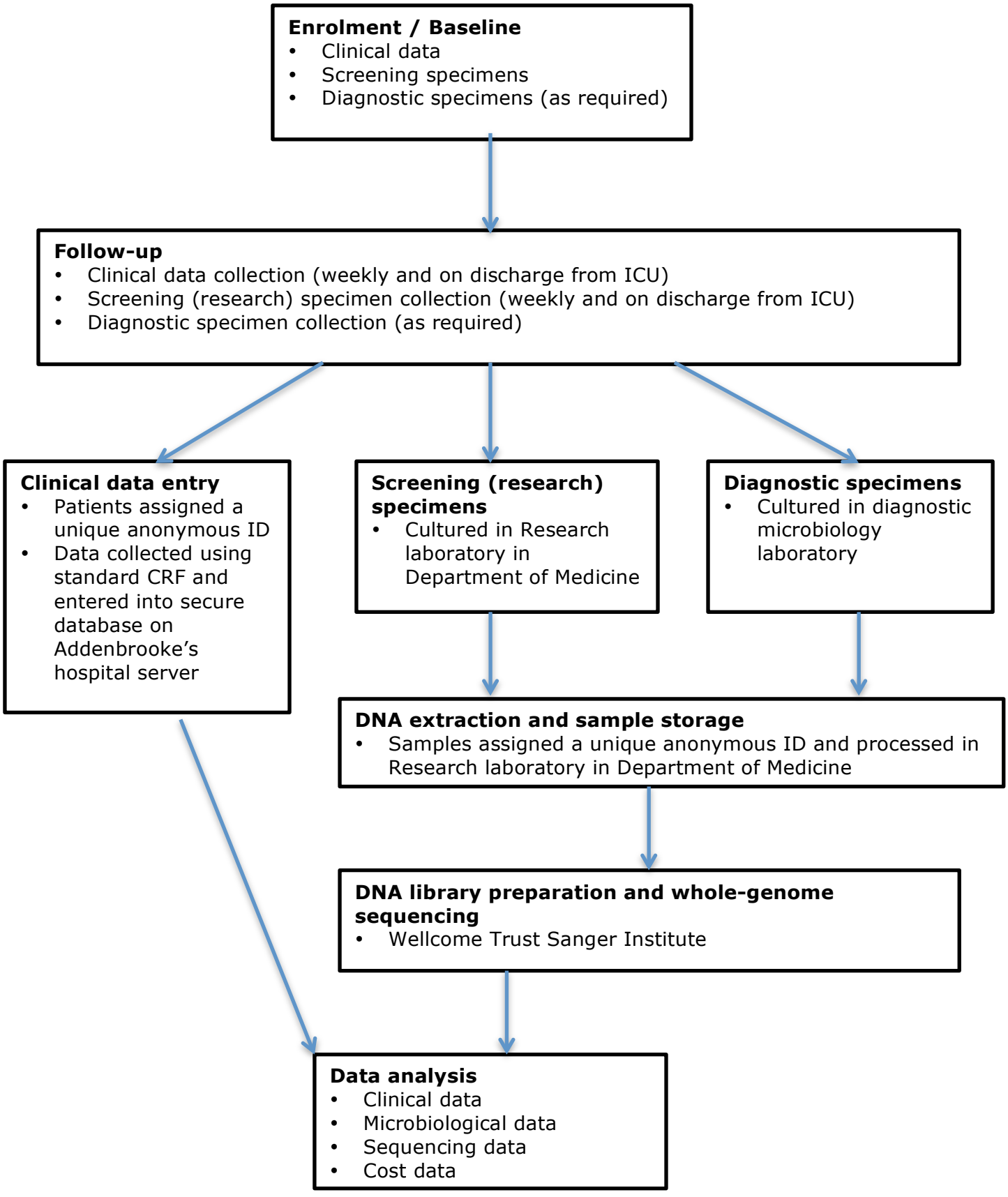
CDI	Clostridium difficile infection
CRE	Carbapenem-resistant Enterobacteriaceae
CRF	Case Report Form
ESBL	Extended spectrum beta-lactamase
GP	General Practitioner
GCP	Good Clinical Practice
GNB	Gram-negative bacilli
HCAI	Healthcare-associated infection
ICT	Infection Control Team
ICU	Intensive Care Unit
JFICU	John Farman ICU
MDR	Multidrug-resistant
MDRO	Multidrug-resistant organism
MRSA	Methicillin-resistant Staphylococcus aureus
NRES	National Research Ethics Service
PHE	Public Health England
R&D	Research and Development
REC	Research Ethics Committee
VRE	Vancomycin-resistant enterococci
WGS	Whole-genome sequencing

5 Study Synopsis

Title of clinical study	Whole-genome sequencing to investigate colonisation and transmission of multidrug-resistant organisms on the adult intensive care unit
Sponsor name	Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge
EudraCT number	Not applicable
Medical condition or disease under investigation	Healthcare-associated infection
Purpose of clinical study	To use whole-genome sequencing to investigate the colonisation and transmission of multidrug-resistant organisms in the adult intensive care unit at Addenbrooke's Hospital
Primary objective	To determine the rates of carriage, infection and transmission of multidrug-resistant Gram-negative organisms in the adult intensive care unit at Addenbrooke's Hospital using bacterial whole-genome sequencing.
Secondary objective (s)	1. To compare standard epidemiological investigation / typing with whole-genome sequencing in the investigation of suspected outbreaks 2. To conduct a health economic evaluation of standard epidemiological investigation / typing with whole-genome sequencing in the surveillance and investigation of suspected outbreaks and the management of confirmed outbreaks
Study Design	Observational cohort study of all patients admitted to the adult intensive care unit at Addenbrooke's hospital during the study period
Primary Outcome Measures	• Number of patients colonised with multidrug-resistant organisms • Number of patients with clinical evidence of infection with multidrug-resistant organisms • Number of transmission events of multidrug-resistant organisms
Secondary Outcome Measures	• Risk factors for colonisation / infection with multidrug-resistant organisms • Outcome of patients colonised / infected with multidrug-resistant organisms • Cost-consequences of whole-genome sequencing versus standard epidemiological investigation / typing for surveillance and investigation of suspected outbreaks and the management of confirmed outbreaks
Sample Size	600 patients
Summary of eligibility criteria	Inclusion Criteria: • Adult (age 18 years older) • Male or Female • Admitted to adult intensive care Exclusion Criteria • Age <18 years • Does not fulfil study inclusion criteria

Screening & enrolment	Participants will be screened for carriage of multidrug-resistant organisms on admission to the adult intensive care unit and enrolled into the study. Written informed consent will not be required prior to study enrolment as this is a surveillance study for healthcare-associated infections. Clinical and antibiotic treatment data will be collected at enrolment
Treatment period	Participants will undergo screening for multidrug-resistant organisms on admission, discharge and weekly throughout their admission to ICU. Clinical data and microbiological specimens will be collected on admission to ICU, on discharge from ICU and weekly during the ICU admission period for patients admitted for 7 days or more.
End of Study	The end of the study will be the date that specimen and data collection are completed
Procedures for safety monitoring during study	Not applicable, as this is an observational cohort study and not an intervention study
Criteria for withdrawal of patients	Not applicable, as this is an observational cohort study and not an intervention study

6 Study Flow Chart



7 Background

The emergence and spread of antimicrobial resistance (AMR) poses a significant challenge to the safe delivery of modern medical care. Indeed AMR has been identified as a major threat to human health by the Chief Medical Officer (1), and has resulted in the development of a five-year Antimicrobial Resistance Strategy Plan by the Government to address this threat (2).

Multidrug-resistant organisms (MDRO) are found more commonly in healthcare settings where they contribute to the burden of healthcare-associated infections (HCAI). In 2011 the estimated prevalence of HCAI was 6.4% (95% confidence interval 4.7 to 8.7%) with the highest prevalence (24.7%) in intensive care units (3). Over the past 15 years considerable efforts have been made to reduce the burden of HCAI in the United Kingdom (UK). These have included screening and decolonisation of patients with methicillin-resistant *Staphyloccocus aureus* (MRSA) (4), and mandatory reporting of MRSA bacteraemia (5) and *Clostridium difficile* infection (CDI) (6). These have resulted in dramatic reductions in MRSA bacteraemia and CDI (3). However, new threats such as vancomycin-resistant enterococci (VRE) (7) and multidrug-resistant Gram-negative bacilli (MDR GNB) have emerged (8). In particular the emergence and spread of resistance to carbapenem drugs, considered to be last-line agents, is a great concern (9). In response to this, Public Health England has recently published a toolkit to enable acute hospital trusts to identify and manage patients who are colonised or infected with carbapenem-resistant Enterobacteriaceae (CRE) (10).

Patients in intensive care units are particularly vulnerable to HCAI as they are critically ill (often with multiorgan failure), have multiple invasive medical devices (e.g. intravascular lines, urinary catheters, mechanical ventilation) and are frequently treated with broad-spectrum antibiotics (which select antibiotic-resistant organisms). Several outbreaks of infection caused by MDROs in intensive care units have been reported (11). They are often recognised late, and are associated with high mortality. Methods to prevent HCAI and improve early detection of MDRO outbreaks in this population are urgently required.

When a suspected outbreak is identified samples are collected from the index patient as well as other patients who may have been affected and, occasionally, from medical staff. Current methods to determine the relatedness of bacterial isolates rely on molecular typing which is often performed in reference laboratories (rather than local laboratories) and take too long to inform outbreak management in real time. Indeed in most cases the results may only be available 1 to 2 weeks later and are used retrospectively to confirm or refute the outbreak.

Microbial whole-genome sequencing (WGS) is an emerging technology that will transform the practice of clinical microbiology (12). It is now possible to sequence a microbial genome in one day and obtain information about organism identity, antimicrobial resistance, genotype, genetic relatedness and virulence determinants (13). This single technology could, therefore, replace several processes that currently occur in diagnostic and reference laboratories. The information produced can be applied to clinical practice in a number of ways. First, it can enhance the epidemiological investigation of suspected outbreaks and inform infection control and public health interventions. Second, it can guide the management of patients with highly drug-resistant organisms, for example multidrug-resistant and extensively drug-resistant tuberculosis. Third, it can be used for the detection and surveillance of antimicrobial resistance, including during the evaluation and use of new antimicrobial agents.

We have previously conducted one of the first studies to demonstrate the potential utility of WGS in nosocomial outbreak investigation at Addenbrooke's Hospital (14). In this study we retrospectively investigated an MRSA outbreak in a neonatal intensive care unit by sequencing MRSA isolates from infants on the unit and from control patients located elsewhere in the hospital. Bacterial sequence data analysis revealed a cluster of outbreak

strains that were clearly separated from the control isolates; we also detected a previously unsuspected transmission event between two of the control patients. In a second study we applied WGS during the course of suspected ongoing MRSA outbreak in Special Care Baby Unit (15). In this study, we sequenced MRSA isolates from infants who were admitted to the special care baby unit (SCBU) at Addenbrooke's hospital over a six-month period. These were found to be a novel MRSA strain (ST2371). Sequencing of additional MRSA isolates with the same antibiotic susceptibility profile identified 24 cases that were epidemiologically linked, indicating spread between infants on the SCBU, mothers on the post-natal ward, and family members in the community. A new case, which occurred 64 days after the previous case, prompted screening of healthcare workers on the unit. One person was found to be carrying the outbreak strain, and no further cases were detected after they were successfully decolonized. This study demonstrated for the first time the ability to use bacterial WGS in real-time to successfully manage an outbreak.

Klebsiella pneumoniae is an important nosocomial pathogen in intensive care units, particularly among immunocompromised patients. In recent years *K. pneumoniae* strains resistant to carbapenems have emerged and spread worldwide. In 2011, the United States National Institutes of Health experienced an outbreak of carbapenem-resistant *K. pneumoniae* that affected 18 patients, 11 of whom died (16). Snitkin and colleagues sequenced *K. pneumoniae* isolates from the affected patients and found that the outbreak was monoclonal despite a three-week gap between the index case and subsequent cases. They identified at least three independent transmission events from the index case, resulting in two major clusters of colonized patients. One patient could be linked to a contaminated ventilator. They also identified putative resistance mutations is isolates that had become resistant to colistin.

A number of other studies have used WGS to investigate nosocomial outbreaks caused by a variety of pathogens including multidrug-resistant *Acinetobacter baumannii* (17), vancomycin-resistant *Enterococcus faecium* (18, 19), carbapenem-resistant *Enterobacter cloacae* (18), and *Pseudomonas aeruginosa* (20). Of note, most of these studies were conducted retrospectively and did not influence individual patient management, or the course of the outbreak.

8 Rationale for Study

One strategy to reduce HCAI in the ICU setting would be to perform prospective surveillance for MDRO in patients admitted to critical care units in order to identify them more quickly and to enable the earlier implementation of appropriate infection control measures and tailored antimicrobial therapy. Current practices for screening for MDRO vary between countries, hospitals and units, reflecting a lack of data and the uncertainty around best practice.

We do not currently perform surveillance for MDRO at Addenbrooke's Hospital, apart from screening patients who fulfil the criteria for CRE screening outlined in the PHE recommendations above (10). We therefore do not have accurate data on the prevalence of these organisms in our hospital, as we rely on samples taken for clinical diagnostic purposes. Furthermore we are unlikely to be able to detect transmission of these organisms between patients as they only come to attention if more than one patient in the same ward develops an infection with the same organism at the same time.

In terms of microbiological data from clinical specimens the prevalence of MRSA colonisation and *C. difficile* infection in the ICU is low at 2.9% and 4% respectively. For blood cultures the frequency of positive cultures is 17% and the most commonly isolated bacteria are *Enterococcus faecium*, coagulase-negative staphylococci, *Elizabethkingia meningoseptica, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Staphylococcus aureus*. In terms of respiratory samples (sputum, tracheal aspirate, broncho-alveolar lavage) the frequency of positive cultures is 57%, and the most commonly

isolated bacteria are *P. aeruginosa, Stenotrophomonas maltophilia, S. aureus, Elizabethkingia meningoseptica, E. coli, K. pneumoniae* and *Enterobacter* spp. In terms of urine samples the frequency of positive cultures is 20.3%, and the most commonly isolated bacteria are *E. coli, K. pneumoniae, E. faecium, P. aeruginosa, Proteus spp, and E. cloacae.*

There are previously published studies on whole-genome sequencing of MRSA prevalence and transmission in an ICU setting (21), and of *C. difficile* prevalence and transmission in a hospital setting (22). We have therefore decided to focus our study on vancomycin-resistant enterococci (VRE) and multidrug-resistant Gram-negative bacilli (MDR GNB) e.g. (*Escherichia coli, Klebsiella pneumoniae, Enterobacter spp, Acinetobacter spp, Pseudomomas aeruginosa and Elizabethkingia meningoseptica*) as they are known to spread readily in an ICU setting, but their prevalence and rates of transmission are unknown. Furthermore they can be very difficult to treat because of the limited number of effective antibiotics available to treat them.

In order to address these limitations, we propose to conduct a prospective surveillance study of MDRO in adults admitted to the John Farman Intensive Care Unit at Addenbrooke's hospital in Cambridge. This will enable us to determine the rates of carriage and infection in this vulnerable patient population, and to identify possible intervention and control strategies. We will compare standard epidemiological investigation / typing methods with WGS to determine transmission of MDRO between patients on the ICU to determine whether WGS is more sensitive than current standard methods of detection MDRO. We will also conduct a parallel health economic evaluation to determine the cost-effectiveness of this strategy.

9 Study design

9.1 Statement of design

This a surveillance study of MDRO and there are no study-specific treatments or interventions. The study design is a prospective observational cohort study of all adults admitted to the John Farman ICU during the six-month study period. Patients will be screened for MDRO on admission to ICU, on discharge from ICU, and weekly during their ICU admission period if the duration of admission is 7 days or longer. Participants will receive routine standard clinical care, including antibiotics to treat infections as clinically indicated.

9.2 Number of Centres

This is a single centre study, which will be conducted in the John Farman Intensive Care Unit at Addenbrooke's Hospital in Cambridge. The JFICU has 20 beds and admits up to 1200 patients per year.

9.3 Number of Subjects

We plan to include 600 patients in this study. This number is based on the estimated number of patients admitted to the John Farman Intensive Care Unit during the six-month study period.

9.4 Participants Study duration

The participants' study duration will be dependent on the duration of their admission to the John Farman Intensive Care Unit. In January 2015 the mean length of stay was 7.19 days (standard deviation ± 8.94 days) and the median was 3.4 days (range 0.2 to 47.2 days). The duration of the whole study is six months.

9.5 Study objectives

9.5.1 Primary objective

To determine the rates of carriage, infection and transmission of MDRO in the John Farman Intensive Care unit at Addenbrooke's Hospital using bacterial whole-genome sequencing.

9.5.2 Secondary objectives

7.5.2.1. To compare standard epidemiological investigation / typing with whole-genome sequencing in the investigation of suspected MDRO outbreaks

9.5.3. To conduct a health economic evaluation of standard epidemiological investigation / typing with whole-genome sequencing in the investigation of suspected MDRO outbreaks

9.6 Study Outcome Measures

9.6.1 Primary outcome measures

The primary outcome measures of the study will be:

- Number of patients colonised with MDRO
- Number of patients with clinical evidence of infection with MDRO
- Number of transmission events of MDRO

9.6.2 Secondary outcome measure

The secondary outcome measures of the study will be:

- Risk factors for colonisation / infection with MDRO
- Outcome of patients colonised / infected with MDRO
- Cost-effectiveness of whole-genome sequencing versus standard epidemiological investigation / typing for suspected MDRO outbreaks

10 Selection and withdrawal of subjects

10.1 Inclusion Criteria

To be included in the study the patient must be:

- Aged 18 years old or older
- Male or female
- Admitted to the John Farman Intensive Care Unit during the study period

10.2 Exclusion Criteria

The presence of any of the following will preclude patient inclusion:

- Age less than 18 years
- Does not fulfil study inclusion criteria

10.3 Treatment

This is a prospective observational cohort study for surveillance of MDRO in intensive care. Participants will receive routine clinical care including antibiotics to treat infections as clinically indicated. There are no study specific interventions or treatments.

10.4 Subject withdrawal criteria

All adult patients admitted to the John Farman Intensive Care Unit during the study period will be enrolled into the study. As this is a surveillance study for healthcare-associated infections, written informed consent from individual participants will not be required prior to study entry. Information about the study will be displayed on posters on the ward and

patients who wish to withdraw from the study may do so by informing the staff on the intensive care unit. This will not affect the treatment that they receive.

11 Procedures and assessments

11.1 Screening

11.1.1 Subject identification

All patients admitted to the John Farman ICU during the study period will be eligible for inclusion in the study

11.1.2 Consent procedures

As this is a surveillance study for MDRO / healthcare-associated infections, written informed consent from individual participants will not be required prior to study entry.

11.1.3 Subject registration

All study participants will be assigned a unique anonymised identification code, which will be used in the case record form, laboratory request forms and the study database.

11.2 Baseline assessments

11.2.1 Screening for multidrug-resistant organisms (research specimens)

Participants will be screened for MDRO on admission to the John Farman Intensive Care Unit. All specimens will be assigned a unique anonymised identification number prior to transfer to the research laboratory the Department of Medicine for processing.

Specimens will be cultured on selective media for the following target organisms:

- Vancomycin-resistant enterococci (VRE)
- Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL)
- Carbapenem-resistant organisms (CRO)

Samples that are positive for suspected target organisms will undergo identification using MALDI TOF MS and antimicrobial susceptibility testing using the Vitek-2 system.

Isolates that are confirmed to be target organisms will undergo DNA extraction and storage at -80°C.

DNA extracts will be transferred in batches to the Wellcome Trust Sanger Institute for DNA library preparation and high-throughput microbial whole-genome sequencing.

11.2.2 Clinical diagnostic specimens

Clinical diagnostic specimens will be collected as clinically indicated and processed in the diagnostic microbiology laboratory at Addenbrooke's hospital according to routine clinical practice.

Samples that are positive for the target organisms will be identified by the laboratory information system and retrieved from the diagnostic microbiology laboratory on weekdays for further processing as detailed above.

11.2.3 Clinical data collection

Clinical and laboratory data will be collected on each patient by a research nurse who is part of the ICU clinical care team and entered into a case record form.

Clinical data collection will be performed on admission to ICU, on discharge from ICU and weekly during the ICU admission if the duration of admission is 7 days or longer.

Data will be entered into a secure electronic database located on the Addenbrooke's hospital server. Data will be anonymised as soon as it is practical to do so.

11.3 Follow-up assessments

11.3.1 Screening for multidrug-resistant organisms (MDRO) (research specimens)

Participants will be screened for MDRO on discharge from ICU, and weekly during their ICU admission if the duration of admission is 7 days or longer.

All specimens will be assigned a unique anonymised identification number prior to transfer to the research laboratory the Department of Medicine for processing.

Specimens will be cultured on selective media for the following target organisms:

- Vancomycin-resistant enterococci (VRE)
- Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL)
- Carbapenem-resistant organisms (CRO)

Samples that are positive for suspected target organisms will undergo identification using MALDI TOF MS and antimicrobial susceptibility using Vitek-2 system.

Isolates that are confirmed to be target organisms will undergo DNA extraction and storage at -80°C.

DNA extracts will be transferred in batches to the Wellcome Trust Sanger Institute for DNA library preparation and high-throughput microbial whole-genome sequencing.

11.3.2 Diagnostic specimens

Diagnostic specimens will be collected as clinically indicated and processed in the diagnostic microbiology laboratory at Addenbrooke's hospital according to routine clinical practice.

Samples that are positive for the target organisms will be identified by the laboratory information system and retrieved daily on weekdays from the diagnostic microbiology laboratory, for further processing as detailed above.

11.3.3 Clinical data collection

Clinical and laboratory data will be collected on each patient by a research nurse who is part of the ICU clinical care team and entered into a case record form.

Clinical data collection will be performed on admission to ICU, on discharge from ICU and weekly during the ICU admission if the duration of admission is ≥7 days.

Data will be entered into a secure electronic database located on the Addenbrooke's hospital server. Data will be anonymised as soon as it is practical to do so.

Cost data for microbiological tests, antibiotic costs and microbial whole-genome sequencing will be obtained from the diagnostic laboratory, the pharmacy

department at Addenbrooke's hospital, and from the Wellcome Trust Sanger Institute.

11.4 End of Study Participation

Subjects will cease to participate in the study when they are discharged from the John Farman Intensive Care Unit.

11.5 Sampling schedule

11.5.1 Clinical data collection and screening for MDRO (research specimens)

Screening specimens will be collected on admission, on discharge, and weekly in patients admitted for ≥7 days according the following schedule:

Study specimens	Admission	Discharge	Follow-up (weekly)
Clinical data collection	√	√	√
Stool sample / rectal swab*	√	√	√
Urine	√	√	√
Sputum or tracheal aspirate	√	√	√
Wound swab (if applicable)	√	√	√

*A rectal swab will only be collected if a stool sample is unavailable. This is standard clinical practice for screening for carbapenem-resistant organisms, as recommended by the Public Health England guidelines.

Specimens will be sent to the research laboratory in the Department of Medicine for processing Monday to Friday.

Samples that are collected at the weekend will be stored at 4°C in a refrigerator on the ICU and processed the following Monday.

11.5.2 Diagnostic specimens

Diagnostic specimens will be taken as clinically indicated and sent to the diagnostic microbiology laboratory for processing. The results of these investigations will be available to the clinical staff on ICU, and any infections will be managed according to routine clinical practice.

If any target organisms are cultured in sterile site specimens (e.g. blood cultures, cerebrospinal fluid, pleural fluid, ascitic fluid, joint aspirate) they will be identified via the laboratory database and collected from the diagnostic laboratory on weekdays. This will enable us to include samples / isolates that are not captured by the regular screening procedures described in section 9.5.1 above.

11.5.3 Environmental sampling

In order to determine the prevalence of the target organisms in the ICU environment as a possible source of infection we will perform environmental sampling prior to study commencement and at monthly intervals throughout the study.

Environmental samples will be collected using swabs from the following areas of the intensive care unit:

- Door handles of patient rooms
- Taps in patient rooms or adjacent to patient beds
- Bed rails
- Ventilation equipment
- Patient tables
- Computer keyboards

Environmental samples will be sent to the research laboratory in the Department of Medicine for processing Monday to Friday.

Samples that are collected at the weekend will be stored at 4°C in a refrigerator on the ICU and processed the following Monday.

12 Assessment of Safety

This is a prospective observational cohort surveillance study of MDRO in the John Farman Intensive Care Unit.

All participants will receive routine clinical care, including antimicrobial therapy for infections, if clinically indicated.

There are no study-specific interventions or treatments and therefore no need for study-specific safety assessments.

If the patient develops an adverse event then this will be managed according to routine clinical care and reported through standard NHS clinical governance and complaints procedures.

13 Outcome measures

13.1 Primary outcome measures

The primary outcome measures are:

- Number of patients colonised with MDRO during the study period
- Number of patients with clinical evidence of infection with MDRO during the study period
- Number of transmission events of MDRO, detected by the current infection control procedures and by analysis of whole genome sequence data during the study period.

13.2 Secondary outcome measures

The secondary outcome measures are:

- Risk factors for colonisation / infection with MDRO
- Outcome of patients colonised / infected with MDRO i.e. death or alive at discharge from ICU
- Cost-effectiveness of whole-genome sequencing versus standard epidemiological investigation / typing for suspected outbreaks of infection

14 Storage and analysis of samples

Study samples will be analysed and stored in the research laboratory in the Department of Medicine at Addenbrooke's Hospital.

The original screening specimens (swabs, urine, stool, respiratory samples) will be stored at -80°C +/- 10°C in secure freezers. Positive culture isolates will also be stored at -80°C +/- 10°C in secure freezers.

DNA extracts will be transferred in batches from the Department of Medicine research laboratory to the Wellcome Trust Sanger Institute for library preparation and bacterial whole-genome sequencing on a high-throughput sequencing platform.

15 Data analysis

15.1 Statistical analysis

A formal sample size calculation is not possible as this is an observational cohort study of adults admitted to the John Farman Intensive Care Unit at Addenbrooke's hospital. The unit admits 1100 to 1200 patients per year, so we would anticipate enrolling 600 patients during the six-month study period.

Clinical and microbiological variables will be analysed using descriptive methods to determine the baseline characteristics and the frequency of carriage with the target organisms.

Clinical and microbiological data will also be analysed to determine the incidence of clinical infections with the target organisms during the study period. Risk factors for carriage and development of clinical infection with MDRO will be determined by univariate and multivariate analysis.

Transmission rates of MDRO based on epidemiological and microbiological data will also determined.

Statistical analysis will be performed under the guidance of Professor Richard Samworth, Statistical Laboratory at the University of Cambridge.

15.2 Bacterial sequence data analysis

Bacterial sequence data will be analysed using bioinformatic pipelines developed at the Wellcome Trust Sanger Institute. Phylogenetic analyses will be performed to determine relatedness of bacterial isolates and to look for evidence of transmission. Sequence data analysis will be performed under the guidance of Professor Julian Parkhill and his team at the Wellcome Trust Sanger Institute.

16 Health economic assessment

Health economic evaluations of current standard microbiological / epidemiological methods versus microbial whole genome sequencing will be performed under the guidance of Dr Gurdeep Sagoo at the PHG Foundation.

The aims of the health economics work are to:

- Determine and monitor levels of resource use in standard epidemiological investigation / typing versus whole-genome sequencing to the NHS in order to determine the cost impact of WGS.
- To undertake preliminary cost-consequences analyses of using whole-genome sequencing versus standard epidemiological investigation / typing.
- To identify drivers of the cost of using whole-genome sequencing versus standard epidemiological investigation / typing.
- To use the cost and outcome data collected to model the cost-effectiveness of using whole-genome sequencing versus standard epidemiological investigation / typing for the diagnosis of MDRO outbreaks with the ICU at Addenbrooke's hospital. The level of uncertainty associated with this analysis will also be estimated.

17 Definition of the end of the study

The end of study will be the date that occurs six months after the start of the study.

18 Data handling and record keeping

18.1 Case record form

All data will be entered into an individual case report form (CRF) for each participant. All participants will be assigned a unique anonymised identification code, which will be used on the CRF and for labelling all study samples. All study data in the CRF will be extracted from and be consistent with the relevant source documents (e.g. the electronic medical records, and the laboratory and pharmacy databases). The CRFs will be completed, dated and signed by the investigator or designee in a timely manner. The CRF will be accessible by the investigators and those monitoring / auditing the study on behalf of the Cambridge University Hospitals NHS Foundation Trust R&D Department.

All CRF pages must be clear and legible. Any errors should be crossed with a single stroke so that the original entry can still be seen. Corrections should be inserted and the change dated and initialled by the investigator or designee. If it is not clear why the change has been made, an explanation should be written next to the change. Typing correction fluid must not be used.

18.2 Source Data

To enable peer review, monitoring, audit and/or inspection the investigator must agree to keep records of all participating patients (sufficient information to link records e.g., CRFs, hospital records and samples), and copies of the CRF pages.

Source data include:

- Paper or electronic medical records
- Paper or electronic laboratory test results
- The case record form (if the information is collected only for the purposes of the study and not recorded elsewhere in the medical records)

18.3 Data Protection & Patient Confidentiality

All investigators and study site staff involved in this study must comply with the requirements of the Data Protection Act 1998 and Trust Policy with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

All documents will be stored securely and only accessible by study staff and authorised personnel. All data will be entered on a secure electronic database located on the Addenbrooke's hospital server.

Personal information will be stored securely and data will be anonymised as soon as it is practical to do so. Study participants will be identified only by their initials and a unique study specific identification number on the case record form, study specimens and in any electronic database. Study participants will not be identifiable from any publications or presentations arising from the study.

19 Study Management

19.1 Data Monitoring Committee

A Data Monitoring Committee is not required for this study as it is an observational cohort study with no study-specific interventions or treatments, and therefore no related safety concerns.

19.2 Study Steering Committee

The Study Steering Committee will comprise all investigators listed in Section 2 of the study protocol. The TSC will review the progress of the study at regular intervals and be responsible for the quality, analysis and publication of the data arising from the study.

20 Ethical considerations

20.1 Consent

Written informed consent will not be obtained from study participants prior to enrolment in the study. This is because this is a surveillance study of multidrug-resistant organisms / healthcare-associated infections. Surveillance for multidrug-resistant organisms / healthcare-associated infections is exempt from requiring individual patient consent as detailed in Section 251 of the Health Act.

In order to be able to determine accurately the prevalence and transmission of pathogens between patients the study will require 100% data capture as excluding even one patient will compromise the study design and analysis, and undermine the ability to detect transmission events. It is also not feasible to consent all patients on admission to ICU as the numbers are high (1200 per year), they can be admitted at any time of the day or night, they are often too unwell to provide consent, and relatives or legal representatives are not available to provide consent on their behalf. Furthermore if subjects were to decline participation this would result in missing data that would jeopardise the ability to conduct the study.

In terms of the risks of participation in the study these are negligible as the study is observational in nature and there are no study-specific interventions or treatments. All specimens will be collected by experienced nursing staff, in accordance with routine clinical practice.

20.2 Ethical committee review

The study protocol will be submitted to the National Research Ethics Service (NRES) Research Ethics Committee (REC) and to the Human Research Authority Confidentiality Advisory Group (HRA CAG) for approval prior to the commencement of the study.

20.3 Protocol Amendments

Protocol amendments will be reviewed and agreement received from the Sponsor for all proposed amendments prior to submission to the REC.

20.4 Peer Review

This study will be conducted as part of a programme of work funded by the Health Innovation Challenge Fund grant awarded to Professor Sharon Peacock and Professor Julian Parkhill, which has already been peer-reviewed as part of the funding application. The study protocol will be peer reviewed by Dr Sani Aliyu, Consultant in Infectious Diseases & Medical Microbiology, Cambridge University Hospitals NHS Foundation Trust

20.5 Declaration of Helsinki and Good Clinical Practice

The study will be performed in accordance with the spirit and the letter of the Declaration of Helsinki, the conditions and principles of Good Clinical Practice, the protocol, and applicable local regulatory requirements and laws.

20.6 Good Clinical Practice (GCP) Training

All clinical staff involved in the study must hold evidence of appropriate GCP training or undergo GCP training prior to undertaking any responsibilities on this study. This training should be updated every two years or in accordance with the Trust's policy.

21 Sponsorship, Financial and Insurance

The study will be sponsored by Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge.

The study is funded by the following organisations / grants:

- the Department of Health, the Wellcome Trust, and the Health Innovation Challenge Fund (HICF-T5-342 and WT098600, awarded to Professor Peacock and Professor Parkhill)
- the Academy of Medical Sciences and the Health Foundation (Clinician Scientist Fellowship awarded to Dr Torok)
- the NIHR Cambridge Biomedical Research Centre.

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the clinical study caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the study, but noone has acted negligently.

The University of Cambridge will arrange insurance for negligent harm caused as a result of protocol design and for non-negligent harm arising through participation in the clinical study.

22 Monitoring, Audit & Inspection

The study will be audited / monitored by the Cambridge University Hospitals NHS Foundation Trust Research and Development Department

23 Protocol Compliance and Breaches of Good Clinical Practice

The study will be conducted in accordance with the ICH Guidelines on Good Clinical Practice. Protocol compliance may be monitored by the Research and Development Department at Cambridge University Hospitals NHS Foundation Trust. Any major deviations to the protocol will be reported to the study sponsor.

24 Publications policy

Ownership of the data arising from this study resides with the study team. On completion of the study the data will be analysed and a final study report prepared. The study data will be prepared for presentation at national and international scientific conferences and publication in peer-reviewed scientific journals. All study investigators will have access to the final data, will contribute to and approve any publications arising. The funding bodies will have review rights and will be acknowledged in any publications arising from the study.

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