Clinical Study Protocol

Full Title of Study:	Evolution of antimicrobial resistance (AMR) in patients receiving Outpatient Parenteral Antimicrobial Therapy (OPAT)
Short Study Title:	Study of AMR in OPAT patients
Protocol Version:	Version 2.0
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Study Sponsors:	Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge

1 Protocol Signatures:

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

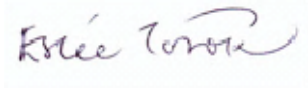
I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:

Date:

Signature:



23/01/2020

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4 Abbreviations

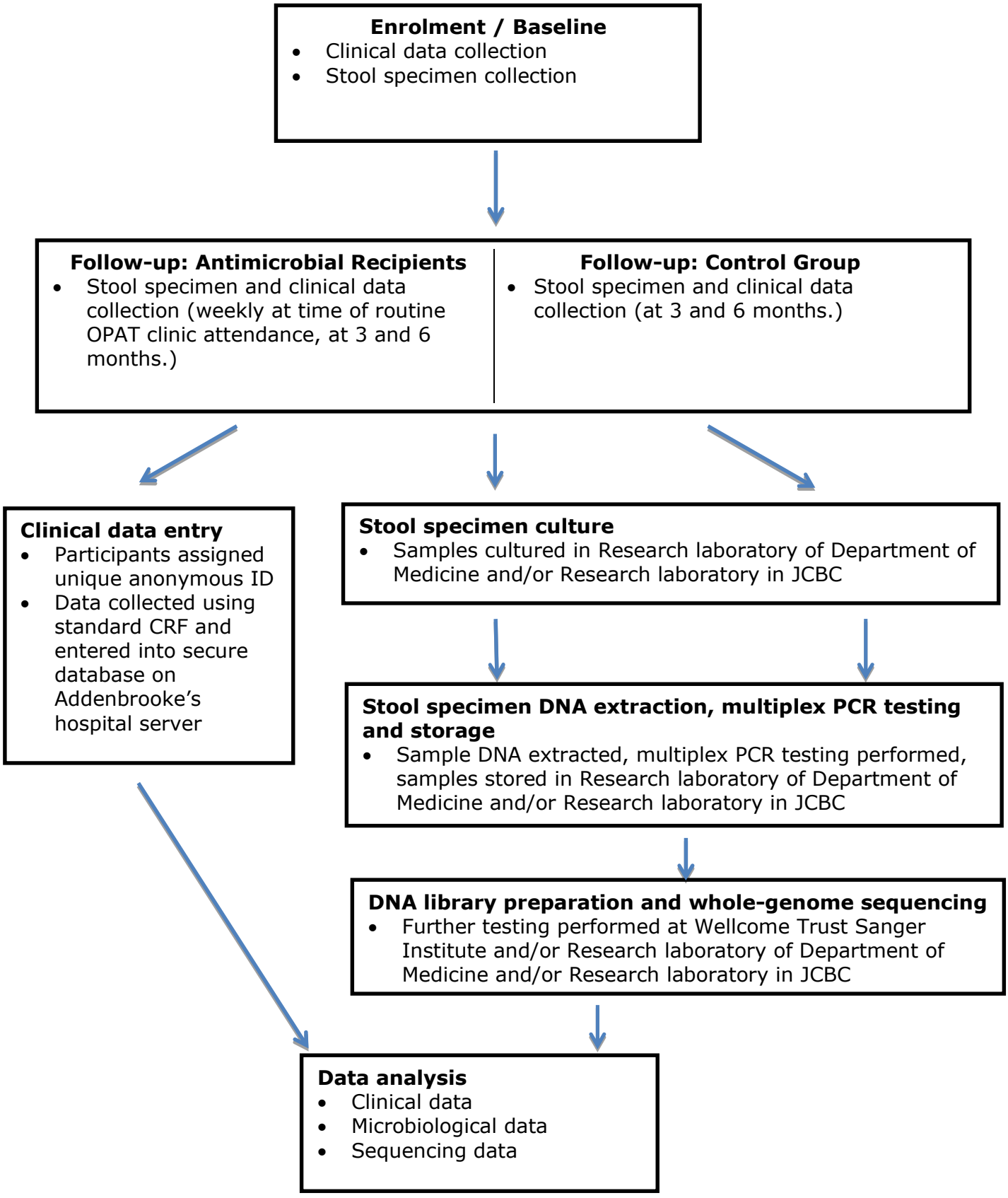
AMR	Antimicrobial resistance
ARG	Antibiotic resistance genes
CPE	Carbapenemase-producing Enterobacteriaceae
CRF	Case Report Form
ESBL	Extended spectrum beta-lactamase
GCP	Good Clinical Practice
MDRO	Multidrug-resistant organism
NRES	National Research Ethics Service
JCBC	Jeffrey Cheah Biomedical Centre
OPAT	Outpatient Parenteral Antimicrobial Therapy
PHE	Public Health England
R&D	Research and Development
REC	Research Ethics Committee
VRE	Vancomycin-resistant enterococci

5 Study Summary

Title of clinical study	Evolution of antimicrobial resistance (AMR) in patients receiving Outpatient Parenteral Antimicrobial Therapy (OPAT)
Sponsor name	Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge.
Medical condition or disease under investigation	Colonisation with multi-drug resistant organisms (MDRO.)
Purpose of clinical study	To use multiplex PCR assays and clinical metagenomic sequencing to investigate the evolution of antimicrobial resistance (AMR) in two patient cohorts (outpatient parenteral antibiotic therapy (OPAT) and controls) at Addenbrooke's hospital.
Primary objective	To determine the evolution of selected antibiotic resistance genes (ARG) in serial stool samples in two patient groups (OPAT versus control) at Addenbrooke's Hospital.
Secondary objective (s)	1. To determine correlation between phenotypic and genotypic testing for AMR. 2. To explore the effects of prolonged specific antimicrobial therapy on faecal microbiome diversity (by looking at the relative abundance of differing taxa) in two patient groups (OPAT versus control.)
Study Design	Prospective observational cohort study of 40 participants: 30 OPAT patients receiving 3 different antibiotic regimens and 10 control patients recruited from the Infectious Diseases ward / clinic.
Primary Outcome Measures	• Number of participants with stool carriage of specific ARGs at baseline, during antimicrobial therapy, at 3 months and 6 months. • Number of participants with phenotypic evidence of AMR organisms at baseline, during antimicrobial therapy, at 3 months and 6 months. • Number of participants with significant changes in the faecal microbiome diversity at selected time points.
Secondary Outcome Measures	• Risk factors for carriage of ARGs • Clinical outcome of patients with AMR organisms e.g. clinical deterioration or death
Sample Size	40 participants – 30 OPAT patients and 10 controls.
Summary of eligibility criteria	Inclusion Criteria: • Adult (age 18 years older) • Male or Female • Cases: Patients expected to receive 2 or more weeks of IV antibiotics • Controls: An Infectious Diseases ward or clinic patient who has received no

	antimicrobial therapy within the last 3 months. Exclusion Criteria: • Patients with known previous MDRO colonisation (e.g. ESBL, CPE, VRE) • Those known to be pregnant • Does not fulfil study inclusion criteria • Declines or are unable to consent.
Screening & enrolment	Participants will be considered for eligibility after referral and acceptance onto the OPAT service or admission to the ID ward/clinic. Written informed consent will be sought prior to study enrolment. At enrolment, a baseline stool sample and clinical data will be collected.
Treatment period	Samples and clinical data will be collected, weekly during any OPAT therapy, at 3 months and 6 months after enrolment.
End of Study	The end of study will be the 6 month follow up visit of the last recruited patient.
Procedures for safety monitoring during study	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 participant develops an adverse event then this will be managed according to routine clinical care and reported through standard NHS clinical governance and complaints procedures.
Criteria for withdrawal of participants	Written information about the study will be provided to the participants at recruitment. Those participants who wish to withdraw from the study may do so at any time by informing the staff during the OPAT clinic visits or contacting them using the contact information provided. This will not affect the treatment that they receive.

6 Study Flow Chart



7 Background

Public Health England recognizes Antimicrobial Resistance (AMR) as a priority and in the 2019 position statement they emphasized the importance of infection prevention and control. They pledged to "develop real time patient level data so that clinicians can see infection, treatment and resistance histories … and to help develop new interventions for AMR". [1] This study seeks to do this in a novel way using Next generation sequencing techniques.

The use of intravenous broad-spectrum antimicrobials in the community has increased with the introduction of the Outpatient parenteral Antimicrobial Therapy (OPAT) service. The service often uses daily administration of well tolerated antimicrobials to allow the early discharge from hospital in selected patients. However, it has been demonstrated that high rates of use of these antimicrobials is a driver in AMR development.

If bacteria develop antibiotic resistance genes (ARGs) they may become multidrug-resistant organisms (MDROs) such as ESBL-producing Enterobacteriaceae, carbapenemase-producing Enterobacteriaceae (CPE) or vancomycin resistant enterococci (VRE.) Carriage of MDROs has implications not only for the treatment of individual patients, limiting the number of effective and non-toxic antimicrobials available to them in the setting of infection, but also for the wider population because of the risk of transmission of MDROs in healthcare settings.

Next generation sequencing is an emerging technology and has the potential to transform the practice of clinical microbiology.[2] Sequencing techniques are currently available to confirm the presence of certain MDROs in clinical samples and can guide physician's antimicrobial choice in real time. For example, the use of PCR can confirm the presence of CPE in clinical samples such as urine samples or pus swabs. However, stool samples are not routinely screened prior to the initiation of antimicrobials to guide choices tailored to the individual patient. As the majority of MDRO (CPE, ESBL and VRE) originate or reside primarily in the gut, the stool sample is an expected high-yield screening sample.

It has been demonstrated that many factors, including antimicrobial courses, reduce the diversity of the faecal microbiome allowing an environmental niche for potential MDRO to flourish. [3] [4] We are interested specifically in the temporal effect that antimicrobial courses have on antimicrobial resistance and the faecal microbiome. There are few studies in adult patients that have explored this previously.

A European dentistry study involving 2 sites showed a single dose of an antimicrobial (ciprofloxacin, clindamycin or minocycline) in 66 healthy individuals transiently reduced the faecal microbiome diversity during a 12 month period. [5]

One US study followed 4 patients after they had received a 6 week course of antimicrobials. It compared these to 5 control patients and showed a non-statistically significant increase in genes involved in resistance to beta lactams, vancomycin, macrolides, tetracyclines and penicillin-binding proteins by examining faecal flora with 16S PCR testing. [6]

Another US study, of 8 patients with diarrhoeal symptoms and 2 healthy faecal-donors, showed recent antibiotic exposure correlated with reduced faecal diversity in the microbiome. 16S PCR testing showed the 2 patients with VRE had enterococcus faecium responsible for 67-84% of their faecal microbiome.[7]

Another US study of 3 patients showed that the majority of taxa within the faecal microbiome recovered to baseline levels within 4 weeks after completion of a 5 day course of ciprofloxacin. [8]

A study comparing 58 Irish elderly care home residents, who had received antibiotics in the month prior to faecal profiling, showed the cultured numbers of Bifidobacterium species were significantly reduced, when compared with 79 patients who had not received antibiotics. [9]

A UK study in 24 ITU patients compared faecal biodiversity, using shotgun metagenomic sequencing, and isolate abundance using quantitative PCR, over time in patients receiving different broad-spectrum antimicrobials. Intravenous meropenem was significantly associated with reduced microbial diversity and the increase in dominance of pathogens, including enterococcal blooms. This was not seen in patients receiving piperacillin/tazobactam and the authors suggested further research was needed to explore which antimicrobial regimes spared changes in gut microbiota. [10]

8 Rationale for Study

We propose that detecting AMR genes in stool samples, before the associated bacteria have opportunity to cause potentially life-threatening difficult-to-treat infection, would allow clinicians to alter antimicrobials and remove selection pressures. This could allow the faecal microbiome to restore equilibrium and potentially clear these bacteria carrying the AMR genes.

The previously published studies are not directly comparable to our cohort for the following reasons. They have recruited differing patient cohorts (those in intensive care, nursing homes, visiting dentists) compared to our community-based clinic cohort. Often, they have a single sampling period or short follow-up sampling period. The individual antimicrobials studied are either not stated or are not comparable to those used in our cohort. The data regarding comorbidities is often not collected, or not published, which makes comparison with our patient group unreliable. Often, they have focused either on ARG, or MDRO, or faecal microbiome changes after antimicrobials but not the three together.

We do not currently collect stool samples to screen for ARG or MDRO in our OPAT clinic. We therefore have no accurate data concerning their incidence or prevalence and no baseline data to compare any future interventions in this group. Local research has shown community carriage of vancomycin resistant *E. faecium* in stool to be 7% (3/45 participants in a long-term care facility in Cambridgeshire in 2014) [11] and ESBL producing *E. Coli* in urine/stool to be up to 38% (17/45 participants in a long-term care facility in Cambridgeshire in 2014.)[12] CPE carriage rates in the region are known to be low compared to a national rate in clinical samples of 0.02%. [13] Research performed at our site shows that ESBL producing *K. pneumoniae* in urine samples, between 2006 and 2012, displayed different MDR lineages approximately every 2 years. This highlights the transient nature of AMR, particularly when carried on genetically mobile elements such as bacterial plasmids, and supports the need for ongoing surveillance such as this study provides. [14]

In order to address these limitations, we propose to conduct a prospective surveillance study of AMR in adults accepted by the OPAT clinic versus a control group at Addenbrooke's Hospital in Cambridge. By studying these high-risk patients, clinicians will be able to quantify the risk of MDRO and the development of AMR *in vivo*. Data generated would inform MDRO screening policies, antimicrobial policies and infection control polices in this setting. Associations between ARGs, MDROs and changes in the faecal microbiome would be explored.

Future applications of this research could lead to the introduction of tailored antimicrobial choices for individual patients; choosing antimicrobials that are shown to have a lower risk

of generating AMR, MDRO and reduced faecal microbiome diversity. Individualized antimicrobial choices could be based on stool sampling prior to embarking on antimicrobial therapy.

Finally, we anticipate this research may identify the types of individuals at high risk of AMR evolution and persistent carriage who may be suitable for potential interventional trials (involving prebiotics, probiotics or faecal transplantation) that attempt to "reset" the faecal microbiome and thus reduce the relative abundance of MDROs and other bacteria associated with AMR. [2][15][16][17]

9 Study design

9.1 Statement of design

This is a prospective, observational, cohort study of carriage of ARGs and associated AMR organisms, of 40 adults under the care of the Infectious Diseases and OPAT Services, at Addenbrookes hospital. This includes 30 participants referred to and accepted by the OPAT service (and thus receiving intravenous antimicrobials) and 10 participants (not receiving antimicrobials) selected from the Infectious Diseases ward or clinic during the study period. It is a surveillance study of carriage of Antimicrobial Resistance genes and associate organisms. There are no study-specific treatments or interventions.

Recruited participants will provide a baseline stool sample within 72 hours of commencing OPAT therapy or at time of recruitment in the control group. Clinical data will be collected at baseline.

Those on OPAT therapy will provide clinical data and stool samples weekly throughout treatment, at 3 and 6 months after enrolment. The control group will provide clinical data and stool samples at 3 and 6 months after enrolment.

Participants will receive routine standard clinical care, including additional antibiotics to treat infections during the follow up periods if necessary, as clinically indicated.

9.2 Number of Centres

This is a single centre study, which will be conducted in the OPAT Clinic and Infectious Diseases department at Addenbrooke's Hospital in Cambridge.

9.3 Number of Subjects

We plan to include 40 participants in this study. This consists of 4 groups of 10 participants. Each group will receive either intravenous cephalosporin (ceftriaxone), carbapenem (ertapenem), cyclic lipopeptide (daptomycin) or no antimicrobials (the control group.) As this is a surveillance study, we are unable to perform a power calculation to determine sample size.

A comparison of 10 participants per antimicrobial group, totalling 30 participants, in addition to 10 controls is larger than most of published studies. The use of multiple sampling of participants over time (at baseline, weekly during therapy, at 3 and 6 months) will allow for additional comparison over at least 4 time points in the 3 antimicrobial groups and at 3 time points in the control group. Therefore, a minimum of 150 stool samples are anticipated: (30x4) + (10x3) = 150.

9.4 Participants Study duration

The participants' study duration will be for 6 months and end at the time of the 6 month follow up appointment.

9.5 Study objectives

9.5.1 Primary objective

To determine the evolution of selected antibiotic resistance genes (ARGs) in serial stool samples in two patient groups (OPAT versus control) at Addenbrooke's Hospital.

9.5.2 Secondary objectives

1. To determine correlation between the phenotypic and genotypic testing for AMR in all patients

2. To explore the effects of prolonged specific antimicrobial therapy on faecal microbiome diversity (by looking at the relative abundance of differing taxa) in two patient cohorts (outpatient parenteral antibiotic therapy (OPAT) and controls) at Addenbrooke's hospital.

9.6 Study Outcome Measures

9.6.1 Primary outcome measures

The primary outcome measures of the study will be:

- Number of participants with stool carriage of specific ARG isolated at baseline, during antimicrobial therapy, at 3 months and 6 months.
- Number of participants with phenotypic evidence of AMR at baseline, during antimicrobial therapy, at 3 months and 6 months.
- Number of participants with significant changes in the faecal microbiome diversity at selected time points.

9.6.2 Secondary outcome measure

The secondary outcome measures of the study will be:

- Risk factors for carriage of ARGs
- Clinical outcomes of patients with AMR organisms e.g. clinical deterioration or death.

10 Selection and withdrawal of subjects

10.1 Inclusion Criteria

To be included in the study the participants must be:

- Aged 18 years old or older
- Male or female
- Cases: Patients accepted by OPAT service and expected to receive 2 or more weeks of intravenous antibiotic
- Controls: An Infectious Diseases ward or clinic patient who has received no antimicrobial therapy within the last 3 months

10.2 Exclusion Criteria

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

- Those known to have multi-drug resistant organisms (MDRO) previously. This will include VRE, CPE or ESBL-producing Enterobacteriaceae
- Those known to be pregnant at enrolment
- Does not fulfil study inclusion criteria
- Declines or unable to consent

10.3 Treatment

This is a prospective observational cohort study involving participants receiving OPAT versus a control group recruited from the Infectious Diseases ward or clinics at Addenbrookes hospital.

Participants will receive routine clinical care, including any necessary subsequent antibiotics to treat infections, as clinically indicated. There are no study specific interventions or treatments.

10.4 Subject withdrawal criteria

Written information about the study will be provided to the participants at recruitment. Those participants who wish to withdraw from the study may do so at any time by informing the staff during the OPAT clinic visits or contacting them using the contact information provided. This will not affect the treatment that they receive.

11 Procedures and assessments

11.1 Screening

11.1.1 Subject identification

All patients referred to and accepted by the OPAT service during the study period will be screened for eligibility. The first 30 eligible patients who give consent will be included in the study. The study requires 10 participants in each antimicrobial group: 10 receiving Ceftriaxone therapy, 10 receiving Ertapenem therapy and 10 receiving Daptomycin therapy. All patients admitted to the Infectious Diseases inpatient ward and Clinic setting during the study period will be screened for eligibility and the first 10 eligible patients who give consent will be included in the study as the control group.

11.1.2 Consent procedures

Written informed consent will be obtained from individual participants 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 Stool sample collection

Participants will provide a self-collected stool sample at time of recruitment to the study. Those receiving antimicrobial therapy will provide this within 72 hours of commencing OPAT.

All stool specimens will be assigned a unique anonymised identification number prior to transfer to the research laboratory of the Department of Medicine and the Jeffrey Cheah Biomedical Centre for processing. This will be on a labelled sample pot given to the patient at recruitment. Replacement individually labelled sample pots will be available if required by contacting the study team.

Specimens will be cultured on selective media (such as MacConkey agar) to identify gram-negative bacteria and MDRO. A sweep of selected bacteria will be plated onto media with serial dilutions of the antimicrobials of interest. Colony counts will be performed to quantify the number of bacteria viable at differing antimicrobial

dilutions. Resistant organisms will undergo identification using MALDI TOF MS (Bruker) and antimicrobial susceptibility testing using the Vitek-2 system (BioMerieux.) The isolates of interest will undergo DNA extraction and storage at -80°C prior to sequencing.

Specimens will also be cultured on chromogenic agar for CPE, ESBL and VRE. Resistant organisms will undergo identification using MALDI TOF MS (Bruker) and antimicrobial susceptibility testing using the Vitek-2 system (BioMerieux.) The isolates of interest will undergo DNA extraction and storage at -80°C prior to sequencing. Any clinically relevant MDROs will be reported to the Infection Control Lead Clinician and communicated to the patient in line with the usual protocols.

Stool samples will also undergo DNA extraction and testing using multiplex PCR for selected ARGs in the research laboratory in the Jeffrey Cheah Biomedical Centre.

The DNA extraction from the stool and from the resistant organisms will be transferred in batches to the Wellcome Trust Sanger Institute for DNA library preparation and high-throughput microbial whole-genome sequencing and/or tested in the research laboratory of the Department of Medicine and/or tested in the research laboratory in the Jeffrey Cheah Biomedical Centre.

11.2.2 Clinical data collection

Clinical data will be collected at recruitment to the study. It will be collected for each participant by the study team clinical member who is part of the OPAT and Infectious Diseases clinical care team. This data will be taken from the hospital's electronic medical records and directly from the patient themselves and will be entered into a case record form.

Data will be collected on the following factors that have been shown to alter faecal microbiome diversity: age, gender, weight, ethnicity, recent travel, smoking status, alcohol intake, specific dietary requirements (including probiotic and prebiotic use,) medical and surgical history (including Faecal Microbiota Transplant,) previous colonisation or infection with multidrug resistant organisms, recent antimicrobial use and current gastrointestinal symptoms (nausea, vomiting, diarrhoea.)

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 Stool sample collection

Participants receiving OPAT will provide a self-collected stool sample weekly during therapy, at 3 and 6 months after completion of therapy, giving a total of least 4 samples. Participants recruited as controls will provide a self-collected stool sample at 3 and 6 months after enrolment, giving a total of 3 samples.

The follow-up samples will be delivered by patients to the clinical staff in the OPAT clinic. They will be placed and stored in a fridge by the OPAT clinical team until same-day transfer to the research laboratory.

All stool specimens will be assigned a unique anonymised identification number prior to transfer to the research laboratory of the Department of Medicine and the Jeffrey Cheah Biomedical Centre for processing. This will be on a labelled sample pot given to the patient at recruitment. Replacement individually labelled sample pots will be available if required by contacting the study team.

Specimens will be cultured on selective media (such as MacConkey agar) to identify gram-negative bacteria and MDRO. A sweep of selected bacteria will be plated onto media with serial dilutions of the antimicrobials of interest. Colony counts will be performed to quantify the number of bacteria viable at differing antimicrobial dilutions. Resistant organisms will undergo identification using MALDI TOF MS (Bruker) and antimicrobial susceptibility testing using the Vitek-2 system (BioMerieux.) The isolates of interest will undergo DNA extraction and storage at -80°C prior to sequencing.

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Stool samples will also undergo DNA extraction and testing using multiplex PCR for selected ARGs in the research laboratory in the Jeffrey Cheah Biomedical Centre.

The DNA extraction from the stool and from the resistant organisms will be transferred in batches to the Wellcome Trust Sanger Institute for DNA library preparation and high-throughput microbial whole-genome sequencing and/or tested in the research laboratory of the Department of Medicine and/or tested in the research laboratory in the Jeffrey Cheah Biomedical Centre.

11.3.2 Clinical data collection

Clinical data will be collected for each participant by the study team clinical members who are part of the OPAT and Infectious Diseases clinical care team. For those receiving OPAT therapy, this data will be collected weekly from the patient's electronic medical records. Limited data collection will include information on the following factors: recent antimicrobial use, recent hospital contact, recent *Clostridium difficile* associated diarrhoea or current gastrointestinal symptoms (nausea, vomiting, diarrhoea.) This data will be entered into a case record form.

Clinical data will be collected for all participants at the 3 and 6 months by the study team member, either in clinic or remotely via telephone, and from the patient's electronic medical records. (This will be collected at the same time as sample collection.) Limited data collection will include information on the following factors: recent antimicrobial use, recent hospital contact, recent *Clostridium difficile* associated diarrhoea or current gastrointestinal symptoms (nausea, vomiting, diarrhoea.) This data will be entered into a case record form.

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.

11.4 End of Study Participation

All study participants will cease to participate in the study at their last follow up visit which is at 6 months post enrolment in the study.

Sampling schedule

11.4.1 Clinical data collection and stool sampling

Clinical data and stool samples will be collected on recruitment, weekly during any OPAT therapy, at 3 and 6 months follow up.

Time point for clinical data and stool sample collection	OPAT therapy groups (30)	Control group (10)
Recruitment	√	√
Weekly during any OPAT therapy	√	Non-applicable
3 months	√	√
6 months	√	√

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

Samples that are collected at the weekend from an inpatient will be stored at 4°C in a refrigerator on the patient's hospital ward and processed the following Monday.

12 Assessment of Safety

This is a prospective, observational, cohort study of carriage of Antimicrobial Resistance genes and associated organisms, of 40 adults under the care of the Infectious Diseases and OPAT Services, at Addenbrookes hospital.

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

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

If the participant 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 Storage and analysis of samples

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

The original stool specimens will be stored at -80°C +/- 10°C in secure freezers. Cultured isolates of interest will also be stored at -80°C +/- 10°C in secure freezers. Extracted DNA from stool samples and isolates of interest 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 and/or testing at the research laboratory of the Department of Medicine and/or testing at the research laboratory in the Jeffrey Cheah Biomedical Centre.

Samples will be stored for 15 years in accordance with Medical Research Council (MRC) Good Research Practice guidelines.

14 Data analysis

14.1 Statistical analysis

A formal sample size calculation is not possible as this is an observational cohort study of adults accepted by the OPAT service and receiving care on the Infectious Diseases ward or in the Infectious Diseases clinic setting.

The OPAT service accepts approximately 12 new patients a week and it is anticipated that the 10 would be eligible for recruitment. Allowing for recruitment of 25% of eligible patients per month, it is anticipated the Antimicrobial group's recruitment period would take a maximum of 3 months.

The Infectious Diseases ward accepts approximately 14 new patients a week and the clinics see in excess of 50 additional new patients a week. It is anticipated that 1 ward patient and 25 additional clinic patients would be eligible for recruitment to the control group. Allowing for recruitment of 25% of eligible patients it is anticipated the control group could be recruited to within a maximum of 1 month.

Clinical variables will be analysed using descriptive methods to determine the baseline characteristics and their relation to stool carriage of ARG.

Risk factors for development of ARG and MDRO will be determined by univariate and multivariate analysis.

14.2 Bacterial sequence data analysis

Bacterial sequence data will be analysed using bioinformatic pipelines developed at the Wellcome Trust Sanger Institute, locally in the research laboratory of the Department of Medicine and in the research laboratory in the Jeffrey Cheah Biomedical Centre.

15 Definition of the end of the study

The end of study will be the date that occurs at the 6 month follow up visit of the last recruited patient.

16 Data handling and record keeping

16.1 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). 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.

16.2 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
- The case record form (if the information is collected only for the purposes of the study and not recorded elsewhere in the medical records)

CRFs will be stored for 15 years in accordance with Medical Research Council (MRC) Good Research Practice guidelines.

16.3 Data Protection & Patient Confidentiality

All investigators and study site staff involved in this study must comply with the requirements of the Data Protection Act 2018 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, date of birth 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.

17 Study Management

17.1 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.

17.2 Study Steering Committee

The Study Steering Committee will comprise all investigators listed in the study protocol. The SSC 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.

18 Ethical considerations

18.1 Consent

Written informed consent will be obtained from study participants prior to enrolment in 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.

18.2 Ethical committee review

The study protocol will be submitted to the National Research Ethics Service (NRES) Research Ethics Committee (REC) for approval prior to the commencement of the study.

18.3 Protocol Amendments

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

18.4 Peer Review

This study will be conducted as part of a programme of work funded by the Academy of Medical Sciences and the Health Foundation awarded to Dr Estee Torok, Clinician Scientist Fellow and Honorary Consultant in Infectious Diseases and Microbiology. The study protocol has been peer reviewed by Dr David Enoch (PHE employee) Consultant in Microbiology.

18.5 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.

18.6 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.

19 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 Academy of Medical Sciences and the Health Foundation (Clinician Scientist Fellowship awarded to Dr Torok)
- the NIHR Cambridge NIHR BRC AMR theme

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 no-one 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.

20 Monitoring, Audit & Inspection

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

21 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.

22 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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