



Full title of study	Relationship of antifungal exposure to emergence of Candida resistance in Intensive Care patients: a multi-site cohort study
Short study title / acronym	Candida ICU resistance (CandiRes)
Protocol version number and date	5.0 , dated 13 July 2026
IRAS number	305864
JRES reference number	2021.0219
Funder reference number	Pfizer 68715765
REC reference number	21/WA/0370
This protocol has regard for the HRA guidance and order of content	

Signature page

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.

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Study summary

Study title	Relationship of antifungal exposure to emergence of <i>Candida</i> resistance in Intensive Care patients: a multi-site cohort study
Internal short title	<i>Candida</i> ICU resistance (CandiRes)
Study design	Prospective observational cohort study

Study setting	Intensive care units across nine NHS Trusts: 1. Guy's and St Thomas' NHS Foundation Trust (GSTT) 2. St George's University Hospitals NHS Foundation Trust (SGH) 3. King's College Hospital NHS Foundation Trust (KCH) 4. Liverpool University Hospitals NHS Foundation Trust (LUH) 5. Royal Free Hospital NHS Foundation Trust (RFH) 6. University Hospital of Wales (UHW) 7. London North West University Healthcare NHS Trust (LNWH) 8. Sheffield Teaching Hospitals NHS Trust (STH) 9. University Hospitals Sussex NHS Foundation Trust (UHS)
Study participants	Adult patients admitted to intensive care (ICU) treated for invasive Candida infection Baseline evaluation: patients on antifungals in ICU or with invasive candidiasis between 2015 – 2020
Inclusion/exclusion criteria	Inclusion criteria 1. Age ≥ 18 years 2. Suspected or confirmed invasive candidiasis Exclusion criteria 1. Expected ICU length of stay from study enrolment < 48h
Planned sample size	n = 520 including 220 invasive candidiasis
Interventions	• Twice weekly oral/ perianal swab for Candida spp. during ICU admission • Serum/ plasma/ whole blood save

- 7 blood draws from CVC at 0h, 6h, 12h, 24h, 36h, 48h, 72h and D7 for serial culture, BDG/Candida PCR, PK (total volume: 65-139 ml above standard of care); BDG/ BDG/Candida PCR D14 and D21 (total volume: 6ml above standard of care)
- Urine sampling at 0h, 24h, 72h and D7 for fungal culture, If drain in situ: drain fluid sampling (10 ml) at 0h, 6h, 12h, 24h, 72h and D7, then every 72h for fungal culture and PK until drain removed
- Stool sampling for faecal mycobiome analysis in patients with confirmed candidaemia

Objectives and outcome Measures	Objectives	Outcome Measures
	To describe the use of antifungal drugs and the epidemiology of invasive candidiasis in the ICU	Incidence of invasive candidiasis in the ICU and rationale and duration of antifungal therapy
	Compare species distribution and resistance profile (MIC, genetic testing) of <i>Candida</i> colonising flora in ICU patients exposed or not exposed to an antifungal.	Incidence rate of colonisation and invasive infection with azole or echinocandin-resistant <i>Candida</i> spp. (e.g. <i>C. glabrata</i> ; <i>C. parapsilosis</i> ; <i>C. auris</i>) in those exposed or not exposed to an antifungal during their ICU stay. Change in isolate MIC and supra-MIC growth (tolerance).
	Elucidate mechanism of resistance evolution in serial clinical isolates and relationship with drug exposure.	Change in MIC and supra-MIC growth Emergence of genetic mutations associated with antifungal resistance
	Evaluate treatment response biomarkers in candidaemia and	Rate of decline in CFU/ BDG/ PCR/ time-to-culture-positivity in candidaemic patients treated with antifungals.

relationship with antifungal drug exposure	Exploratory: relationship between <i>Candida</i> resistance phenotype (MIC, tolerance) and genotype, antifungal (echinocandin) drug levels and pharmacokinetics and mycological clearance in candidaemia. In-hospital mortality
Evaluate contribution of faecal mycobiome to invasive infection	Stool sample for faecal mycobiome analysis on days 1, 7 and 14

Follow up duration Until ICU discharge or day 30 (whichever is longer).

Study timelines 5th January 2022 – 31st July 2027: patient enrolment, prospective clinical data and sample collection.
1st July – 31st July 2027: Data analysis and dissemination.

Research aims 1. To assess the *Candida* resistance ecology of ICU patients (colonising and invasive isolates), and its relationship with antifungal exposure. 2. To evaluate mycological clearance as a treatment response biomarker and its relationship with resistance evolution and antifungal exposure in patients with candidaemia.

Funding and support

<i>Funder</i>	<i>Financial and non-financial support given</i>
Pfizer	Grant number 68715765 - £107K total budget, including 12 months lab technician salary, sample courier and consumables for study.

Medical Research Foundation UK	£90K Emerging Leaders in Antimicrobial Resistance Award to Dr T Bicanic: clinical research fellow salary support 12 months plus £25K consumables
Gilead Fellowship	Grant number 17437 (£124635) 12 months clinical research fellow salary; 6 months lab tech salary; courier, consumables and PK analyses
Medical Research Council	Grant code MR/Y002164/1 – Extending the utility and durability of antifungal agents via innovative treatment regimens that minimise drug resistance.

Patient and public involvement

City St George's University of London Research Expert Group/ with lived experience of ICU admission and suggested edits King's College Hospital ICU PPI incorporated (see section 7.5. Patient & Public group Involvement).

Protocol contributors

This study protocol was designed by the Chief Investigator in conjunction with the site principal Investigators, co-investigators, clinical research fellows and the statistician.

The funders of this study (Pfizer/ Medical Research Foundation/ Gilead) had no role in the study design, and will play no role in the conduct of the study, data analysis, presentation or publication of findings

The sponsor (CSGUL) will be responsible for study conduct, data analysis and interpretation, manuscript writing, and dissemination of results.

There has been patient and public involvement in the design of the study and patient information sheets (see section 7.5. Patient & Public Involvement).

This study is also participating in the NIHR Associate PI Scheme. Associate PIs at participating sites will support study delivery under the supervision of the local Principal Investigator. This is an NIHR initiative to develop research capacity and does not impact study design, participant involvement or data collection procedures.

Abbreviations

AUC	Area under the (drug concentration-time) curve
BC	Blood culture
BDG	Beta-D-glucan
BMT	Bone marrow transplant
BSI	Bloodstream infection
CFU	Colony-forming units
CI	Chief Investigator
CQUIN	Commissioning for quality and innovation
CVC	Central venous catheter
ECMO	Extra corporeal membrane oxygenation
EORTC	European Organization for Research and Treatment of Cancer/ Invasive Fungal Infections Cooperative Group
GCP	Good Clinical Practice
HRA	Health Research Authority
IC	Invasive candidiasis
ICF	Informed Consent Form
ICU	Intensive Care Unit

IRAS	Integrated Research Application system
JRES	(City St George's) Joint Research and Enterprise Services
MDR	Multi-drug resistant/ resistance
MIC	Minimum inhibitory concentration
NHS	National Health Service
PI	Principal Investigator
PIS	Patient Information Sheet
PK/PD	Pharmacokinetics/ pharmacodynamics
PSN	Participant Study Number
REC	Research Ethics Committee
R&D	NHS Trust Research & Development Department

1. Background

1.1. Invasive candidiasis

Invasive candidiasis (IC) is the most common invasive fungal infection in the UK, with an estimated 5,000 cases each year¹. The term 'IC' comprises three clinical conditions; candidaemia; deep seated candidiasis (most frequently intra-abdominal); and deep-seated candidiasis with associated candidaemia². Critically ill patients managed in Intensive Care Units (ICU) are particularly vulnerable, with an estimated 30-40% of all candidaemia occurring in this setting³. Unfortunately, even with the use of antifungal therapy, the overall crude mortality for invasive candidiasis is estimated to be between 40-60%, with attributable mortality in the range of 15-25%⁴⁻⁶. Treatment options for IC are limited with just four antifungal drug classes available; azoles (fluconazole, voriconazole); echinocandins (anidulafungin, caspofungin, micafungin); polyenes (amphotericin); and the pyrimidine analogue flucytosine. In critical care, antifungal prescribing is dominated by the use of fluconazole

and echinocandins which account for three-quarters of all consumption, much (>40%) of which is empiric (unpublished data), i.e. directed at suspected but not confirmed fungal infection. This empiricism coupled with lack of heterogeneity in antifungal use risks propelling the development of antifungal resistance. Preserving the effectiveness of our limited antifungal armamentarium is vital to ensuring we have viable treatment options to manage invasive candidiasis in the future.

1.2. Epidemiology of *Candida* resistance

Antifungal resistance in *Candida* spp. is a growing problem. Over the last two decades, there has been a global shift in *Candida* epidemiology; while *C. albicans*, which is largely fluconazole sensitive, accounted for most infections historically, wider use of azoles in clinical practice has led to the selection for less azole susceptible species such as *C. glabrata* and *C. krusei*⁷. Echinocandin resistant *Candida* spp. remain rare as a cause of bloodstream infection in the UK (<2%)⁸, however, in settings of high echinocandin use in the US, incidence has increased (~6-12%)⁹⁻¹¹. Concerningly, approximately a third of these isolates have cross resistance to fluconazole¹². Moreover, the recently emerged, multi-drug resistant hospital-acquired pathogen *C. auris* has caused large outbreaks in a number of UK critical care units^{13,14}. *C. auris* is usually fluconazole-resistant, with variable amphotericin and echinocandin susceptibility, and pan-fungal resistance reported. The significant clinical, operational and financial implications of *C. auris* outbreaks in the UK have been well published, with refurbishment of entire critical care units and introduction of costly infection control measures¹⁵. The emergence of *C. auris* as a persistent coloniser of both patients and the ICU environment has highlighted the lack of epidemiological data on resistance profiles in *Candida* spp. in ICU, as hitherto it has been standard laboratory practice to only characterise invasive *Candida* isolates to species level and undertake resistance profiling for national surveillance.

1.3. Evolution of resistance

Our understanding of the evolution of antifungal resistance in *Candida* is limited. *Candida* spp. are commensals in the human gut, skin and mucous membranes. Patients develop invasive infection following breaches in barrier

defences such as the gut (perforation or surgery) or skin (line insertion) allowing entry of *Candida* into a sterile site¹⁶. Exposure to broad spectrum antibiotics facilitates *Candida* overgrowth: a recent faecal microbiome study demonstrated that expansion of the *Candida* population in the gut predates candidaemia in bone marrow transplant recipients, but the skin may also be a source of candidaemia^{17,18}. The gastrointestinal tract is a key reservoir for multi-drug resistant (MDR) pathogens; indeed, there is good evidence that antimicrobial exposure allows for the selection and evolution of MDR Gram-negative bacteria which can persist long after antimicrobials are stopped¹⁹. These colonising MDR organisms can cause infection in the individual, but can also contaminate the hospital environment leading to patient-to-patient transmission of resistant organisms. It is likely that the same phenomenon occurs with *Candida*, particularly given that penetration of widely used antifungal drugs (particularly echinocandins) to sites such as the skin, gut and urinary tract is sub-optimal²⁰. Recent outbreaks of *C. auris* in ICUs highlight our lack of understanding of the extent of intra-hospital transmission of fungal resistance between patients on ICU, despite being demonstrated in bacterial pathogens such as *Klebsiella*²¹. Furthermore, evidence of how antifungal drug exposure impacts fungal ecology at relevant body sites and drives the emergence of resistance in the clinical setting is lacking.

A study examining the gastrointestinal tract in mice exposed to antifungals demonstrated that the gut is a major source of echinocandin resistance in *C. glabrata*²². Additionally, a retrospective study in France between 2005-2012, matching prescribing data to the epidemiology of colonising fungal species in critical care patients, demonstrated an increased incidence of *C. glabrata* colonisation with the use of pre-emptive fluconazole²³. There was little echinocandin use over the study period therefore impact of echinocandin use could not be rigorously assessed. Given echinocandin use has significantly increased, particularly in ICU where it is now recommended as first-line therapy in international guidelines^{24,25}, a prospective study to assess the impact of antifungals currently in widespread use on fungal ecology is urgently needed.

In the subset of *Candida*-colonised patients who go on to develop invasive candidiasis, resistance can also emerge during antifungal treatment. While multiple mechanisms of resistance in *Candida* spp. (discussed below) have been described, most studies on evolution of *Candida* resistance have been performed *in vitro* and are not understood in the context of drug exposure. Assessment of resistance development from the time point of antifungal commencement in patients with invasive candidiasis has not been explored. Insight into the dynamics

of how the pathogen responds to the pressure of drug therapy in clinical practice is important in furthering our understanding of antifungal resistance.

Additionally, reliable and reproducible biomarkers of treatment response in candidaemia are needed²⁶ for evaluating the effectiveness of new drug therapies, antifungal combinations and antifungal stewardship interventions. Validation of these biomarkers must incorporate careful measurement of antifungal resistance emergence as well as mycological clearance.

1.4. Mechanisms of resistance

Like in bacteria, antifungal resistance in *Candida* spp. can be intrinsic or acquired, and occur via single or multiple mechanisms affecting expression of or binding to the drug target (encoded by the ERG11 gene for azoles and FKS gene for echinocandins), or activation of drug efflux via membrane transporters. This can occur through classical point mutations e.g. gain-of-function mutations in the ERG11 transcription factor Upc2; gain-of-function mutations in transcription factors Pdr1 or Tac1 regulating expression of CDR efflux pumps; point mutations in ERG11 (azole resistance), or point mutations in two hot spot regions of FKS (echinocandin resistance). In contrast to bacteria, fungi also have the ability to transiently duplicate all or parts of chromosomes containing drug targets or efflux pump genes (aneuploidy) in response to drug stress, again resulting in overexpression (e.g. chromosome 5- containing ERG11 and TAC genes- disomy in *C. albicans* and *C. auris* and azole resistance²⁷). Moreover, some of these genetic mechanisms are present in a very small subpopulation of cells in the fungal population, termed *heteroresistance*, that subsequently emerges under antifungal selection pressure: this has been described in *Candida glabrata* as well as in another human fungal pathogen, *Cryptococcus*^{28,29}. Unlike in bacteriology (e.g. MRSA, MDR TB), genetic testing is not currently in routine use to look for fungal resistance markers, even in mycology reference laboratories.

Cellular processes promoting the formation of resistant strains involve complex stress response pathways yielding a variety of adaptive compensatory responses- collectively known as *tolerance*, which is likely a prelude to development of genetic resistance. Tolerance to both azoles and echinocandins is measurable and reproducible and has been shown to occur in clinical isolates of *Candida* spp. and impact fungal clearance in

animal models^{22,30}; however its relevance to fungal clearance and evolution of resistance in human infection is yet to be demonstrated.

In the diagnostic hospital laboratory, antifungal resistance is usually determined through phenotypic methods (Minimum Inhibitory Concentration, MIC) missing many of the above mechanisms, including tolerance and heteroresistance, which can currently only be evaluated in real-time using in vitro research methods.

Using serial plating onto non-drug and drug-containing media, we have previously successfully applied *ex vivo* real-time methods to track the emergence and mechanism of resistance in HIV infected African patients with cryptococcal meningitis treated with fluconazole or combination fluconazole with flucytosine²⁸, and demonstrated a relationship between drug exposure (pharmacokinetics), fungal clearance (pharmacodynamics) and resistance emergence³¹. We now propose to apply these same principles to UK Intensive care patients with candidaemia. Echinocandins (micafungin, caspofungin, anidulafungin) are the first-line treatment for candidaemia and are highly protein-bound drugs. Critically ill patients may exhibit alterations in drug PK due to the systemic inflammatory response and interventions such as renal replacement therapy and ECMO, leading to changes in volume of distribution or clearance, with inter-individual as well as inter-drug variability^{32,33}. As echinocandin efficacy is related to the AUC/MIC ratio, this in turn could affect microbial clearance and resistance evolution.

1.5. Treatment response biomarkers in candidaemia

In cryptococcal meningitis, early fungicidal activity of drugs using the rate of decline in serial quantitative cultures from patient cerebrospinal fluid is an established treatment response biomarker, facilitating smaller-scale phase II clinical trials to prioritise novel and existing agents to take forward to phase III trials^{34,35}. No such biomarker has been validated for candidaemia. This is in part due to a much lower fungal burden (10-1000-fold lower) in the bloodstream in candidaemia compared to that in cerebrospinal fluid in cryptococcal infection, resulting in a reduced sensitivity of blood cultures (BC) for diagnosis of candidaemia (50-60%)².

A 2011 study used lysis centrifugation BC systems to evaluate the fungal burden in 152 patients with candidaemia: median CFU/ml was 1, range 0.1 to >1000, with higher fungal burdens in children, patients with a CVC and candidaemia due to non-albicans spp, consistent with findings in earlier studies in the 1980s and on a par with organism burden in bacterial BSIs (median bacterial load 0.01-1CFU/ml). This was a retrospective study with no record of or standardisation of timing and site of BC collection (peripheral or central venous catheter, CVC) or inoculum volume, demonstrated by multiple studies to be a key determinant of BC yield, with volumes of ≥ 30 ml generating a sensitivity of 95-99% for BSI³⁶: UK and International guidelines recommend a draw of 4-6 blood culture bottles (40-60ml; or 8-10ml per bottle) in evaluation of suspected sepsis, although in practice a single BC is often performed, and bottles are often underfilled³⁷. Lysis centrifugation has now been largely replaced by automated liquid culture platforms in diagnostic laboratories (BD Bactec or BACT/ALERT, BioMerieux). These do not permit fungal burden quantitation, but allow recording of time-to-culture positivity, an indirect correlate of organism burden, demonstrated to be a useful marker of catheter-related BSI, including candidaemia³⁸. Development of antigen-based (beta-D glucan, BDG) and molecular-based (T2MR) diagnostics for candidaemia opens the prospect of non-culture-based biomarkers. The T2MR, a rapid diagnostic test (4h), combining MR with nanotechnology to identify *Candida* DNA in whole-blood, detects *Candida* with greater sensitivity than BC and stays positive for longer than BC in candidaemia^{39,40}. This assay requires expensive equipment and kits and does not detect all *Candida* spp. (including *C. auris*), thus is unlikely to be implementable as a treatment response biomarker.

BDG, measuring a component of fungal cell walls in blood, is a test widely used in the UK. The largest retrospective analysis of BDG kinetics in candidaemia included 203 patients enrolled in an IC clinical trial with ≥ 2 serial BDG values (Fungitell)⁴¹. Mean BDG levels at diagnosis in those with treatment success or failure (composite clinical/ microbiologic endpoint) were 600 and 1318 pg/ml respectively, well above the test positivity threshold of 80 pg/ml. A negative slope between initial and final BDG was associated with a successful outcome (positive predictive value, PPV 90%), whilst a positive slope was associated with treatment failure (negative predictive value, NPV 90%). Recent retrospective studies of candidaemia showed that persistently negative BDG were associated with higher rates of infection clearance, fewer complications and lower 30d mortality^{42,43}. The kinetics of BDG during invasive fungal infection thus warrant prospective study.

2. Rationale

2.1. Potential for improved patient care & need for this study

Understanding link between antifungal drug exposure and colonising and invasive *Candida* resistance ecology underpins antifungal stewardship

The 2019/2020 introduction of the NHS CQUIN to optimise the use of antifungals and implement antifungal stewardship nationally highlights this as a UK health priority⁴⁴. The most common type of antifungal prescribing in critical care is empirical therapy when *Candida* infection is suspected but not proven. Understanding the effect of antifungal exposure on the fungal microbiome is essential to gain understanding of how prescribing practice impacts on resistance development in the clinical setting, underpinning prescribing and stewardship policies.

Understanding micro-evolution of resistance in clinical practice is essential to evaluating diagnostic tools for resistance

Our understanding of the micro-evolution of *Candida* resistance to date largely comes from animal studies and small clinical isolate collections with limited accompanying clinical data. A well-designed, prospective study collecting clinical metadata designed specifically to examine *Candida* resistance ecology and in vivo microevolution and link this to antifungal exposure will extend our existing knowledge of resistance epidemiology and mechanism, giving it clinical context and greater relevance. In the case of candidaemia, echinocandin exposure, isolate MIC, presence of aneuploidy or *FKS* mutations and *in vitro* measures of tolerance could be examined to determine

the best tools to predict whether a given isolate will respond to antifungal treatment

Treatment response biomarkers are needed to understand the link between microbial clearance and resistance development in vivo, and better assess the effect of novel therapeutic strategies to prevent emergence of resistance

Biomarkers are needed for assessment of treatment effectiveness and monitoring clinical response in candidaemia. Repeated daily blood cultures to ensure microbiologic clearance are recommended clinical practice^{24,45}. Quantitative measure of serial *Candida* CFU/ml or BDG values in blood provide a linear (rate of decline) rather than binary outcome measure (culture sterility yes/no). Phase II trials using such biomarkers could be sufficiently powered to show significant differences in treatment response with relatively small numbers of patients per arm, as successfully applied in another invasive fungal infection, cryptococcosis⁴⁶.

Serial quantitative measurements of fungal load using *Candida* CFU/ml³⁶, T2MR⁴⁷ and BDG⁴⁸ have been shown to be feasible in patients with candidaemia, but have not yet been validated as treatment response biomarkers, nor correlated with antifungal drug exposure. Validation of such a marker, in comparison to the current binary outcomes currently used in clinical trials, could facilitate wider and more cost-effective implementation to evaluate the effectiveness of drug therapies in phase II clinical trials, identifying the most promising compounds and combinations to take forward into much larger phase III trials powered for clinical endpoints.

2.2. Study hypotheses

Antifungal use impacts local fungal ecology, and acts a driver for the micro-evolution of resistance. Patients exposed to azoles or echinocandins during their ICU stay will be more likely to become colonised with non-*albicans Candida* spp. in the skin or gut, with isolates showing increased MICs to the drug of exposure, associated with evidence of stress adaptation and/or genetic changes (point mutations/ genomic rearrangements) associated with antifungal resistance. The widely used echinocandin antifungals do not penetrate skin, urine or gut well: these may be sentinel sites for resistance emergence in the ICU.

Patients develop invasive candidiasis in critical care with their colonising flora.

Candida spp. colonise the gut as well as the skin of patients on ICU. Exposure to antibiotics leads to expansion of *Candida* spp, and exposure to antifungals drives selection of more resistant species. Sequencing will allow us to confirm whether patients' own colonising flora is the source of their *Candida* BSI.

Serial quantitative measurements (*Candida* CFU/ml, time to culture positivity, BDG) are a feasible marker of microbiologic treatment response.

We will enrol a small number of patients with candidaemia in a pilot study to determine whether a quantitative biomarker can be plotted to calculate a slope of treatment response. We will assess variability in treatment response with drug exposure using PK/PD modelling. If validated such a marker could be extremely useful in future phase 2 trials comparing efficacy of antifungals.

3. Research question and aims

3.1. Aims

1. To assess the *Candida* resistance ecology of ICU patients (colonising and invasive isolates), and its relationship with antifungal exposure.
2. To evaluate mycological clearance as a treatment response biomarker and its relationship with resistance evolution and antifungal exposure in patients with candidaemia.

3.1.1. Specific objectives

1. To describe the use of antifungal drugs and the epidemiology of invasive candidiasis in the ICU
2. Compare species distribution and resistance profile (MIC, genetic testing) of *Candida* colonising flora in ICU patients exposed or not exposed to an antifungal.
3. Evaluate treatment response biomarkers in IC and relationship with antifungal drug exposure.
4. Elucidate mechanism of resistance evolution in serial clinical isolates and relationship with drug exposure.
5. Evaluate association between faecal mycobiome and candidaemia.

3.2. Outcome measures

Primary outcome

Incidence rate of colonisation and invasive infection with azole or echinocandin-resistant *Candida* spp. (e.g. *C. glabrata*; *C. parapsilosis*; *C. auris*) in those exposed or not exposed to an antifungal during their ICU stay.

Secondary outcomes

- Incidence of invasive candidiasis in the ICU
- Rationale and duration of antifungal therapy
- Change in Isolate MIC and supra-MIC growth (tolerance);
- Emergence of genetic mutations associated with antifungal resistance in serial colonising and invasive isolates;
- Rate of decline in *Candida* CFU/ BDG/ PCR/ time-to-culture-positivity in candidaemic patients treated with antifungals;
- In-hospital mortality;
- Exploratory: relationship between *Candida* resistance phenotype (MIC, tolerance) and genotype, antifungal (echinocandin) drug levels PK, mycological clearance (PD) and faecal mycobiome in candidaemia

4 Study design, methods, data collection and analysis

4.1. Study design

CandiRes is a multi-centre prospective observational cohort study assessing the impact of antifungal use on the evolution of fungal resistance in colonising and invasive *Candida* isolates in the ICU, with a non-interventional treatment response biomarker pathway for participants who develop invasive candidiasis.

In this observational study taking place over five years at five ICUs in London teaching hospitals and ICUs in Aintree University Hospital, Liverpool, University Hospital of Wales, Sheffield Teaching Hospitals and University Hospitals Sussex, we aim to enrol 520 adult patients admitted to the ICU and at higher risk of *Candida* infection, including 220 patients treated for invasive candidiasis. Of these, a proportion will be receiving antifungal treatment. Following informed consent, we will collect clinical information (see section 4.3. Data collection) and take twice weekly swabs from the mouth and perianal skin, profiling the *Candida* isolates identified in the

laboratory for antifungal resistance (see section 4.2.3. Isolate collection and testing). We will then compare the development and mechanisms of resistance in those who did or did not receive an antifungal during their ICU stay.

We will take serial samples of blood or drain fluid to measure how quickly the *Candida* infection is cleared from the bloodstream, and the impact that fungal resistance and drug level in that individual has on the speed of this clearance (see section 4.2.5. Invasive candidiasis).

Participants are considered to have completed the study once they have been discharged from ICU or after 30 days since enrolment, whichever is later. No further data or samples will be collected and no further participation is required. Once study results are published, site teams will contact the participants to disseminate the findings, for participants who opted into this.

Retrospective critical care antifungal prescribing data and adult candidaemia epidemiology from the previous 5 years at the three sites will be collated to understand the antifungal prescribing practices and the epidemiology and incidence of invasive candidiasis in the ICU (see section 4.2.1 Baseline evaluation).

4.2. Outline of methods

Study procedures are listed in Table 4 and Table 5 in the Appendix (page 65).

4.2.1 Baseline evaluation

Study sites have previously audited antifungal prescribing and the management of invasive candidiasis between Jan 2015- Dec 2020 according to the *European Confederation of Medical Mycology (ECMM)* criteria to evaluate local prescribing and invasive candidiasis management. This retrospective data (n≈200 patients at each site) will be anonymised and collated to evaluate rationale and indication for antifungal use in critical care, and the distribution by species and incidence of invasive candidiasis in the ICU. This knowledge will be used to contextualise results and underpin the prospective observational study.

4.2.2. Baseline blood draw

A baseline blood sample will be collected, from the CVC or arterial line whenever possible. Of this, 3 ml will be spun down and serum and plasma aliquoted and stored at -80°C for measurement of baseline BDG and Candida PCR. Unless the participant opted out of DNA sampling, 2.5 ml will be inoculated directly into a whole blood EDTA tube for future research. See also 6.3.4. Biological sample handling.

4.2.3. Isolate collection and testing

As part of routine ICU care, all ICU patients at the three sites have throat, skin and rectal screening swabs sent to microbiology laboratories to look for MRSA and resistant gram-negative bacteria on admission and then at regular intervals during ICU admission (varies between sites). Clinical isolates from patients with candidaemia are routinely stored in the local laboratory or sent to the Reference laboratory.

Study participants will have an additional mouth and perianal swab taken twice weekly throughout their ICU stay, whenever possible at the same time as these screening swabs. If a participant is discharged from ICU after less than 30 days, a final swab will be taken between ICU discharge and day 30. If a participant is readmitted to ICU before day 30, regular swabbing will be restarted.

If a participant has been in the study for more than 30 days, the frequency of swabbing whilst in ICU will be reduced to once weekly.

Urine samples (10 mL) will be collected for culture at 0h, 24h, 72h and D7. Further urine samples will be taken every 72h if urine culture remains positive and the patient has a urinary source of candidaemia.

Participants who have a drain in-situ – we will take serial samples of fluid: 10 ml at 0h, 6h, 12h, 24h, 72h, D7 then every 72 hours until the drain is removed or the patient is discharged from ICU.

Upon lab receipt, urine, abdominal fluid and the swabs will be streaked onto *Candida* chromogenic agars and incubated at 30°C for up to 5 days. Cultured fungi will be identified using MALDI-ToF mass spectrometry (Bruker Daltonics, Germany) and stored at -80°C for subsequent resistance profiling.

Patients with candidaemia will have a stool sample collected on day 1, 7 and 14. These will be stored in RNALater at -80°C for subsequent analysis of faecal mycobiome.

4.2.4. Resistance profiling

Candida isolates will undergo MIC testing using a standard broth microdilution method. Plates will be read at 24h to determine MIC and at 72h to determine the fraction of the population growing above the MIC (supra-MIC growth, a marker of tolerance)³⁰. CLSI guidelines will be used to interpret MIC values (i.e. resistant, susceptible, susceptible-dose dependant). *Candida* isolates with MICs in the resistant range and any serial isolates from a single participant with rising MICs following antifungal (echinocandin/ azole) exposure will undergo whole genome sequencing for relevant resistance-associated mutations and partial or whole gene copy changes (e.g. *FKS1* and *FKS2* for echinocandins; *ERG11*, *UPC2*, *TAC1*, chromosome 5 duplications for azoles).

4.2.5. Invasive candidiasis

Participants will be flagged via routine Microbiology systems or screening of antifungal prescriptions and have the following procedures, summarised in the Appendix tables (Appendix 1: Schedule of study procedures). Once started, sampling should continue regardless of antifungal drugs being stopped, or negative results.

Serial blood samples will be taken at 0, 12, 24, 36, 48, 72h and day 7 (refer to Table 5, page 68 for volumes); the D7 culture sample is only to be performed in the minority of participants whose 72h blood culture is still positive for *Candida*). As ESCMID guidelines recommend daily blood cultures in routine clinical management of candidaemia in the first 48-72h of treatment until cultures are negative²⁵, only 3 of these samples are over- and-above routine sampling as part of clinical care.

10 ml of the BC sample will be injected directly into BACTEC BC bottles, transferred promptly to the laboratory and incubated at 37°C for 5 days in the automated instrument as per routine microbiology practice, and time-to-culture-positivity recorded. The 3ml serum sample, and 8ml of whole blood (only to be taken if most recent haemoglobin (Hb) level is $\geq 80\text{g/dL}$) will be collected for quantitative assessment of candida burden using quantitative culture techniques, BDG and candida PCR. BDG and PCR will be batch-tested and performed at the end of the study. Antifungal drug concentrations in serum will be quantified using high-performance liquid chromatography.

To more accurately model drug exposure in this ICU population, a small number of participants (up to 20) will have 2ml of blood taken at additional timepoints within the first 24h of antifungal therapy (1, 2, 4 and 6h). Abdominal drain fluid samples (if present) will be taken at 1, 2, 4 and 6h.

We will also culture and store all *Candida* bloodstream isolates collected as well as any accompanying isolates from sterile/ non-sterile sites (e.g. urine, wound swab, drain fluid, line tip).

All above assays will be performed on pseudo-anonymised samples (labelled with participant study number [PSN] only) by a member of the research team or collaborators blinded to any participant clinical data.

4.3. Data collection

Participants will be allocated a PSN on enrolment. Prospective clinical and microbiological data will be entered into an NHS-compatible REDCap™ database using the PSN. A separate 'key' log will be kept securely at each site linking the PSN to participant identifiable information (name, date of birth, NHS number); this will take the form of a paper log stored in a locked cabinet at site. Research blood samples will be stored using the PSN. The PSN will not reveal the identity of the participant or the clinical outcome to laboratory staff undertaking the subsequent laboratory diagnostic evaluation (see 6.3.3. Data collection tool). Table 1 lists the data fields to be collected and entered into the REDCap database at baseline, during and following the ICU stay.

For the baseline evaluation, the data collection has already been completed at each site by the clinical team, who will anonymise the data before it can be transferred onto REDCap (see also section 4.2.1 Baseline evaluation).

Table 1. Data collection items

Timing	Type	Data points
Baseline	<i>Demographics</i>	Age, gender
	<i>Eligibility criteria</i>	Date and time of ICU admission, date and time of enrolment
	<i>Candida risk factors</i>	Use of intravenous antibiotics, abdominal surgery in the previous 4 weeks, upper gastrointestinal/mediastinal perforation or surgery in the last 4 weeks, liver failure, haematological malignancy, previous bone marrow or solid organ transplant, neutropenia, receipt of an immunosuppressive drug, TPN, renal replacement therapy, ECMO
	<i>Severity of illness</i>	SOFA, APACHE II score at ICU admission
	<i>Candida colonisation</i>	Date and time of sampling, sample site, species identification
Through ICU stay (weekly)	<i>Blood sample</i>	Date and time of sampling
	<i>Antifungal and antibiotic use</i>	Drug, dose, indication, start and end dates and times
	<i>Swabs</i>	Date and time of twice-weekly swabbing
	<i>Invasive candidiasis</i>	Date and time of culture positivity, sample site, species identification, antifungal susceptibility profile, EORTC diagnostic classification, evidence of disseminated infection, date and time of serial blood sampling, date and time of stool sampling
End of study	Date of ICU discharge, ICU readmission, in-hospital mortality assessments	

4.4. Data analysis

4.4.1 Descriptive analyses

The number of participants with colonisation or bloodstream infection with less susceptible *Candida* species will be divided by the total participant-days to calculate an incidence rate.

We will calculate the percentage of participants exposed to antifungals at the start of the study, as well as the rate of new antifungal prescriptions during the ICU admission. We will summarise antifungal classes and individual drugs using proportions.

We will summarise continuous variables using median and interquartile range (illness severity scores, length of ICU stay, time to culture positivity). Exploratory mortality analyses will be performed (overall in-hospital mortality rate). Serial CFU counts, BDG, *Candida* PCR and exploratory biomarker (mannan antigen, mannan antibody, CAGTA) measurements over time in participants with invasive candidiasis will be visualised using line graphs. The presence and emergence of fungal genetic mutations associated with antifungal resistance will be summarised.

4.4.2. Regression modelling

In univariable analysis, the effect of previous antifungal exposure on colonisation or bloodstream infection with less susceptible *Candida* species will be modelled using Poisson or Cox regression, to calculate a hazard ratio (HR) and its 95% confidence interval (95% CI).

In the primary multivariable analysis, age, illness severity, length of ICU stay, and other possible confounders will be listed as covariates in the Poisson or Cox model above, to calculate adjusted HR and 95% CI. We will conduct a likelihood ratio test to test the null hypothesis that, after adjusting for the listed covariates, there is no association between antifungal exposure and incidence of colonisation or infection with less susceptible *Candida* species.

4.4.3. Biomarkers

The rate of decline over 7-21 days in time-to-culture positivity/ BDG / Candida PCR / mannan antigen and antibody / CAGTA (mycologic clearance) in candidaemic participants will be calculated using linear mixed effects models (as used in prior studies in cryptococcal meningitis). Further models will be constructed to explore the relationship between baseline isolate MIC and tolerance levels and presence of relevant genetic mutations with fungal clearance.

Information on echinocandin dosing, more detailed PK measurements from up to 20 participants, together with published pharmacokinetic (PK) models will be used to model mycological clearance (PD) data incorporating the infecting *Candida* isolate MIC. This combined PK-PD model will be used to in an exploratory analysis to estimate the proportion of candidaemic ICU patients achieving sterilisation of blood with echinocandin therapy. Simulations of alternative dosing regimens will be used to predict optimal dosing strategies that maximise *Candida* clearance. These simulations could be used to inform subsequent clinical trials. These analyses will be conducted in collaboration with Dr Dagan Lonsdale (site PI, SGH and CSGUL), Prof Joe Standing (collaborator, UCL) both of whom have extensive experience in antimicrobial PK/PD modelling, and Dr Eric Decloedt (Stellenbosch University). We will analyse the association between faecal mycobiome markers and invasive infection.

6. Study sites

CandiRes is a multicentric study; it will take place in ICUs across nine Trusts:

- Guy's and St Thomas' NHS Foundation Trust (GSTT)
- St George's University Hospitals NHS Foundation Trust (SGH)
- King's College Hospital NHS Foundation Trust (KCH)
- Liverpool University Hospitals NHS Foundation Trust (LUH)
- Royal Free London NHS Foundation Trust (RFH)
- University Hospital of Wales (UHW)

- London North West University Healthcare NHS Trust (LNWH)
- Sheffield Teaching Hospitals NHS Trust (STH)
- University Hospitals Sussex NHS Foundation Trust (UHS)

At each site potential participants will be identified and enrolled by site PIs or delegated members of their team (see section 6.3. Recruitment).

The research teams, under the oversight of the site PI, will undertake:

- Data entry into the REDCap™ database (see section 6.3.3. Data collection tool)
- Twice weekly *Candida* screening swab culture and isolate storage (see section 4.2.3. Isolate collection and testing)
- Blood sampling, serial cultures, processing and storage (see section 4.2.5.)
- Approaching participants with Recovered capacity (see section 6.3.2. Consent)

6. Sample and recruitment

6.1. Eligibility criteria

On ICU admission, patients will be screened for eligibility based on the inclusion/exclusion criteria outlined below.

6.1.1. Inclusion criteria

- 1) Age ≥ 18 years

2) Suspected or confirmed invasive candidiasis

6.1.2. Exclusion criteria

1. Expected ICU length of stay from study enrolment <48h

6.2. Sampling

6.2.1. Size of sample

For the retrospective evaluation, all patients prescribed an antifungal on ICU or who had invasive candidiasis antifungal prescribing and invasive candidiasis between 2015 – 2020 at the three sites were included.

Over a 5-year enrolment period we expect approximately 60000 ICU admissions, of which 6% of admissions (~3600 patients) will receive systemic antifungal therapy during their ICU stay, with patients with the risk factors listed in other criteria above more likely to receive an antifungal and develop IC. An enrolment target of up to 300 patients over 6 months (100 per site, i.e. 3-4 per week per site) was originally considered feasible by ICU research teams, though enrolment was slower, at 1-2 per week, so continued for 14 months in order to achieve this sample size. A sample size of 300 calculated (150 exposed + 150 unexposed) gives ≥80% power to identify a proportion ratio (PR) ≥1.36 in those exposed to antifungals, and 91% power to identify a PR ≥1.42. Power calculations were performed using R package *pwr* (version 1.3).

Over the same period, based on audits from Microbiology databases at each of the sites, we would expect 300-400 candidaemias in patients in ICU: enrolment of half these patients in CandiRes would allow us to meet our recruitment target of 150-200 participants for the invasive candidiasis pathway. If an ICU patient developing candidaemia is not already enrolled in CandiRes, there will be scope to enrol these patients at the point of developing invasive candidiasis.

6.2.2. Sampling technique

All potentially eligible patients who consent to be enrolled will be included in the study.

6.3. Recruitment

6.3.1. Participant identification

Adults admitted to ICU will be screened for eligibility by the PI's team at site. Each potential participant must satisfy all the approved inclusion and exclusion criteria of the protocol to be eligible for enrolment.

Patients with candidaemia will be prospectively identified by the on-site microbiology laboratories and patients flagged to the site research team, to ensure timely sampling of patients enrolled in CandiRes.

6.3.2. Consent

The consent process will be undertaken by a delegated member of the PI's team at site.

In many cases it will not be possible to obtain prospective informed consent from the patient at the time of enrolment. This is because many patients will have a reduced level of consciousness due to their illness or due to sedative medications used as part of their treatment.

Patient consent

If possible, informed consent will be obtained directly from the patient. The patient will be informed about the study and given a copy of the Participant Information Sheet (PIS). Patients will be given an adequate amount of time to consider their participation in the study. If the patient decides to participate in the study they will be asked to sign the Patient Consent Form, together with the responsible clinician/researcher. The participant will retain one copy of the signed Consent Form. Another copy will be placed in the participant's medical records whilst the original will be retained in the study Site File. Participants are expected to have been transferred

and/or discharged up until the 30-day data collection point and will be assumed (if not withdrawn before this point) to have given consent up to this data collection point. Fluctuating capacity is anticipated for this patient group. Any participants who are initially able to consent will be asked to identify a person who knows them well in order to support with monitoring of their ongoing consent, alongside standard care.

Personal Consultee Declaration

If the patient is unable to give consent then advice will be sought from the patient's Personal Consultee, who may be a relative, partner or close friend. This is in line with the legal requirements for obtaining consent in patients without capacity in England and Wales (Mental Capacity Act 2005). The Personal Consultee will be informed about the study, provided with a copy of the Consultee Information Sheet, and asked to give an opinion as to whether the patient would object to taking part in medical research. The Consultee will be given adequate time to consider the patient's participation in the study. If the Consultee decides that the patient would have no objection to participating in the study, they will be asked to sign the Consultee Declaration Form together with the consenting clinician / researcher. The Consultee will retain a copy of the signed Declaration/Assent Form. A second copy will be placed in the participants' medical records whilst the original will be retained in the Study Site File.

If the Personal Consultee advises that, in their opinion, the patient would not choose to participate in CandiRes, please refer to the "Withdrawal of participants" section on page 42.

Remote consent procedure

It is expected that visitation to the ICU (by friends and family) could be restricted as an infection control measure. This would make it more challenging for the research team to obtain a personal consultee or patient declaration in person. As a result, remote consent may be taken following the steps below:

- PIS discussed by telephone with personal consultee.
- PIS posted or emailed to personal consultee.

- The outcome of the consultation will be documented and signed by the person seeking opinion on the Personal Consultee Telephone Declaration, countersigned by a witness.
- A copy of this document will be sent to the Consultee via email or post; a second copy will be placed in the participants' medical records; the original will be retained in the Study Site File.

If it is not possible to provide PIS and declaration to personal consultee via email or post in the required time then nominated consultee consent can be taken and research team will follow up with personal consultee at the earliest opportunity.

Nominated Consultee Declaration

If the patient is unable to give informed consent and attempts to meet and discuss with a Personal Consultee have failed then consent will be sought from a Nominated Consultee, i.e., an ICU Consultant who is not an Investigator on the study.

The opinion of the Nominated Consultee will be sought in the same manner as for the Personal Consultee (see section above).

Deferred consent

Deferred consent will be an option for the small number of patients treated for invasive candidiasis but are not already enrolled in CandiRes.

Candidaemia is a complication of critical illness requiring immediate antifungal therapy. The majority of these patients will have an altered level of consciousness and lack capacity to consent to research. Moreover, the emergency clinical situation can cause further distress for anxious relatives, raising ethical concerns both about the burden of trying to understand the study and the ability of a Personal Consultee or Nominated Consultee to provide an opinion about participation on a very tight timescale, as blood samples need to be drawn before the first dose of antifungal is administered. For these reasons there may be some cases where attempts to obtain

prior informed consent from either a Personal or Nominated Consultee, are not possible in the circumstance of candidaemia/invasive candidiasis.

Patients with candidaemia/invasive candidiasis will therefore be recruited as soon as possible, without *prior* consent; deferred consent will be sought upon recovery of capacity. This is an accepted consent model in adult emergency and critical care research in which participants lack mental capacity; it minimises the additional burden on families during a distressing time. It is covered by HRA guidance (<https://www.hra.nhs.uk/planning-and-improving-research/policiesstandards-legislation/research-emergency-settings/>) and the Mental Capacity Act, Section 30.

Before a patient regains capacity to consent, a delegated member of the site team will obtain Personal or Nominated Consultee consent as soon as appropriate and practically possible. Ideally, this approach would take place once the patient's medical situation is no longer an emergency, and within 24-48 hours of recruitment.

Once a participant is deemed to have full capacity to provide informed deferred consent, they will be approached by a delegated member of the site research team (see "Recovered Capacity" section below).

Recovered Capacity

Patients, for whom an opinion was given by a Consultee or who were recruited without prior consent at the time of candidaemia or invasive candidiasis will be monitored in line with GCP and if they gain capacity by the time of primary hospital discharge, they will be informed of their participation in the study. The clinician / researcher will discuss the study with the patient and the patient will be given a copy of the Recovered Capacity PIS to keep. The patient will be asked for consent to continue follow-up in the study or will be supported if they wish to withdraw, it will be confirmed that data already collected will be retained by default unless the participant or their Guardian/Welfare Attorney or Relative requests otherwise. If consent is given, the patient will be asked to sign the Recovered Capacity Consent Form. The patient will retain one copy of the signed Consent Form. Another copy will be placed in the patient's medical records whilst the original will be retained in the Study Site File. If

the patient does not want to continue follow-up in the study, no further clinical data beyond that time-point or new samples will be collected.

The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment.

Withdrawal of participants

Participants may withdraw from the study at any time without prejudice. This could be because the participant, or their Consultee/Guardian/Welfare Attorney withdraws consent, or because of Lead clinician opinion.

According to the design of the study, participants may have the following two options for withdrawal:

- Participants may withdraw from any further communication but allow the study team to continue to access their medical records and any relevant hospital data that is recorded as part of routine standard of care; i.e., CT-Scans, blood results and disease progression data etc. No further samples will be taken/stored but any samples already taken/stored will still be included in the study.
- Participants can withdraw completely from the study and withdraw the data and samples collected up until the point of withdrawal. The data and samples already collected would not be used in the final study analysis. Stored samples would be destroyed.

The type of withdrawal and reason for withdrawal will be recorded.

Retrospective baseline audit data

For patients included in the retrospective baseline audit data (candidaemia and ICU antifungal use) informed consent is not needed; all data was collected as part of routine clinical care by the clinical team; it will be anonymised and collated by the clinical care team. Additionally, given it is retrospective, informed consent would not be feasible.

Study amendments

If changes to the protocol are made such that further informed consent is required at a later date, this will be sought and PIS and IFC updated.

6.3.3. Data collection tool

The site team will collect clinical and microbiological data from source (electronic notes systems and hospital databases) and enter it into electronic Case Report Forms in an NHS compatible REDCap™ database (Research Electronic Data Capture). This database will only be accessible by the site research team and CSGUL research team.

Only the PSN will be used to enter data into REDCap. The informed consent forms and a 'key' log linking this study number to the participant's medical record will be kept at each site.

Any information collected during the study will be kept strictly confidential and will only be used for research purposes (see section 7.7. Data protection and patient confidentiality).

6.3.4. Biological sample handling

Fungal (*Candida*) samples

Candida screening swabs: Swabs will be transported in sealed non-sterile bags and processed at site Microbiology or research laboratories by trained laboratory or research personnel following established protocols and risk assessments. *Candida* spp. Are Hazard Group 2 pathogens as well as human gut commensals: invasive infection is rare in immunocompetent individuals and outside of the hospital environment and treatment is available. *Candida* spp. Can be handled on the open bench wearing gloves and laboratory coats. Isolates will be stored on microbeads in dedicated 80°C freezers. See also 4.2.3. Isolate collection and testing.

Isolates from sterile/ non-sterile sites: Isolates will be harvested from culture plates from swabs sent to the Microbiology laboratory as part of routine clinical care and stored as above.

Blood and drain fluid samples

All research staff handling blood will be Hepatitis B vaccinated and fully trained in laboratory procedures. Participants will have a baseline blood draw taken by research nurses via a CVC using aseptic technique: 2.5ml will be injected into whole blood DNA tubes (providing not opted out) and immediately refrigerated; the remaining 3 ml will be centrifuged in on site research labs and serum aliquoted into 3 cryovials and stored in -80°C freezers for subsequent baseline BDG measurement (see also 4.2.2. Baseline blood draw).

Serial blood draws will be taken via the CVC or arterial line whenever possible using aseptic technique by research nurses or the clinical research fellows. 10ml will be inoculated into BC bottles and incubated following local microbiology laboratory standard operating procedures and 8ml for quantitating the Candida load using culture and microscopy techniques (unless participant's Hb <80mg/dl, see section 4.2.5. Invasive candidiasis. The remaining 5ml will be divided into aliquots and stored in CSGUL research freezers at -80°C in HTA-compliant facilities. Serial 5 mL urine sample will be collected for culture and PK at 0h, 24h, 72h and D7. For participants with a drain in situ, serial samples of drain fluid will be collected and similarly processed – see section 4.2.5. Invasive candidiasis. Stored plasma aliquots will subsequently be transported from CSGUL to undergo batch testing for antifungal drug levels (PK collaboration J Standing UCL and E Decloedt Stellenbosch University), BDG levels and Candida PCR (to be batch tested post-study end at the Mycology Reference Unit in Cardiff (collaborator Lewis White) and at Radboud University Medical Centre (collaborator Frank van der Veerdonk). De-identified DNA samples from whole blood will be analysed to assess genome patterns in patients at risk of invasive candidiasis, in collaboration with F van der Veerdonk at Radboud University Medical Centre. De-identified stool samples will be centrifuged to homogenisation with 9ml of RNALater then stored at -80°C for subsequent shipping to collaborator A Alanio at Hôpital Saint-Louis for faecal mycobiome analysis.

Only members of the site research team will have access to the stored biological samples. Analyses will only be performed within the scope described in this protocol. If there is a desire to use the stored samples for a subsequent research project a new IRAS application will be made.

7. Ethical and regulatory considerations

7.1. Ethical considerations

Most patients on ICU will be unable to give informed consent due to alterations in consciousness and cognition caused by illness and, in some cases, therapeutic sedation. Consent will therefore be obtained in line with legal requirements for obtaining consent in patients without capacity in England & Wales (Mental Capacity Act 2005) (see section 6.3.2. Consent).

Some patients will have capacity to consent, either during their acute illness or after they recover. Under these circumstances the consent process will include: assessment and documentation of capacity by a trained member of a research team; providing written information about the study; allowing enough time for the patient to understand the material and ask questions; obtaining written informed consent.

7.1.1. Declaration of Helsinki

The Chief Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

7.1.2. Guidelines for Good Clinical Practice

The Chief Investigator will ensure that this study is conducted in accordance with Good Clinical Practice and that local PIs, co-investigators and clinical research fellows are up-to-date with GCP training.

7.1.3. Approvals

Ethical approval for the study was sought using the Integrated Research Application System (IRAS). For more information, please see section 7.3. Research Ethics Committee and other regulatory review & reports.

Before enrolling patients into the study, each study site must ensure that the local conduct of the study has the agreement of each NHS Trust Research & Development (R&D) department.

The CI will submit a final report to the required authorities with the results, including any publications, within one year of the end of the study.

7.2. Assessment and management of risk

This study is largely observational in nature using routinely collected hospital data: the management of patients will be unchanged, therefore no issues related to the medical management of participants are expected to arise. The only intervention for all patients will be the clinical sampling: for all patients, a single baseline blood draw of 5.5 ml and collection of twice weekly oral and perianal swabs which are non-invasive and pose very little risk.

There will be 7–13 blood draws (113–145ml total above standard of care) which will be taken via a vascular device whenever possible (ICU patients usually have indwelling lines) precluding the need for venepuncture – with the option to reduce volumes in patients in whom anaemia is a clinical concern (65–75 ml total above standard of care).

This will be undertaken by trained, experienced personnel using aseptic technique. Patients' blood counts are monitored daily on the ICU- we would not expect a significant drop in haemoglobin from this quantity of blood removed. In most cases ICU patients will be sedated, however a blood draw procedure would be expected to cause only minor and transient discomfort even in conscious patients.

Patients with candidaemia or invasive candidiasis will be managed by the ICU Physicians according to local and international treatment guidelines, with no direction from the study team.

There will be no direct information gathering from participants. All information will be gathered from the electronic medical notes and limited to that information outlined in section 4.3. Data collection. This means there is minimal scope that a researcher could come into information that has safeguarding implications. If

during the enrolment process the potential participant reveals information that has safeguarding implications this would be brought to the attention of the clinical team caring for the patient on the ICU.

7.3. Research Ethics Committee and other regulatory review & reports

This study received NHS Health Research Authority and REC Approval on 23 November 2021 (REC reference 21/WA/0370).

For HRA- NHS REC reviewed research

- Substantial amendments that require review by NHS REC will not be implemented until that review is in place and other mechanisms are in place to implement at site.
- It is the Chief Investigator's responsibility to produce the annual reports and submit the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.
- The Chief Investigator will notify the REC of the end of the study within one year after the end of the study.
- If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.

7.3.1. Regulatory Review & Compliance

Before any site can enrol patients into the study, the Chief Investigator/Principal Investigator or designee will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance.

7.3.2. Amendments

For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body for them to issue approval for the amendment. The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment and confirm their support for the study as amended.

7.4. Peer review

The research protocol was written and reviewed by members of Study steering group (CI, Principal Investigators and Co-investigators above). This group comprises at least one Infection and one Intensive care specialist for each site.

As part of the Pfizer grant application, the protocol was approved via independent peer review.

The protocol (version 1.2) also underwent external expert peer review using a standardised feedback form. The reviewer reported that the study aims and questions are clear and pertinent, as it is an important health issue with a lack of evidence in this area. They accepted the proposal, with some helpful comments that were addressed (Table 2).

Table 2. Peer review feedback and action taken.

Peer review feedback	Action taken
Eligibility criteria could lead to a discrepant control group with low Candida burden.	Eligibility criteria reviewed to have a single cohort and no selection bias of control group.
Unclear time points for mortality assessment.	Clarified.

Blood sampling in candidaemia pathway not frequent enough for pharmacokinetics.	Increased frequency of sampling in a small subgroup of participants.
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Need to include organ transplant and immunosuppressants as risk factors.	Added as data collection points and in inclusion criteria.
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7.5. Patient & Public Involvement

Patient and public involvement was sought from the CSGUL Research Expert Group, a lay panel within the Centre for Public Engagement.

Two members of the public kindly participated and will be reimbursed for their time using recommended NIHR rates. One of them had previous lived experience of Intensive Care and is also a relative of a patient who required Intensive Care.

A copy of participant information sheet (PIS), informed consent form (ICF) and personal consultee information and declaration (all version 0.1), as well as study protocol version 1.3, were sent to both PPI members for review.

Both found the PIS very comprehensive and easy to read and understand, with one participant reporting that she had “participated in a few programmes where [she] had to read and give consent [...] I found these much easier to understand”. Both reported that they would take part in the study after reading the PIS.

The study was deemed worthwhile, with one PPI member mentioning that “it is really essential to do this – drug resistance is an essential issue especially for people in the ICU”.

PPI members provided some excellent feedback which helped improve the study documents, as reported in Table 3.

Table 3. PPI feedback and action taken

PPI advice	Action taken
PIS felt almost too comprehensive, so participants and relatives might find it a bit daunting.	Clarified that participants will be able to receive support from the staff taking consent.
From the PIS it seemed like all participants required serial blood samples.	Clarified that only a small minority of participants (who develop candidaemia) would require those samples, and that for almost all participants a single blood sample is required.
The first item in the Personal Consultee declaration was found to be unclear	Rephrased the sentence.

7.6. Protocol compliance

Protocol deviations, non-compliances, or breaches are departures from the approved protocol. All protocol deviations must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately. Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

7.7. Data protection and patient confidentiality

All data will be handled in accordance with the Data Protection Act 2018 (UK implementation of the EU General Data Protection Regulation, GDPR).

Participants will be allocated a personal study number (PSN) which will be used to protect their identity. The secure (password protected) REDCap™ database will only be accessed by members of the research team. The REDCap™ database will be coded with the PSN only. A separate 'key' log will be kept securely at each site linking the PSN to patient identifiable information (name, date of birth, NHS number); this will take the form of a paper

log stored in a locked cabinet at each site. This pseudonymised system ensures that confidentiality is protected during routine collection of data. Only the pseudonymised database will be used during analysis.

All data will be stored in line with the Data Protection Act (2018) and Caldicott principles. City St. George's University of London (CSGUL) is the data controller for this study. City St. George's University of London (CSGUL) is a REDCap™ consortium partner with a dedicated secure server hosting the database. The legal basis we are applying in order to process personal data under GDPR regulations is that 'processing is necessary for the performance of a task carried out in the public interest' (Article 6(1)f). The following web address will be provided on the PIS: www.hra.nhs.uk/information-about-patients/

The electronic database will be kept securely in pseudonymised form for 5 years following the end of the study in accordance with relevant legislation and the CSGUL Research Data Management Policy. All information will be held in compliance with NHS Digital data confidentiality and security arrangements, the Data Protection Act (1998) and the GDPR.

Physical patient identifiable information will be stored in areas where there is restricted access including only people involved in research. For example, the paper patient consent and Consultee declaration forms will be stored securely in the research team's office at each site. This information will be kept securely at each site for 5 years following the end of the study.

7.8. Indemnity

City St George's University of London holds insurance to cover participants for injury caused by their participation in a clinical study. Participants may be able to claim compensation if they can prove that City St George's has been negligent. This includes negligence in the writing of the protocol, or selection of study resources.

Where the study is conducted in a hospital, the hospital has a duty of care to participants. City St George's University of London will not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees.

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of City St George's University of London immediately.

Failure to alert City St George's University of London without delay and to comply with requests for information by the sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

7.9. Access to the final study dataset

Only the Chief Investigator, full time clinical research fellows and postdoctoral research assistant responsible for data entry, and the statistician will have access to the full dataset. Only the site team will have access to the separate database linking the PSN to patient identifiable information

8. Dissemination

All PIs and co-investigators will be involved as co-authors of the main study manuscript. Any other publications arising from the scientific sub-studies will include the CI, site PIs, collaborating scientists as well as any co-investigators meeting authorship criteria. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. Authors will acknowledge that the study was funded by the Medical Research Foundation and Pfizer, but the funder will have no role in study design analysis, presentation or publication of findings.

The study findings will be presented at national and relevant international meetings with abstract publication, and published in high impact peer-reviewed journals, with media coverage where applicable, so that findings and their implications quickly reach all relevant UK clinical communities and can be translated into policy. This will be facilitated by our investigator group which includes key individuals linked to Infection, Mycology and Intensive Care societies, laboratory networks, professional bodies, NHS England antifungal CQUIN committee and patient/relative groups across a wide range of responsibilities relevant to the management of fungal infection and intensive care in the NHS.

8.1. Dissemination policy

Publication: “Any activity that discloses, outside of the circle of study investigators, any final or interim data or results of the study, or any details of the study methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations.”

All scientific contributors to the study have a responsibility to ensure that results of scientific interest arising from study are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the study in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the study, data shall be consolidated over the duration of the study, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the study shall lie with the Sponsor in the first instance.

8.1.1. Before the official completion of the study

All publications during this period are subject to permission by the Sponsor. If an investigator wishes to publish a subset of data without permission by the Sponsor during this period, the Steering Committee shall have the final say.

Exempt from this requirement are student theses that can be submitted for confidential evaluation but are subject to embargo for a period not shorter than the anticipated remaining duration of the study.

8.1.2. Up to 180 days after the official completion of the study

During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer-review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

- The Chief Investigator shall be senior and corresponding author of the Main Publication.
- Insofar as compatible with the policies of the publication outlet and good academic practice, the other Investigators shall be listed in alphabetic order.
- Providers of analytical or technical services shall be acknowledged but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.
- Members of the Steering Group shall only be acknowledged as co-authors if they contributed in other capacities as well.
- If there are disagreements about the substance, content, style, conclusions, or author list of the Main Publication, the Chief Investigator shall ask the Steering Group to arbitrate.

8.1.3. Beyond 180 days after the official completion of the study

After the Main Publication or after 180 days from study end date any Investigator or group of investigators may prepare further publications. In order to ensure that the Sponsor will be able to make comments and suggestions where pertinent, material for public dissemination will be submitted to the Sponsor for review at least sixty (60) days prior to submission for publication, public dissemination, or review by a publication committee. Sponsor's reasonable comments shall be reflected. All publications related to the study shall credit the Chief and Co-Investigators as coauthors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

8.2. Archiving arrangements

Each site will be responsible for their onsite level study archiving. The study essential TMF along with any central study database will be archived in accordance with the sponsor SOP.

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Appendix 1: Schedule of study procedures

Table 4. Schedule of study procedures.

	SAMPLING SCHEDULE ...hr Labels in CandiRes folder, please use this on all samples (except blood cultures) with date/time and PSN number								
Timepoint – please write exact time of sampling on the samples	 Whole blood EDTA (DNA)	 Lithium/heparin plasma (PK)	 Whole blood EDTA (plasma)	 Blood culture	 Serum	 Drain fluid	 Urine	 Mouth and perianal swabs	 Stool sample (1g)
Patients	All	On echinocandin (anidulafungin, caspofungin, micafungin)	All	All	All	Has drains in situ	All	All	London sites only Confirmed candidaemia only
Sampling notes	Collected by research team	Processed on site by nurses	Collected by research team	- Aerobic bottle - Send to micro lab as usual	Collected by research team	- Fresh – take direct from drain or clean bag. - Use highest output drain - Record bag volume 2 samples: 1. Collected by research team (culture) 2. If on echinocandin, processed on site (PK)	- Catheter samples only – exclude if no catheter - Collected by research team	Collected by research team	Scoop 1g of faeces into the tube using the attached spoon
0h									
1h	X	*if Hb >80	X	X	X	X	X	X	X

2h	X	*if Hb >80	X	X	X	X	X	X	X
4h	X	*if Hb >80	X	X	X	X	X	X	X
6h	X	*if Hb >80	X	X	X	*if on an echinocandin	X	X	X
12h	X			*if Hb >80		*if on an echinocandin	X	X	X
24h	X							X	X
36h	X			*if Hb >80		X	X	X	X
48h	X					X	X	X	X
72h	X								X
D7	X			*if 72hr culture +					

D10	X	X	X	X	X		*if D7 culture +		X
D14	X	X	X	X			*if last culture +		
D17	X	X	X	X	X		*if last culture +		X
D21	X	X		X			*if last culture +		X
D24	X	X	X	X	X		*if last culture +		X
D27	X	X	X	X	X		*if last culture +		X
D30	X	X	X	X	X		*if last culture +		X

Appendix 2: Amendment log

Any amendments will be logged here.

Amendment #	Protocol version #	Date issued	Authors changes	of	Details of changes made
1 (SA1)	2.0	5/4/22	A Mazzella, T Bicanic		Sites and study group - Added Liverpool site and its investigators. - Removed VP as collaborator. Timeline - Extended recruitment. Consent - Added deferred consent option for candidaemia. - Introduced Personal Consultee Telephone Declaration form. Interventions 1. Changed blood volumes (removed immunology sample, reduced BDG/PK volumes, increased culture volumes). 2. Added urine and drain fluid sampling for all patients with candidaemia. 3. Reduced frequency of swabbing to once weekly for patients who have been in the study for more than 30 days. Inclusion criteria - Clarified wording of inclusion criteria Throughout the protocol Minor clarifications.

2 (NSA1)	2.5	11/10/22	C Logan, P Allebone-Salt, T Bicanic	Timeline - Extended recruitment.
3 (NSA 2)	*UPDATE WHEN STUDY FOLDER ONLINE*	7/11/22	P Allebone-Salt	Added Liverpool tick boxes to study documents
4 (SA2)	3.0	25/11/22	A Mazzella,	Sites and study group

			T Bicanic, C Logan, P Allebone-Salt	Changed PI at Liverpool site Interventions 1) For participants with invasive candidiasis, Candida PCR to be performed (no change in blood volumes collected) and added urine and drain fluid collection on D7 2) Clarified that if a participant is readmitted to ICU before day 30, regular swabbing will be restarted. Throughout the protocol Minor clarifications.
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5 (NSA3)	3.5	26/1/23	P Allebone-Salt	Sites and study group Changed clinical research fellow name Funding Added Gilead Fellowship Interventions 1) For participants with invasive candidiasis and abdominal drains, the frequency of sampling for culture is increased to add samples at 12h and 24h, and every 72h after day 7 until drain is removed or they are discharged from ICU. 2) For patients with invasive candidiasis, another urine sample has been added at 24h. If the candidiasis is of a urinary source and the urine culture remains positive at D7, urine samples will continue to be collected every 72h until negative. 3) For participants with invasive candidiasis, two additional serum samples (3ml each) for BDG and Candida PCR will be taken at day 14 and day 21. These will be taken from CVC if still in ICU. If on the ward, if possible they will be taken when the patient is having other clinically required blood tests.
				Throughout the protocol Minor clarifications of sampling timepoints and volumes (10ml of drain fluid and urine)

6 (NSA4)	3.6	23/3/23	P Allebone-Salt	Sample number Increased to 320 including 15-20 with strongly suspected/confirmed invasive candidiasis. Recruitment criteria Participants started on an antifungal for suspected IC. Recruitment extension Extended to 31st November 2023 to allow recruitment of a further 5 patients with IC, in keeping with funding. Increased sampling frequency Increased drain fluid and plasma sampling for PK at 1, 2 and 4h in minority of patients with suspected IC at St George's, and 6h for any patients with abdominal drain.
7 (NSA5)	3.65	20/11/23	P Allebone-Salt	Sample processing Samples may be processed in collaborating laboratories in the UK and internationally Collaborators Added Eric Decloedt, divisional head and Associate Professor of the Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa Recruitment extension Extended to 31st August 2024 to allow recruitment of a further patients, in keeping with funding (Gilead).
8 (SA3)	4.0	1/2/24	P Allebone-Salt, B. England, H Davidson	New sites Royal Free Hospital NHS Foundation Trust University Hospital of Wales Change to Principal Investigators/research officer/collaborators KCH PI changed from Rohit Saha to Pervez Khan

				PIs and Co-Is for RFH and UHW added Change to research officer at sponsor site, to Alisa Azhar Frank van de Veerdonk at Radboud UMC and Alexandre Alanio at Hôpital Saint-Louis added as collaborators End date Study extended until 2027, in keeping with additional funding and outcomes to date Study documents Added RFH and Cardiff as sites on all documents.
9 (NSA6)	4.1	6/8/24	H Davidson	Change to Principal Investigators/research officer/collaborators RFH PI changed from Brian Hogan to Rohit Saha Sample number Intensive PK sampling to be performed on 20 participants Study documents Added RFH & Cardiff as sites on outstanding documents
10	4.2		H Davidson	Change in sponsor Sponsor changed to City St Georges University of London
11 (NSA7)	4.3	15/11/24	H Davidson	Change to Co-Investigators KCH CoIs changed from Ali Abdolrasouli and Silke Schelenz to Anita Verma and Jasmin Islam

12 (NSA8(	4.4	23/1/25	H Davidson	Protocol: clarified wording regarding recruitment of patients with invasive candidiasis. Updated and clarified sampling schedule, sample size calculation and expanded data analysis section. PIS: clarified wording of PIS to remove references to development of invasive candidiasis.
13 (NSA9)	4.5	8/5/25	P Allebone-Salt	Change sample for DNA collection to whole blood EDTA tube from PAXgene (cheaper, as effective for same purpose) Plasma samples in EDTA to be collected from all patients at pre-stated timepoints (0h,12h,24h,36h,48h,72h,7d) and analysed for Candida PCR at Hôpital Saint-Louis by collaborator Alexandre Alanio Stool samples collected on days 1, 7 and 14 from patients with confirmed candidaemia at London sites for faecal mycobiome analysis at Hôpital Saint-Louis by collaborator Alexandre Alanio PK to be collected from anyone on an echinocandin, changed from only those on anidulafungin Added Sarah Jawad and Jennifer Shuttleworth as clinical research fellows/coordinators, removed Harriet Davidson
14 (NSA10)		2/8/25	C Dines	Addition of London North West University Healthcare NHS Trust as a trial site, with Dr Michael Dean as site PI

15 (NSA11)		18/9/25	S Jawad	Inclusion of NIHR Associate PI Scheme participation
16 (SA4)	5	13/7/26	H Davidson	Sites: - Added Sheffield Teaching Hospitals and University Hospitals Sussex NHS Foundation Trust Study Team: - Removed Jenni Shuttleworth, added Harriet Davidson and Charlotte Dines Study Documents: - Updated to reflect new sites

Appendix 3: Research Data Protection Impact Assessment

Study Title/Acronym	Relationship of antifungal exposure to emergence of <i>Candida</i> resistance in Intensive Care patients: a multi-site cohort study (CandiRes)
JRES Reference Number	2021.0219
Chief Investigator Name	Professor Tihana Bicanic
Chief Investigator Email Address	tbicanic@sgul.ac.uk

PROJECT DETAILS

Project / process description:

CandiRes is a prospective observational study that will collect personal health data following informed consent from participants in order to address research questions around antifungal resistance in Candida.

The Data Flow Diagram is presented in Figure 1.

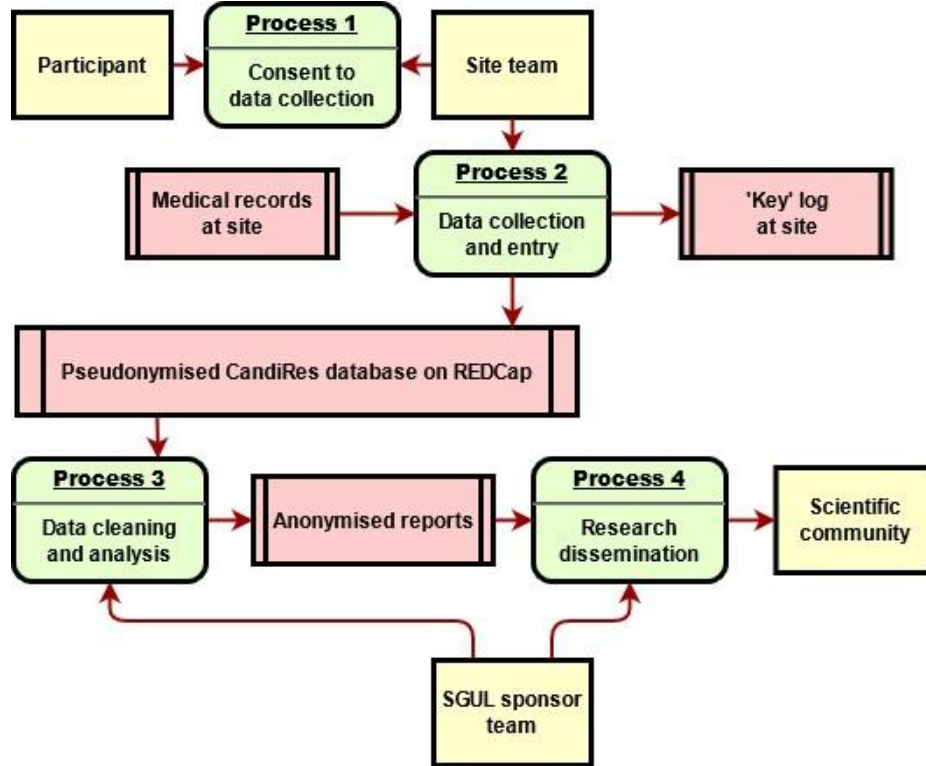


Figure 1. Data flow diagram for CandiRes.

What personal data do you intend to use, and why? (List all categories)

- Basic demographic data (age, gender)
- Date and time of ICU admission and study enrolment
- Risk factors for *Candida* colonisation or infection • Severity of illness

- Date and time of collection of research samples (blood, swabs, drain fluid)
- Clinical and microbiological data on invasive candidiasis
- Date of ICU discharge and study end, in-hospital mortality

All these data points are collected exclusively for the purposes of this research, and they are required to answer the research questions. For more details, please refer to section 4.3. Data collection.

Will the personal data be identifiable, pseudonymised or anonymised (if a mix tick accordingly)

Identifiable		
Pseudonymised	X	The key to this data is kept in a separate log, securely away from the main database, at each site, with strict controlled access.
Anonymised		

List all organisations / agencies which will have access to the personal data collection used for this project / process

- Site team at St George's site: access to 'key' log and pseudonymised data for St George's.
- Site team at King's site: access to 'key' log and pseudonymised data for King's.
- Site team at GSTT site: access to 'key' log and pseudonymised data for GSTT.
- Site team at Liverpool site: access to 'key' log and pseudonymised data for Liverpool.
- Site team at RFH site: access to 'key' log and pseudonymised data for RFH.
- Site team at University Hospital of Wales site: access to 'key' log and pseudonymised data for UHW.
- CSGUL sponsor team: access to the full REDCap pseudonymised data.

Length of the study

– include an assessment of the necessity and proportionality of the processing in use. Also include who, internally & externally, has been consulted in the preparation

of this DPIA.

The study is expected to recruit over the course of five years. This is the estimated time required to enrol the sample size to answer the research questions, as explained in section 6.2.1. Size of sample. The data procedures and confidentiality protection were established by the study steering group along with Research Governance and Facilitation Officer and the PPI group.

If external organisations / agencies are involved, is there a contract or information sharing agreement in place with suitable clauses for data protection and data incident reporting? If not why not?

Samples will be shared with collaborating laboratories in the UK and internationally to perform analysis. Samples will be shared in non-identifiable form and there will be an agreement in place with relevant data protection and material transfer clauses.

RISK

Can you achieve your objectives using anonymised data? – see ICO Code of Practice on Anonymisation

Yes

No

X

Why not?

This is a prospective study, therefore keeping a log of participants is required to complete data entry and address later data queries.

What are the benefits to the individual of their personal data being used for this purpose?

There are no direct benefits to participants. Some participants might take pleasure in knowing that their data is contributing to the advancement of scientific knowledge in the field.

What are the organisational benefits of the individual's personal data being used for this purpose?

These data will allow the Sponsor team to conduct this research study (see also section 2.1. Potential for improved patient care & need for this study).

What are potential negative impacts to the individual of their personal data being used for this purpose in the event of a Data Breach occurring?

In the unlikely event that any part of the pseudonymised dataset were breached, there would be minimal risk of a negative impact for participants, as that dataset does not contain any directly identifiable personal information.

If a 'key' log at site were breached, the participation in CandiRes of all participants in that site would be revealed. This would not reveal any other personal information unless the pseudonymised dataset were also breached, which would be an extremely unlikely coincidence.

How will you avoid causing unwarranted or substantial damage/distress to the individual when using their personal data for this purpose?

All data will be collected from the participants' medical records by members of the site team. Patients or designated consultees will be fully informed of the study and explicit consent will be given prior to any data being collected.

Only pseudonymised data will be used for analysis and dissemination of results.

Is the data already held by City St George's?

Yes		
No	X	This project will gather these data.

Is it held by one of the partner organisations / agencies involved in this process/project?

Yes		
No	X	Which agency will be collecting the data
		The study sites will collect the data.

Have you told the individuals whose personal data you want to use for this purpose, how and why you intend to use their data?

Yes	X	This is all explained in the Participant Information Sheet.
No		

Do you already have the individual's consent to use their data for this purpose?

Yes		
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No	X	Why not?	We will seek consent prospectively.
If not, are you going to ask for their permission?			
Yes	X		
No		Why not?	
Have individuals been given the opportunity to refuse us permission to use their data for this purpose?			
Yes	X	Approached patients may decide not to consent to the study, in which case no data will be collected.	
No			
How will you make sure that the personal data you are using is kept accurate and up to date?			
Prospective data collection.			
What steps or controls are you taking to minimise risks to privacy? Please tick those which apply and provide details of how each is ensured			
✓ Risks to individual privacy are minimal ○ Limited personal data is collected ✓ Personal data is pseudonymised ○ 'Key' log linking PSN and participant identity kept securely at each site. ✗ Encryption of data at rest, i.e. when stored ○ REDCap does not employ any kind of encryption at rest – it is not required, as the database server is held on secure CSGUL servers. ✓ Encryption used in transfers			

- The REDCap database is hosted on a secure CSGUL server.
- ✓ Information compliance training for staff has been completed - data protection, information security, FOI ○ This is part of mandatory training at CSGUL and at each site and the CI/PIs will ensure all team members have completed this.
- ✓ Adherence to privacy by design principles ○ The protocol details the procedures required to respect confidentiality.
- ✗ Special category personal data is not used ○ Data concerning health are classified as special category data. It is not possible to conduct health research without using special category data.
- ✓ Participant opt out at any stage of the research ○ This is clearly explained in the PIS.
- ✓ Personal data kept in the UK ○ No data transferred abroad.
- ✓ Research is not used to make decisions directly affecting individuals ○ This is observational research; it will not lead to any change in the medical care of the participants.
- ✓ Short retention limits ○ Data will not be kept after 5 years.
- ✓ Restricted access controls ○ REDCap can only be accessed with a personal username and password, and it allows for data access control by group (sponsor team, site team). The key log will be kept securely at each site.

How long will you need to hold the personal data for after the study has completed?

The electronic database will be kept securely in pseudonymised form for 5 years following the end of the study in accordance with relevant legislation and the CSGUL Research Data Management Policy. The paper patient consent and consultee declaration forms will be stored securely in a locked cupboard in the research nurses' office at each site for 5 years following the end of the study.

Once the main publication is published, the unique samples and data collected in this study will form an excellent research resource for future scientific projects (for which separate Ethics applications would be made); these could extend the scientific contribution of this study.

How will you make sure that you are holding data for the appropriate length of time and no longer?

The dataset will be removed from the CSGUL servers at the end of the time period agreed in this protocol, unless there is an extension approved by the ethics committee.

How will the data be held /stored?

The pseudonymised dataset will be stored on a password-protected REDCap database on a secure CSGUL server.

Will you be using any electronic and/or paper Case Report Forms (CRFs) to collect data? If so what are these and how will they be held securely and managed at the end of the project?

Only electronic CRFs will be used on the REDCap database mentioned above.

Will personal data be transferred/shared between the organisations involved in this project? If so how?

Each site team will only enter pseudonymised data onto REDCap; they will not share the 'key' log. The Sponsor team will have access to the pseudonymised database only.

Will you be transferring personal data to a country or territory outside of the UK? *If yes, name countries and receiving parties.*

Yes – within EEA		
Yes – outside of EEA		
No	X	

How will you ensure that third parties will comply with data protection obligations?

Information compliance training for staff has been completed and will be monitored at each site by the PI and by the CI for CSGUL.

What measures are in place to ensure only appropriate and authorised access to and use of, personal data?

All electronic data is only accessible via unique IT logins and passwords. Only authorised individuals will be able to access and use personal data – this will be ensured by the REDCap data access groups feature, and will be monitored by the CI and the PIs at site.

How will technical and organisational security be monitored/audited?

The CI and PIs at each site will monitor their research teams. Technical security is monitored by the IT team at each site and at CSGUL.

Declaration I confirm that the information recorded on this form is, to the best of my knowledge, an accurate and complete assessment of the potential privacy impacts of this study.

Name: Tihana Bicanic

Signature: 

Date: 05 Apr 2022

Institute Director (CSGUL) Name: Prof Julian Ma

Signature: 

Date: 05 Apr 2022

JRES Reviewer

Name: Sam Hollingworth Signature:

A handwritten signature in black ink, appearing to read 'Sam H', with a long horizontal stroke extending to the left.

Date: 05 Apr 2022