Protocol

Antimicrobial resistance gene and pathogen burden in sinks in UK hospitals and associations with health-care associated infections, sink design and sink usage (SinkBug project)

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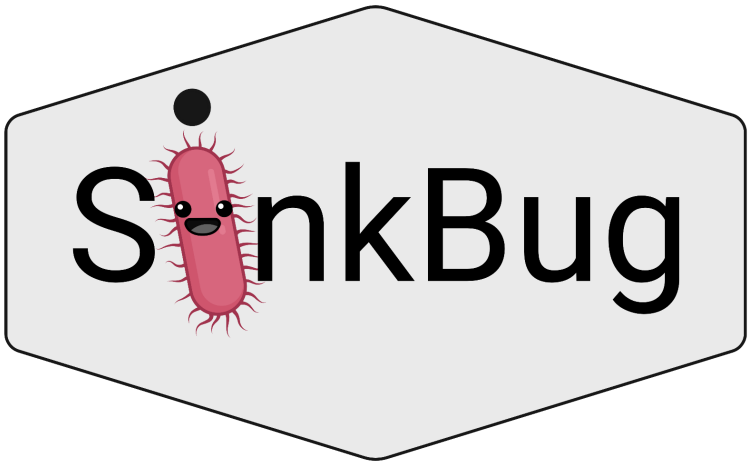


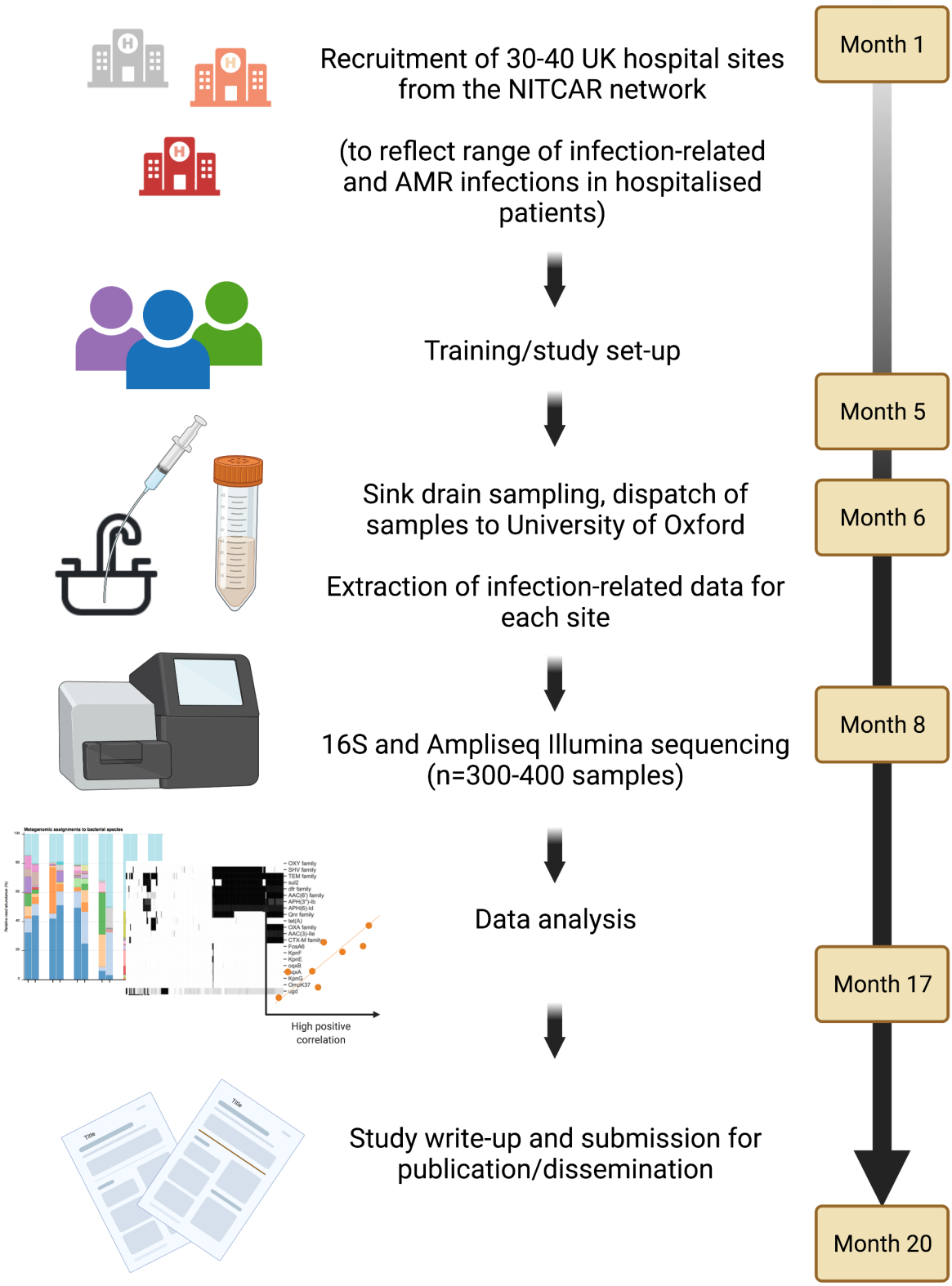
NITCAR
National Infection Teams Collaborative for Audit and Research

UK Health Security Agency

Modernising Medical Microbiology

Table of Contents

Schematic of study workflow



Background and rationale

Healthcare-associated infections, especially those caused by Gram-negative bacilli (e.g. Enterobacterales, *Pseudomonas aeruginosa*) represent a major cause of morbidity and mortality in the UK and other settings[1]. Antimicrobial resistance (AMR) in these organisms has become a significant global problem, with the emergence of broad-spectrum AMR mechanisms (e.g. extended-spectrum beta-lactamases, carbapenemases) rendering infections caused by these bacteria increasingly difficult to treat[2]. Marked regional differences in the detected prevalence of major AMR mechanisms occurs, as has been shown in the latest English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) report, with the rate of acquired carbapenemase-associated Gram negative infections nearly twice as high in London and the Northwest as in the UK in general[1].

Hospital sink drains, p-traps and other wastewater sites may represent a sentinel site for the dissemination of AMR mechanisms and "high-risk" bacterial species in hospitals in the absence of dedicated screening programs, given that in many cases most dissemination occurs "under-the-radar" in asymptomatic, colonised patients, rather than in infected cases[3-5]. The use of wastewater for pathogen and AMR surveillance has been successfully deployed at larger regional and national scales, as in the Global Sewage Surveillance project[6], and for population-level monitoring of SARS-CoV-2 and other viruses[7, 8]. Several single-centre studies have shown associations between drain-level and patient-associated AMR/pathogen burden, but typically these have been evaluating only a few gene mechanisms or species[3, 9].

Sinks and drain sites in healthcare settings are also potentially significant contributors to healthcare-associated infections (HAIs), as has been evidenced by a number of observational and interventional studies, where sink/drain-related interventions such as decontamination or removal have resulted in a lower incidence of Gram-negative infections and/or terminations of clonal outbreaks[3, 10-12]. Sinks and wastewater sites may also act as important niches for the exchange of AMR genes, reflected in horizontal gene transfer amongst strains and species[13]. However, much more limited information on the impact of sink design features and usage to the development of AMR and pathogen burden in these niches is available from "real-life" hospital settings, and this could be useful in designing interventions (e.g. modified sink design, specific cleaning protocols).

We propose a UK-wide survey of sink drains in UK hospitals, using genomics to evaluate the taxonomic and AMR gene distributions in these settings, and investigating associations with:

(i) Infection-associated data derived from national surveillance datasets, and from aggregate ward-level data where this is available
(ii) Sink design, water characteristics and infection prevention and control interventions, including cleaning protocols at each site.

If strong associations between sink colonisation, sink design/usage and infection/AMR burden are identified, we will explore future interventional studies designed to reduce healthcare infections associated with Gram-negative bacilli and other pathogens, and with AMR in these organisms.

Aims and Objectives

Aims

Employing the National Infection Teams Collaborative for Audit and Research (NITCAR) network (http://nitcollaborative.org.uk/wp/), we will conduct a multicentre point-prevalence study in the UK to evaluate whether microbiome differences in sinks between wards within the same hospital and between different hospitals reflect ward- and hospital-level data on pathogen and AMR prevalence in invasive infections.

As a secondary aim, we will determine whether specific sink design/features and usage characteristics are associated with sink wastewater burden of pathogens and AMR.

Specific objectives

1. To characterise, quantify and compare pathogen and AMR gene abundance and diversity in sinks stratified by:
    a. Water quality and biochemical features (e.g. hardness, pH, antibiotic residues)
    b. Sink design type (e.g. horizontal versus plughole drainage)
    c. Use type (patient/visitor use, sluice use, treatment room/staff)
    d. Ward type (medical, surgical, critical care)
    e. Hospital

    We will focus initially on Enterobacterales species, but will consider *Pseudomonas aeruginosa*, *Enterococcus* spp. and *Clostridioides difficile* as secondary analyses
2. To investigate associations between sink wastewater pathogen and AMR prevalence with that from invasive infections within the same setting, using second generation surveillance system (SGSS) and mandatory surveillance data on bloodstream infections (available for certain species) and *C. difficile*, and data on hospital admissions from national Hospital Episode Statistics (HES). Where possible (based on local capacity), we will also investigate associations with aggregate ward-level data on infections.
3. To evaluate the association between sink design and cleaning regimens (e.g. products, cleaning frequency/practice) and sink wastewater pathogen and AMR gene abundance and diversity.

This unique project will also substantially contribute to training and development of a network of researchers linking NITCAR, the University of Oxford, and the UK Health Security Agency (UKHSA), to foster high-quality, inter-disciplinary research training amongst scientists and healthcare professionals with different and complementary skills.

Project design and sample and data collection

Design

This is a prospective, systematic, point prevalence sampling survey across 30-40 UK hospitals within the NITCAR network. Samples will be collected over a two-month period by local investigators (trainees/healthcare professionals affiliated with

NITCAR). Participating sites will be asked to submit samples from 10 sink p-traps within a 2-week window, alongside p-trap/tap water biochemistry analysis and data on sink usage and cleaning protocols using a standardised data collection platform. A total of 300-400 sink p-trap samples will be collected.

Ideally, participating sites will reflect the UK geography and regional variation in AMR and invasive healthcare-associated infection prevalence.

Sample and data collection

Standardised sampling kits and an instructional video demonstrating the sink p-trap sampling and biochemical testing will be sent out to all local investigators prior to the sampling window. P-trap samples will be returned to researchers at the University of Oxford for processing, DNA extraction, sequencing and sequence data analysis.

Local investigators will sample sink p-traps using a stratified approach. Sampled sinks will be purposively sampled by ward type, namely critical care (i.e. general, adult ICU; n=4 sinks in one ward), a general medical ward (i.e. with acutely unwell, unselected inpatients; n=3 sinks in one ward), and a general surgical ward (n=3 sinks in one ward). For the critical care unit, samples will be taken from a treatment/medicines room sink, two patient bay sinks, and a sluice room sink. From the medical and surgical wards, samples will be taken from a treatment/medicines room sink, a patient bay sink, and a sluice room sink.

Local dipstick-based tests (like urine testing strips) will be performed to evaluate sink p-trap sample biochemistry; a small sample of tap water collected at the same time from each sink will be similarly tested. Samples and dipstick results will be recorded on a custom data collection platform.

Photographs of each sink will be taken and uploaded to the data collection platform, enabling us to classify sink design features. Similarly, data on sink usage and cleaning protocols will be uploaded to the same platform, based on self-reports from ward staff and cleaners.

Samples can be collected by any infection specialty doctor, biomedical scientist or infection control-related healthcare professional, or by any individual under the supervision of these experts.

Clinical/infection-related data associated with each participating site will be extracted in an anonymised manner from SGSS, mandatory surveillance and HES platforms by University of Oxford/UKHSA researchers through access associated with an established UKHSA/University of Oxford partnership that is funding this project (an NIHR funded Health Protection Research Unit in AMR and HAI [2020-2025]). Monthly data will be collected for 6 month time periods either side of the sampling window. Where possible, equivalent aggregate monthly data at the level of the sampled ward will be collected from local investigators, based on local microbiology systems.

No specific additional ethical approvals are required for this project as it does not involve human participants, tissue or personal data (https://researchsupport.admin.ox.ac.uk/governance/ethics/apply).

We anticipate that it will take approximately 200 minutes in total to sample 10 sink p-traps, perform the chemical analyses on paired p-trap and tap water samples and prepare the p-trap samples for shipping.

Laboratory and sequencing processes/analyses

Sink p-trap sample analyses

The shipment to and receipt of p-trap samples will be coordinated in Oxford and metagenomic DNA extractions will be conducted using the MoBio PowerSoil DNA isolation kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Sample pellets will be stored at −80 °C for subsequent batch processing.

Microbiome/resistome sequencing methods

Metagenomic extracts will undergo a joint sequencing strategy to profile species diversity and AMR content. For this, we currently anticipate using 16S sequencing and a highly multiplexed PCR-based approach (AmpliSeq for Illumina Antimicrobial Resistance Research Panel [AmpliSeq™]) respectively; however, profiling methods are continuously improving and we will adopt the most relevant, cost-effective strategy available at the time of the study. The AmpliSeq™ AMR panel targets >800 AMR gene variants conferring resistance to >20 antibiotic classes, and facilitates semi-quantitative analysis of AMR gene abundance differences between sink samples. This is a novel strategy, which has been recently optimised by the University of Oxford research team and validated on a mock and real-life polymicrobial communities.

Indicative project timeline – summary

- Set-up: hospital site recruitment, training and sampling kit dispatch - months 1-5
- Sampling: Sink sampling, sample return to Oxford - months 6-7
- Sample processing: DNA extraction, sequencing and sequence data analysis - months 8-15
- Clinical data extraction: to month 12
- Study analysis: months 12-17
- Study write-up: months 18-20

We anticipate starting with the study set-up from August 2022.

Data analysis plan

To analyse the taxonomic and AMR sequencing data, classical ecological measures of diversity will be used, including metrics describing alpha- (richness, evenness) and beta-diversity (variation of community composition between samples). As an example, species and AMR gene richness, relative abundance, Shannon indices and species wUniFrac measures will be calculated using the phyloseq and vegan packages in R. Species diversity metrics (16S) will be used to normalise AMR gene abundance results and allow direct measurement of species diversity and AMR gene content at the individual sink-level, as well as stratified evaluation of these metrics by sink function, ward- and site-level, and local AMR prevalence further related to antibiotic type and treatment. Initial models will exploit the nested structure of the

data (sinks nested within wards nested within hospitals) using random effects models; testing the effect of factors such as sink design type, sink usage type, ward type, cleaning protocols as main effects.

Extracted clinical data will be used to evaluate infection rates per 1000 admissions and bed days over the 12-month window covering the sampling period, stratified by hospital site and ward-level (where these data are available), and evaluating antimicrobial-resistant infections for these cases. The latter will be evaluated at the species and antibiotic class-level, and using a standard definition of multi-drug resistance, representing resistance to ≥3 classes of antimicrobial.

Sample size

The degree of clustering within ward, hospital or sink is unknown, but may be relatively low given more intensive work in a large hospital in Manchester (https://www.medrxiv.org/content/10.1101/2021.11.26.21266267v1). The impact of different features may also vary across outcomes, e.g. there may be larger effects on taxonomic outcomes than AMR outcomes. As an indication, comparing 100 samples from each of two wards would give at least 90% power to detect differences in outcomes of 0.5 times the outcome's standard deviation (two-sided alpha=0.025 to account for multiple testing across three ward types). Given the unknown magnitude of variation, in itself a scientifically interesting parameter to estimate, sampling 30-40 hospitals will enable a thorough assessment of the impact of a range of exposures on a range of relevant outcomes.

Publication/Data sharing policy

The project leads fully support collaborative research and inclusion. This study will adhere to the principles and stipulations summarised in NITCAR's authorship policy, available on the NITCAR website (http://nitcollaborative.org.uk/wp/documents/).

All local investigators submitting sink samples and relevant metadata will be listed as authors on any manuscript/outputs written by the University of Oxford/UKHSA research team as long as they remain contactable. Manuscripts will be submitted to preprint servers and peer-reviewed, open access journals to facilitate research dissemination as soon as possible. Abstracts describing intermediate analysis outputs may also be submitted to relevant conferences. In order to comply with submission guidelines a corporate authorship may be required; in this case, authors will be listed individually in the Appendix/acknowledgements section and these listings will be indexed so that credit is given at the individual-level.

The ordering of authors will be at the discretion of the project leads, but will reflect contribution/input. Any issues will be resolved by discussion with the project leads until consensus is reached.

Any study data (e.g. sequencing data) will be made available at the project end or the time of preprinting/publication, whichever is sooner.

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