Change history

Version Nr	Version date	Modified without version change	Description, comments	Control
1.0	26.11.2021		Initial version	AS
1.1	10.01.2022		Updated secondary outcomes in synopsis to match main study protocol Addressed Ethics Committees comments: - Clarified goal of rectal swabs in synopsis and section 3.4.1 - Clarified patient selection for rectal swabs (3.4.1) - Clarified consent procedure and included participants in cohort study (section 4.2.3) - Deleted Regulatory sections not applicable to this study (Section 6) - Clarified data handling and record numbering for cohort study (Section 8.2)	AS
1.2	11.03.2022		Updated study duration in summary table to reflect different registration and study timing for nested cohort study Updated sentence on rectal swabs (Section 3.4) to be consistent with Section 3.4.1 Updated follow up procedure for cohort study and provided more detail on the cohort study (Section 3.4.6) Updated inclusion/ exclusion criteria for cohort study (Section 4.1)	

REVERSE: pREVention and management tools for rEducing antibiotic Resistance in high prevalence SEttings

Study Type:	Other Clinical Trial according to ClinO, Chapter 4
Risk Categorisation:	Risk category A
Study Registration:	International Standard Registered Clinical/soCial sTudy Number (ISRCTN)
Sponsor:	University Hospital Zurich
Principal Investigator	**PD Dr. med. Walter Zingg** Leiter Spitalhygiene Klinik für Infektionskrankheiten und Spitalhygiene UniversitätsSpital Zürich Rämistrasse 100 8091 Zürich Email: walter.zingg@usz.ch Tel. +41 43 253 03 52
Investigated Intervention:	Prospective multi-centre, cluster-randomised, stepped-wedge trial to evaluate the effectiveness of diagnostic stewardship, infection prevention and control, and antibiotic stewardship programmes on antimicrobial resistance in acute care.
Protocol ID	Protocol 1
Version and Date:	Version 1.2 (dated 14/03/2022)

CONFIDENTIALITY STATEMENT

PROTOCOL SIGNATURE FORM

Study Title	REVERSE: pREVention and management tools for rEducing antibiotic Resistance in high prevalence SEttings
Study ID	REVERSE

The Principal Investigator has approved the protocol version 1.2 (dated 14/03/2022) and confirms hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, and ICH-GCP guidelines as well as the local legally applicable requirements.

Sponsor:

Name: *PD Dr Walter Zingg*

Date: _____ Signature: _____

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GLOSSARY OF ABBREVATIONS

ABS	*Antibiotic Stewardship*
ABHR	*Alcohol Based Hand Rub*
AE	*Adverse Event*
AMR	*Antimicrobial Resistance*
API	*Application Programming Interface*
ASR/DSUR	*Annual Safety Repot / Development Safety Report*
BASEC	*Business Administration System for Ethical Committees*
BSI	*Bloodstream Infection*
CFIR	*Consolidated Framework for Implementation Research*
CRAB	*Carbapenem-Resistant Acinetobacter baumannii*
CRE	*Carbapenem-Resistant Enterobacterales*
CRF	*Case Report Form*
CRPA	*Carbapenem-Resistant Pseudomonas aeruginosa*
CTCAE	*Common Terminology Criteria for Adverse Events*
DCE	*Discrete Choice Experiment*
FADP	*Federal Act on Data Protection (in German: DSG, in French: LPD, in Italian: LPD)*
eCRF	*electronic Case Report Form*
ECDC	*European Centre for Disease Prevention and Control*
ERIC	*Expert Recommendations for Implementing Change*
EQ-D	*EuroQol-Dimension*
FOPH	*Federal Office of Public Health*
GCP	*Good Clinical Practice*
HA	*Healthcare acquired*
HAI	*Healthcare Acquired Infections*
HRA	*Human Research Act (in German: HFG, in French: LRH, in Italian: LRUm)*
HTTPS	*Hypertext Transfer Protocol Secure*
ICH	*International Conference on Harmonisation*
IPC	*Infection Prevention and Control*
LMIC	*Low- and Middle-Income countries*
LRTI	*Lower Respiratory Tract Infection*
MALDI-ToF	*Matrix-Assisted Laser Desorption/Ionization – Time of Flight*
MDRO	*Multi-Drug Resistant Organism*
MDS	*Microbiology and Diagnostic Stewardship*
ClinO	*Ordinance on Clinical Trials in Human Research (in German: KlinV, in French: OClin, in Italian: OSRUm)*
PROHIBIT	*Prevention Of Hospital Infection By Intervention and Training*
RT	*Room Temperature*
QALY	*Quality Adjusted Life Year*
RCT	*Randomised controlled trial*
SAE	*Serious Adverse Event*
SF-12	*12 Item Short Form survey*
SSL	*Secure Sockets Layer*

STEP-UP	*SusTainability of Effective interventions to promote Prudent antibiotic Use in Primary care*
TAT	*Turn Around Time*
UMCU	*University Medical Centre Utrecht*
UNIFI	*University of Florence*
UOXF	*University of Oxford*
USZ	*University Hospital Zurich*
UZH	*University of Zurich*
WGS	*Whole Genome Sequencing*
WHO	*World Health Organisation*

1 STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	PD Dr. med. Walter Zingg Leiter Spitalhygiene Klinik für Infektionskrankheiten und Spitalhygiene UniversitätsSpital Zürich Rämistrasse 100 8091 Zürich Email: walter.zingg@usz.ch Tel. +41 43 253 03 52
Study Title	Prevention and Management Tools for Reducing Antibiotic Resistance in High Prevalence Settings
Short Title / Study ID	REVERSE
Protocol Version and Date	Version 1.2 (dated: 11.03.2022)
Study Registration	Main study Registration: International Standard Registered Clinical/soCial sTudy Number (ISRCTN) ISRCTN12956554 Nested Cohort Study Registration: pending
Study Category and Rationale	Risk category A Rationale: This is a multi-national randomized clinical trial with preventive interventions implemented by healthcare providers, and randomization occurring at the hospital level. The swabs collected from patients are part of routine Infection Prevention and Control (IPC) practices that are standard of care at most European hospitals. The interventions included in the Infection Prevention and Control (IPC), Antibiotic Stewardship (ABS), and Microbiology and Diagnostic Stewardship (MDS) bundles are targeting health care providers and have individually shown to improve patient outcomes.
Background and Rationale	Antimicrobial resistance (AMR) results in increased morbidity, mortality, and cost. IPC, ABS, and MDS interventions target AMR in different ways, and have individually been shown to be safe and effective. It is still unknown - but hypothesized - that these interventions would have a synergistic effect, with the combination of interventions reducing AMR more than any one intervention. The REVERSE trial will address this knowledge gap.
Risk / Benefit Assessment	There is no additional risk to patients above the risk of a usual hospital admission. The potential benefits to patients include reduced rates of colonization and infection with antibiotic-resistant bacteria (and therefore reduced morbidity and mortality usually associated with these infections), and reduced rates of *Clostridioides difficile* infection. The potential benefits to the hospital include decreased cost, increased capability and knowledge to prevent HAIs, and expert help in programme implementation.
Overarching Objective(s)	Develop and implement cost-effective strategies and tools for the prevention and clinical management of healthcare-associated infections (HAIs) due to multidrug-resistant pathogens, and to reduce the burden of antimicrobial resistance (AMR) in high prevalence care settings.
Endpoint(s)	Primary Endpoint: - Incidence density of HAIs due to CRE, CRPA, and CRAB Secondary Endpoints: - Quarterly proportions of HAI due CRE, CRPA, and CRAB - Incidence density (N/1000 patient-days) of healthcare-associated bloodstream of any type (to obtain a proxy for the overall burden of HAI) - Incidence density (N/1000 patient-days) and quarterly proportions of HAI due to other clinically important multidrug-resistant organisms such as ESBL-producing Klebsiella pneumoniae, methicillin-resistant Staphyhlococcus aureus, and vancomycin-resistant enterococci (to assess the overall impact of the interventions on HAI) - Incidence density (N/10'000 patient-days) of nosocomial Clostridioides difficile infection (as a proxy for the consumption of broad-spectrum antibiotics) - Performed blood culture sets per 1000 patient-days (to assess detection bias for HAI)

	- Performed stool tests for Clostridioides difficile per 1000 patient-days (to assess detection bias for Clostridioides difficile infection) - Consumption of alcohol-based handrub solution per 1000 patient-days (to assess compliance with the infection prevention and control programme) - Antimicrobial consumption in daily-defined doses (to assess compliance with the antibiotic stewardship programme) - Prevalence of CRE colonisation at the end of baseline, at the end of the infection prevention and control programme, and at the end of the antibiotic stewardship programme (to assess impact on antimicrobial resistance outside HAI) - Resistance-mechanisms of the isolated CRE in the three prevalence surveys (to understand CRE spread) - Clonality of the isolated CRE in the four prevalence surveys (to understand CRE spread) - In-hospital all-cause mortality (to assess harmlessness of the antibiotic stewardship programme) - (Re-) admissions density (N / month) of any type - Length of hospital stay for admissions of any type - Intervention (MDS, IPC and ABS) and tailoring (enhanced implementation condition) fidelity, feasibility, and sustainability - Implementation determinants (qualitative assessment)
Study Design	Prospective multi-centre, cluster-randomised, stepped-wedge trial. Hybrid type 2 effectiveness-implementation trial.
Statistical Considerations	Statistical Model: Generalized mixed-effects models with log-link function Sample size: Twenty-four acute care hospitals from high AMR prevalence areas
Inclusion- / Exclusion Criteria	Inclusion Criteria: • All adult inpatients in participating centres in intensive care, internal medicine, haematology-oncology, and surgery (including transplant units) Exclusion criteria: • Patients in settings other than mentioned above • Children, infants, or neonates • Outpatients
Number of Participants with Rationale	Number of randomized hospitals: 24 (likely at least 2.5 million in-patients over the study period) Based on the data for HAI in the participating hospitals, and the hypothesized efficacy of the interventions, this number will give us sufficient power to detect differences between: - IPC to baseline - ABS to IPC - IPC and ABS combined to baseline - IPC and ABS under conditions of enhanced implementation practice to ABS and IPC under conditions of basic implementation practice
Study Intervention	Three bundled programmes will be sequentially implemented after a 6-month baseline monitoring period - microbiology and diagnostic stewardship (MDS), infection prevention and control (IPC), and antimicrobial stewardship (ABS). These interventions target the institutions and health professionals. The data will be collected throughout the baseline and intervention periods. All centres will conduct a point prevalence survey for CRE, CRAB, and CRPA colonization at three pre-defined time points. These swabs are in addition to any routine surveillance swabs done by the hospital and will be sent to a centralised European laboratory (UMCU) for analysis. Positive swabs may be sequenced to assess for clonality and to establish transmission links. At two time points in the study, an audit will be done to assess microbiology capabilities. In addition, before the IPC module, hospitals will be randomised to either basic (12 BASIC study sites) or enhanced implementation (12 ENHANCE study sites) as part of the hybrid

	approach. This randomization applies only to the implementation part of this study for both the IPC and ABS bundles. A cost-effectiveness analysis will be done at the end to assess the feasibility of expanding such an initiative. This cost effectiveness analysis will also include a nested cohort study to study quality of life in patients with and without infection.
Control Intervention	Each centre will serve as a control during the baseline period.
Study procedures	The planned study period is 51 months, with the data collection and implementation period lasting 45 months. Please see Appendix 1 for the schematic. The bundles will be implemented in a sequential manner every 6-12 months. After a baseline of 6 months, 6 participating hospitals will be randomised to start with the first programme, until all 24 hospitals have started the intervention 9 months later. Randomisation is stratified by country. Workshops with the site prior to the IPC and ABS intervention will be organised. All workshops will be organised in collaboration with a team of implementation experts. Twelve randomised hospitals, also stratified by country, will receive additional support from these experts to tailor their implementation of both interventions to local conditions. Quarterly videoconferences with the participating hospitals and national coordinating centres will support implementation. Audits will be performed by local focal points in collaboration with MDS, IPC, and ABS content experts.
Study Duration and Schedule	51 months Planned 03/2022 (M0) for start of baseline period of first six hospitals Planned 06/2026 for end Planned recruitment start for nested cohort study: 04/2022 Planned recruitment end for nested cohort study: 02/2025
Investigator(s)	UZH: Dr. med. Walter Zingg UniversitätsSpital Zürich Rämistrasse 100 8091 Zürich Italy: Prof Evelina Tacconelli Università di Verona Via Dell Artigliere 8 000, 37129, Verona, Italy Spain: Prof Dr Jesús Rodríguez-Baño Servicio Andaluz de Salud Avenida de la Constitucion 18 000, 41071, Sevilla, Spain Greece: Prof Dr George L. Daikos Ethniko Kai Kapodistriako Panepistimio Athinon 6 Christou Lada Str 000, 10561, Athina, Greece Romania: Prof Dr Adriana Hristea Universitatea de Medicina si Farmacie Carol Davila Din Bucuresti Dionisie Lupu 37 000, 020021, Bucuresti, Romania
Study Center(s)	Multicentre study: 24 centres involved Multinational study: 4 countries

Data privacy	The data will be collected at the centre level. 4500 patients will be consented for the nested cohort study. The data will be entered into a secure Redcap database, stored at the University Hospital Zurich. The data gathered by the implementation experts (interviews, surveys, readiness audit) will be stored on a Redcap database hosted by the University of Zurich.
Ethical consideration	MDR infections result in significant morbidity and mortality for patients, and increased length of stay and cost for hospital systems. These interventions, targeted at healthcare providers, have shown to be effective separately. Proper implementation and evaluation of these programs together is essential to prevent spread of MDR organisms and improve patient outcomes. Since these interventions are targeted at the providers, all patients under their care will benefit. The nested cohort study comparing the difference in quality of life between patient with and without CRO infection is observational only.
GCP Statement	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP, the HRA as well as other locally relevant legal and regulatory requirements.

2 BACKGROUND AND RATIONALE

Resistance in bacteria is driven by selection pressure after exposure to antimicrobials. In healthcare settings, it can be further transmitted from one patient to another (from lapses in IPC) or from the transmission of resistance genes between pathogenic bacteria (1). Infections caused by antimicrobial resistant organisms represent a serious challenge to healthcare in Europe. For example, infections caused by carbapenem resistant gram negatives result in increased morbidity and mortality due to limited options for treatment (2). Therefore, there is an urgent need to limit development and further spread of antimicrobial resistance.

Infection Prevention and Control and Antibiotic Stewardship Interventions have addressed these issues in isolation, but healthcare systems are complex and often transmission is related to multiple issues rather than a single problem (3).

The REVERSE project seeks to address these interdependent systematic issues in hospitals by implementing three bundled interventions focused on Microbiology and Diagnostic Stewardship, Infection Prevention and Control, and Antibiotic Stewardship. REVERSE will use a mixed-methods approach, using a type-2 hybrid effectiveness-implementation trial and economic analysis, aiming to better demonstrate the impact of these programmes collectively.

3 STUDY OBJECTIVES AND DESIGN

3.1 Hypothesis and primary objective

We hypothesise the joint implementation of these programs will result in a greater reduction of AMR and HAIs than each program individually. We further hypothesise that enhanced implementation support will have an added effect on the primary outcome compared to basic implementation.

The primary objective of this study is to develop and implement cost-effective strategies and tools for the prevention and clinical management of HAIs due to multidrug-resistant pathogens, and to reduce the burden of AMR in high prevalence care settings.

Specific objectives:

1. To design and evaluate an integrated, modular strategy of evidence-based intervention programmes that can be implemented in the clinical management of hospitalised patients in high AMR prevalence settings
2. To design and evaluate a tailored enhanced implementation strategy versus a standard basic implementation strategy to introduce evidence-based interventions in high AMR prevalence settings
3. To estimate the cost-effectiveness of the intervention programmes and their implementation for the prevention and clinical management of infections and colonization due to AMR pathogens
4. To develop recommendations and implementation strategies on AMR prevention and clinical management strategies in high AMR prevalence settings in Europe, and to explore transferability of the proposed intervention programmes to LMIC outside Europe
5. To obtain a change of the local organisational way of working in the participating hospitals and to engage them as European reference hospitals for sustainability and further dissemination.

3.2 Primary and secondary endpoints

Primary outcome:
Incidence density (N/1000 patient-days) of HAIs due to CRE, carbapenem-resistant CRPA, and CRAB, combined in a composite index; measured during baseline and during the infection

prevention and control- and antibiotic stewardship programmes.

Secondary outcomes:

1. Quarterly proportions of HAI due CRE, CRPA, and CRAB
2. Incidence density (N/1000 patient-days) of healthcare-associated bloodstream of any type (to obtain a proxy for the overall burden of HAI)
3. Incidence density (N/1000 patient-days) and quarterly proportions of HAI due to other clinically important multidrug-resistant organisms such as ESBL-producing Klebsiella pneumoniae, methicillin-resistant Staphyhlococcus aureus, and vancomycin-resistant enterococci (to assess the overall impact of the interventions on HAI)
4. Incidence density (N/10'000 patient-days) of nosocomial Clostridioides difficile infection (as a proxy for the consumption of broad-spectrum antibiotics)
5. Performed blood culture sets per 1000 patient-days (to assess detection bias for HAI)
6. Performed stool tests for Clostridioides difficile per 1000 patient-days (to assess detection bias for Clostridioides difficile infection)
7. Consumption of alcohol-based handrub solution per 1000 patient-days (to assess compliance with the infection prevention and control programme)
8. Antimicrobial consumption in daily-defined doses (to assess compliance with the antibiotic stewardship programme)
9. Prevalence of CRE colonisation at the end of baseline, at the end of the infection prevention and control programme, and at the end of the antibiotic stewardship programme (to assess impact on antimicrobial resistance outside HAI)
10. Resistance-mechanisms of the isolated CRE in the three prevalence surveys (to understand CRE spread)
11. Clonality of the isolated CRE in the four prevalence surveys (to understand CRE spread)
12. In-hospital all-cause mortality (to assess harmlessness of the antibiotic stewardship programme)
13. (Re-) admissions density (N / month) of any type
14. Length of hospital stay for admissions of any type
15. Intervention (MDS, IPC and ABS) and tailoring (enhanced implementation condition) fidelity, feasibility, and sustainability
16. Implementation determinants (qualitative assessment)

3.3 Study design

The study is a prospective multi-centre, cluster-randomised, stepped-wedge trial and a type 2 effectiveness-implementation trial in 24 hospitals of four European countries (Greece, Italy, Romania, Spain). The hospitals are located in high prevalence settings for multidrug-resistant microorganisms, particularly carbapenem-resistant Gram-negative bacteria.

The intervention programmes – MDS, IPC, and ABS– will be implemented as bundles and based on professional implementation support; they are multifaceted and tiered in best practice procedures and technology interventions. All hospitals will benefit from all interventions. Control is provided by the stepped-wedge design.

Randomisation is on the hospital level, and the interventions address institutions as a whole and particularly healthcare professionals.

All 24 hospitals will start their baseline period in month 0. After a baseline of six months, six participating hospitals will be randomised (stratified by country) to begin with the first programme, until all 24 hospitals have adopted the intervention nine months later. The three intervention programmes will be implemented sequentially and build on each other. The total observation time will be 45 months.

12 hospitals will also receive enhanced implementation support. After randomising hospitals for programme start, hospitals in each cohort will also be randomised to either the basic or enhanced implementation condition. Each cohort will therefore include three hospitals in the enhanced and three in the basic condition.

(*Please see Appendix 1*)
Start of trial: M0
First 6 hospitals start MDS programme: M6
Last 6 hospitals start MDS programme: M15
First 6 hospitals start IPC programme: M12
Last 6 hospitals start IPC programme: M21
First 6 hospitals start ABS programme: M24
Last 6 hospitals start ABS programme: M33
End of trial: M45
Analysis and cost effectiveness calculations M45-51

3.4. Study intervention

At three time points in the study (before IPC intervention, before ABS intervention and at the end), all centres will undergo a point prevalence survey for CRE, CRAB, and CRPA colonization. These swabs are in addition to any routine surveillance swabs done in hospital and to surveillance swabs suggested in the IPC bundle of interventions (see Section 3.4.3). The three point prevalence surveys mentioned here will be analysed in a central European laboratory (UMCU, see section 3.4.1). Positive swabs may be sequenced to assess for clonality and to establish transmission links. At two time points in the study, an audit will be done to assess microbiology capabilities (further details of both in Section 3.4.1 and 3.4.2 below; see Appendix 1 for timing).

After the baseline period, hospitals will implement the MDS bundle, followed by the IPC and ABS bundles (6-12 months between each implementation; see Appendix 1). Section 3.4.3 presents the detailed interventions in each bundle.

If there is an outbreak of carbapenem resistant organisms, outbreak strains will be sent for WGS to assist in outbreak characterisation and control (see section 3.4.4).

In addition to the interventions described in each bundle, REVERSE will use implementation science to evaluate whether and to what degree hospital implementation activities influence primary and secondary endpoints. Based on a mixed-methods type 2 hybrid effectiveness-implementation trial, we will assess the readiness of all REVERSE hospitals to implement the IPC and ABS interventions. Prior to the adoption of the IPC module, hospitals will then be randomised to either basic implementation practice (12 BASIC study sites) or enhanced (tailored) implementation practice (12 ENHANCE study sites). In working with hospitals in the enhanced implementation condition, we will use qualitative techniques to understand contextual factors of importance to the implementation of REVERSE interventions and – based on this understanding – support the development of locally tailored implementation strategies. We will also apply quantitative measures to assess how both clinical interventions (all REVERSE hospitals) and implementation strategies (hospitals in the enhanced implementation condition) are perceived by hospital staff.

Details of the implementation assessment are presented in Section 3.4.5.

Lastly, we will conduct a cost-effectiveness analysis to assess the feasibility of expanding an initiative such as REVERSE. See section 3.4.6 for details.

3.4.1 Point Prevalence Surveys

CRO screening:

All patients hospitalised in intensive care, haematology-oncology, general surgery, and internal medicine (general internal medicine and subspecialty wards including infectious diseases) in the REVERSE hospitals will be eligible for CRE, CRAB, and CRPA screening by rectal swab. However, only 250 patients (200 patients with length of stay 3 days or more; 50 patients with length of stay 1-2 days) will be screened. The duration of hospital stay on the day of the survey is recorded. These swabs will be performed in addition to any routine, hospital level surveillance swabs.

The centralized REVERSE laboratory (UMCU) will provide the included hospitals with transwabs containing liquid Amies for sampling and all necessary medical packing for international shipping of biological samples in order to reach the UMCU for testing. Swabs will be stored at room temperature (RT) before use. After the swab, samples will be stored at 4C and shipped at RT to the Central REVERSE laboratory (UMCU), within two weeks of sampling. Sampling and shipping will be done within the month scheduled for screening according to Appendix 1.

The swabs will be processed at a centralized REVERSE laboratory (UMCU) and results will not be identifiable on a patient level. When more than 250 swabs are received from a single survey, the Central REVERSE lab (UMCU) will take a weighted sample of 200 patients with a length of stay of 3 days or more. The remaining 50 will be a random sample of the patients with a length of stay with a maximum of 2 days. A total of 250 swabs will be included per time point per centre.

Sampling will be performed at three time points: before the IPC intervention, before the ABS intervention and at the end of the intervention period. See the schedule included in Appendix 1.

Whole genome sequencing

The swabs are cultured using selective agarplates. Growth of colonies suspected for *Enterobaceriaceae*, *P. aeruginosa* or *A. baumanii* will be further processed using mass spectrophotometry and a phenotypic susceptibility test. Phenotypically confirmed CRE isolates stored at -80°C are plated onto blood agar, incubated overnight at 37°C and checked for purity. One colony is used to inoculate 3 mL LB followed by overnight incubation at 37°C. The bacteria in 1.6 mL suspension are pelleted in an Eppendorf tube. The fluid is decanted and after a brief spin the remaining fluid is removed by using a pipette. The pellet is suspended in 300 µl microbead solution, which is subjected to DNA extraction with the Ultraclean Microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA). The DNA concentration is measured using the Qubit double-stranded DNA (dsDNA) BR assay kit (Life Technologies, Carlsbad, CA, USA) or picogreen. One nanogram of bacterial DNA is used for library preparation. The DNA library is prepared using the Nextera XT library preparation kit with the Nextera XT v2 index kit (Illumina, San Diego, CA, USA). Subsequently, the library is sequenced on either a MiSeq sequencer, using the MiSeq reagent kit v2 generating 250-bp paired-end reads, or on a NextSeq with 2x 150 bp paired-end reads(Illumina, San Diego, CA, USA). Sequencing is aimed at a coverage of at least 50-fold. MiSeq data are processed with MiSeq control software v2.4.0.4 and MiSeq Reporter v2.4 and HiSeq data with bcl2fastq2 conversion software v1.8.4 (Illumina, San Diego, CA, USA). Bioinformatics analyses are performed with the snakemake pipeline bactofidia, release Coniophis. It includes quality check with FastQC (version 0.11.9) and trimming and adaptor removal with trim-galore (version 0.6.2) with a phred score cut-off of 20. *De novo* assembly is performed with SPAdes version 3.11.1 with a k-mer range of

57,97,127 for miseq data and 37,57,77 for hiseq data. Contigs with a length smaller than 500 basepairs or a k-mer coverage of less than 10 are excluded from further analysis to remove low-level contamination. For each assembled genome, the number of scaffolds, the N50, the maximum scaffold length, and the percentage of the expected genome size are determined with Quast and coverage of each contig and mean depth is determined with bbmap2 version 37.62. WGS-based species identification and MLST typing is done by scanning contigs against the whole PubMLST database with [mlst](http://bioconda.github.io/recipes/mlst/README.html), version 2.16.2. Resistance gene determination is performed with abricate version 0.8 using the resfinder database, downloaded on 2019-Apr-23. Potential target genes need to show a minimum DNA identity of 90%. All quality parameters are visualized in a summarizing report with multiqc, version 1.6a0.

Assembled genomes should have a coverage of at least 20x, contain less than 600 contigs and the genome size should be within the range reported for the species.

WGS-based typing

The MLST target definer function of Ridom SeqSphere (Ridom, Münster, Germany) is used to define target gene sets for all species with at least 10 isolates available. Depending on the number of available complete reference genomes, typing schemes are developed at the genus level (*Citrobacter* spp.), genetic-complex level (*E. cloacae* complex), or species level (*E. coli*, *K. oxy- toca*, and *K. pneumoniae*). For each scheme, one annotated and publicly available complete genome (chromosome) is used as a reference genome. For *K. pneumoniae* and *E. coli* schemes are available.

New schemes will be developed based on a reference genome, preferably of the type strain or a complete circular genome. Additional genomes are selected to define the gene set. These additional genes should be from well-defined isolates and of high quality. Repeat isolates should be avoided.

Of those, genes that are present in all query genomes with a sequence identity of at least 90% and an alignment of 100% are classified as core genome targets; the remaining genes are classified as accessory genome targets. wgMLST schemes include all whole-genome targets; core genome MLST (cgMLST) schemes include only the core genome targets.
All-to-all distance matrices, describing pairwise genetic distances, are constructed separately for core, accessory, and whole-genome target gene sets. The pairwise genetic distance is defined as the proportion of allele differences and is calculated by dividing the number of allele differences by the total number of good targets shared by both sequences, i.e., pairwise ignoring missing values.

Clonal relatedness will be based on the genetic distance and cut offs as defined previously based on a combination of epidemiological and molecular data in a similar setting (4).

Default SeqSphere settings are used for the development of schemes and the analysis of sequences. Samples that deviate more than 10% from the others are removed for generating a Neighbour Joining Tree and missing values are an own category.

A detail protocol is available at the UMCU under document name: Assessing relatedness between bacteria of the same species using NGS data and Ridom SeqSphere+. Document number: BAC-wi-071.

3.4.2 Microbiology Audits

Specifically, this will cover the practices and workflow for CRE screening and for routine microbiological diagnosis of BSI and LRTI. For CRE screening, auditing will cover the screening

methodology (by culture or molecular), the diagnostic systems used (selective media, identification systems, molecular platforms), the methods for identification of resistance mechanisms (if any), the reporting format and the turn-around-time (TAT).

For diagnostics with clinical specimens (blood cultures and LRT specimens), auditing will cover the diagnostic workflows, the identification systems (e. g. MALDI-ToF, semi-automated systems), the phenotypic susceptibility testing systems, the breakpoint systems used, the molecular diagnostic platforms used, the reporting format and TAT. For blood cultures, thresholds for adequate sampling will also be considered.

There will also be a review of the internal control processes of each laboratory (already present as part of accreditation criteria) and an external control (known MRDO strains) will also be used to assess their proficiency.

3.4.3 MDS, IPC, ABS interventions

Microbiology and Diagnostic Stewardship Interventions

The MDS intervention will include the following components:

- Guidance document on usage of diagnostics for suspected bacterial infection
- Audit and feedback on compliance to guidance
- Universal screening in high-risk settings (intensive care, haemato-oncology, transplant units)

Once the above interventions have started, we will determine the feasibility of starting the interventions below (based on hospital infrastructure and laboratory capacity):

- Universal screening in abdominal surgery patients
- Molecular characterization of blood cultures and samples from lower respiratory tracts (HAP) to inform ABS
- Rapid tests if molecular tests unavailable (e.g. CARBA-5 or beta-LACTA)
- Molecular characterization of isolated CRE from repetitive colonisation surveys to inform IPC.

Infection Prevention and Control Interventions

The basic best practice intervention bundle of the IPC module will include the following elements:

- Enhanced standard precautions (e.g., use of gloves for contacts with wounds and body fluids) and improved hand hygiene, with special emphasis on the use of alcohol-based hand rub (ABHR) solutions
- Regular point prevalence surveys to detect previously unknown MDRO carriers and identify hidden hot spots of MDRO transmission in the concerned institution in collaboration with MDS
- Reinforced basic environmental hygiene (e.g. surface decontamination, biofilm eradication)
- Targeted MDRO screening at admission for selected high-risk populations (e.g., previously known MDRO carriers)
- Audits and feedback on the basic IPC components in regular time intervals

Once the basic best practice intervention is implemented, regular audits will determine the feasibility of implementing the advanced best practice bundle.

The advanced best practice intervention bundle of the IPC-module will include the following elements:

- Enhanced, universal MDRO screening at admission in ICUs and other high risk units
- Reinforced contact precautions for identified MDRO carriers
- Enhanced cleaning in high-risk settings with targeted environmental point prevalence sampling surveys
- Improved information transfer on MDRO's carriage status within the hospital and along the referral pathways
- Root-cause analysis of newly detected cases to direct infection control measures

Organisational and pharmaceutical interventions of the IPC module will include the following elements:

- Setup and implementation of advanced cohorting facilities for selected highly resistant MDROs (e.g., CRE)
- Dedicating nursing staff for patient care with highly resistant MDROs (if operationally feasible)
- Decolonization or decontamination of colonized patients or patients in high-risk units using chlorhexidine body wash
- Molecular analysis and sequencing of isolates for outbreak investigation

The organisational and pharmaceutical interventions will be started with the basic best practices bundle.

Antibiotic Stewardship Interventions

The basic best practice intervention bundle of the antibiotic stewardship (ABS) module will include the following elements:

- Establishment of a multidisciplinary stewardship committee with regular meetings
- Guidance document on syndrome-specific treatment pathways
- Dedicated recommendations for new drugs
- Training on judicious antibiotic prescription
- Audit and feedback on compliance to guidance on antibiotic use
- Stewardship rounds 2 times a week in high-risk settings (intensive care, haematology-oncology, transplant units)
- Pathways for integration of antibiotic consumption reporting to the stewardship policies
- Weekly stewardship rounds in wards other than high-risk, but with a high prevalence of AMR

After the above interventions, a review of the implemented hospital ABS programme will be completed. Based on the assessment the following interventions (in collaboration with MDS) will be explored for implementation and sustainability.

- Integration of screening results in the decision-making process for empiric therapy for bloodstream infections in immunocompromised patients
- Integration of screening results before abdominal surgery for personalised prophylaxis
- Integration of molecular characterization of blood cultures to drive targeted therapy of BSI.

3.4.4 Outbreak characterization

An outbreak eligible for characterization of carbapenem-resistant Gram-negatives (CRGN, including CRE, CRAB or CRPA) includes at least one of the following criteria:

i. an increase, in the number of cases (above the local epidemiology) caused by the same species or

ii. an increase of specific resistance-mechanisms (or resistance profiles) in a short time frame compared to local epidemiology.

For each patient only the first clinical and/or surveillance isolate will be characterized. Clinical data required for each patient will include date of admission, length of stay, ward, clinical specimens, age, and sex.

Each isolate should be stored in BHI medium with 15% glycerol or in dedicated cryovials at -80°C until shipment with dedicated transport swabs (organized by UNIFI).

Bacterial DNA of all isolates will be extracted using the Qiagen DNeasy PowerLyzer PowerSoil Kit by UNIFI. Genomic DNA will be subjected to WGS with an Illumina MiSeq platform, using a 2 × 250 bp or a 2 × 150 bp paired-end approach, and reads will be assembled using SPAdes. In selected cases, WGS will be also performed by the Oxford Nanopore MinION system in order to generate a *de novo* hybrid assembly using Unicycler.
In silico identification of antimicrobial resistance genes, plasmid replicons, and bacterial clonality will be carried out using dedicated tools available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). Comparative analysis of resistance genes sequences will be carried out using the BLASTn Tool and annotation of *IS* elements will be performed using the *IS*Finder database (http://www-is.biotoul.fr/). SNPs on core genome will be evaluated using snippy (https://github.com/tseemann/snippy).

Phenotypic antimicrobial susceptibility testing will be carried out in selected cases (e.g. detection of unusual resistance mechanisms or resistance gene variants). AST will be performed with reference methods (ISO 20776:1-2019) and interpreted according to the most updated EUCAST clinical breakpoints.

3.4.5 Implementation evaluation and assessment

Organisational Culture and Implementation readiness

Information about all hospitals' implementation readiness will be obtained using two questionnaires and one telephone interview per site. The first questionnaire will focus on information about the existing organisation and structure of IPC and ABS in REVERSE hospitals. It will follow the concept of the ECDC 10 key components (5) and the WHO 8 key components(6). The second questionnaire aims at obtaining data on hospital organisational culture, previous implementation experiences, as well as facilitators and barriers in the day-to-day functioning of the hospitals and potentially influencing implementation readiness. The questionnaire will build on the CFIR framework (7), and the findings of the PROHIBIT study (8). Telephone interviews will be organised with the onsite investigators upon submission of the two questionnaires. These will be aimed at verifying the content of the questionnaires and further exploring implementation readiness using semi-structured interview guides. Onsite investigators will be specifically asked about their planned implementation strategies for both the IPC- and the ABS-module.

Basic implementation support

All hospitals will receive basic implementation support, delivered at the kick-off meeting and in workshops preceding the IPC- and ABS modules. Given the stepped-wedge design, six hospitals from different countries will attend each of the workshops. Each hospital will identify a group of clinicians responsible for local implementation of the clinical intervention programmes in their respective hospitals. Representatives of these local implementation teams will attend the kick-off meeting and two workshops, one before the IPC-module, and one before the ABS-module. The workshops will provide information on the upcoming module and summarised information from the analysis of the questionnaires and telephone interviews during baseline.

Enhanced implementation support

In addition to basic implementation support, the ENHANCE study sites will receive enhanced implementation support. This support will consist of building hospitals staff's capacity to develop a prospectively tailored set of implementation strategies adapted to the implementation conditions at each individual hospital. Information of relevance to tailoring the implementation strategies will be collected by the REVERSE implementation science team during two site visits. One visit will be conducted prior to the IPC-module and one before the ABS-module. Both visits will include interviews and observations.

For the tailoring of implementation strategies at hospitals in the ENHANCED study condition, local implementation determinants will be identified using the CFIR framework (7) and mapped to the ERIC compilation of implementation strategies(9). Strategies will be co-produced in collaboration with local implementation teams, refined based on their local expertise, and then documented. Two videoconferences will be organised six months into the IPC- and the ABS modules to check in on the intervention implementation progress and to provide formative evaluation. This feedback will further refine and guide the implementation strategy to enhance the likeliness of implementation success.

Interview partners

Interview partners chosen for each institution could include hospital CEO, director of nursing, medical director, microbiology or infectious diseases specialists, pharmacists, quality officers, and the IPC team. Interviews will be performed by teams of two members of the REVERSE study team. The two videoconferences will be organised with the local implementation teams.

Qualitative analysis

The qualitative analysis will contribute to the mixed-method summative analysis together with the quantitative findings. The methodology will follow rigorous qualitative research combining interviews and observations. Interviews will be performed in the local language. Professional translators will be hired to attend the interviews, and all interviews will be audio-recorded (with written informed consent provided by the interviewees). The translated passages will be transcribed verbatim by a professional company. Transcriptions will be analysed with a qualitative data management system, allowing to identify, manage and map themes and quotes from interviews. Analysis will identify themes (elements driving the functioning of the organisation), which will be validated by triangulation (unsolicited expression of a theme by different stakeholders). We will use a deductive approach to data analysis during which the CFIR will serve as a coding framework to guide interpretation of interview and observation data. Analysis will initially take place at the hospital level where each hospital represents a case and in-depth case description allows understanding of rich local phenomena. Cross-case analyses will then be conducted using stacked matrices to identify transversal themes.

Quantitative implementation research

The ENHANCE study sites will be compared to the BASIC study sites on the primary outcome of REVERSE (composite index incorporating healthcare-associated infection due to CRE, CRPA, CRAB), and the following secondary outcomes: prevalence of CRE colonisation, microbiological testing (blood cultures, stool cultures for *Clostridioides difficile*), consumption of alcohol-based hand rub, the use of the ABS APP, and implementation outcomes.

Implementation outcomes will include MDS, IPC and ABS feasibility (*are interventions perceived as easily usable/ implementable?*), sustainability (*are interventions maintained over time?*), and fidelity (*are interventions applied and implemented as intended*?), the latter for

which criteria will be defined in collaboration with MDS, IPC and ABS content leads. For hospitals in the enhanced implementation condition, the feasibility, fidelity and sustainability of tailoring will also be assessed.

3.4.6 Cost effectiveness analysis

Systematic reviews on cost-effectiveness of ABS, IPC and MDS programmes

Existing systematic reviews will be updated using the same search terms and databases such as EMBASE, Medline, EconLit, CINAHL, NHS EED, CEA Registry. This work will provide an up-to-date overview of cost-effectiveness estimates and, importantly, will be used to inform specific parameters and modelling approaches for both model-based cost-effectiveness analyses (from the hospital perspective and the societal perspective).

Micro-costing of interventions and implementation

Previous studies have failed to adequately identify the cost of the intervention itself and associated implementation costs - an important limitation highlighted by previous reviews. To overcome this, we will perform a micro-costing study collecting costs on all intervention components as well as complete pathways of intervention implementation in the different settings. Because micro-costing is a labour-intensive task, two hospitals will be selected from each country for a detailed micro-costing analysis (n=8). A standardized reporting form will be created in the local language to collect data on resources and prices. The collected data will be uploaded into REDcap. .

The standardized reporting form will be created in close collaboration with WP2-5. First, the components relevant for each intervention and its implementation will be determined, including average staff time for each activity (including both bench work as well as education/training) and related average salary data, use of equipment (initial costs, maintenance costs, and proportion of time used for the intervention(s)), (increases in) consumables (e.g. laboratory supplies, software licences, service contracts).

Examples of the standardised reporting forms - for resource use as well as unit costs where appropriate – that will be used are shown in Table 1-6 (See Appendix 2). Forms will be completed by the study nurse and local staff implementing the intervention that are directly approached by the study nurse to complete the standardized forms. Where appropriate, resource use will be recorded per time-unit (e.g. time spent on audits per week/month) or per infected case (e.g. DNA extraction for whole genome sequencing). It is also important to understand and quantify what resource use and costs are trial-specific and what would be needed beyond REVERSE to sustainably implement programmes in practice. The standardised reporting forms will also collect information on what percentage of time or resources used are specific to the trial and not needed beyond REVERSE to sustainably implement programmes in practice. The reported estimates will be checked for face-validity by co-investigators.

Equipment and consumable costs will be extracted from purchasing records where possible. Where not possible, unit costs will be obtained from commercial laboratory equipment suppliers. When cost data are only available for a kit as a whole, kit costs will be apportioned equally across all items in the kit.

Estimates of the lifespan of equipment will be based on information provided by laboratory staff. Staff costs will be based on national data on salaries for the respective job titles for the specific countries, taking the midpoint where bands are provided. These costs will be verified by obtaining estimated midpoint salaries for each job of interest from finance/human resource departments of the respective hospitals. To enable quantification of intervention-related

changes in expenditure on specific elements that do exist to a certain extent before intervention implementation, such as time spend on audits, the standardized reporting form will also be used before interventions are implemented in the hospitals (baseline). This enables to quantify whether the (cost-)effectiveness of depends on the baseline levels of infection prevention and control, diagnostics and antibiotic stewardship before intervention implementation.

Outcome data collection for economic evaluations

To enable assessment of the cost-effectiveness of the interventions, data on outcomes that are associated with costs and life-years lost need to be collected. The following outcomes need to be collected:

- Infection status (date of sample)
- Admission and discharge dates (length of stay)
- Dates admitted to and discharged from ICU (length of stay in ICU)
- Readmissions
- Infection related treatment (type and duration of treatment)
- Death

Cost associated with above resource use will be obtained using national reference cost where available, supplemented with extensive searches of published and unpublished literature and databases (such as WHO-CHOICE) along with consultation with co-investigators and finance departments of the respective hospitals.

Quality of life estimation using nested matched cohort study

To enable comparisons between different, potentially unrelated, interventions competing for the same budget ideally health-economic analyses would be expressed in terms of cost per QALY. This allows for maximising the quality of life of the population given a fixed budget by prioritising interventions that cost less per QALY and are affordable given the budget. QALYs represent a measure of both morbidity and mortality. However, there is a severe lack of data on impact of different infections of interest on morbidity, as measured by health-related quality of life.

To address this knowledge gap, a matched cohort study (REVERSE-QoL) will be nested in the randomised trail with the primary objective of estimating the impact of hospital-acquired infections caused by carbapenem-resistant enterobacteriales (CRE), carbapenem-resistant Pseudomonas aeruginosa (CRPA), or carbapenem-resistant Acinetobacter baumannii (CRAB) on patients' health-related quality of life (HRQoL) during their hospitalisations and 1-, 3-, 6-, and 12-months after their infection.

Using a matched cohort study nested in the RCT we will compare quality of life among patients acquiring key pathogens of interest versus patients with a similar reason for admission (randomly sampled from the same ward) to estimate the impact of acquiring these infections on quality of life during the hospitalisation and 1-, 3-, 6-, and 12-months post discharge using EuroQol-5D (EQ-5D) and 36-Item Short Form Health Survey (SF-36) health-related quality of life questionnaires. These validated questionnaires are available in local languages (Italian, Romanian, Greek and Spanish) for patients and proxies.

The primary outcome of this study will be health-related quality of life over time as measured using the EQ-5D questionnaire. Secondary outcomes include i) health-related quality of life over time as measured using the SF-36 questionnaire, and differences in separate domains of both health-related quality of life questionnaires (EQ-5D and SF-36).

We aim to recruit consenting adult patients (REVERSE only recruits adult patients) that acquire a CRE, CRPA, or CRAB hospital acquired infection (main outcome of the RCT) during their hospital stay. Specific inclusion and exclusion criteria for the nested matched cohort study are in Section 4.1.

For each infected patient 2 eligible hospitalised patients without a CRE, CRPA, or CRAB infection at the time of recruitment will be included. This nested matched cohort design is needed to assess the impact of healthcare-associated infections on health-related quality of life in a feasible manner. These estimates will subsequently be used to model the impact of the interventions on health-related quality of life mediated by reductions in the density of healthcare-associated infections, and subsequently cost per QALY.

Uninfected patients will be selected at random from the cohort of patients without CRE, CRPA, or CRAB infection who are being cared for on the same ward as the infected cases. Uninfected patients will be selected using a random number generator from the same ward as the case at the same time period and matched by age and time in the hospital before the index date (day of infection or day of being matched).

The study nurses will approach the clinical teams to ensure that patients meet the inclusion criteria, and if being recruited as a case, they must be aware of their infection and considered well enough to be invited to take part. The initial approach to the patient will be by the clinical team caring for the patient. Patients will be asked to provide consent after reviewing the patient information sheet provided. Study nurses will seek a guardian, welfare attorney or family member to provide consent for patients who are unable to consent for themselves. This approach will ensure that the matched cohort study also includes the most unwell and patients potentially at risk of acquiring a CRE, CRPA, or CRAB infection.

Patients or their proxies who have provided informed consent will be asked to fill in a short paper or online questionnaire (EQ-5D and SF-36) when recruited and before discharge, and data will be subsequently recorded in REDCap by the study nurses. Consenting patients will also be asked to provide their contact details in order to provide them with a unique link to a REDCap survey for online completion of follow-up questionnaires at 1, 3, 6, and 12 months after the index date, at which points the participants would receive a reminder by email. Those that consent, but are not able/willing to complete the questionnaires online, will be provided with prepaid return envelopes (up to 2,500 patients to remain within the budget). Where possible, National Death Registries will be contacted in order to identify any patients who have died since discharge to i) ensure that their families will not be contacted after their relatives have died and ii) incorporate the fact that patients do not have a quality of life after dying. The study nurses will also undertake a case note review collecting clinical data which is not routinely available. If no follow up survey is recorded >2 weeks from the expected date, the study nurse will follow up with the patient via telephone. During this telephone follow up only the EQ-5D survey will be filled out. If the patient withdraws their consent or has since passed away, this will be recorded in REDCap and no further surveys will be sent.

The analyses of the nested matched cohort study will match on the following covariates: age, hospital, ward (e.g. internal medicine (or subspecialty), general surgery, intensive care, or haemotology-oncology), and time in the hospital before infection (or day of matching for uninfected patients).

Additional covariates that will be recorded and adjusted for in mixed effects regression analysis, but not matched on, include sex, comorbidities (Charlson Comorbidity Index), surgical procedure within 30 days before the index date (date of matching), antibiotic use within 30 days before the index date.

Potential effect modification will be assessed by age, sex, and country. Potential differential effects by infection type (e.g. bloodstream, pneumonia, surgical site infection) and causative pathogen (CRE, CRPA, or CRAB) will be assessed.

Composite outcome

In addition to the primary study outcome, we will generate a composite outcome measure consisting of a weighted cumulative incidence of all included key pathogens. This will be developed with the relative importance of different components of the composite outcome estimated using swing weighting, acknowledging that prevention of infection caused by pathogen A may be more valuable/important than prevention of an infection by pathogen B. Swing weighting is increasingly used to evaluate the relative importance of different criteria. Respondents receive a description of a hypothetical programme that has the worst possible level of performance on all outcome criteria (e.g., incidence of specific infections of interests). They are asked which criterion they would improve first (i.e., swing) from the worst to the best level to improve the overall situation the most. This criterion is subsequently removed from the set of criteria and the respondents are again asked which criterion they would select next. This process is repeated until all criteria are ranked. The criterion that was chosen first is assigned 100 points, subsequently respondents are asked on a scale from 0 to 100, what the weight of a swing on the criterion ranked second would be. This is repeated for all criteria, and the scores are subsequently normalized into weights for the composite outcome. This swing weighting study will involve multiple experts from high-endemic settings and relevant stakeholders and networks such as the European Union Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI), WHO and ECDC included in or associated to the Advisory Board.

Cost-effectiveness analysis from the hospital perspective

The cost-effectiveness of the different intervention bundles will be assessed from the hospital perspective over defined time horizons, using a health economic model informed by data from the study trials. Specifically, a decision analytic model will be used to conduct the cost-effectiveness analysis of the trial interventions and accompanying implementation. Data on:

a) all cost-causing events (including both intervention and infection related costs) and associated resource use and relative differences, combined with appropriate setting-specific unit costs
b) relevant clinical outcomes and relative effectiveness estimates, including modelled quality of life impact and other relevant outcome measures, such as the weighted composite outcome of the cumulative incidence of infections caused by different key pathogens, synthesised with additional data and evidence as necessary

will be used to parameterize the decision model to assess incremental cost-effectiveness of the different interventions in defined settings from the hospital perspective.

This model structure will enable numerous ‘levels’ of outcome to be assessed. The incremental cost-effectiveness of the sequential intervention components, as well as an entire package of interventions will be expressed as, for example cost per a) reduction in incidence of clinical samples positive for specific indicator pathogens, (or infection averted); b) change in the composite (both primary and weighted) outcome measure; c) unit reduction in antimicrobial use; d) life-year gained; and e) QALY gained. Incremental cost per QALY estimated will be presented as incremental cost-effectiveness ratios (ICERs), these will reflect uncertainty in model parameters, propagated through the model, which are important for decision-makers to be aware of, and will be explored under alternative values of *willingness to pay for health benefits*, thus providing a net monetary benefit (with associated uncertainty) for the intervention components as well as the intervention package as a whole.

We will develop a Graphical User Interface for this cost-effectiveness model that may be used as a 'tool' for hospitals in particular settings to evaluate cost-effectiveness, given particular epidemiological and cost parameter inputs.

Cost-effectiveness analysis from a societal perspective

IPC, ABS, and MDS intervention programmes likely have relevant effects on societal health and economy that are not adequately captured in a cost-effectiveness analysis from the hospital perspective. We will extend the hospital-perspective model developed by incorporating costs due to absence of work and model potential longer-term impact of interventions on antibiotic resistance and the health and economic consequences of this change in resistance beyond the trial observation period. For the former, we will evaluate country-specific, age- and sex-specific work participation rates and predict influence of longer/shorter hospital stays on absenteeism. For the latter, we will further develop methods for estimation of long-term population effects, and integration into cost-effectiveness evaluations. This will build further upon 1) state-of-the-art Bayesian spatio-temporal models evaluating the relationship between changes in antibiotic prescribing and prevalence of resistance (developed by UOXF within the Economic and Social Research Council-funded STEP-UP project), and 2) mathematical models extrapolating effects observed during the trial beyond the trial observation period.

Budget impact analysis

Budget impact analyses will be performed to assess affordability in different settings. The estimated uptake of the interventions along with the economic inputs used in the cost-effectiveness models will be incorporated. These budget impact analyses will show the financial implications of implementing the different interventions. A shiny app will be developed to allow decision makers in various settings, including in low- and middle-income countries where available budgets are often limited, to effortlessly assess the affordability of the trialled interventions at varying levels of uptake and local costs in their own setting.

4 STUDY POPULATION AND STUDY PROCEDURES

4.1 Inclusion and exclusion criteria, justification of study population

Inclusion criteria:
All adult inpatients in the participating hospitals are included in the study when hospitalized in intensive care, internal medicine (including all sub-specialty wards including infectious diseases), haematology-oncology, and surgery (including transplant units).

Exclusion criteria:
Patients in settings other than the ones mentioned above, outpatients, and neonates, infants, children, and adolescents.

Justification of study population:
REVERSE will focus on antibiotic resistance in acute care hospitals because MDROs often have their origin there. Compared to the community, the burden of antibiotic resistance is higher in hospitals; and within hospitals, the burden of antibiotic resistance is higher in healthcare-associated than in community-acquired infections.

The 24 hospital sites were selected because they have a high prevalence of MDROs (specifically CRE, CRPA, CRAB) based on a point prevalence survey conducted in 2016/17.

Inclusion criteria for the nested cohort study:

- Adult patient (>/= 18) admitted to a participating hospital on a participating ward

- Able to speak/ understand the local language or English well enough to fill out the surveys
- Hospital-acquired infection caused by CRE/CRPA/CRAB or control from the same ward

Exclusion criteria for the nested cohort study:
- Unable to speak/ understand one of the survey languages or English
- Admitted to a ward or hospital not participating in REVERSE
- Under 18 years of age
- Admitted with infection caused by CRE/CRPA/CRAB (community-acquired infection)

4.2 Recruitment, screening, and informed consent procedure

4.2.1 MDS, IPC, and ABS interventions (as outlined in section 3.4.1-3.4.4)

We are asking for a waiver of consent given REVERSE is a quality improvement project and the designed interventions are for healthcare workers and not patients. The measures outlined in Section 3.4.3 have previously individually been shown to improve patient outcomes, therefore there is no additional risk to the patients.

There will be three screening tests performed on patients as part of the MDRO prevalence surveys (as detailed in Section 3.4.1). Such tests are not invasive, and the harm from microbiological screening for MDROs is very low, compared to a high benefit for both the institution and individual patients if colonisation with multidrug-resistant organisms is confirmed or ruled out. This is valid for all screening activities within REVERSE.

Furthermore, it would be impossible to exclude a patient in a participating hospital once the interventions are implemented as they are directed at healthcare workers caring for multiple patients.

4.2.2 Implementation analysis (as outlined in Section 3.4.5)

The implementation readiness surveys will be done by the site investigator at the hospital. The site investigator is part of the clinical trial team and therefore no consent is needed. The quantitative implementation measures (feasibility, sustainability, fidelity) will be measured via surveys taken by health care workers. Survey data will be collected anonymously (emails used for survey links will not be connected to responses). Electronic consent will be collected for these surveys.

For qualitative research as outlined in Section 3.4.5, informed written or verbal consent will be obtained from the healthcare personnel when interviews are conducted and audio-recorded. Only adult persons capable of consenting will be interviewed. No vulnerable individuals will be included in the interviews. If individuals are unable to give informed consent, they will not be involved in the interviews.

For the interviews, the investigators will explain to each participant the nature of the interview, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the interview/study is voluntary and that he or she may withdraw at any time and that withdrawal of consent will not affect his or her subsequent employment or duties. The participant will be informed that his or her interview will be recorded and reviewed by authorised individuals.

All participants for the interview will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an

informed decision about their participation in the study. All participants will be given up to sufficient time (1-2 weeks) to decide if they would like to participate. The formal consent of a participant, using the approved consent form, will be obtained before the participant is submitted to any study procedure.

The consent form will be signed and dated by the investigator or his designee at the same time as the participant signature. A copy of the signed informed consent will be given to the participant. The consent form will be retained as part of the study records.

No remuneration will be given to the healthcare personnel for participating in the interviews.

4.2.3 Economic analysis (as outlined in Section 3.4.6)

Micro costing and cost effectiveness analysis

The micro costing analysis will be done on a hospital level with administrative data. No patient level data will be collected and therefore we ask for a consent waiver for this analysis.

QALY cohort study

For the QALY cohort study, we ask for a waiver of consent for patient identification. Patients with infections of interest will be identified by the microbiology department and forwarded to investigators. For control patients, a chart review of patients on the ward will be done by study personnel. Patients meeting study criteria (infection with organism of interest or matched control) will then be contacted by study personnel for consent. Written or verbal informed consent will be obtained from a patient or alternate decision maker (if the patient is unable to provide consent).Only permanently incapacitated patients will be included based on consent of alternate decision maker. Temporarily incapacitated patients will be included when they regain capacity.

The waiver of consent for chart review is needed as it would be impossible to identify matched controls otherwise. Furthermore, there is no intervention planned for these patients as the QALY analysis includes only observational data from questionnaires.

For the cohort study, the investigators will explain to each participant (or alternate decision maker) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant (or alternate decision maker) will be informed that the participation in the study is voluntary and that he or she may withdraw at any time and that withdrawal of consent will not affect his or her subsequent care. The participant (or alternate decision maker) will be informed that their questionnaire answers will be reviewed by other authorised persons involved in REVERSE.

All participants (or alternate decision makers) for the cohort study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. All participants will be given sufficient time to decide if they would like to participate. The study nurses will follow up 48 hours after the initial contact. The formal consent of a participant (or alternate decision maker), using the approved consent form, will be obtained before the participant is submitted to any study procedure.

The consent form will be signed and dated by the investigator or his designee at the same time as the participant (or alternate decision maker) signature. A copy of the signed informed consent will be given to the participant or alternate decision maker. The consent form will be

retained as part of the study records and a copy will be placed in the chart.

No remuneration will be given to the participants.

4.3 Study procedures

The planned study period is 60 months, with the data collection and implementation period lasting 45 months. Please see Appendix 1 for a schematic representation of the study timeline.

The interventions are outlined in Section 3.4, these will be implemented in a sequential manner every six months. After a baseline of six months, six participating hospitals will be randomised to start with the first programme, until all 24 hospitals have started the intervention nine months later. Randomisation will be stratified by country.

Data collection will begin in the baseline line period - specifically HAI surveillance, antimicrobial data utilization, HAI and laboratory data, and hospital data. HAI surveillace will be done prospectively for CRE, CRAB, CRPA, ESBL organisms, MRSA, and VRE. All other data will be collected from admistrative systems every 3 months.

During the MDS audit periods, the audit tool will be completed by members of the MDS team. This may occur in person or via teleconference (depending on travel regulations). External strains for validation will be sent to the hospitals in advance.

CRE, CRAB, and CRPA rectal screens will occur at 3 time points as noted in Section 3.4 and Appendix 1. Swabs will be sent to the hospitals by the central laboratory and shipped back within the same month.

A total of eight workshops will be organised with participating hospitals: a first set before the IPC interventions, and a second set before the ABS intervention. Every hospital will participate twice, once before the IPC intervention and once before the ABS intervention. The first four workshops will be organised in collaboration with MDS and IPC content experts, the second four workshops will be organised in collaboration with MDS and ABS content experts. All workshops will be organised in collaboration with implementation experts. These workshops can be organised as videoconferences if required.

Quarterly videoconferences with the participating hospitals and national focal points will support implementation. Twelve randomised hospitals will receive support for tailoring implementation by the REVERSE implementation science team (see Section 3.4.5). Audits will be performed by local focal points in collaboration with MDS content experts (to check on microbiological capacity), IPC content experts (for education and training in IPC), and ABS content experts (to establish local ABS groups). Twelve of the 24 participating hospitals will also have visits by the REVERSE implementation science team (See section 3.4.2-3.4.5).

Throughout the intervention period, regular assessments of the programme will be made to determine whether additional MDS, IPC, and ABS interventions can be implemented (see sections 3.4.2-3.4.4).

After the last 6 hospitals complete their intervention period, a wrap-up meeting with all participating hospitals will be organised. This last meeting will gather all partners of REVERSE together with national and international stakeholders in healthcare, and particularly infection prevention and control and antimicrobial resistance.

After data collection is completed at the hospitals, a cost effectiveness study will be done based on the data gathered, including a QALY analysis based on the nested cohort study (see Section 3.4.6).

4.4 Withdrawal and discontinuation

Not applicable for sections 3.4.1-3.4.4 since we are requesting a waiver of consent.

Implementation analysis

The consent for interviews can be withdrawn at any point up to end of the interview. If consent for an interview is withdrawn, the participant will be asked if the interview data up to that point can be used. If yes, the incomplete transcript will be analysed. If no, the interview data will not be used in the analysis and any identifying information (contact details of staff etc.) will be deleted.

Likewise consent for surveys can be withdrawn by exiting the survey prior to completion.

Economic analysis

The consent for the QALY cohort study can be withdrawn at any point up to the end of the study period (12 months post discharge). If consent is withdrawn, the participant will be asked if the survey data up to that point can be used. If yes, their answers up to the point of withdrawal will be used. If no, the data will not be used in the analysis.

5 STATISTICS AND METHODOLOGY

5.1. Statistical analysis plan and sample size calculation

Statistical analysis plan

Generalized mixed-effects models with log-link function will be used to analyse the primary outcome. The fixed effects will be the interventions, the country, and the time (to account for the partial confounding of the interventions with time). A cluster-specific random effect will be considered to model the repeated measurements on the same cluster. In the presence of over-dispersion, negative-binomial mixed-effects models with the same parametrization will be used instead. Model-based intervention effects will be reported. Supportive analyses considering more complex random effects structures will also be investigated. (e.g., time within clusters, wards within hospitals). The interaction between time and interventions will also be added as a fixed effect to model a possible time-varying intervention effect.

Sample size calculation for main RCT

Based on findings and modelling from the ECDC point prevalence survey of 2016/2017, mean estimated incidence densities of HAI due to a composite index incorporating CRE, CRPA and CRAB combined for Greece, Italy, Romania, Spain, were 2.99/1000 patient-days, 0.73, 0.62, and 0.51, respectively. Considering the lowest incidence density of 0.5/1000 patient-days, an intra-cluster correlation of 0.9, four randomisation steps, and 25'000 admissions per year in average, the following estimations were calculated for hypothesized effects of the intervention programmes:

- Reduction of 25% of HAI by IPC alone (IPC compared to baseline): 2.3 required hospitals
- Reduction of 35% of HAI by IPC and ABS combined (IPC plus ABS compared to baseline): 1.1 required hospitals

- Reduction of 10% HAI by ABS on top of IPC (ABS compared to IPC): 19.9 required hospitals
- Reduction of 15% HAI by enhanced implementation support on top of 35% reduction by IPC and ABS combined (as compared to basic implementation support): 9.8 required hospitals

Twenty-four acute care hospitals from high AMR prevalence areas provide sufficient power to perform all relevant comparisons for the primary outcome as specified by REVERSE: 1) IPC to baseline; 2) ABS to IPC; 3) IPC and ABS combined to baseline; and 4) enhanced implementation support to basic implementation support.

Additionally, we hypothesise that enhanced implementation will have an added effect on the primary outcome of 15% on top of the IPC- and ABS-modules combined (additional 15% to a 35% reduction). A total of 9.8 hospitals would need to be included in each group considering the same parameters as outlined above. Analysis will be done using a generalized mixed-effects models with log-link function as described. The fixed effects will be the enhanced implementation support, the country, and the time (to account for the partial confounding of the interventions with time).

Statistical analysis plan for nested cohort study:
Mixed effects models with optimal type of mixed-effects model (e.g. mixed-effects linear model or mixed-effects beta-regression) determined by model fit. Exposed patients (infected with organism of interest) and unexposed patients will be matched on ward, time in hospital before index date, and age (categorical: 18-44, 45-64, 65-74, 75+ years).

The analysis will include fixed effects for the matching variables and the following additional covariates: sex, comorbidities (Charlson Comorbidity Index), surgical procedure within 30 days before the index date (date of matching), antibiotic use within 30 days before the index date. Time will also be included as a covariate to model changes over time, with an interaction with the exposures of interest to model potential time-varying effects of the exposure. Total quality of life losses will be estimated and compared by obtaining the area under the curves for exposed and unexposed groups using Simpson's rule (quadratic interpolation).

A cluster-specific and patient-specific random effect will be considered to model the repeated measurements on the same cluster and patient. Supportive analyses considering more complex random effects structures will also be investigated. (e.g., time within clusters, wards within hospitals). The interaction between time and interventions will also be added as a fixed effect to model a possible time-varying intervention effect.

It is possible that a limited number of individuals that are recruited as uninfected controls will attract a CRE/CRPA/CRAB infection at a later point during their hospitalisation. This is necessary to avoid bias introduced when selecting controls that will never be infected (conditioning on the future). In expectation, the number of people acquiring such infections is small and measurements on or after the day of infection in those patients originally assigned to the control group will be censored.

Sample size calculation for nested cohort study
The RCT this matched cohort study will be nested in, will be conducted in 24 acute care hospitals with ~75 infections per year caused by carbapenem-resistant enterobacteriales (CRE), carbapenem-resistant Pseudomonas aeruginosa
(CRPA), and carbapenem-resistant Acinetobacter baumannii (CRAB). The study will have a duration of 4 years, leading to approximately 24*75*4 = 7,200 CRE/CRPA/CRAB infections. Assuming a similar distribution of types of infections – bloodstream, urinary tract infection, etc. – as observed in point prevalence surveys among hospitals across Europe, a drop-out of 10% over time, and at least 80% power to detect a difference in utility of 0.05 at each time-point for all HAI of interest and a difference of 0.1 for bloodstream infections one would need to recruit

Commented [KP1]: Have added more detail now, so it is clear how to use age for matching.

Rationale:
Population-norms of HR-QoL generally available at following age-brackets:
<25, 25-34, 35-44, 45-54, 55-64, 65-74, 75+

Charlson comorbidity index gives age-points based on following categorisation (with no points below 50):
50-59, 60-69, 70-79, 80-89, 90-99

Given similar HRQoL for healthy adults in general population – especially after considering comorbidities, can use relatively broad age-categories for matching:

18-44, 45-64, 65-74, 75+

approximately 4,500 participants (189 – 63 infected and 126 uninfected – patients per hospital over 4 years).

5.2. Handling of missing data and drop-outs

Missing data
Quarterly checks on data completeness with feedback to the centres will be organised. Delays or errors of data collection will be discussed in the quarterly videoconferences with the hospitals.

Drop-outs
Hospitals dropping out of the study will be replaced until month 9, which allows a minimum baseline of 6 months. Thereafter, hospitals dropping out will not be replaced. The power calculation is conservative, and the primary outcome can still be analysed.

6 REGULATORY ASPECTS AND SAFETY

6.1 Amendments

Substantial changes to the study setup and study organization, the protocol and relevant study documents will be submitted to the Ethics Committee for approval before implementation. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Ethics Committee. Such deviations shall be documented and reported to the Ethics Committee as soon as possible.

A list of all non-substantial amendments will be submitted once a year to the competent EC together with the ASR.

6.2 Termination of study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, e.g.

- Ethical concerns,
- When the safety of the participants is doubtful or at risk (e.g. when the benefit-risk assessment is no longer positive),
- Alterations in accepted clinical practice that make the continuation of the study unwise, or
- Early evidence of harm or benefit of the experimental intervention

Upon regular study termination, the Ethics Committee will be notified via BASEC within 90 days (ClinO, Art. 38 (13)).

Upon premature study termination or study interruption, the Ethics Committee will be notified via BASEC within 15 days (ClinO, Art. 38 (13)).

All samples submitted to external laboratories will be anonymized.Health related data will be collected as anonymized where possible (for incidence of MDROs or antibiotic data) or anonymized at the end of the study (for nested cohort study).

7 FURTHER ASPECTS

7.1 Overall ethical considerations

At the end of REVERSE, four European countries with high prevalence of carbapenem-resistant Gram-negative bacteria will have six highly experienced hospitals to expand a national network for combatting antibiotic resistance. These hospitals will have experience in implementing integral interventions on improving microbiology, infection prevention and control, and antibiotic stewardship and can promote similar programmes to peer institutions.

REVERSE will also produce tools and bundles on infection prevention and control, antibiotic stewardship and implementation support that have a wider impact, beyond the 24 hospitals and countries participating in REVERSE. These tools will be field-tested for ease of implementation and cost-effectiveness. The effectiveness analysis in REVERSE will allow tailoring of best practice interventions in different contexts. Knowledge on what works with reasonable investment in terms of human, financial and social capital can be used and applied by stakeholders and hospitals around the globe, including low-and-middle income countries.

REVERSE will find answers to study questions for which there is low or only fractioned evidence. This will impact best practice guidelines in both infection prevention and control and antibiotic stewardship. It will also impact future intervention studies if enhanced tailored implementation support is effective in the hybrid implementation-intervention study. Enhanced implementation support will then become a mandatory part of clinical trial management.

7.2 Risk-benefit assessment

Since REVERSE is an observational quality improvement project, there is no additional risk to patients beyond those of a usual admission. As outlined in Section 4.2, the three additional swabs are not invasive, part of regular screening activities within hospitals, and will benefit patients.

The other benefits apply to all future patients – ongoing MDS, IPC, ABS programs will help curb antimicrobial resistance, and in turn, reduce morbidity and mortality for patients.

For the nested cohort study:

The risks are small as this is a non-interventional cohort study. Patients may experience some anxiety as they remember their illness and reflect on how it has impacted their life.

Patients may experience an overall positive feeling in that their illness is being researched and their quality of life is being given consideration. Entering this study may not directly help them, but the information we get from the REVERSE-QoL study should help similar patients in the future and would be useful for informing cost-effectiveness evaluations that determine whether potential expensive interventions preventing (antibiotic resistant) infections will be implemented in routine practice.

8 QUALITY CONTROL AND DATA PROTECTION

8.1 Quality measures

For quality assurance the sponsor, the Ethics Committee, or a trial monitor (focal national points) may visit the research sites. Direct access to the source data and all study related files is granted on such occasions. All involved parties will keep the participant data strictly confidential.

8.2 Data recording and source data

Data recording

Data collection will be done by study nurses using the REDCap platform (https://www.project-REDCap.org/). The REDcap platform will be accessed through USZ, and each site will have their own login. UZH will be able to access data from all sites (24 hospitals); the national focal points will only be able to access data from their country (6 hospitals). All information related to individual patients will always be entered into REDCap directly (HAI surveillance, Outbreak information). Hospital data (average length of stay, number of blood cultures and stool tests, antimicrobial utilization etc.) will be sent via excel spreadsheet and uploaded into REDCap by USZ for all 24 sites. Data used for micro-costing and cost effectiveness (see Appendix 2) will be given directly to the cost effectiveness team by the hospitals and not entered into REDCap.

For HAI surveillance and Outbreak information each infection or outbreak sample will be assigned a neutral ID by REDCap.

The REVERSE Implementation science team will have their own database using the REDCap platform hosted through UZH. They will have access to interview and survey data from all sites (24 hospitals).

For the nested cohort study, data will be recorded in REDCap under the site the patient(s) were originally admitted to. The data will be entered by the study nurses and non-identifiable data will be accessible to UZH and the cost-effectiveness team. Each site will only be able to access their data (including identifiers), the national focal points will be able to access non-identifiable data in their country (6 hospitals), and USZ will be able to access non-identifiable data from all sites. The cost effectiveness team will be able to access and download de-identified data from all sites.

Each cohort study participant will be assigned a neutral ID by REDCap (composed of the REDCap site ID (4 digit number) followed by subject number (sequential starting at 1).

Source data

Clinical Study:
- Clinical data (as aggregate: age, gender, length-of-stay, all-cause mortality)
- Microbiology data (CRE, CRPA, CRAB, MRSA, ESBL organisms, or VRE isolated in clinical or screening samples; results of bloodstream infections)
- HAI data (type of infection and organism, antimicrobials used, patient data (age, location (ward and hospital), devices present)

MDS:
- Hospital data (detection and testing capacity of microbiology; blood culture samples per 1000 patient-days; stool tests for Clostridioides difficile per 1000 patient-days); Microbiology data (WGS of CRE, isolated in repeated point prevalence surveys; WGS of MDRO isolated during local outbreaks)

IPC:
- Hospital data (results from audits on IPC practices)
- Microbiology data (results from targeted or universal screening)
- Literature (recommendations, guidelines, policies on IPC)
- Performance data (alcohol-based handrub consumption per 1000 patient-days)

ABS:
- Epidemiological data (age, gender, country; risk factors for bacterial infections; epidemiological data of hospitals and patient case mix)
- Clinical data (comorbidities, clinical manifestation of infections, empiric and targeted antimicrobial therapy)

- Outcome data (antibiotic consumption, use of broad-spectrum antibiotics, surgical site infections)
- Microbiological data (colonising and infecting bacteria; concomitant infection; susceptibility pattern)

Implementation:
- Site data including infrastructure, organisational structures and processes
- Interview notes, contact details of local staff (study group), artefacts
- Site specific implementation data (feasibility, fidelity, sustainability)

Cost effectiveness:
- Data on clinical outcomes (incidence of clinical samples of indicator pathogens, Clostridioides difficile infection, mortality)
- Relevant resource use (length of hospital stay, re-admissions, type and duration of antibiotic use)
- health outcomes (QALY, EuroQol-5D [EQ-5D], 36-Item Short Form Health Survey [SF-36], health-related quality of life questionnaires).
- Nested cohort study only: information on covariates from patient notes and answers to first questionnaire. Follow-up questionnaires will only include the health-related quality of life questionnaires (EQ-5D and SF-36) and will not ask again about covariates.
- Information on study health facilities in LMIC (name, place, position within the health system, services offered and main indicators – e.g., number of beds, number of deaths/year, catchment population, periodic number of patients seen by each service)
- Information on interviewees/healthcare personnel (job position and tasks, age, gender, level of education, years of experience overall and within the health facility

8.3 Confidentiality and coding

Trial and participant data will be handled with uttermost discretion and is only accessible to authorised personnel who require the data to fulfil their duties within the scope of the study. On the CRFs and other study specific documents, participants are only identified by a unique participant number.

Patient data will be stored in the REDCap database, behind the UZH or USZ firewall, and institutional data protection standards of UZH and USZ will apply. This is a password protected, secure database that can track changes. The server has an SSL security certificate that allows encrypting the data that is transferred between the client and the server. Regular back-ups of data can be created from REDCap database. Each site will be given their own login with no access to data from other sites. Focal national points will have access to data from all sites in their country, and UZH will have access to data from all sites. The Implementation team will have access to their own data from all sites.

MDS, IPC and ABS interventions

REVERSE will collect some identifiable information in outbreak investigations (ward and hospital information, length of stay, age, sex). However, each sample will be coded prior to being sent out, with the identifiable data stored in REDCap with the sample code. Identifiable information is also collected as part of the HAI surveillance (e.g.: age, sex, length of stay, ward) but stored on the REDCap database and analysed as aggregate data. No patient names or health-care numbers will be recorded.

For the type of samples to be collected, please refer to section 3.4. REVERSE will only collect samples from the three prevalence surveys on CRE colonisation and if molecular typing is required to support local outbreak investigation. Any other clinical samples are performed in the

hospitals by local microbiology, and results are collected in non-identifiable manner from the hospitals.

Hospital level data (average length of stay, number of tests performed, patient days, antimicrobial utilization) will be uploaded into REDCap by USZ for all 24 sites every 3 months. This data will be provided by each hospital in a password protected excel spreadsheet.

REVERSE will not utilise other samples that are collected for reasons unrelated to the project. Material Transport Agreements between the partners will be prepared for the transport of samples. In addition, we will use standard protocols for the collection, packaging, and shipment of diagnostic samples in accordance with international guidelines and regulations should the need arise to send samples for bio-banking or additional tests within the network. The International Air Transport Association regulations will be followed with an IATA qualified technician packing the samples. Only an approved courier will carry the samples following IATA protocols. No category A infectious substances (UN2814) will be collected. All samples will be processed, transported, and stored according to strict standard operation procedures. The sample specific data will be kept in the central repository under the supervision of UMCU (for the colonisation surveys) and UNIFI (for outbreak investigation samples).

Implementation analysis
For quantitative implementation data (feasibility, fidelity, and sustainability), survey respondents will be assigned a unique identifier in the RedCap database, and all survey data uploaded. Interview subject data will be collected during the interview and uploaded to the RedCap database with the interview and the transcript.

Economic analysis
Participants in the nested cohort study will be assigned a unique identifier in the database. Names and email addresses are required for follow up surveys and will be collected only after consent. These identifying information will be removed from the dataset that will be used for analysis and replaced by an anonymous unique identifier.. All data will be stored in the REDCap database. For the cost-effectiveness analyses only aggregated data will be analysed. Microcosting data (see Appendix 2) will be provided by hospitals to the cost effectiveness team directly. This data does not contain any patient information.

8.4 Retention and destruction of study data and biological material

All study data are archived for 5 years (or as per regional requirements if longer than 5 years) after study termination or premature termination of the study.

The rectal screens (as described in section 3.4.1) sent to UMCU will be stored as per standard guidelines without patient level identifying information (as per Section 8.3). These will be destroyed by the end of the study period. The outbreak strains will be sent to UNIFI with a unique code, and all identifying information (age, sex, ward location) will be stored on the REDCap database. All outbreak strains will be destroyed by the end of the study period.

9 MONITORING AND REGISTRATION

UZH will be the trial co-ordination site for all countries. The national focal points will be the coordination centres within each country. The national focal points will be responsible for site visits and to ensure completeness of data.

The MDS, IPC, ABS, and implementation teams will also visit the sites (in person or virtual depending on travel regulations) for workshops or interviews (see section 3.4 and 4.3).

Additionally, a quarterly meeting (videoconference) will be set up with the national focal points and UZH to ensure data completeness and accuracy. Any questions can be clarified at this time.

This trial will be registered in the Swiss National Clinical trial Portal (SNCTP via BASEC) as is required. In addition, the study will be registered with International Standard Registered Clinical/soCial sTudy Number (ISRCTN) - a registry listed in the WHO International Clinical Trials Registry Platform (ICTRP; http://www.who.int/ictrp/en/).

10. FUNDING / PUBLICATION / DECLARATION OF INTEREST

REVERSE is funded by the European Commission (Grant number 965265). The grant funding will be distributed through UZH to the national focal points and from there to the participating sites. Contracts between the national focal points and the participating sites outlining the responsibilities of each will be signed before funding is distributed further.

The results of REVERSE will be published in peer reviewed journals and presented at regional and international conferences after the end of the trial. We plan to host a meeting to disseminate results in month 45 after the end of the trial.

Final authorship will require the fulfilment of the Uniform Requirements for Authorship and Contributorship from the International Committee of Medical Journal Editors (www.icmje.org): *"Authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to the published. Authors should meet conditions 1, 2, and 3."* No publication should be submitted before all co-authors have been informed and have given their formal approval by any written media.

For all papers, reference has to be made to the REVERSE team. However, two different types of papers can be distinguished:

- Papers including results directly or indirectly gathered from the clinical trial of REVERSE: authored "…and the REVERSE Study Group". If the Editor specifically refuses this mention in the authorship, it should be added in acknowledgements that "the results published have been obtained on behalf of the REVERSE Study Group". The REVERSE Study Group includes the REVERSE consortium as well as the hospital site investigators.
- Other papers produced in REVERSE (e.g. methodology papers) authored "…and the REVERSE Consortium". If the Editor specifically refuses this mention in the authorship, it should be added in acknowledgements that "the results published have been obtained on behalf of the REVERSE Consortium". The REVERSE consortium includes all beneficiary leads in the project.

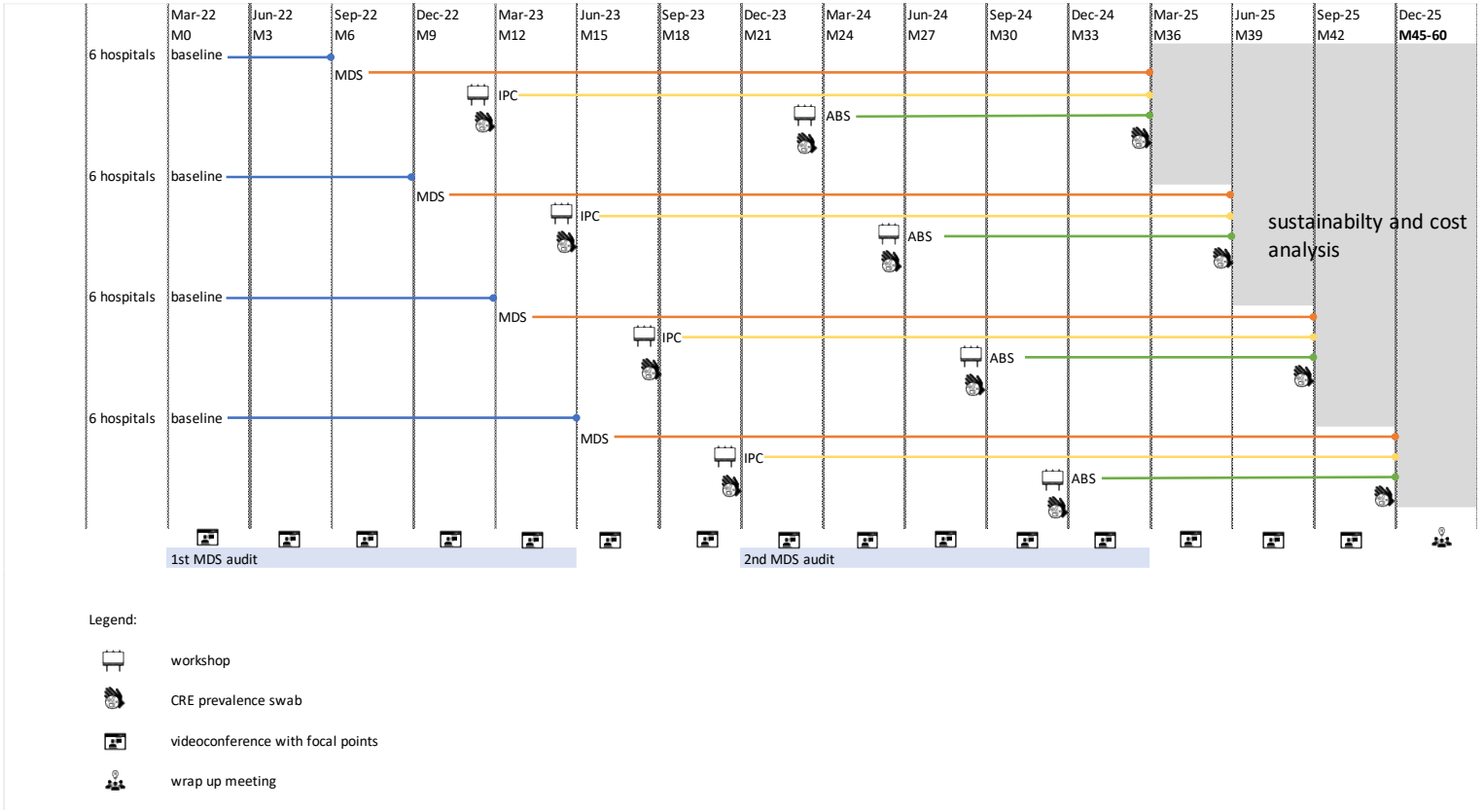
In case of disputes related to authorship and/or content, the REVERSE coordinator will be informed and will serve to solve these issues, taking in consideration REVERSE consortium interests and the Uniform Requirements for Authorship listed above.

11. REFERENCES

1. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet. 2016 Jan 9;387(10014):176–87.
2. A C, LD H, D P, A Q, A H, GS S, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. The Lancet Infectious diseases [Internet]. 2019 Jan 1 [cited 2021 Oct 7];19(1):56–66. Available from: https://pubmed.ncbi.nlm.nih.gov/30409683/
3. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathogens and Global Health [Internet]. 2015 Oct 1 [cited 2021 Oct 7];109(7):309. Available from: /pmc/articles/PMC4768623/
4. MF K den B, JW R, PC B-V, MJ B, AW F, CM V-G, et al. Whole-Genome Multilocus Sequence Typing of Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae. Journal of clinical microbiology [Internet]. 2016 Dec 1 [cited 2021 Oct 7];54(12):2919–27. Available from: https://pubmed.ncbi.nlm.nih.gov/27629900/
5. W Z, A H, M D, T G, F S, L C, et al. Hospital organisation, management, and structure for prevention of health-care-associated infection: a systematic review and expert consensus. The Lancet Infectious diseases [Internet]. 2015 Feb 1 [cited 2021 Oct 7];15(2):212–24. Available from: https://pubmed.ncbi.nlm.nih.gov/25467650/
6. J S, A T, W Z, N D, C K, J R, et al. Core components for effective infection prevention and control programmes: new WHO evidence-based recommendations. Antimicrobial resistance and infection control [Internet]. 2017 Jan 10 [cited 2021 Oct 7];6(1). Available from: https://pubmed.ncbi.nlm.nih.gov/28078082/
7. Damschroder LJ, Aron DC, Keith RE, Kirsh SR, Alexander JA, Lowery JC. Fostering implementation of health services research findings into practice: a consolidated framework for advancing implementation science. Implementation Science 2009 4:1 [Internet]. 2009 Aug 7 [cited 2021 Oct 7];4(1):1–15. Available from: https://implementationscience.biomedcentral.com/articles/10.1186/1748-5908-4-50
8. L C, W Z, S S, A C, S T, F da LJ, et al. Implementing infection prevention practices across European hospitals: an in-depth qualitative assessment. BMJ quality & safety [Internet]. 2018 Oct 1 [cited 2021 Oct 7];27(10):771–80. Available from: https://pubmed.ncbi.nlm.nih.gov/29950324/
9. Powell BJ, Waltz TJ, Chinman MJ, Damschroder LJ, Smith JL, Matthieu MM, et al. A refined compilation of implementation strategies: results from the Expert Recommendations for Implementing Change (ERIC) project. Implementation Science 2015 10:1 [Internet]. 2015 Feb 12 [cited 2021 Oct 7];10(1):1–14. Available from: https://implementationscience.biomedcentral.com/articles/10.1186/s13012-015-0209-1
10. WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects – WMA – The World Medical Association [Internet]. [cited 2021 Oct 7]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/
11. INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE (ICH) ICH HARMONISED GUIDELINE INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE E6(R2). 2016;
12. SR 810.30 - Bundesgesetz vom 30. September 2011 über die Forschung am Menschen (Humanforschungsgesetz, HFG) [Internet]. [cited 2021 Oct 7]. Available from: https://www.fedlex.admin.ch/eli/cc/2013/617/de
13. SR 810.305 - Verordnung vom 20. September 2013 über klinische Versuche mit Ausnahme klinischer Versuche mit Medizinprodukten (Verordnung über klinische Versuche; KlinV) [Internet]. [cited 2021 Oct 7]. Available from: https://www.fedlex.admin.ch/eli/cc/2013/643/de

12. APPENDICES

Appendix 1: Trial Schedule



Appendix 2: Cost effectiveness data collection

Table 1. Task list and staff time by intervention example

Intervention	Task	Staff job title	Time required (minutes)	Per infected case or time-unit (e.g. day/week/month)	% of time that is specific to the trial and not needed when implementing the same intervention outside the trial-setting
Basic IPC module	Audit on basic IPC component	Nurse	20	week	10%

Table 2. Unit costs applied to staff times example

Job title	Employer (country)	Salary mid-point	Cost per hour at this mid-point
Nurse	Hospital A, (Spain)	€28,000	€16.97

Note: Only the actual staff time used to complete a task will be costed. No assumptions will be made regarding the number of each type of staff member that would need to be employed in a laboratory in order to process the assumed sample throughput.

Table 3. Use of equipment by intervention example

Intervention	Task	Type of equipment (manufacturer)	Quantity used	% of equipment time used for intervention	% of time used for intervention (previous column) that is specific to the trial and not needed when implementing the same intervention outside the trial-setting
Advanced IPC module	DNA extraction	Vortexer	1	3%	0%

Table 4. Equipment costs and life-time example

Type of equipment (manufacturer and type)	Purchase costs (€)	Maintenance fee per year (€)	Lifespan (years)

Centrifuge (Thermo Scientific Sorvall ST4R Plus)	€9126	€210	5

Table 5. Use of consumables by intervention example

Intervention	Task	Type of consumable	Units used	Per infected case or time-unit (day/week)	% of units that are specific to the trial and not needed when implementing the same intervention outside the trial-setting
Basic IPC module	Hand hygiene	Alcohol-based hand rub	3 liter	Per patient-facing healthcare-worker per month	0%

Table 6. Consumable costs example

Consumable	Price (€)	Pack size	Unit cost (€)
Alcohol-based hand rub	19	1 liter	19