Decontamination strategies in Intensive Care Units

a cluster-randomized cross-over study

Supplement: study protocol and statistical analysis plan

This supplement contains the following items:

Decolonisation strategies in Intensive Care

Study Protocol

Part of

R-GNOSIS: Resistance in Gram-Negative Organisms: Studying Intervention Strategies

March 2012

Short title: WP6	**R-GNOSIS: Decolonisation strategies in ICU**
FP7-HEALTH-single-stage grant agreement no. 282512	
Date	March 2012
Coordinating investigator	**B.H.J. Wittekamp** E-mail: b.h.j.wittekamp@umcutrecht.nl
Principle investigators	**Prof C. Brun-Buisson** [redacted] **Prof M.J.M. Bonten** [redacted]
R-GNOSIS coordinator	**Prof M.J.M. Bonten** [redacted]

Protocol signature sheet

Name	Date	Signature
Head of R-GNOSIS: Prof. M.J.M. Bonten, MD, PhD Professor of Molecular Epidemiology of Infectious Diseases		
Coordinating Investigator: B.H.J. Wittekamp, MD		
Principal Site Investigator: ……. …….		
Physician / ICU director ……. …….		
Nursing Director ……. …….		

Table of contents

List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
CHX	Chlorhexidine
CPE	Carbapenemase producing Enterobacteriaceae
CRAB	Carbapenem-resistant Acinetobacter baumannii
cRCT	Cluster Randomised Controlled Trial
DDD	Defined Daily Dose
DSC	Data Safety Committee
e-CRF	Electronic Case Report Form
ESBL	Extended Spectrum Beta Lactamase
GCP	Good Clinical Practice
GNB	Gram Negative Bacteria
GRE	Glycopeptide Resistant Enterococci
HRMO	Highly Resistant Micro Organism
ICU	Intensive Care Unit
IRB	Institutional Review Board
KPC	Klebsiella Pneumoniae Carbapenemases
MDR	Multi Drug Resistant
MDR-GNB	Multi Drug Resistant Gram Negative Bacteria
MDR-PA	Multi Drug Resistant Pseudomonas aeruginosa
MRSA	Methicillin-Resistant Staphylococcus aureus
Oro-CHX	Oropharyngeal chlorhexidine
R-GNOSIS	Resistance in Gram-Negative Organisms: Studying Intervention Strategies
SC	Standard Care
SDD	Selective Digestive Tract Decontamination
SOD	Selective Oropharyngeal Decontamination
UMC	University Medical Centre
VAP	Ventilator-Associated Pneumonia
VRE	Vancomycin Resistant Enterococcus
WHO	World Health Organisation
WP	Work package

1. Introduction, rationale and objectives

Introduction

The “R-GNOSIS: Decolonisation strategies in Intensive Care” study is part of the R-GNOSIS project.

The R-GNOSIS (**R**esistance in **G**ram-**N**egative **O**rganisms: **S**tudying **I**ntervention **S**trategies) project combines 5 international clinical intervention studies, all supported by highly innovative microbiology and mathematical modelling, to determine - in the most relevant patient populations - the efficacy and effectiveness of interventions to reduce acquisition, carriage, infection and spread of Multi-Drug Resistant Gram-negative Bacteria (MDR-GNB). The studies and analyses proposed in R-GNOSIS will generate a step-change in identifying evidence-based preventive measures and clinical guidance for primary care and hospital-based physicians, as well as health-care authorities, to combat the spread and impact of the unprecedented rise of infections caused by MDR-GNB in Europe.

Unless explicitly stated otherwise throughout this protocol the abbreviation MDR-GNB will include:

- extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae
- carbapenem-resistant Enterobacteriaceae, such as Klebsiella pneumoniae carbapenemases (KPC)
- non fermenting MDR-GNB such as carbapenem-resistant Acinetobacter baumannii (CRAB) and Multi drug resistant Pseudomonas aeruginosa (MDR-PA).

Within health-care settings, MDR-GNB are most prevalent in specialized wards, such as Intensive Care Units (ICUs). Such units are characterized by a high antibiotic selective pressure, by the presence of patients highly susceptible to acquire colonisation and infection, and by frequent contacts between health care workers and patients, – factors that could highly facilitate cross-transmission of MDR-GNB.

The “R-GNOSIS: Decolonisation strategies in Intensive Care” study will investigate the safety and efficacy of 3 decontamination regimens on clinical outcome, infection rates and cross-transmission rates in ICUs. This is a cluster randomised controlled trial (cRCT) with cross-over preceded by a baseline period of standard care. The scientific goals (5

hypotheses) and the interaction with microbiology and mathematical modelling will progress well beyond the current state-of-the-art in intensive care medicine and hospital infection control. The 3 regimens (selective digestive tract decontamination (SDD), selective oropharyngeal decontamination (SOD) with antibiotics and SOD with chlorhexidine (Oro-CHX) have been previously tested, but mostly in studies that were underpowered. Decontamination with antibiotics or antiseptics has never been evaluated in a head-to-head comparison.

Rationale

Despite a large number of controlled trials and meta-analyses supporting its use, SDD has never gained full acceptance in European ICUs for three major reasons. First, the antibiotics used to achieve SDD are often not totally selective for Enterobacteriaceae and thus they also harm the rest of the microbiota and associated colonisation resistance. Second, for long times beneficial effects of SDD on patient outcome were only demonstrated in meta-analyses (1) and not in individual studies. Third, the antibiotics used in SDD may create some degree of selective pressure for bacterial resistance. This could be particularly problematic if SDD is used over a prolonged time, as in patients in ICU units.

In contrast, in the most recent and by far largest SDD trials performed in ICU units with low levels of antibiotic resistance, (i.e. in Dutch ICU units) decolonisation strategies were associated with statistically significant improvements in patient outcome. In a single-centre study of 934 patients, those treated in a unit where all received SDD had a 35% lower risk of dying in ICU when compared to those treated in the unit without SDD usage (2). In a multi-centre cluster-randomised trial (cRCT) with cross-over over interventions comparing SDD to SOD and to standard care (no SDD/no SOD) day-28 mortality rates were 13% and 11% lower (absolute reductions being 3.5% and 2.9%) for patients receiving SDD and SOD, respectively, when compared to patients receiving standard care (3). Moreover, SDD and SOD were associated with a 10% reduction in total systemic antibiotic use, when compared to patients receiving standard care (3). During SDD and SOD study periods, prevalence rates for resistant gram negative bacteria were lower than during standard care. Based on these newest insights SDD and SOD are safe and beneficial to patients. Also, the oral application of Chlorhexidine 2% seems a simple and effective measure to reduce the incidence of Ventilator-Associated Pneumonia (VAP) in ICU patients (4) and of nosocomial infections after cardiac surgery (5).

Successful suppression of MDR-GNB carriage could be a very important infection control measure, as it may not only protect the individual patient by reducing the risk of infection, but also the population at large by reducing transmission of MDR-GNB. Even transient suppression of ESBL carriage might reduce the likelihood of transmission and thus still be beneficial from an ecologic perspective. However, no validated strategy of MDR-GNB decontamination has currently been identified for widespread use.

Considering the paucity of alternatives for the ever-increasing problem of antibiotic resistance, it is appropriate and warranted to carefully determine the safety and efficacy of using decontamination strategies for elimination of MDR-GNB, including ESBL, in ICU populations across Europe.

Objectives

Primary objective

- To determine the effectiveness of 3 decolonisation regimens (SDD, SOD and Oro-CHX) in ICU patients in reducing ICU-acquired MDR-GNB bacteraemia when compared to standard care (SC).

Secondary objectives

- To determine the effectiveness of 3 decolonisation regimens (SDD, SOD and Oro-CHX) in ICU patients in reducing acquired respiratory tract colonisation with MDR-GNB when compared to standard care.
- To determine the effectiveness of 3 decolonisation regimens (SDD, SOD and Oro-CHX) in ICU patients in reducing day-28 mortality when compared to standard care.
- To quantify the effects of 3 decolonisation regimens (SDD, SOD and Oro-CHX) in ICU patients on overall systemic antibiotic use when compared to standard care.
- To quantify the associations between intestinal and respiratory tract colonisation with GNB and the occurrence of ICU-acquired GNB bacteraemia.
- To quantify species-specific nosocomial transmission capacities (reproductive number per hospital admission, R_A) of MDR-GNB during 3 decolonisation regimens and during standard care.
- To quantify cross-transmission rates with MDR-GNB during 3 decolonisation regimens and during standard care.

- To determine the ecological safety of 3 decolonisation regimens (SDD, SOD and Oro-CHX) in ICU patients.
- To determine ICU-acquired bacteraemia rates caused by any multi-drug resistant micro organism, including MRSA, VRE, MDR-GNB, Acinetobacter, *S. maltophilia*, and ceftazidime- and/or carbapenem resistant *P. aeruginosa,* during each phase of the trial.

R-GNOSIS study partners involved

As part of the R-GNOSIS project, MDR-GNB from point prevalence and surveillance cultures will be sent to the central laboratory for microbiological analyses. To determine species-specific nosocomial transmission capacities of MDR-GNB and cross-transmission rates during each regimen, data on MDR-GNB carrier status will be sent to workpackage (WP) 8 for mathematical modelling. WP 9 partners will provide an electronic database based on e-crf's developed specifically for this trial.

2. Study design

The study is a prospective cluster randomised controlled trial. Each site will complete 4 study periods, starting with the baseline period.

- Baseline period: 6 months, consisting of standard care: daily bathing with chlorhexidine (CHX) and implementing a hand hygiene improvement programme based on the program designed by the World Health Organisation (WHO).

Followed by (in randomised order) and added to standard care:

- SDD regimen: 6 months, consisting of:
    - oropharyngeal application of a paste containing colistin, tobramycin and nystatin each in a 2% concentration and
    - administration of a 10 ml suspension containing 100 mg colistin, 80 mg tobramycin and nystatin 2 x 10^6 i.u. via a nasogastric tube.

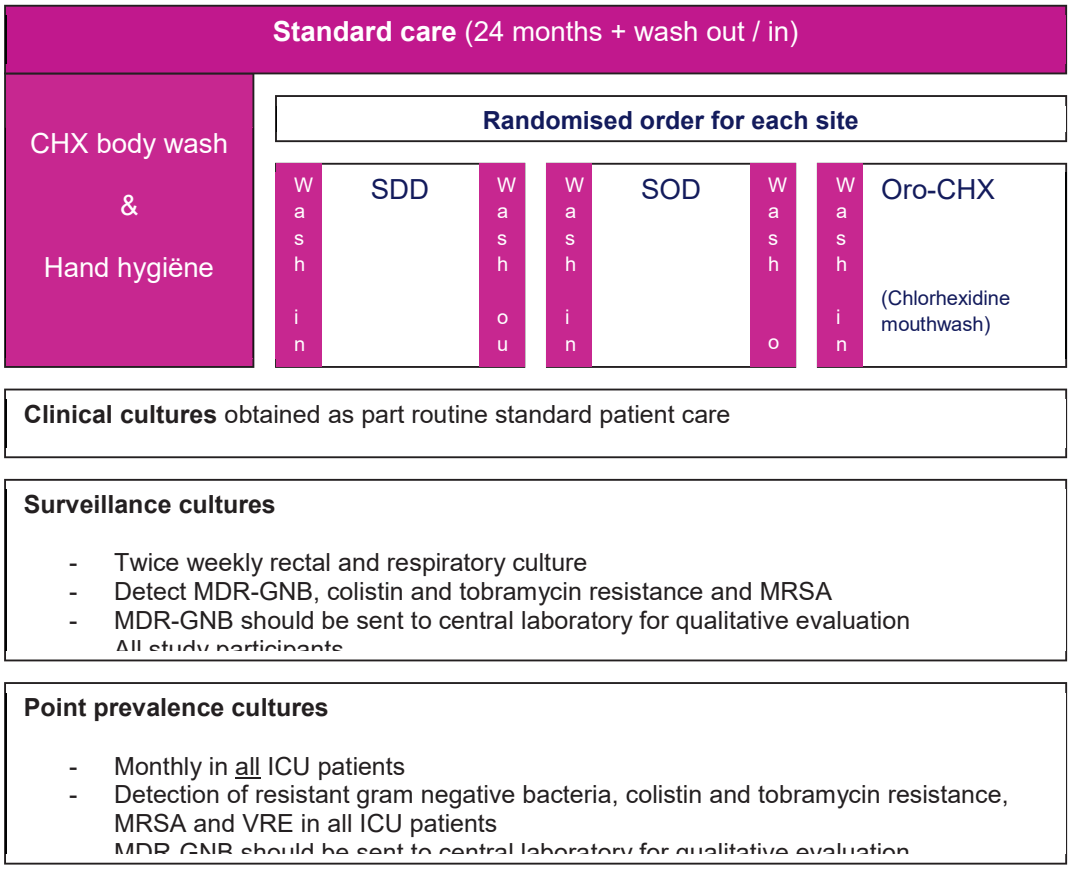
  SDD will be applied 4 times daily until extubation.
- SOD regimen: 6 months, consisting of 4 times daily oropharyngeal application of the paste only (containing colistin, tobramycin and nystatin each in a 2% concentration). SOD will be applied 4 times daily until extubation.
- Oro-CHX regimen: 6 months, consisting of 4 times daily oropharyngeal application of CHX in a 2% concentration. Chlorhexidine mouthwash should be performed 4 times daily until extubation.

A one month wash-out / wash-in period will separate intervention periods, as a transitional period between two interventions. There will be no wash in before the baseline period. Surveillance cultures and point prevalence cultures for ecological safety will be collected during all regimens and wash-out / wash-in periods.

In contrast to other SDD studies, systemic prophylaxis with Cefotaxim (or other broad spectrum cephalosporins) will not be implemented in the SDD regimen. Cefotaxim is considered inappropriate for prophylaxis in settings with endemic levels of antibiotic resistance. Moreover, most ICU patients already receive systemic antibiotics due to the nature of their severity of disease.

The cross over design is necessary to prevent confounding by relevant (and non-avoidable) differences between ICUs (such as differences in bacterial ecology, local practices, patient case-mix). The chlorhexidine body wash ensures state of the art standard of care to prevent carriage and transmission of (resistant) gram positive bacteria commonly residing on body surface, such as Staphylococcus aureus and Enterococci. Implementation of the hand hygiene program derived from the WHO hand hygiene program ensures state of the art standard of care for transmission prevention of all relevant pathogens.

Study Scheme per site



ICU inclusion criteria

ICU units can be included if:

- The ICU has endemic levels of ESBL, defined as a proportion of ESBL among ICU-acquired GNB-bacteraemia of >5% in 2011.
- The ICU consists of at least 8 beds with possibility for mechanical ventilation, with an average bed-occupancy of at least 80%.

- Have the ability of at least one dedicated health care worker for patient monitoring, compliance, monitoring and instruction of health care workers regarding interventions. In the following this person will be called “research-nurse”.
- A signed protocol signature page is present, indicating willingness from the ICU physician and the ICU nursing directors to enrol the ICU in the study.

In addition, ICU units should be able to:

- Adhere to the study protocol, collect required data and complete the study regimens.
- Obtain, analyze and store (colonisation) cultures at -70ºC and facilitate transport through a courier.
- Implement daily chlorhexidine bathing as standard care.
- Implement the WHO hand hygiene improvement program.
- Extract data in a pre-defined format from an operational digital patient-information system. Specifically, there should be an automated process for digital data-collection regarding microbiological culture-results (from swabs and bacteraemias), patient demographics and illness severity-scores.
- Obtain written approval for the study from the institutional review board (IRB) with a waiver for patient informed consent.

The following ICU units will be <u>excluded</u>:

- ICU units with endemic levels of carbapenem-resistant Enterobacteriaceae, MDR-Pseudomonas or Acinetobacter species (defined as >5% of ICU-acquired GNB-bacteraemia, see textbox 1) or having endemic VRE (defined as > 10% of all enterococci, see textbox 2).
- ICU units planning to enrol subjects in studies testing investigational agents for the purpose of eradicating or preventing colonisation with MDR-GNB, MRSA or VRE or devices or practice management strategies that have colonisation and/or infection with resistant organisms as an outcome.
- ICU units that will use chlorhexidine in standard oropharyngeal care during the study period.
- Burn units (due to the specific nature of the care provided and the patients admitted).
- Cardiothoracic surgery units (because of the expected small number of patients admitted for three days or more).
- Paediatric and neonatal ICU units.

Selection of eligible ICU units

ICUs will be selected on their capacity to generate high-quality data. This includes a suitable environment for electronic clinical and microbiological data collection and performing on-site observations. As part of EU legislation, putative subcontractors will be given the opportunity to apply for a position as a subcontractor in the trial. ICUs will be informed through the websites of the Julius Center (UMC Utrecht) and the European Society of Intensive Care Medicine (ESICM). Through a procedure legislatively outlined by the EU, candidate-institutions will be allowed to submit their application. After initial selection by the principal and coordinating investigators, selected ICUs will undergo on-site audits before final selection. From these ICUs, fifteen units will be selected for trial participation. If possible, ICUs will be included in an equal ratio of older and new member states.

Textbox 1. Endemic levels of carbapenem resistant micro-organisms

In 2011, did the ICU have >10 cases of carbapenem resistant bacteraemia with

1. Enterobacteriaceae (i.e. *E. Coli, Klebsiella p.*) y / n
2. Acinetobacter y / n
3. Pseudomonas y / n

If >10 bacteremias of one or more of these species occurred in 2011 (one or more yes), what was the proportion of carbapenem resistant isolates from the total amount of bacteremias with that species in 2011:

$$\frac{\text{Number of bacteremia with resistant isolates of the species (divided by)}}{\text{Total number of bacteremia of that species}} \times 100\% = \ldots\ \%$$

If **> 5%** consider the resistant form of the species endemic

Textbox 2. Endemic levels of Vancomycin Resistant Enterococci

In 2011, did the ICU have >10 cases of bacteraemia with VRE?

If >10 VRE bacteremias occurred in 2011, what was the proportion of VRE isolates from the total amount of enterococci bacteremias in 2011:

$$\frac{\text{Number of VRE bacteremia (divided by)}}{\text{Total number of enterococci bacteremia}} \times 100\% = \ldots\ \%$$

If **> 10%** consider the resistant form of the species endemic

3. Study population

Population

All mechanically ventilated adult patients, surgical or non surgical, admitted to the participating ICUs, with no planned extubation within 24 hours.
The reasons for excluding non ventilated (and “short stay”) patients are:

- the presumed negligible contribution of these patients to transmission dynamics
- the presumed small effect on outcome of study regimens administered for less than 48 hours

Inclusion and exclusion criteria

Inclusion criteria:

- mechanical ventilation

and

- no planned extubation within 24 hours

Exclusion criteria:

- patients under the age of 18
- patients with known allergy to any of the medications or agents used (i.e. colistin, tobramycin and nystatin, chlorhexidine)
- pregnancy

Sample size calculations and enrolment numbers

Sample size calculations (where relevant) and assumptions regarding outcome, are given per study outcome. The quantification of the effects of decolonisation regimens on overall systemic antibiotic use and the occurrence of ICU acquired MDR bacteraemia are exploratory and therefore not based on a formal power calculation.

Association between intestinal and respiratory colonisation and occurrence of ICU-acquired GNB bacteraemia

Presence or absence of intestinal and respiratory tract carriage will be determined upon centrally stored colonisation cultures in patients receiving SC, SOD, SDD and Oro-CHX. Using an incidence of 4.5/1,000 patient days in the presence of carriage at both sites 3,000

patient days are needed to demonstrate a statistically significant reduction to 1/1,000 patient days in the absence of carriage at both sites. Assuming GNB at both sites during 75% of patient days with SC and absence of carriage at both sites during 25% of patient days with SDD we would need two groups of 500 patients with SC and SDD. The percentages and incidence rates are based on analyses of patient data in the before-mentioned Dutch multi-centre trial (3).

28-Day mortality

The study sample size has been determined on one of the secondary outcomes: day-28 mortality. In a Dutch SDD ICU trial day-28 mortality rates during SC was 27.5% (3). Assuming a low level of cluster-effects, 2016 patients are needed in each study period to demonstrate a 10% reduction in day-28 mortality as compared to SC (alpha=0.05; beta=0.8). We expect to include 2,700 patients per study arm. The margin of 600 patients per study arm is included to allow for adjustment for differences in baseline characteristics in a random-effects logistic regression model if needed, or to include cluster-effects. Of note, assuming day-28 mortality in SC to be 27%, the absolute reduction that can be demonstrated is 2.7%. The statistical analysis will be based on a random effects logistic regression model, incorporating all relevant covariates. Day-28 mortality data will be derived from clinical data obtained as part of routine standard care.

MDR-GNB ICU acquired bacteraemia

For the primary outcome the following assumptions were made. In the Dutch ICU trial incidences of ICU-acquired bacteraemia with GNB were 7.1%, 5.0% and 2.3% during SC, SOD and SDD, respectively. Assuming that 20% of the episodes of ICU-acquired GNB-bacteraemia during SC will be caused by MDR-GNB this would yield an incidence of 1.4%. A 50% reduction to 0.7% can be demonstrated with 1,675 patients in each study arm (alpha=0.05; beta=0.8).

Acquired respiratory tract colonisation during each study phase

Incidences of ICU-acquired respiratory tract colonisation with MDR-GNB will be derived from respiratory tract cultures (endotracheal aspirates or throat swabs) obtained twice weekly (on average 4 per patient). Results from samples obtained for clinical reasons will be derived from the clinical laboratories. In the Dutch ICU trial incidences of ICU-acquired respiratory tract colonisation with Enterobacteriaceae was 70%, 47% and 24% during SC, SOD and SDD, respectively. Assuming that 20% of these episodes during SC will be caused by MDR-GNB this would yield an incidence of 14%. A 30% reduction to 10% can be demonstrated with 521 patients in each study arm (alpha=0.05; beta=0.8). This analysis will be performed

on respiratory tract samples. From all ICUs, swabs from patients with LOS of at least 7 days will be selected to create 4 groups of 550 patients.

Enrolment numbers

The study aims to enrol on average 30 patients per month per participating ICU. Estimated ICU stay is 10 days. These numbers are based on previous ICU trials, including the MOSAR trial (to be published) (3). With 15 participating ICU this would lead to at least 2700 patients per study group, each group with 27.000 consecutive patient days.

4. Interventions

Standard care

Each participating ICU will start with the baseline period of 6 months in which standard care is implemented. Standard care consists of daily chlorhexidine body wash and oropharyngeal care. Standard oropharyngeal care consists of oral washing with sterile water (3-4 times daily) and tooth brush twice daily. During baseline no form of SDD or SOD or chlorhexidine mouth wash should be applied to patients included in the study.
During the baseline period, all ventilated patients should be checked for eligibility to participate in the study. Eligible patients should be included in the study. Inclusion is necessary to form comparable groups of participants during all regimens.

During all regimens, all units will use daily body wash with Chlorhexidine gluconate 2% concentration (e.g. Hibiscrub). The bathing solution is prepared by adding a standardized amount of Chlorhexidine to the water used to bathe patients. The face and neck of the patient will not be cleansed with Chlorhexidine to prevent irritation of the eyes and face. Patients' skin will be moisturized with a lotion that does not interfere with the biocidal activity of Chlorhexidine gluconate.
At the start of the baseline period a hand hygiene protocol will be introduced. This protocol is derived from the WHO hand hygiene programme and consists of education of health care workers and monitoring of hand hygiene compliance. The protocol will be used during all regimens and is part of standard care.

SDD regimen

SDD consists of:

- standard care
- oropharyngeal application of a paste containing colistin, tobramycin and nystatin every 6 hours until extubation
- administration of a 10 ml suspension containing 100 mg colistin, 80 mg tobramycin and nystatin (2×10^6 units) via a nasogastric tube every 6 hours.

Topical antibiotics will be applied 4 times daily until extubation. Application modifications for patients with tracheostomy and jejunostomy will be specified in the investigator brochure.

SOD regimen

SOD consists of standard care and the application of an antimicrobial mouthpaste every 6 hours. The paste contains colistin and tobramycin in a 2% concentration and nystatin 1 x 10^5 units. Topical antibiotics will be applied 4 times daily until extubation. Application modifications for patients with tracheostomy will be specified in the investigator brochure.

Oro-CHX regimen

Oro-CHX consists of standard care and oropharyngeal application of 10 ml chlorhexidine in a 2% concentration every 6 hours. Chlorhexidine will be applied daily until extubation.

Wash out / wash in period

A one month wash out / wash in period will precede each study regimen. During the first two weeks of this period patients following the old regimen will finish this regimen, while newly admitted patients will already follow the new regimen. After the first two weeks, patients following the old regimen switch to the new regimen. Surveillance cultures should be obtained on the regular fixed twice weekly basis (see below). Point prevalence cultures will also be taken once monthly during the wash out / in period.

Cultures

Surveillance cultures

Surveillance cultures will be taken twice weekly, every Monday and Thursday. These cultures will determine whether a study participant is colonised with gram negative bacteria and if this micro organism is resistant to antimicrobial agents. Thus, changes in antibiotic resistance in study participants will be monitored by surveillance cultures.
Samples will be inoculated on ESBL- and MRSA chromagar media and colistin and tobramycin containing media. Growing isolates (per definition resistant) will be further determined (including susceptibility patterns) by the local microbiology laboratories.
Surveillance cultures will start in the baseline period. During wash out / in periods, surveillance cultures should also be obtained. Any MDR-GNB cultured should be stored and sent to the central laboratory for qualitative determination.

Clinical cultures

It is at the discretion of the treating physicians to perform (blood) cultures on clinical ground, i.e. on suspicion of infection. Culture results from participants will be collected. These data will be sent periodically (in digital format) to the UMCU. Data will include type of sample, culture result and antibiotic sensitivity pattern.

Results from clinical cultures include all (but not exclusively):

- blood cultures
- sputum cultures
- wound cultures
- faecal cultures
- urinary cultures
- skin cultures
- catheter / drain tip cultures

Point prevalence cultures

Point prevalence cultures will be taken once a month from all patients in the ICU (study participants and non participants) to determine ecological safety. Samples will be inoculated on MRSA, VRE and ESBL chromagar plates, as well as colistin and tobramycin containing media. Rectal and respiratory tract (either sputum, throat swab of tracheal aspirate) cultures will be obtained. Growing isolates (per definition resistant) will be further determined (including susceptibility patterns) by the local microbiology laboratories. The external DSC will review these results on a monthly basis to follow ecological changes during the trial.

<table>
<tr><td colspan="9">Cultures</td></tr>
<tr><td></td><td>SC
(6 mo)</td><td>Wash out / in</td><td>SDD
(6 mo)</td><td>Wash out / in</td><td>SOD
(6 mo)</td><td>Wash out / in</td><td>Oro-CHX
(6 mo)</td><td></td></tr>
<tr><td>Surveillance</td><td colspan="7">Study participants: Respiratory and rectal samples - twice weekly</td><td rowspan="3">Local lab</td></tr>
<tr><td>Point prevalence</td><td colspan="7">All ICU patients: Respiratory and rectal samples - monthly</td></tr>
<tr><td>Clinical</td><td colspan="7">All ICU patients: At the discretion of treating physician</td></tr>
</table>

5. Methods

The following section elaborates on study methods. Information on interventions and cultures can be found in chapter four.

Study procedures and data collection

Randomisation of regimens

Randomisation will occur at the ICU level. Randomisation of regimens will be computer generated and performed centrally by an independent pharmacist, who will be not involved in patient care.

Inclusion

Inclusion will be performed by the local research nurse, within 24 hours after admittance of a patient to the ICU. Each ventilated patient should be checked for eligibility and meeting in- and exclusion criteria.

Data registration

Preferably, data will be directly extracted from a digital database and sent to the UMCU in a universal digital format. Otherwise data will be extracted manually by the Research nurse (RN) and entered in the web based database “research online”. Data will be anonymised by recoding the patient ID. The following data will be recorded:

ICU related data:

- The amount of beds, with and without options for mechanical ventilation
- Teaching or non/teaching hospital
- Name of physician director of the ICU and primary specialty (changes herein will be recorded)
- Name of nurse director (manager) of the ICU (changes herein will be recorded)
- Antibiotic use per regiment per type of antibiotic

Data from all patients admitted to the ICU

- Sex
- Age
- Date of admission to and discharge from the ICU
- Mechanical ventilation (invasive or not) and duration
- Disposition of the patient at ICU discharge (alive or deceased)

- APACHE II or SAPS II scores

Additional data for all study participants:

- Date of hospital admittance
- Place from which patient was admitted to ICU:
    - This hospital [#]
    - Another hospital [#]
    - Home or Emergency Room
- [#] If patient was already admitted to a hospital: the patient came from:
    - Operating room
    - Hospital ward:
        - Other ICU
        - Acute care
        - Rehabilitation or long-term care
- Principle reason for ICU admission
    - Medical
    - Trauma
        - Patient needed surgery
        - No surgery needed
    - Surgical
        - Scheduled
        - Unscheduled / emergency / complications after surgery
- Acute illness:
    - Yes
    - No (routine observation after surgery and routine monitoring)
- Reason for ICU admission, site of organ failure (if applicable) (more than one option)
    - Respiratory
    - Cardiovascular
    - Neurologic
    - Renal
    - Hepatic
    - Hematologic
    - Metabolic
- Antibiotic on admission:
    - Yes
    - No

- Previous or pre-existing condition
    - Malignant solid tumor
    - Metastatic cancer
    - Hematologic cancer
    - Immunodepression or AIDS
    - Alcohol or drug abuse
    - Cardiovascular disease
    - Chronic heart failure
    - Pulmonary disease / chronic respiratory failure
    - Diabetes Mellitus
    - Chronic renal failure
- Date of start and cessation of mechanical ventilation
- Status at day 28 (alive of deceased)
- Disposition of the patient at ICU discharge (alive or deceased)
- Date of death (if within 29 days of ICU admission)
- Isolation precautions (barrier precautions: need for mask, gown, gloves)
    - Yes
        - Due to surveillance cultures during hospital stay
        - Due to known MDR micro organism on admission
    - No
- Single room >50% of ICU stay?

Antibiotic use:
To monitor the antibiotic use on an ICU level, antibiotic use per site per regimen will be recorded. Antibiotic use will be expressed as defined daily dose (DDD). The DDD per antibiotic is defined by the world health organisation. It is the assumed average maintenance dose per day for a drug used (6). By using the DDD system antibiotic use per site can be compared.

Culture results

Culture results that should be recorded include results from all:
Clinical cultures:

- date of obtaining the culture sample
- qualitative determination of isolated micro organism
- susceptibility pattern

Surveillance cultures (twice weekly) and point prevalence cultures (monthly):

- date of obtaining the culture sample
- determination on the species levels of the micro organism cultured on chromogenic (MRSA, ESBL) plates, colistin and tobramycin plates
- susceptibility pattern of these isolated micro organisms

MDR-GNB isolates from point prevalence, clinical and surveillance cultures should be sent to the UMCU for further determination. Detailed information will be available in the microbiology protocol.

Financial compensation

R-GNOSIS will compensate the department of the principal site investigator for the value of 0.5 FTE of a Research Nurse position. The compensation will be divided into roughly 4 terms and compensation for the next term will only proceed if data has been delivered for the previous term. Definite failure to provide qualitative and quantitative data as described in this protocol will result in discontinuation of the ICU as subcontractor within the trial and the compensation received for data not delivered will be reclaimed by the WP6 coordinating centre: the Julius Center / UMC Utrecht.
Patients will not be compensated for their participation in the trial.

Site withdrawal or discontinuation of trial

The ICU physician or ICU nursing director or the site investigator may withdraw his or her site from the study for any reason at any time. In case of an unexpected change in MDR-GNB or other resistant bacterial epidemiology in a certain ICU, leading to a situation in which adherence to protocol can no longer be recommended; the site will temporarily be withdrawn. In such case, the external DSC will be notified. If withdrawal exceeds >3 months, participation of the site will be terminated.

The *RGNOSIS WP6 trial investigators* may discontinue the participation of a study site for the following reasons:

- The site does not follow the study protocol with respect to obtaining the surveillance or point prevalence cultures
- The site does not implement the assigned regimen satisfactorily
- The site does not collect the required patient information or

- The site fails to report culture results or fails to send cultures within reasonable time to the study coordinator or central laboratory
- The ICU enrols subjects in any other study of an intervention or investigational agent administered for the purpose of eradicating or preventing colonisation with MRSA, VRE or ESBL or MDR-GNB; or for the purpose of reducing the likelihood of transmission of these bacteria

Withdrawal of individual subjects

Subjects can withdraw from the study at any time for any reason if they wish to do so without any consequences. See the chapter *ethical considerations* for further information.

6. Safety reporting

The primary safety measure to detect an increase of anti-microbial drug resistant bacteria is performing point prevalence cultures. The purpose of these cultures is to ensure ecologic safety. Analysis will detect any Carbapenemase Producing Enterobacteriaceae (CPE), ESBL, CRAB, VRE or MRSA isolates. Also, susceptibility of bacteria to colistin and tobramycin, both used in SDD and SOD, will be tested.

This way, any increase in the number of resistant isolates will be detected early. An independent data safety committee consisting of three external experts will monitor results of point prevalence cultures and judge whether resistance levels have increased to such extent that the trial should be interrupted. Stopping rules will be determined before start of the study.

An adverse drug reaction for a marketed drug is defined as: “a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function”, according to good clinical practice (GCP) (7). Because of the large experience with and broad use of medication used in this study, no new or unknown adverse drug reactions are to be expected.

Study-related procedures applied to human subjects are considered to be no more than minimal risk procedures. SDD, SOD and Oropharyngeal application of chlorhexidine require no invasive procedures, are considered safe and have been used extensively. SDD has been widely applied on ICUs in The Netherlands as well as in other countries.
Cultures taken during the study are obtained from either endotracheal aspirates (regularly performed in ventilated patients), tracheal cultures or sputum cultures (non invasive) and rectal cultures. All these are minimal risk procedures frequently performed in ICU patients causing minimum discomfort.

7. Statistical analysis

Statistical analysis plan

Analysis will determine the effect of each intervention on colonisation rates, the occurrence of bacteraemia, mortality and the use of antibiotics. Statistical analysis of the primary objective and secondary objectives regarding mortality, antibiotic use and rate of colonisation and bacteraemia will include the use of random-effect logistic regression models to account for ICU-level clustering. Measured confounding factors will be fitted as covariates. Outcomes will be presented as odds ratios.

All available data on patient colonisation with MDR-GNB (participant data, surveillance and clinical cultures) will be used to determine, as carefully as possible, the colonisation status of each patient on every study day. Together with work package 8 (mathematical modelling, WP8), nosocomial transmission capacities (R_A values) for different species of MDR-GNB during study regimens will be quantified. As a secondary aim species-specific R_A values will be compared between wards. Available data will be used to quantify incidences of cross-transmission in each study periods, using sophisticated modelling approaches to be developed in WP8.

Missing data

Study investigators and the R-GNOSIS staff will make every attempt to collect complete data from all subjects enrolled in the study. There will be regular contact between the study coordinating centre and study sites to track and retrieve missing data. Should culture results be inadvertently lost, those data will be treated as missing at random. All inferential analyses will be based on available data. Details of the procedures for addressing missing cultures will be provided in the Statistical Analysis Plan.

Changes to the Statistical Analysis Plan

Details of the analysis methods and changes in the analyses from those described in the protocol will be documented in the statistical analysis plan prior to database lock. Those changes and the reasons for the changes will be described in detail in the final study report.

8. Ethical considerations

Regulation statement

This study is conducted in agreement with the declaration of Helsinki (Seoul, October 2008) and with the guidelines of GCP issued by the European Union (7-9).

Recruitment and consent

Study-related procedures involving subjects or subjects' medical records shall not be initiated prior to initial IRB review and approval.

The ICU is a participant of the study upon documented agreement from the ICU director(s) or equivalent. The ICU director(s) will be provided with a copy of the protocol and with enough time to assess the impact of the study on the established procedures of the ICU.
Compliance with GCP guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements of the Good Clinical Practice guidelines of the European Union (7). By signing this protocol, the investigator agrees to adhere to these requirements.

The study protocol will be reviewed and approved by the local IRB or ethics committee. Changes to the protocol will be initiated by the primary investigator and approved by the IRB. The ethical principles and norms of research involving human beings apply to cluster randomised trials, although there is much less published information on the application of these principles and norms to this type of study. A recent publication by the United Kingdom Medical Research Council, "Cluster Randomised Trials: Methodological and Ethical Considerations," provides guidance in this regard (10). The approach described below is consistent with the recommendations of this guideline.

The physician and nurse director (manager) of the ICU will be required to provide written approval for the participation of the ICU in the trial by signing the protocol signature page. This requirement is consistent with the fact that the ICU is the site of inclusion and with the leadership role these individuals play in determining policies and procedures that apply to patients and healthcare workers in the ICU. These leaders will consider the risks and benefits of the regimens and will be required to provide their approval and support for:

- participation of the ICU in the study

- obtaining cultures from ICU patients according to the prescribed schedule in the protocol;
- the implementation of the study regimens, including the standard care regimen, throughout the entire study period
- the use of contact precautions in the care of patients who are known to be colonised with MRSA, VRE or MDR-GNB

Considering the minimal risks of the intervention for the individual patient and the necessity of evaluating decolonisation strategies in a clustered design a waiver of informed consent from patients in the participating ICUs is both important and appropriate for the proper conduct and analysis of this trial. The justification for a waiver of informed consent from patients in the participating ICUs meets the criteria outlined below:

1. The study will involve no more than minimal risk of harm to patients.
2. A waiver will not adversely affect the rights and welfare of patients.
3. The trial cannot practicably be carried out without a waiver.
4. Whenever appropriate, subjects can be provided with additional pertinent information.

For similar study purposes (unit-wide infection prevention interventions with minimal risks for individual patients) waivers have been provided in a Dutch multi-centre cluster-randomised study on SDD and SOD (3), and two ongoing multi-centre studies as part of the European funded projects MOSAR (rapid diagnostic testing in ICUs) and SATURN (mixing versus cycling of antibiotics in ICUs), both lead by the coordinator of R-GNOSIS (M. Bonten).

All patients admitted to a participating ICU (or when not possible their spouse or relatives) will be informed through a written document (written in the first language of the country were the study ICU is located), posted in a visible place in the ICU. The document will state the purpose and possible risks of the study. More specifically, this document will state that:

- A study is taking place on the ICU and all eligible patients are participants in this study because the interventions are applied to all patients, as were it standard care.
- Anonymised patient related data will be collected for study purposes
- Patients can at all times object to data collection or the intervention for any reason. This has no consequence for further patient care.

If a patient, spouse, family member or representative (in this order of availability) objects to data collection an opt out form can be signed by the patient or representative and data collection for this patient will not take place. If a patient objects to or refuses study medication used in the SDD, SOD or chlorhexidine regimen this will not be administered.

Benefits and risks assessment

The study uses a clustered randomised design for the reason of ecological changes on the ICU level brought by the study regimens. Applying SDD, SOD or Oro-CHX influences colonisation not only in the patient to whom the regimen is applied, but also affects ecology of other patients in the unit.

Clinical cultures, point prevalence cultures and surveillance cultures will be obtained in each regimen to determine secondary outcome objectives. Surveillance cultures are already standard of care for some patient groups in most ICUs, as are clinical cultures for all patients when infection is suspected. Monthly point prevalence cultures will be performed in all ICU patients to monitor ecological safety on a study level. These rectal swabs and respiratory samples (either endotracheal aspirates or sputum cultures) are easy and safe to perform, cause minimal discomfort to the patient and have been frequently used in previous studies as well as in normal clinical practice.

During the entire trial, all patients admitted to the participating ICUs will be washed daily using a solution containing chlorhexidine gluconate 2%. This is a procedure with minimal burden, which is already widely used throughout Europe and the United States and is considered safe. Recent studies have demonstrated that body washings with chlorhexidine effectively reduce the bacterial burden of Gram-positive skin colonisers, including MRSA and VRE, and infections caused by these pathogens (11-13). By implementing chlorhexidine body wash as standard care, the effect of the SDD, SOD and Oro-CHX on colonisation and infection with MDR-GNB can be investigated, while at the same time efforts are made to reduce the risk of emergence and spread of resistant skin colonisers.

Benefits for the patients individually and as a group are to be expected primarily in terms of prevention of GNB acquisition, nosocomial infection, cross transmission, and in improvement in patient outcome as previous studies have shown.

The risk of increased antibiotic resistance as a consequence of SDD and SOD is expected to be small, since the prevalence of resistant bacteria in two trials using SDD and SOD decreased (2-3). SDD did not cause an increase in resistance rates in long term follow up studies (14-16).

Compensation for injury

ICU patients will not be reimbursed for participating in this study. The hospitals will be subcontractors of the R-GNOSIS project. The principal investigators (UMC Utrecht and Université Paris-Est Créteil) cannot be held responsible for any damage to research study subjects through injury or death caused by the study. The participating centres will provide appropriate insurance which is in accordance with the legal requirements of each country.

9. ADMINISTRATIVE ASPECTS AND PUBLICATION

Confidentiality

Information linking the patient's medical data to study materials, including the CRF, will be maintained in a secure location at the participating site. This information will not be transmitted to the R-GNOSIS management or the principal investigator or coordinating investigator. ICU and (anonymised) individual subject data and records will be held in strictest confidence by the investigator and healthcare staff and by the R-GNOSIS project and trial management representatives as permitted by law.
Information contained in this protocol and data and results from the trial may not be disclosed without the written permission of the principal investigator and the management of the R-GNOSIS project. If results from this study are published individual subject's identity will remain confidential.

Handling and storage of data and documents

All worksheets will be kept at the sites. A sample of these forms will be copied and sent to R-GNOSIS staff regularly for quality control reasons. All anonymised electronic data will be stored in the web based electronic database used for the trial (ResearchOnline), to be developed by work package 9 (WP9) of the R-GNOSIS consortium.

Amendments

Amendments are changes made to the research after a favourable opinion by the accredited IRB has been given. All substantial amendments will be notified to the IRB(s) that gave a favourable opinion. A 'substantial amendment' is defined as an amendment to the terms of the IRB application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

Non-substantial amendments will not be notified to the accredited IRB(s), but will be recorded and filed by the principal investigator and coordinating investigator.

Annual progress report

The site investigator will submit a summary of the progress of the trial to the accredited IRB(s) once a year. Information will be provided on the date of inclusion of the first cluster, numbers of subjects included and numbers of subjects that have completed the trial, other problems, and amendments.

Public disclosure and publication policy

Manuscript(s) and abstract(s) resulting from the data collected during this trial will be prepared through the study investigators (principal investigator and coordinating investigator) and the R-GNOSIS management. Site investigators will not publish or present interim or definite results, including but not restricted to oral presentations, without written consent of the principal investigator. They will be allowed to participate in publications regarding this trial.

Site investigators will provide R-GNOSIS management with publication or presentation materials in advance of publication/ presentation to allow for review and comment as a means of ensuring confidentiality, accuracy, and objectivity. According to the rules of the International Committee of Medical Journal Editors, this trial will be registered in a public trial registry.

10 References

(1) Silvestri L, van Saene HK, Milanese M, Gregori D, Gullo A. Selective decontamination of the digestive tract reduces bacterial bloodstream infection and mortality in critically ill patients. Systematic review of randomised, controlled trials. J Hosp Infect 2007 Mar;65(3):187-203.

(2) de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. Lancet 2003 Sep 27;362(9389):1011-6.

(3) de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med 2009 Jan 1;360(1):20-31.

(4) Labeau SO, Van d, V, Brusselaers N, Vogelaers D, Blot SI. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. Lancet Infect Dis 2011 Nov;11(11):845-54.

(5) Segers P, Speekenbrink RG, Ubbink DT, van Ogtrop ML, de Mol BA. Prevention of nosocomial infection in cardiac surgery by decontamination of the nasopharynx and oropharynx with chlorhexidine gluconate: a randomised controlled trial. JAMA 2006 Nov 22;296(20):2460-6.

(6) WHO Collaborating Centre for Drug Statistics and Methodology. DDD: Definition and general considerations. WHOCC website 2011 [cited 2011 Nov 15];Available from: URL: http://www.whocc.no/ddd/definition_and_general_considera/

(7) EMEA. Note for Guidance on Good Clinical Practice. EMEA website 2011 [cited 2011 Nov 7];Available from: URL: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002874.pdf

(8) World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. WMA website 2008 [cited 2011 Nov 7];Available from: URL: http://www.wma.net/en/30publications/10policies/b3/index.html

(9) Ministerie van Volksgezondheid WeSN. The Medical Research Involving Human Subjects Act (WMO). Healthlaw website 2000 [cited 2011 Nov 7];Available from: URL: http://www.healthlaw.nl/humsub.pdf

(10) Medical Research Council. Cluster randomised trials: methodological and ethical considerations. MRS Clinical Trials Series 2002 [cited 2011 Nov 7];Available from: URL: http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002406

(11) Vernon MO, Hayden MK, Trick WE, Hayes RA, Blom DW, Weinstein RA. Chlorhexidine gluconate to cleanse patients in a medical intensive care unit: the effectiveness of source control to reduce the bioburden of vancomycin-resistant enterococci. Arch Intern Med 2006 Feb 13;166(3):306-12.

(12) Bleasdale SC, Trick WE, Gonzalez IM, Lyles RD, Hayden MK, Weinstein RA. Effectiveness of chlorhexidine bathing to reduce catheter-associated bloodstream infections in medical intensive care unit patients. Arch Intern Med 2007 Oct 22;167(19):2073-9.

(13) Climo MW, Sepkowitz KA, Zuccotti G, Fraser VJ, Warren DK, Perl TM, et al. The effect of daily bathing with chlorhexidine on the acquisition of methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus, and healthcare-associated bloodstream infections: results of a quasi-experimental multicenter trial. Crit Care Med 2009 Jun;37(6):1858-65.

(14) Heininger A, Meyer E, Schwab F, Marschal M, Unertl K, Krueger WA. Effects of long-term routine use of selective digestive decontamination on antimicrobial resistance. Intensive Care Med 2006 Oct;32(10):1569-76.

(15) Leone M, Albanese J, Antonini F, Nguyen-Michel A, Martin C. Long-term (6-year) effect of selective digestive decontamination on antimicrobial resistance in intensive care, multiple-trauma patients. Crit Care Med 2003 Aug;31(8):2090-5.

(16) Ochoa-Ardila ME, Garcia-Canas A, Gomez-Mediavilla K, Gonzalez-Torralba A, Alia I, Garcia-Hierro P, et al. Long-term use of selective decontamination of the digestive tract does not increase antibiotic resistance: a 5-year prospective cohort study. Intensive Care Med 2011 Sep;37(9):1458-65.

Appendix: Definition (Multi) Drug Resistant Gram negative Bacteria

Highly Resistant Micro Organisms (HRMO) comprises (multidrug) resistant gram negative bacteria as well as other important resistant bacteria.

HRMO are defined as micro organisms which:

1) are known to cause disease
2) have acquired an antimicrobial resistance pattern that hampers (empirical) therapy
3) have the potential to spread if – in addition to standard precautions – no transmission-based precautions are taken.*

Three main groups of HRMO are distinguished:

- highly resistant **Enterobacteriaceae** (Table 1), including (resistant) species of
  - ESBLs (all ESBL producing gram negative micro organisms)
  - All Carbapenemases
  - E. Coli
  - Klebsiella pneumonia
- highly resistant **gram-negative nonfermenters** (Table 2), including resistant species:
  - Pseudomonas aeruginosa
  - Acinetobacter spp.
  - Stenotrophomonas maltophilia
- highly resistant **gram-positive bacteria** (Table 3), including
  - MRSA
  - Glycopeptide resistant Enterococci (GRE)

Table 1. Definition of Highly Resistant Enterobacteriaceae*

	ESBL	**Quinolones**	**Amino-glycosides**	**Carbapenems**	**Co-trimoxazole**
Escherichia coli	A	B	B	A	*na*
Klebsiella *spp.*	A	B	B	A	*na*
Other	A	B	B	A	B

A: resistance against an antibacterial agent from **one of the indicated groups** of this category is sufficient to define the microorganism as highly resistant;
B: resistance against antibacterial agents from at least **two of the indicated groups** of this category is required to define the microorganism as highly resistant;
na: not applicable

Table 2. Definition of highly resistant gram-negative nonfermenters*

	Ceftazidime	**Quinolone**	**Amino-glycosides**	**Carbapenems**	**Piperacillin**	**Co-trimoxazole**
Acinetobacter *spp.*	B	B	B	A	*na*	*na*
Pseudomonas *spp.* and other	C	C	C	C	C	*na*
Stenotrophomonas maltophilia	*na*	*na*	*na*	*na*	*na*	A

A: resistance against an antibacterial agent from **one of the indicated groups** of this category is sufficient to define the microorganism as highly resistant;
B: resistance against antibacterial agents from at least **two of the indicated groups** of this category is required to define the microorganism as highly resistant;
C: resistance against antibacterial agents from at least **three of the indicated groups** of this category is required to define the microorganism as highly resistant;
na: not applicable

Table 3. Definition of highly resistant Enterococcus faecium*

	Penicillins	**Glycopeptides**
Enterococcus spp.		A

A: resistance against an antibacterial agent from **one of the indicated groups** of this category is sufficient to define the microorganism as highly resistant

Reference:

* : Kluytmans-Vandenbergh MF, Kluytmans JA, Voss A. Dutch guideline for preventing nosocomial transmission of highly resistant microorganisms (HRMO). Infection 2005 Oct;33(5-6):309-13 (partially adjusted)

R-GNOSIS

Ecological Effects of Decolonisation Strategies in Intensive Care

Study protocol

(version 8, February 2015)

Part of

R-GNOSIS: Resistance in Gram-Negative Organisms: Studying Intervention Strategies

R-GNOSIS: Ecological effects of Decolonisation strategies in ICU EudraCT: 2012-002604-41	
FP7-HEALTH-single-stage grant agreement no. 282512	
Version	Version 8 , February 2015
Sponsor	University Medical Centre Utrecht, code: WP6-282512
Primary investigators (coordinators)	**Prof C. Brun-Buisson** M.D., Ph.D. Director medical Intensive Care unit; head of Infection Control Hospital Henri Mondor, Créteil Assistance Publique – Hopitaux de Paris Université Paris-Est Créteil E-mail : christian.brun-buisson@hmn.aphp.fr **Prof M.J.M. Bonten** M.D. Ph.D. Professor of Molecular Epidemiology of Infectious Diseases University Medical Centre Utrecht Heidelberglaan 100 E-mail: mbonten@umcutrecht.nl
Coordinating investigator (coordinators) Project Manager Institution	**B.H.J. Wittekamp,** MD, PhD Candidate **N.L. Plantinga,** MD, PhD Candidate Phone: E-mail: b.h.j.wittekamp@umcutrecht.nl n.l.plantinga@umcutrecht.nl **M.C. Hopman** Phone Email: m.c.hopman@umcutrecht.nl ***Julius Center for Health Sciences and Primary Care*** HP Stratenum 6.131 PO Box 85500 3508 GA Utrecht, The Netherlands
Principle site investigator (investigator)	*Name* *Address*

Amendments Study Protocol V8

Version 8 of the Study Protocol replaces the seventh version (Feb 2014) and differs from the previous versions on the following points:

- Due to an unexpected rate of adverse events in patients receiving chlorhexidine 2% mouthwash, the intervention has been adapted to a chlorhexidine gel with a lower concentration (1%). Accordingly, the following items – all part of the study protocol - have been adapted:
    - The patient information leaflet
    - The ECRF
    - The SUSAR report form
- The randomization procedure has been adapted: there is now random allocation concealment of all three infection prevention strategies (the order in which CHX, SOD and SDD are implemented is randomized per ICU), where SOD was previously always followed by SDD. There are now six different orders possible for the randomisation scheme.
- Appendix (II) - which provides an advice concerning the refrainment from SDD/SOD in patients that carry a certain type of resistant micro-organism - has been adapted slightly.
    - The advice applies only to patients that have such a micro-organism in a clinical culture
    - The type of micro-organisms for which this advice applies now also includes MO's with co-resistance to a carbapenem and colistin.
- A secondary endpoint has been adapted: hospital mortality has been replaced by ICU-mortality.
- Ethical concerns:
    - Implementation of any of the three infection prevention strategies shall not be initiated prior to IRB review and approval, unless the intervention has been part of previous regular care in that hospital. The baseline period may now be started before ethical approval for implementation of one of the interventions, as it is merely observational.
    - ICUs should be able to obtain written approval for the study from their institutional review board (IRB) with a waiver for patient informed consent. The option to "obtain consent by a guardian for the intensive care population, where applicable, e.g. in the United Kingdom" has been omitted

- Sample size estimation: due to a lower inclusion rate than anticipated, the aim to include 12 hospitals has been adapted to 13.

Protocol signature sheet

Protocol version 8, February 2015

Name	Date	Signature
Study management		
Principle investigator and supervising coordinator: Prof. M.J.M. Bonten, MD, PhD University Medical Centre Utrecht, Netherlands		
Principle site investigator		

Table of contents

List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
BAL	Broncho Alveolar Lavage
CHX	Chlorhexidine
CHX-BW	Chlorhexidine body-wash
CHX-Oro	Oropharyngeal chlorhexidine
CPE	Carbapenemase producing Enterobacteriaceae
CRAB	Carbapenem-resistant *Acinetobacter baumannii*
DDD	Defined Daily Dose
e-CRF	Electronic Case Report Form
ESBL	Extended Spectrum Beta Lactamase
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GNB	Gram Negative Bacteria
GRE	Glycopeptide Resistant *Enterococci*
HRMO	Highly Resistant Micro Organism
ICU	Intensive Care Unit
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
KPC	Klebsiella Pneumoniae Carbapenemases
MDR	Multi Drug Resistant
MDR-GNB	Multi Drug Resistant Gram Negative Bacteria
MDR-PA	Multi Drug Resistant *Pseudomonas aeruginosa*
MRSA	Methicillin-Resistant *Staphylococcus aureus*
R-GNOSIS	Resistance in Gram-Negative Organisms: Studying Intervention Strategies
SC	Standard Care
SCom	Safety Committee
SDD	Selective Digestive Tract Decontamination
SOD	Selective Oropharyngeal Decontamination
SOP	Standard Operating Procedure
UMC(U)	University Medical Centre (Utrecht)
VAP	Ventilator-Associated Pneumonia
VRE	Vancomycin Resistant *Enterococcus*
WHO	World Health Organisation
WP	Work package

Summary

Rationale – Modulation of colonization of the digestive tract has been associated with reduced mortality and infectious complications in ICU patients. Regimens evaluated extensively include Selective Digestive tract Decontamination (SDD), Selective Oropharyngeal Decontamination (SOD) and oral decontamination with Chlorhexidine (CHX-ORO). However, most trials with SDD and SOD were performed in settings with low levels of antibiotic-resistant bacteria, and CHX and antibiotics (SOD/SDD) have never been compared head to head.

Study objective – We hypothesise that CHX, SOD and SDD will reduce the prevalence of multi drug resistant gram negative bacteria (MDR-GNB). We, therefore, aim to determine the ecological effects and ecological safety of the three decolonisation strategies.

Study design – Prospective cluster randomised controlled trial in 12 European ICUs.

Study participants – Adult ICU patients receiving invasive mechanical ventilation and who are not expected to be extubated within 24 hours.

Intervention – Three decolonisation interventions will be compared in this study:

- Oropharyngeal decolonisation with CHX (CHX-Oro) consists of 4 times daily application of a 1% chlorhexidine oral gel until extubation.
- SOD: consists of oral application of tobramycin, colistin and nystatin mouth paste 4 times daily until extubation.
- SDD: consists of SOD *plus* administration of the same antimicrobial agents through the nasogastric tube 4 times daily until extubation.

These interventions will be applied for the duration of mechanical ventilation.

The study is divided in four periods, each lasting 6 months. The ICU is a cluster in which one strategy is applied to all eligible patients admitted in that period. The first 6 months consist of the baseline period (standard care only): universal daily chlorhexidine body washing (CHX-BW) and a hand hygiene improvement protocol will be implemented. Standard care will be continued throughout the entire study. If CHX mouthwashes are already part of standard care these will not be abandoned during the baseline period. After the six months baseline period, the first decontamination intervention will be implemented. The order of the three decontamination interventions per ICU is determined by randomisation. To prevent carry over effects, interventions are separated by a one month washout period.

Main outcomes – The primary endpoint is to determine the ecological effects of 3 decolonisation regimens (SDD, SOD and Oro-CHX) in reducing (MDR-GNB) ICU-acquired bacteraemia when compared to standard care (SC). Secondary endpoints include rates of ICU acquired rectal and respiratory tract colonization with MDR-GNB, 28th day mortality, ICU-mortality and systemic antibiotic use during each regimen. Mathematical modelling will be used to calculate transmission capacities and cross transmission of MDR-GNB.

Risks and benefits: All three decontamination interventions evaluated in this study are standards of care in different hospitals and are widely used in ICUs across Europe. The risk to participants is no more than the risk in patients receiving standard care.

1. Introduction, rationale and objectives

Introduction

The “R-GNOSIS: Ecological Effects of Decolonisation Strategies in Intensive Care” study is part of the R-GNOSIS project.

The R-GNOSIS (**R**esistance in **G**ram-**N**egative **O**rganisms: **S**tudying **I**ntervention **S**trategies) project combines 5 international clinical intervention projects, all supported by highly innovative microbiology and mathematical modelling, to determine - in the most relevant patient populations - the efficacy and effectiveness of interventions to reduce acquisition, carriage, infection and spread of Multi-Drug Resistant Gram-negative Bacteria (MDR-GNB). The studies and analyses proposed in R-GNOSIS will generate a step-change in identifying evidence-based preventive measures and clinical guidance for primary care and hospital-based physicians, as well as health-care authorities, to combat the spread and impact of the unprecedented rise of infections caused by MDR-GNB in Europe.

Unless explicitly stated otherwise throughout this protocol the abbreviation MDR-GNB will include:

- extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*
- carbapenem-resistant Enterobacteriaceae, such as *Klebsiella pneumoniae* carbapenemases (KPC)
- non-fermenting MDR-GNB such as carbapenem-resistant *Acinetobacter baumannii* (CRAB) and Multi drug resistant *Pseudomonas aeruginosa* (MDR-PA).

Within health-care settings, MDR-GNB are most prevalent in specialised wards, such as Intensive Care Units (ICUs). The latter are characterized by a high antibiotic selective pressure, by the presence of patients highly susceptible to acquire colonisation and infection, and by frequent contacts between health care workers and patients; factors that facilitate cross-transmission of MDR-GNB.

The “R-GNOSIS: Ecologic Effects of Decolonisation Strategies in Intensive Care” study will investigate the safety and efficacy of 3 decontamination regimens on colonisation with resistant bacteria and cross-transmission rates in ICUs. Data concerning clinical outcome and infection rates that are regularly gathered will also be evaluated. The effectiveness of 3 decontamination regimens will be compared, while simultaneously performing standard care

including the use of state-of-the-art evidence based infection prevention measures in all patients.

The scientific goals (5 hypotheses) and the interaction with microbiology and mathematical modelling will progress well beyond the current state-of-the-art in intensive care medicine and hospital infection control. The 3 regimens (selective digestive tract decontamination (SDD) with antibiotics, selective oropharyngeal decontamination (SOD) with antibiotics and SOD with chlorhexidine (CHX-Oro) have been previously tested, but mostly in studies that were underpowered. Decolonisation with antibiotics or antiseptics have never been evaluated in a head-to-head comparison, despite their routine use in ICUs across Europe.

Rationale

Despite a large number of controlled trials and meta-analyses supporting its use, SDD has never gained full acceptance in European ICUs for three major reasons. First, the antibiotics used to achieve SDD are often not totally selective in suppressing *Enterobacteriaceae* and thus also influence the rest of the microbiota and associated colonisation resistance. Second, for a long period of time the beneficial effects of SDD on patient outcome were only demonstrated in meta-analyses (1) and not in individual studies. Third, the antibiotics used in SDD may create some degree of selective pressure for bacterial resistance. This could be particularly problematic if SDD is used over a prolonged time, especially in areas where the prevalence of antimicrobial resistance is already high.

In contrast, in the two most recent and largest SDD trials performed in ICUs with low levels of antibiotic resistance (i.e. in Dutch ICUs), decolonisation strategies were associated with statistically significant improvements in patient outcome. In a single-centre study of 934 patients in two intensive care units, those treated in a unit where all received SDD had a 35% lower risk of dying in ICU when compared to those treated in the unit without SDD usage (2). In a multi-centre cluster-randomised trial with cross-over of interventions comparing SDD to SOD and to standard care (no SDD/no SOD) day-28 mortality rates were 13% and 11% lower (absolute reductions being 3.5% and 2.9%) for patients receiving SDD and SOD, respectively, when compared to patients receiving standard care (3). Moreover, SDD and SOD were associated with a 10% reduction in total systemic antibiotic use, when compared to patients receiving standard care (3). During SDD and SOD study periods, prevalence rates for resistant gram negative bacteria were lower than during standard care. Based on these newest insights, SDD and SOD appear safe and beneficial to patients.

The oral application of chlorhexidine seems a simple and effective measure to reduce the incidence of Ventilator-Associated Pneumonia (VAP) in ICU patients (4) and of nosocomial infections after cardiac surgery (5). Although oral care with chlorhexidine in ventilated ICU patients is currently recommended by guidelines, the most effective concentration and frequency of administration have not yet been determined. A recent meta-analysis found that chlorhexidine 2% is more effective than lower concentrations in preventing VAP (4), although the 0.12%, 0.20% or 1% concentrations are commercially available and often used in ICUs. Because of unexpected mucosal sides effects recorded in early experience with a 2% CHX solution, we will use a commercially available 1% CHX oral gel in this study
The effectiveness of oral chlorhexidine compared to decolonisation with antibiotics has never been tested in a single study. Depending on their efficacy in reducing MDR-GNB, antiseptics could prove to be a cheaper decontamination strategy than decontamination with antibiotics.

Successful suppression of MDR-GNB carriage could be a very important infection control measure, as it may not only protect the individual patient by reducing the risk of infection, but also the population at large by reducing transmission of MDR-GNB. Even transient suppression of ESBL carriage might reduce the likelihood of transmission and thus still be beneficial from an ecologic perspective (6). However, no validated strategy of MDR-GNB decontamination has currently been identified for widespread use.

Considering the paucity of alternatives, such as new antibiotics, for the ever-increasing problem of antibiotic resistance, it is appropriate and warranted to carefully determine the ecological safety and efficacy of using decontamination strategies for elimination of MDR-GNB, including ESBL, in ICU populations across Europe. Identifying the most effective decolonisation strategy would help to improve the quality of care for critically ill patients by reducing the spread of MDR-GNB and associated morbidity.

Objectives

The overall objective of the study is to determine the ecological effect of 3 decontamination regimens, especially on MDR-GNB rates.

Primary objective

- To determine the ecological effects of decolonisation regimens (SDD, SOD and CHX-Oro) in reducing (MDR-GNB) ICU-acquired bacteraemia when compared to standard care.

Secondary objectives

- To quantify cross-transmission rates with MDR-GNB during 3 decolonisation regimens and during standard care.
- To determine the effectiveness of 3 decolonisation regimens (SDD, SOD and CHX-Oro) in reducing acquired respiratory tract colonisation with MDR-GNB when compared to standard care.
- To quantify the effects of 3 decolonisation regimens (SDD, SOD and CHX-Oro) in ICU patients on overall systemic antibiotic use when compared to standard care.
- To quantify on ICU level the associations between intestinal and respiratory tract colonisation with GNB and the occurrence of ICU-acquired GNB bacteraemia.
- To quantify species-specific nosocomial transmission capacities (reproductive number per hospital admission, R_A) of MDR-GNB during 3 decolonisation regimens and during standard care.
- To determine the effectiveness of 3 decolonisation regimens (SDD, SOD and CHX-Oro) in reducing day-28 and in-ICU mortality when compared to standard care.
    - Optional for sites is the collection of hospital and 6 months mortality
- To determine ICU-acquired bacteraemia rates caused by any multi-drug resistant micro-organism, including MRSA, VRE, MDR-GNB, Acinetobacter, *S. maltophilia*, and ceftazidime- and/or carbapenem resistant *P. aeruginosa,* during each phase of the study.

R-GNOSIS partners involved

As part of the R-GNOSIS project, MDR-GNB recovered from point prevalence and surveillance cultures will be sent to the central laboratory for microbiological analyses and to work package (WP) 7 for further analysis. To determine species-specific nosocomial transmission capacities of MDR-GNB and cross-transmission rates during each regimen, data on MDR-GNB carrier status will be sent to WP8 for mathematical modelling. WP9 partners, or datamanagement, will provide an electronic database based on electronic case report forms (ECRF's) developed specifically for this study.

2. Study design

The R-GNOSIS: Ecological Effects of Decolonisation Strategies in Intensive Care study assesses three decolonisation interventions against standard care to evaluate unit wide ecological effects and compare effectiveness.

Previous studies have demonstrated that decontamination interventions were beneficial to individual patients but also influence ICU ecology, affecting patients who do not receive the intervention. Decolonisation with antibiotics have been shown to reduce the prevalence of resistant bacteria during treatment (3). Reducing the presence of these bacteria in some patients (that are decolonized), reduces cross transmission and is therefore beneficial to all patients in the unit. The decolonisation strategies therefore represent an intensive care unit population rather than an individual patient intervention. In this respect the study represents a cluster-cluster randomised clinical trial which requires the intervention is undertaken on the whole ICU population (7).

As decolonisation strategies represent an ecological intervention on the whole critical care population, all patients meeting inclusion/exclusion criteria will be entered into the study according to ethics approval in each participating country. Each participating ICU will use three decolonisation strategies in a randomised order. The interventions are administered four times daily to ventilated patients until extubation. The interventions will be compared to a 6 month baseline period consisting of standard care only.

The baseline period is the first period and will be used to implement universal standard care:

1. Chlorhexidine body washings (CHX-BW) for all ICU patients
2. A hand hygiene improvement program (HHIP) based on the program designed by the World Health Organisation (WHO).

CHX-BW ensures state of the art standard of care to prevent carriage and transmission of (resistant) gram positive bacteria commonly residing on body surface, such as *Staphylococcus aureus* and Enterococci.

Implementation of the hand hygiene program derived from the WHO hand hygiene program ensures state of the art standard of care for transmission prevention of all relevant pathogens.

“Standard care” (CHX-BW and HHIP) will be the only protocolised intervention in the baseline period and will be used throughout the entire study. However, sites already using

CHX-Oro as standard care can choose to continue CHX-Oro during the baseline period, provided the maximum concentration used is 0.20%.

After the baseline period, (i.e. the first six months of the study), the first decolonisation regimen will be implemented. The order of regimens per ICU is decided by randomisation (see chapter 5). The three regimens are:

- CHX-Oro with chlorhexidine 1%, administered 4 times daily, will be added to standard care. The CHX-Oro phase will last six months. CHX-Oro should be applied to all eligible patients receiving mechanical ventilation until the moment of extubation.

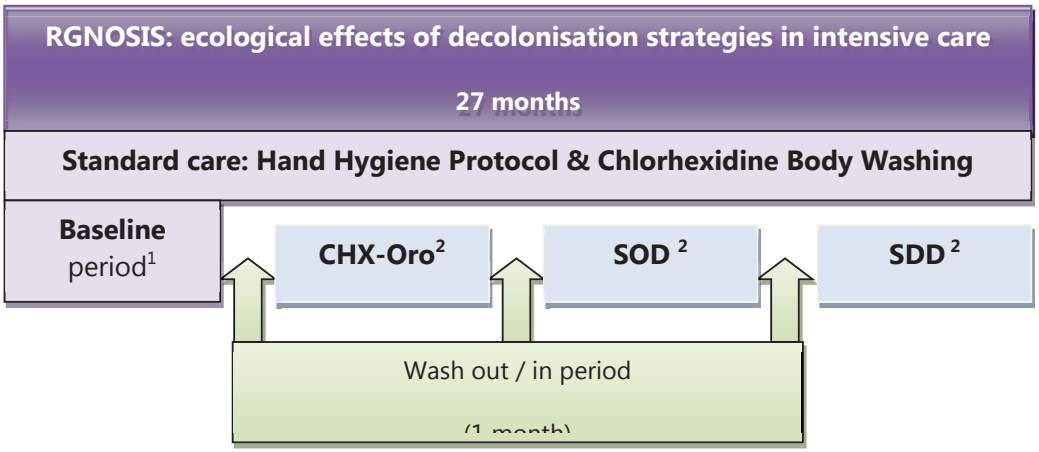
- SOD with antibiotics. SOD consists of application of a paste containing colistin and tobramycin in a 2% concentration and nystatin 1 x 10^5 units. SOD will be applied to the mouth 4 times daily until extubation. Like the other decolonisation interventions, this regimen will last 6 months.

- SDD, in which a 10 ml suspension via the nasogastric tube containing 100 mg colistin, 80 mg tobramycin and nystatin 2 x 10^6 i.u. will be added to application of SOD paste. The combination of SOD and this enteral suspension is called SDD and is applied 4 times daily.

Surveillance cultures and point prevalence cultures to monitor ecological changes will be collected during all regimens. Also, results from regularly obtained cultures for clinical purposes (blood and respiratory cultures) will be recorded.

In contrast to some other SDD studies, systemic prophylaxis with Cefotaxime (or other broad spectrum cephalosporins) will not be implemented as part of SDD. Cefotaxime is considered inappropriate for prophylaxis in settings with endemic levels of antibiotic resistance. Moreover, most ICU patients already receive systemic antibiotics in the first few days of their ICU stay due to the nature and severity of disease.

Study scheme per site



[1] May include chlorhexidine mouthwashes if this was standard care before the study

[2] Randomised order per ICU

Clinical cultures

- Blood and respiratory samples obtained as part of routine standard patient care

Surveillance cultures

- Twice weekly in participants: peri-anal and respiratory culture
- Detect MDR-GNB and colistin resistance

Point prevalence cultures

- Monthly in all ICU patients: peri-anal and respiratory cultures
- Detection of MDR-GNB, colistin resistance, MRSA and VRE in all ICU patients.

**HRMO: Highly Resistant Micro Organism; MDR-GNB: multi-drug resistant gram negative bacteria, Per patient only 1st isolates of each HRMO from each body site will be stored and shipped*

ICU inclusion criteria

ICU units can be included if:

- The ICU has endemic levels of MDR-GNB, defined as a proportion of MDR-GNB among ICU-acquired GNB-bacteraemia of >5% in 2011.
- The ICU consists of at least 8 beds with possibility for mechanical ventilation, with an average bed-occupancy of at least 80%.
- The ICU has the availability of at least one dedicated health care worker for patient monitoring, compliance, supervision and instruction of health care workers regarding interventions. In the following this person will be called “research-nurse”.

In addition, ICU units should be able to:

- Adhere to the study protocol, collect required data and complete the study regimens.
- Obtain, analyse and store (colonisation) cultures at -70ºC and facilitate transport through a courier.
- Implement daily chlorhexidine 2% bathing as standard care.
- Implement the WHO hand hygiene improvement program as standard care.
- Extract data in a pre-defined format from an operational digital patient-information system. Preferably, there should be an automated process for digital data-collection regarding microbiological culture-results (from swabs and bacteraemias), patient demographics and illness severity-scores.
- Obtain written approval for the study from their institutional review board (IRB) with a waiver for patient informed consent

The following ICU units will be excluded:

- ICU units with endemic levels of carbapenem-resistant Enterobacteriaceae, MDR-Pseudomonas or Acinetobacter species (defined as >10% of ICU-acquired GNB-bacteraemia, see textbox 1) or having endemic VRE (defined as > 10% of all enterococci bacteraemia, see textbox 2).
- ICU units planning to enrol subjects in studies testing investigational agents for the purpose of eradicating or preventing colonisation with MDR-GNB, MRSA or VRE and/or ICUs that are planning to use devices or practice management strategies that have colonisation and/or infection with resistant organisms as an outcome.
- Burn units (due to the specific nature of the care provided and the patients admitted).
- Cardiothoracic surgery units (because of the expected small number of patients admitted for three days or more).
- Paediatric and neonatal ICU units.

Selection of eligible ICU units

Textbox 1. Endemic levels of carbapenem resistant micro-organisms

In the year before study start, did the ICU have >10 cases of carbapenem resistant bacteraemia with

4. Enterobacteriaceae (i.e. *E. Coli, Klebsiella p*.) y / n
5. Acinetobacter y / n
6. Pseudomonas y / n

If >10 bacteremias of one or more of these species occurred in the year before study start (one or more yes), what was the proportion of carbapenem resistant isolates from the total amount of bacteremias with that species:

1. Number of bacteremia with carbapenem resistant enterobacteriaceae (divided by)

Total number of bacteremia of that species x 100% = … %

2. Number of bacteremia with carbapenem resistant acinetobacter (divided by)

Total number of bacteremia of that species x 100% = … %

3. Number of bacteremia with carbapenem resistant pseudomonas (divided by)

Total number of bacteremia of that species x 100% = … %

If **> 10%** consider the resistant form of the species endemic

ICUs will be selected on their capacity to generate high-quality data. This includes a suitable environment for electronic clinical and microbiological data collection and performing on-site observations.

As part of EU legislation, putative subcontractors will be given the opportunity to apply for a position as a subcontractor in the study. Through a procedure legislatively outlined by the EU, candidate-institutions will be allowed to submit their application. After initial selection by the principal and coordinating investigators, selected ICUs will undergo on-site audits before final selection. From these ICUs, thirteen units will be selected for study participation. If possible, ICUs will be included in an equal ratio of older and new member states.

Textbox 2. Endemic levels of Vancomycin Resistant Enterococci
In the year before study start, did the ICU have >10 cases of bacteraemia with VRE?

If >10 VRE bacteremias occurred in the year before study start, what was the proportion of VRE isolates from the total amount of enterococci bacteremias:

Number of VRE bacteremia (divided by)

Total number of enterococci bacteremia x 100% = … %

If >10% consider the resistant form of the species endemic

3. Population

Population

Patients eligible to receive the interventions: All mechanically ventilated adult patients (surgical or non-surgical) admitted to the participating ICUs, with no planned extubation within 24 hours.

The reasons for excluding non-ventilated (and "short stay") patients are:

- the presumed negligible contribution of these patients to transmission dynamics
- the presumed small effect on outcome of regimens administered for less than 24 hours

Participant inclusion and exclusion criteria

Inclusion criteria:

- mechanical ventilation (only invasive ventilation: i.e. intubated patients or patients with tracheostomal ventilation)
- no planned extubation within 24 hours

When mechanical ventilation is not started directly after admission but later in the course of their ICU stay, patients are still eligible to participate.

Exclusion criteria:

- patients under the age of 18
- patients with known allergy to any of the medications or agents used (i.e. colistin, tobramycin, nystatin or chlorhexidine[1])
- pregnancy

Participation ends as soon as the patient is extubated or after tracheostomal ventilation has stopped (weaning completed). The discharge data can only be completed after ICU-discharge. If a re-inbutation occurs during the same ICU-admission, participation should be re-initiated, including surveillance sampling and administration of study medication. If re-intubation occurs during the first 15 days of the wash-out, the same regimen is applied as during the previous ventilation period (page 15).

[1] Anaphylactic reactions to chlorhexidine have been described. For more information see the investigator brochure.

Sample size calculations and enrolment numbers

Sample size calculations (where relevant) and assumptions regarding outcome are given per specific outcome. The quantification of the effects of decolonisation regimens on overall systemic antibiotic use and the determination of the ecological safety of 3 decolonisation regimens are exploratory and therefore not based on a formal power calculation.

To adequately determine the effects of the decolonisation strategies, 13 hospitals from different ecological settings will be contracted to perform this study. Selecting hospitals from different European countries ensures a representative mix of European ICU ecology and increases external validity of results.

MDR-GNB ICU acquired bacteraemia

The following assumptions were made for the evaluation of the occurrence of MDR-GNB ICU acquired bacteraemia. In the Dutch ICU trial incidences of ICU-acquired bacteraemia with GNB were 7.1%, 5.0% and 2.3% during SC, SOD and SDD, respectively. Assuming that 20% of the episodes of ICU-acquired GNB-bacteraemia during SC will be caused by MDR-GNB this would yield an incidence of 1.4%. A 50% reduction to 0.7% can be demonstrated with 1,675 patients in each arm (alpha=0.05; beta=0.8).

Acquired respiratory tract colonisation during each study phase

Incidences of ICU-acquired respiratory tract colonisation with MDR-GNB will be derived from respiratory tract cultures (endotracheal aspirates, sputum or BAL) obtained twice weekly (on average 3 per patient if length of stay on the ICU is estimated at 10 days). Results from samples obtained for clinical reasons will be derived from the clinical laboratories. In the Dutch SOD / SDD trial incidences of ICU-acquired respiratory tract colonisation with Enterobacteriaceae was 70%, 47% and 24% during SC, SOD and SDD, respectively. Assuming that 20% of these episodes during SC will be caused by MDR-GNB this would yield an incidence of 14%. A 30% reduction to 10% can be demonstrated with 521 patients in each period (arm) (alpha=0.05; beta=0.8). This analysis will be performed on respiratory tract samples.

Association between intestinal and respiratory colonisation and occurrence of ICU-acquired GNB bacteraemia

Presence or absence of intestinal and respiratory tract carriage will be determined upon surveillance cultures from patients receiving SC, SOD, SDD and CHX-Oro. Using an incidence of 4.5/1,000 patient days in the presence of carriage at both sites 3,000 patient days are needed to demonstrate a statistically significant reduction to 1/1,000 patient days in

the absence of carriage at both sites. Assuming GNB at both sites during 75% of patient days with SC and absence of carriage at both sites during 25% of patient days with SDD we would need two groups of 500 patients with SC and SDD. The percentages and incidence rates are based on analyses of patient data in the before-mentioned Dutch SOD / SDD trial (3).

28-Day mortality

In a Dutch SDD ICU trial the day-28 mortality rate during the baseline period was 27.5% (3). Assuming a low level of cluster-effects, 2016 patients are needed in each phase to demonstrate a 10% relative reduction in day-28 mortality as compared to Standard Care (alpha=0.05; beta=0.8). We intend to include 2700 patients per arm. The margin of 600 patients per arm is included to allow for adjustment for differences in baseline characteristics in a random-effects logistic regression model if needed, or to include cluster-effects. Of note, assuming day-28 mortality in standard care to be 27%, the absolute reduction that can be demonstrated is 2.7%.

The statistical analysis will be based on a random effects logistic regression model, incorporating all relevant covariates. Day-28 mortality data will be derived from clinical data obtained as part of routine standard care.

Additional secondary mortality outcomes will be in hospital mortality and day 180 mortality (6 months), the latter in a subset of patients, as the collection of this data is optional to sites.

Enrolment numbers

With a total sample size of 2700 patients per study period, the study aims to enrol on average 450 patients per month. When planning the study, estimated ICU stay was 10 days and the estimated inclusion rate was 2 patients per bed each month. Therefore, we aimed to include a total of at least 225 beds, through inclusion of 12 hospitals. After 6 months of patient enrolment, it was concluded that the inclusion rate was lower than 2 patients per bed per moment, and additional beds were recruited. We aim to include 13 ICUS and at least 2700 patients per decontamination phase, each group with 27.000 consecutive patient days.

4. Interventions

Standard care

Each participating ICU will start with the baseline period of 6 months consisting of standard care only. Standard care consists of daily chlorhexidine body washing, a hand hygiene improvement program and regular oropharyngeal care. During the baseline period, all ventilated patients should be checked for eligibility to participate in the study and all eligible patients should be formally included. This will ensure comparability of all study periods.

Standard oropharyngeal care consists of oral washing with sterile water (3-4 times daily) and tooth brush twice daily. We will allow chlorhexidine mouthwashes in ICUs in which this already belongs to standard care. If sites choose to use chlorhexidine mouthwashes in the baseline period it is recommended that sites use chlorhexidine [0.12%] 4 times daily, and the maximum concentration allowed is [0.20%]. During the baseline period no form of SDD or SOD should be applied to patients included in the study.

During all phases of the study, all units will use daily body washing with Chlorhexidine gluconate 2% concentration (e.g. Hibiscrub). The bathing solution is prepared by adding a standardized amount of Chlorhexidine to the water used to bathe patients. The face and neck of the patient will not be cleansed with Chlorhexidine to prevent irritation of the eyes and face. Patients' skin will be moisturized with a lotion that does not interfere with the biocidal activity of Chlorhexidine gluconate.
At the start of the baseline period a hand hygiene protocol will be introduced. This protocol is derived from the WHO hand hygiene program and consists of education of health care workers and monitoring of hand hygiene compliance. The hand hygiene protocol will be used during all regimens as it is part of standard care.

CHX-Oro regimen

CHX-Oro is a decolonisation intervention using chlorhexidine. The CHX-Oro regimen consists of standard care and oropharyngeal application of 2 cm of chlorhexidine gel in a 1% concentration every 6 hours. (25) CHX-Oro will be applied daily until extubation. The regimen will last 6 months. The switch to the new regimen will take place during the wash out / in period (see below).

SOD regimen

SOD consists of standard care and the application of an antimicrobial mouthpaste every 6 hours. The paste contains colistin and tobramycin in a 2% concentration and nystatin 1 x 10^5 units and will be applied 4 times daily until extubation. Application modifications for patients with tracheostomy will be specified in the standard operating procedure (SOP).

SDD regimen

SDD consists of:

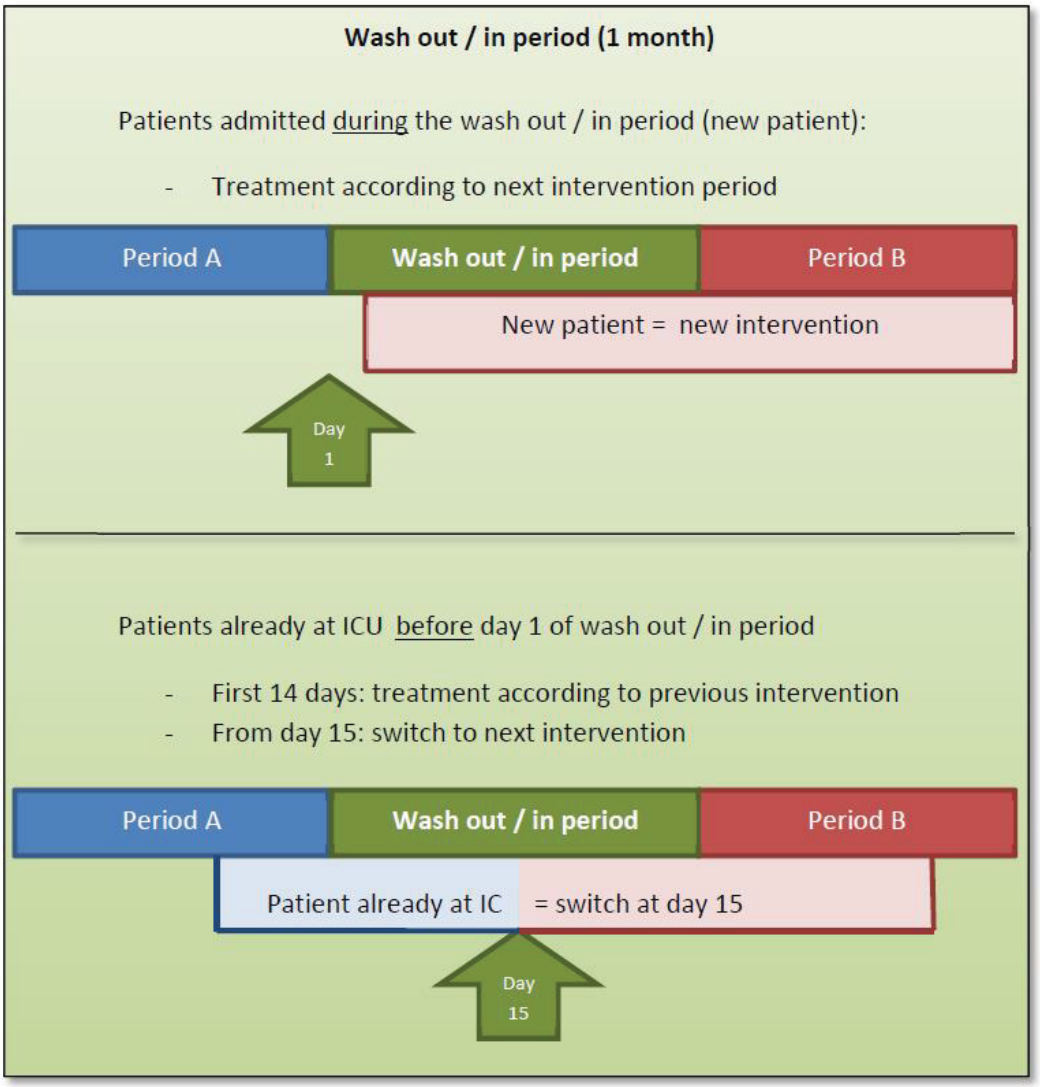
- standard care, AND
- oropharyngeal application of a paste containing colistin, tobramycin and nystatin every 6 hours (SOD), AND
- administration of a 10 ml suspension containing 100 mg colistin, 80 mg tobramycin and nystatin (2 x 10^6 units) via a nasogastric tube every 6 hours (SDD).

SDD will be administered 4 times daily in participants until extubation. Application modifications for patients with tracheostomy and jejunostomy will be specified in the SOP.

More information on the investigational products, including clinical data and a risk benefit analysis can be found in the investigator brochure.

Wash out / Wash in period

To prevent carry-over effects from preceding interventions a one month wash out / in period will separate the different intervention periods. Patients included during the wash out / in period will start directly with the new regimen. Participants using the old regimen have two weeks to finish the old regimen and will switch to the new regimen at the start of the third week.



5. Methods

Study procedures and data collection

Randomisation procedure

Before start of the study participating ICUs will be randomised to either of six orders of decolonisation strategies:

- CHX-Oro → SOD → SDD
- CHX-Oro → SDD → SOD
- SOD → SDD → CHX-Oro
- SOD →CHX-Oro → SDD
- SDD → CHX-Oro → SOD
- SDD → SOD → CHX-Oro

Randomisation of the order of interventions can correct for the effects that occur by natural time trends in antibiotic resistance rates. Furthermore, any persistent effect - or carry over effect - of a preceding intervention on the subsequent regimen would be corrected for by randomisation. Nevertheless, carry over effects are expected to be minimal, if any, because of the wash out / wash in period between interventions.

Random allocation of ICUs to treatment orders will be performed by a computer. The randomisation procedure will be executed by data management, based at the UMC Utrecht, also responsible for producing the electronic case report form, database design and storage of patient data. Data management will be blinded to the names of the sites to ensure a random allocation of treatment orders.

Inclusion

Screening of eligible patients will be performed by the local research nurse within 24 hours after admittance of a patient to the ICU. Each ventilated patient should be checked for eligibility and meeting in- and exclusion criteria, including patients admitted during the baseline period.

Cultures

Point prevalence cultures

To monitor and safeguard ICU ecology, Point Prevalence Cultures will be taken once a month from all patients in the ICU (included and non-included patients). Samples will be inoculated on MRSA, VRE and ESBL chromagar plates. Peri-anal and respiratory tract cultures (either sputum, throat swab of tracheal aspirate) will be obtained. Growing isolates (per definition resistant) will be further determined including susceptibility patterns by the local microbiology laboratories. These results will be available to participating sites. Once every three months, point prevalence samples will be inoculated on Mac Conkey media, to select three colonies for colistin resistance testing in the central laboratory at UMC Utrecht. In addition, an independent Safety Committee (SC) will review these results on a three monthly basis to follow ecological changes during the study and intervene when necessary according to the SC protocol. Any highly resistant micro-organism (HRMO) cultured, including MDR-GNB, should be stored and sent to the central laboratory for qualitative determination. Only first isolates of a unique highly resistant micro-organism (HRMO) per patient will be stored and shipped.

Surveillance cultures

Surveillance cultures will be taken twice weekly, every Monday and Thursday, from participants in the study. These cultures will determine whether a participant is colonised with gram negative bacteria and if this micro-organism is resistant to antimicrobial agents. Thus, changes in antibiotic resistance in participants will be monitored by surveillance cultures.

Samples will be inoculated on ESBL chromagar medium. Growing isolates (per definition resistant) will be further determined (including susceptibility patterns) by the local microbiology laboratories.

Surveillance cultures will start in the baseline period. Any first isolate of a unique HRMO cultured from each body site per patient should be stored and sent to the central laboratory for qualitative determination.

Clinical cultures

It is at the discretion of the treating physicians to perform (blood) cultures on clinical ground, i.e. on suspicion of infection. Culture results from participants will be collected. These data will be recorded in the electronic case report form (e-crf). Data will include type of sample, culture result and antibiotic susceptibility pattern.

Results from the following type of clinical cultures will be recorded:

- blood cultures
- respiratory samples
    - Sputum cultures
    - Broncho Alveolar Lavage (BAL) samples / endotracheal aspirates

In addition, all HRMO from the above mentioned clinical samples in participants should be recorded and the first isolate of each HRMO from each body site per patient should be stored and shipped to the UMC Utrecht.

Clinical cultures

- Blood and respiratory samples obtained as part of routine standard patient care

Surveillance cultures

- Twice weekly in participants: peri-anal and respiratory culture
- Detect MDR-GNB and colistin resistance
- Identified MDR-GNB* should be sent to central laboratory for qualitative evaluation

Point prevalence cultures

- Monthly in <u>all</u> ICU patients: peri-anal and respiratory cultures
- Detection of MDR-GNB, colistin resistance, MRSA and VRE in all ICU patients.
- HRMO* should be sent to central laboratory for qualitative evaluation

**HRMO: Highly Resistant Micro Organism; MDR-GNB: multi-drug resistant gram negative bacteria, Per patient only 1st isolates of each unique HRMO from each body site will be stored and shipped*

Data registration

Preferably, data will be directly extracted from a digital database and sent to the UMCU in a universal digital format. Otherwise data will be extracted manually by the Research nurse and entered in the web based database "Research Online" by completing the e-crf. Data will be "anonymised" by recoding the patient ID and removing all other identifiers. A summary of the data to be collected is described below, details can be found in the Dataset Protocol.

General ICU related data:

At the initiation of the study

- The amount of beds, with and without options for mechanical ventilation
- Teaching or non/teaching hospital
- Name of physician director of the ICU and primary specialty (changes herein will be recorded)
- Name of nurse director (manager) of the ICU (changes herein will be recorded)

Data from all patients admitted to the ICU

- Sex
- Age
- Date of admission to and discharge from the ICU
- Mechanical ventilation (invasive or not) and duration
- Disposition of the patient at ICU discharge (alive or deceased)
- APACHE II/IVor SAPS II/III scores

Additional data for all included patients:

- Date of hospital admittance
- Place from which patient was admitted to ICU:
    - This hospital #
    - Another hospital #
    - Home or Emergency Room
- # If patient was already admitted to a hospital: the patient came from:
    - Operating room
    - Hospital ward:
        - Other ICU
        - Acute care
        - Rehabilitation or long-term care
- Principle reason for ICU admission

    - Medical
    - Trauma
        - Patient needed surgery
        - No surgery needed
    - Surgical
        - Scheduled
        - Unscheduled / emergency / complications after surgery
- Acute illness:
    - Yes
    - No (routine observation after surgery and routine monitoring)
- Reason for ICU admission (more than one option possible)
    - Respiratory
    - Cardiovascular
    - Neurologic
    - Renal
    - Hepatic
    - Hematologic
    - Metabolic
- Antibiotic on admission
    - Yes
    - No
- Previous or pre-existing condition, as defined by the Charlson comorbidity index.
    - Malignant solid tumor
    - Metastatic cancer
    - Hematologic cancer
    - Immunodepression or AIDS
    - Alcohol or drug abuse
    - Cardiovascular disease
        - Chronic heart failure
    - Pulmonary disease / chronic respiratory failure
    - Diabetes Mellitus
    - Chronic renal failure
- Date of start and cessation of invasive mechanical ventilation (including ventilation via a tracheostoma, multiple episodes possible)
- Status at day 28 (alive of deceased)
    - *Optional: status at day 180 (alive or deceased)**

- Status at hospital discharge (alive or deceased)
- Status at ICU discharge (alive or deceased)
- Date of death (if within 29 days of ICU admission or if patient died in hospital)
    - **Also record date of death if day 180 mortality is recorded*
- Isolation precautions (barrier precautions: need for mask, gown, gloves)
    - Yes
        - Due to surveillance cultures during hospital stay
        - Due to known MDR micro-organism on admission
        - Due to other reason (e.g., tuberculosis, neutropenia, clostridium, etc…)
    - No

Culture data

Culture results that should be recorded include results from all:
Clinical cultures in participants:

- date of obtaining the culture sample
- qualitative determination of isolated micro-organism (*species*)
- susceptibility pattern of these isolated micro-organisms

Surveillance cultures (twice weekly) in participants and point prevalence cultures (monthly) in all ICU patients:

- date of obtaining the culture sample
- determination on the species levels of the micro-organism cultured on chromogenic ESBL plate
- susceptibility pattern of these isolated micro-organisms

HRMO (1st unique isolate per HRMO per patient) obtained from point prevalence, clinical and surveillance cultures should be sent to the UMCU for further determination. Detailed information will be available in the microbiology protocol.

Antibiotic use

To monitor other antibiotic use on an ICU level (other than the investigational products), total antibiotic use per site per drug will be recorded during each study period (i.e. from all patients). Antibiotic use will be expressed as defined daily dose (DDD). The DDD per antibiotic is defined by the world health organisation. It is the assumed average maintenance dose per day for a drug used (8). By using the DDD system antibiotic use per site can be compared.

Decontamination compliance

Once monthly a compliance survey should be completed for the ICU. This survey will record the proportion of mechanically ventilated patients in the ICU who indeed received decontamination.

Drug accountability

All antibiotics for decontamination purposes will be produced by the pharmacy of the UMC Utrecht according to Good Manufacturing Practice (GMP) and delivered by the sponsor. Chlorhexidine 1% oral gel will be bought from an external party. When sites are running out of medication, extra deliveries can be requested by contacting the study management.
Since the study uses medication which is already used as standard care in different EU countries and because of the ecological nature of the study, drug accountability will be recorded on a ward level, rather than on individual patient level. Drug accountability of the study medication will be kept by recording (1) the amount of decontamination medication supplied to the ICU by the local pharmacy and (2) the batch number of medication supplied to the ICU. By calculating the dose per patient day, the use of medication by participants will be calculated. Furthermore, monthly compliance surveys will determine the proportion of eligible patients receiving study medication, which allows for monitoring of compliance.

Financial compensation

R-GNOSIS will compensate the department of the principal site investigator for the value of 0.5 FTE of a Research Nurse position. The compensation will be roughly divided into 4 terms and compensation for the next term will only proceed if data has been delivered for the previous term. Definite failure to provide qualitative and quantitative data as described in this protocol will result in discontinuation of the ICU as subcontractor within the study and the compensation received for data not delivered will be reclaimed by the WP6 coordinating centre: the Julius Center / UMC Utrecht.
Patients will not be compensated for their participation in the study.

Site withdrawal or discontinuation of participation

The site investigator may withdraw his or her site from the study for any reason at any time. In case of an unexpected change in MDR-GNB or other resistant bacterial epidemiology in a certain ICU, leading to a situation in which adherence to the study protocol can no longer be recommended, the site will temporarily be withdrawn. In such case, the external safety committee will be notified. If withdrawal exceeds >3 months, participation of the site will be terminated.

The *R-GNOSIS WP6 investigators* may discontinue the participation of a site for the following reasons:

- The site does not follow the study protocol with respect to obtaining the surveillance or point prevalence cultures
- The site does not implement the assigned regimen satisfactorily
- The site does not collect the required patient information
- The site fails to report culture results or fails to send culture (results) within reasonable time to the study coordinator, SC or central laboratory
- The ICU enrols subjects in any other study of an intervention or investigational agent administered for the purpose of eradicating or preventing colonisation with MRSA, VRE or ESBL or MDR-GNB; or for the purpose of reducing the likelihood of transmission of these bacteria.

Withdrawal of individual subjects

Subjects can withdraw their anonymised data from the study at any time for any reason if they wish to do so without any consequences. See the chapter *ethical considerations* for further information.

6. Safety reporting

SDD, SOD and Oropharyngeal application of chlorhexidine are considered safe and have been used extensively in ICU patients for over 20 years. In fact, SDD is standard care in ICUs in The Netherlands and certain ICUs in Spain and France. Oral chlorhexidine is standard of care in ICUs across Europe, based on ICU guidelines for VAP prevention. Therefore, risks for patients that receive the decontamination medication is expected to be comparable to ICU patients receiving standard care.

Definitions:

- **Adverse event** (AE): Any untoward medical occurrence in a participant during the course of the study, which does not necessarily have a causal relationship with this treatment.
- **Serious adverse event** (SAE): A serious adverse event is any untoward medical occurrence that at any dose:

  (a) results in death

  (b) is life-threatening

  (c) requires hospitalisation or prolongation of existing hospitalisation

  (d) results in persistent or significant disability or incapacity

  (e) consists of a congenital anomaly or birth defect.

  Medical and scientific judgement should be exercised in deciding whether an event is 'serious'.

- **Adverse reaction** (AR): An adverse event when there is a least a possibility that it is causally linked to a study drug or intervention.
- **Serious adverse reaction** (SAR): SAE that is thought to be causally linked to a study drug or intervention.
- **Suspected Unexpected Serious Adverse Reaction** (SUSAR): An unexpected occurrence of a SAR; there need only to be an index of suspicion that the event is a previously unreported reaction to a decontamination drug (not reported in the investigator brochure) or a previously reported but exaggerated or unexpectedly frequent adverse drug reaction. The definition implies a reasonable possibility of a causal relationship with the medication used, is unexpected and serious.

Recording and reporting of adverse events during the study

This study is conducted in ICUs, using drugs in common use in E.U. member states. The risks for patients that receive decontamination medication are expected to be comparable to ICU patients receiving standard care without decontamination medication. Application form and dosage of the medicinal products are identical to regimens that are currently being used for decontamination in ICU patients. In the scientific literature, only one serious adverse event consisting of blockage of the oesophagus by SDD paste in two patients has been described. The amount of paste that had been used in these patients was not reported. In the current study 0.5 grams of paste will be used 4 times daily, a quantity which is unlikely to cause blockage (9). Other than this, no serious adverse events associated with SOD and SDD have been reported.

Chlorhexidine 1% oral gel is being used in European ICUs as standard care. Based on broad experience with chlorhexidine 1% no serious adverse events are to be expected.

In ICU patients, co-morbidity and the natural history of the underlying critical illness can cause events which would meet the definition of (serious) adverse events. Given the natural occurrence of these events and the low risk for adverse drug reactions based on the broad experience with the decontamination medication, only the following adverse events will be recorded:

- Adverse events possibly related to the medication (as judged by medical and scientific judgement) AND
- Deemed serious by medical or scientific judgement (as judged by either the investigator of treating physician) AND
- Not part of the natural history of the underlying critical illness

This includes SUSARs. All other adverse events will not be recorded and reported.

Timelines for Reporting

Adverse events meeting these criteria (SUSARs) should be reported by the local investigator within the following time limits:

- to the coordinating investigator within 24 hours
- to the accredited IRB that has approved the protocol in that country
    - within 7 days if the event is life-threatening or fatal
    - within 15 days if the event is not life-threatening or fatal
- to the competent authority of that country
    - within 7 days if the event is life-threatening or fatal

- within 15 days if the event is not life-threatening or fatal

An example of the SUSAR report form can be found in the appendix.

Laboratory abnormalities originating from decontamination medication are not to be expected, since the medication used is not, or at most poorly, absorbed systemically and is not expected to cause systemic effects. In accordance, no systemic interactions with other medications are to be expected.

Annual progress and safety report

The local site investigator will submit a summary of the progress of the study to the accredited IRB(s) and competent authority of the concerned member state once a year in the form of a development safety update report (DSUR) and progress report.
The progress report will include information on the date of inclusion of the first patient, numbers of subjects included and numbers of subjects that have completed the study, development of resistance rates and amendments.
The DSUR consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions
- a report concerning the safety of the subjects, consisting of a safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicinal products used in the study until that time

A template will be provided yearly by the study management.

Safety committee (Scom)

The SCom consists of three external experts and has the objective to guard the ecological safety during the study. The SCom will have an active role during interventions (SDD, SOD, CHX-Oro). During the intervention period, the SCom will issue recommendations to continue or stop the study on a quarterly basis (three monthly), based on the results of monthly point prevalence cultures and input by participating ICUs. A detailed description of the tasks of the SCom is provided in the Safety Committee protocol (appendix).

The primary safety measure to detect an increase of anti-microbial drug resistant bacteria is performing point prevalence cultures in all patients in the participating ICUs. The purpose of these cultures is to evaluate and ensure ecologic safety. Analysis will detect any Carbapenemase Producing Enterobacteriaceae (CPE), ESBL, CRAB, VRE or MRSA isolates. Also, susceptibility of /MDR-GNB to colistin, both used in SDD and SOD, will be

tested. This way, any increase in the number of resistant isolates will be detected early. Stopping rules are defined in the SCom protocol.

7. Statistical analysis

Statistical analysis plan

Analysis will determine the effect of each intervention on colonization rates, the occurrence of bacteraemia and the use of antibiotics. Statistical analysis of the primary objective and secondary objectives regarding mortality, antibiotic use and rate of colonisation and bacteraemia will include the use of random-effect logistic regression models to account for ICU-level clustering. Measured confounding factors will be fitted as covariates. Outcome will be presented as odds ratios.

All available data on patient colonization with MDR-GNB (both from screening and clinical cultures) will be used to determine, as carefully as possible, the ESBL-colonization status of each patient on every study day. Together with work package 8 (mathematical modelling, WP8), nosocomial transmission capacities (R_A values) for different species of MDR-GNB during study regimens will be quantified. As a secondary aim species-specific R_A values will be compared between wards. Available data will be used to quantify incidences of cross-transmission in both study periods, using sophisticated modelling approaches to be developed in WP8.

Missing data

Investigators and the R-GNOSIS staff will make every attempt to collect complete data from all subjects enrolled in the study. Where possible, automatic extraction of data from hospital information systems will be used (without disclosing patient identifiers). There will be regular contact between the study coordinating centre and study sites to track and retrieve missing data. Should culture results be inadvertently lost, those data will be treated as missing at random. All inferential analyses will be based on available data. Details of the procedures for addressing missing cultures will be provided in the Statistical Analysis Plan.

Changes to the Statistical Analysis Plan

Details of the analysis methods and changes in the analyses from those described in the protocol will be documented in the statistical analysis plan prior to database lock. Those

changes and the reasons for the changes will be described in detail in the final study report, but not presented to the regulation authorities or ethical committee as substantial amendments.

8. Ethical considerations

Regulation statement

This study is conducted in agreement with the declaration of Helsinki (Seoul, October 2008) and with the guidelines of GCP issued by the European Union (10-12) as far as these apply to this type of cluster randomised trial.

Recruitment and consent

Implementation of any of the three infection prevention strategies shall not be initiated prior to IRB review and approval, unless this has been part of previous regular care in that hospital.

The ICU is a participant of the 'R-GNOSIS: Ecological Effects of Decolonisation Strategies in Intensive Care study' and the decolonisation strategies apply to the whole ventilated ICU population.

The study protocol will be reviewed and approved by the local IRB (or ethics committee). Changes to the protocol (substantial amendments) will be initiated by the primary investigator and approved by the IRB. The ethical principles and norms of research involving human beings apply to trials with clustered interventions, although there is much less published information on the application of these principles and norms to this type of study. A recent publication by the United Kingdom Medical Research Council, "Cluster Randomised Trials: Methodological and Ethical Considerations," provides guidance in this regard (13). Ethical and design issues of cluster clinical trials where the intervention affects individual patients or populations of patients has also been reviewed by Edwards et al (7). The approach described below is consistent with the recommendations of this guideline.

Decontamination interventions not only affect individual patients but also influence ICU ecology, affecting patients who do not receive the intervention. Decolonisation with antibiotics reduces the prevalence of resistant bacteria during treatment (3). Decreasing resistant bacteria in decolonized patients reduces cross transmission and is therefore beneficial to all patients in the unit. The trial represents a cluster-cluster clinical trial (7) and requires the intervention to be applied to all eligible patients in order to assess the impact on ICU ecology. All the interventions represent a current standard of care in ICUs throughout

Europe, are considered safe and have been in clinical practice for several years. However, the three interventions have not previously been directly compared to one another in a trial. Ethical approval and consent will be according to local and national regulations. Similar studies performed in the Netherlands have obtained a waiver for consent. In two Dutch multi-centre cluster-randomised studies on SDD and SOD (3, 26), and two ongoing multi-centre studies as part of the European funded projects MOSAR (rapid diagnostic testing in ICUs) and SATURN (mixing versus cycling of antibiotics in ICUs), both lead by the coordinator of R-GNOSIS (prof. M.J.M. Bonten), unit-wide infection prevention interventions with minimal risks for individual patients were provided with waivers of consent.

All patients admitted to a participating ICU (or when not possible their spouse or relatives) will be informed through a written document (written in the first language of the country were the ICU is located), posted in a visible place in the ICU. The document will state the purpose and possible risks of the study. More specifically, this document will state that:

- A study is taking place on the ICU and all eligible patients are participants in this study because the interventions are applied to all patients, as a standard of care.
- Anonymised patient related data will be collected for study purposes

Benefits and risks assessment

As stated above, the interventions (SDD, SOD and CHX-Oro) are associated with no more risk than that of standard care and have been widely used in European ICUs over the past two decades, and are considered standard care in the Netherlands and certain ICUs across Europe.

Previous experiences with SDD/SOD and chlorhexidine

The antimicrobial agents used in SDD and SOD have been used extensively in topical application to prevent bacterial infections (colistin, tobramycin). Nystatin has been used frequently as part of SDD/SOD and is a registered and established product for the treatment of oral candidiasis, including prophylactic treatment. The antimicrobial agents used are not or at most poorly absorbed from the gut lumen and no systemic effects are to be expected. Dose, indication and length of treatment in the current protocol are comparable to previous studies and to the most commonly used SDD/SOD regimen which is standard care in The Netherlands and certain ICUs in Spain, France and Germany.

Chlorhexidine 1% oral gel has a marketing authorisation in several European countries and is being used as standard oral care in different European ICUs. Side effects associated with

the use of chlorhexidine oral gel are limited to local side effects. Side effects include (reversible) teeth discoloration, a burning sensation of the tongue and oral mucosal irritation, according to the summary of product characteristics.

Adverse events and safety

Adverse events reported in the past include:

- Clotting of SOD paste in the gastro-intestinal tract in three cases. In two of these, SOD paste obstructed the oesophagus.

Dose of SDD medication was not reported in this case series (9) and this is the only report of such complication to date. In the current study 0.5 grams of paste will be used 4 times daily, a quantity which is unlikely to cause blockage.

- The detection of systemic traces of tobramycin in ICU patients treated with SDD.

However, levels of tobramycin found with the regimen that is comparable to that used in the current study were low and toxic effects have not been reported (14).

Given the extensive experience and the low prevalence of adverse events SDD and SOD are safe for use in intensive care patients.

Chlorhexidine 2% mouthwashes have been successfully used in previous studies: no serious adverse events were reported with the use of this concentration. However, in the current study, side-effects were reported in a high proportion of patients during the use of chlorhexidine 2%, consisting of oromucosal irritation and in some cases desquamation. Therefore, it was decided to stop with this intervention. The side-effects were most probably attributable to the concentration of chlorhexidine, as the excipients used in the R-GNOSIS product are also used in Corsodyl (0.2% chlorhexidine mouthwash, marketing authorization). Therefore, it was decided to adapt the intervention to chlorhexidine 1% oral gel.

Chlorhexidine 1% oral gel has successfully been used in ICU patients and is commonly used in the field of dental care: no serious adverse events were reported with the use of this concentration. Moreover, in a study in which more than 500 ventilated ICU-patients were treated with chlorhexidine 1% gel, no side-effects were reported. (27) We refer to the investigator brochure for a further analysis of this risk.

Risks and discomfort associated with other study related procedures

Clinical cultures, point prevalence cultures and surveillance cultures will be obtained in each regimen to determine primary and secondary outcome objectives. These cultures are

standard of care in most ICUs, as are clinical cultures for all patients when infection is suspected. Monthly point prevalence cultures will be performed in all ICU patients to monitor ecological safety. These peri-anal swabs and respiratory samples (either endotracheal aspirates, sputum cultures or throat swabs) are easy and safe to perform, cause minimal discomfort to the patient and have been frequently used in previous studies as well as in normal clinical practice.

During the entire study, all patients admitted to the participating ICUs will be washed daily using a solution containing chlorhexidine gluconate 2%. This is a procedure with minimal burden, using a product registered for this purpose which is already widely used throughout Europe and the United States and is considered safe. Recent studies have demonstrated that body washings with chlorhexidine effectively reduce the bacterial burden of Gram-positive skin colonisers, including MRSA and VRE, and infections caused by these pathogens (16-18). By implementing chlorhexidine body washing as standard care, the effect of the SDD, SOD and Oro-CHX on colonisation and infection with MDR-GNB can be investigated, while at the same time efforts are made to reduce the risk of emergence and spread of resistant skin colonisers.

Antibiotic resistance

A large part of controversy surrounding SDD/SOD concerns resistance that might occur following prophylactic administration of antibiotics in decolonisation strategies. Indeed, some studies found overgrowth of gram positive bacteria, including MRSA (19;20). Also, selection of tobramycin resistant gram negative bacteria might occur (20). After stopping SDD, the prevalence of ceftazidime resistant bacteria in the ICU increased in one study from the Netherlands (21). In contrast, the largest studies so far, performed in the Netherlands, showed a reduction in colonisation with resistant gram negative bacteria in ICU patients during the intervention (2;3). Increases in colistin resistance during SDD/SOD have not been reported. Obviously, the reported effects of SDD/SOD on antibiotic resistance are conflicting, and reductions in resistance rates have been attributed to the local settings with low baseline rates of antibiotic resistance. Elucidation of the effects of these interventions on the prevalence of antibiotic resistant bacteria in ICUs, with other baseline ecology than Dutch ICUs, is the reason of this study.

Resistance will be monitored by two means:

- twice weekly surveillance cultures in participants
- monthly point prevalence cultures in all patients admitted to the participating ICU.

Results of the point prevalence cultures will be evaluated by an independent safety committee (SCom). This will ensure early detection of any increase in the prevalence of resistant bacteria. Stopping rules in case of an increase of resistance have been defined. Detailed information on this process can be found in the Safety Committee protocol.

Benefits

CHX-Oro, SDD and SOD are evidence based interventions.

SDD and SOD have been associated with:

- lower day-28 mortality (3)
- lower in hospital mortality (2)
- a lower incidence of ICU acquired bacteremia (3)
- a lower incidence of respiratory tract infections in ICU patients (22)
- a reduction in colonisation with (multi drug resistant) gram negative bacteria (2;3)

CHX-Oro has been associated with:

- a lower incidence of VAP (4, 27)

Recent studies showed a reduction in bacteremia rates and infectious complications and mortality in ICU patients (2;3;23) . In the largest study so far numbers needed to treat to prevent one casualty at day 28 after ICU admission were 29 patients for SDD and 34 patients for SOD (3).

Oral chlorhexidine is currently recommended by guidelines as strategy to prevent VAP and standard of care in many European ICUs. (25)

The current study uses these interventions to evaluate unit wide ecological effects and compare effectiveness.

The decontamination interventions were beneficial to individual patients but also influence ICU ecology, affecting patients who do not receive the intervention.

Decolonisation with antibiotics have shown to reduce the prevalence of resistant bacteria during treatment (3). Reducing the presence of these bacteria in some patients (that are decolonized), reduces cross transmission and is therefore beneficial to all patients in the unit. SDD / SOD could therefore prove to be important new strategies to prevent emergence and reduce spread of resistant bacteria.

Conclusion

Considering the broad experience with the medication used and:

- the documented effectiveness of the decolonisation interventions in ICU patients and the use of all regimens as standard care in different ICUs
- the low prevalence of adverse events reported with either of the medications used
- the existing equipoise regarding the relative effectiveness of different interventions
- the non-absorbable nature of all medicinal products used
- the surveillance of antibiotic resistance during the entire study

the risk to study participants is comparable to ICU patients receiving standard care. Benefits in terms of reductions in infectious complications, mortality and transmission of resistant gram negative bacteria – in line with previous findings – are to be expected. The expected reduction of transmission of resistant bacteria is also beneficial to patients in the ICU who do not receive the intervention, illustrating the presumed ecological effects.

Compensation for injury

ICU patients will not be compensated for participating in this study. The hospitals will be subcontractors of the R-GNOSIS project. The principal investigators (UMC Utrecht and Université Paris-Est Créteil) cannot be held responsible for any damage to subjects through injury or death caused by the study or negligence by local site investigators. The participating centres will provide appropriate insurance which is in accordance with the legal requirements of each country. The UMC Utrecht will provide insurance for the manufactured decontamination medication.

9. Administrative aspects, monitoring and publication

Monitoring

The study will use a monitoring plan which is developed on a risk based approach, based on a method described by the UK medicines and healthcare products regulatory agency (MHRA) (24). The risk assessment and further details on monitoring can be found in the monitoring plan.

Confidentiality

Information linking the patient's medical data to database materials will be maintained in a secure location at the participating site. This information will not be transmitted to the R-GNOSIS management or the principal investigator or coordinating investigator. The key to code and recode patient identifiers will only be accessible to local site investigators (research nurse and principle investigator) but not to the study management. ICU and coded individual subject data and records will be held in strictest confidence by the site investigator and healthcare staff and by the R-GNOSIS project and study management representatives as permitted by law.

Information contained in this protocol and data and results may not be disclosed without the written permission of the principal investigator and the management of the R-GNOSIS project. If results from this study are published individual subject's identity will remain confidential.

Handling and storage of data and documents

All worksheets will be kept at the sites. All coded electronic data will be stored in the web based electronic database used for the trial (Research Online), to be developed by work package 9 (WP9) of the R-GNOSIS consortium. Partners of WP9 (*datamanagement*) are specialised in design and dedicated to management of study databases.

Amendments

Amendments are changes made to the research after a favourable opinion by the accredited IRB has been given. All substantial amendments will be notified to the IRB(s) that gave a favourable opinion. A 'substantial amendment' is defined as an amendment to the terms of the IRB application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the study;
- the scientific value of the study;
- the conduct or management of the study; or
- the quality or safety of any intervention used in the study.

Non-substantial amendments will not be notified to the accredited IRB(s), but will be recorded and filed by the principal investigator and coordinating investigator.

Progress report

The yearly study progress report will be combined with the annual safety report (DSUR, see chapter 6).

End of study report

The local site investigator will notify the national accredited IRB and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the end of the last regimen in the last participating site.
In case the study is ended prematurely, the local site investigator will notify the accredited IRB and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigators will submit a final report with the results of the study, including any publications/abstracts of the study, to the accredited ethics committee and the Competent Authority.

Public disclosure and publication policy

Manuscript(s) and abstract(s) resulting from the data collected during this study will be prepared by the study investigators (principal investigator and coordinating investigator) and the R-GNOSIS management. Site investigators will not publish or present interim or definite results, including but not restricted to oral presentations, without written consent of the principal investigator. They will be allowed to participate in publications regarding this study.
Site investigators will provide R-GNOSIS management with publication or presentation materials in advance of publication/ presentation to allow for review and comment as a means of ensuring confidentiality, accuracy, and objectivity. According to the rules of the International Committee of Medical Journal Editors, this study will be registered in a public trial registry.

10 References

(1) Silvestri L, van Saene HK, Milanese M, Gregori D, Gullo A. Selective decontamination of the digestive tract reduces bacterial bloodstream infection and mortality in critically ill patients. Systematic review of randomized, controlled trials. J Hosp Infect 2007 Mar;65(3):187-203.

(2) de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. Lancet 2003 Sep 27;362(9389):1011-6.

(3) de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med 2009 Jan 1;360(1):20-31.

(4) Labeau SO, Van d, V, Brusselaers N, Vogelaers D, Blot SI. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. Lancet Infect Dis 2011 Nov;11(11):845-54.

(5) Segers P, Speekenbrink RG, Ubbink DT, van Ogtrop ML, de Mol BA. Prevention of nosocomial infection in cardiac surgery by decontamination of the nasopharynx and oropharynx with chlorhexidine gluconate: a randomized controlled trial. JAMA 2006 Nov 22;296(20):2460-6.

(6) Brun-Buisson C, Legrand P, Rauss A, Richard C, Montravers F, Besbes M, et al. Intestinal decontamination for control of nosocomial multiresistant gram-negative bacilli. Study of an outbreak in an intensive care unit. Ann Intern Med 1989 Jun 1;110(11):873-81.

(7) Edwards SJ, Braunholtz DA, Lilford RJ, Stevens AJ. Ethical issues in the design and conduct of cluster randomised controlled trials. BMJ 1999 May 22;318(7195):1407-9.

(8) WHO Collaborating Centre for Drug Statistics and Methodology. DDD: Definition and general considerations. WHOCC website 2011 [cited 2011 Nov 15];Available from: URL: http://www.whocc.no/ddd/definition_and_general_considera/

(9) Smit MJ, van der Spoel JI, de Smet AM, de JE, Kuiper RA, van Lieshout EJ. Accumulation of oral antibiotics as an adverse effect of selective decontamination of the digestive tract: a series of three cases. Intensive Care Med 2007 Nov;33(11):2025-6.

(10) World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. WMA website 2008 [cited 2011 Nov 7];Available from: URL: http://www.wma.net/en/30publications/10policies/b3/index.html

(11) Ministerie van Volksgezondheid WeSN. The Medical Research Involving Human Subjects Act (WMO). Healthlaw website 2000 [cited 2011 Nov 7];Available from: URL: http://www.healthlaw.nl/humsub.pdf

(12) EMEA. Note for Guidance on Good Clinical Practice. EMEA website 2011 [cited 2011 Nov 7];Available from: URL: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002874.pdf

(13) Medical Research Council. Cluster randomized trials: methodological and ethical considerations. MRS Clinical Trials Series 2002 [cited 2011 Nov 7];Available from: URL: http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002406

(14) Oudemans-van Straaten HM, Endeman H, Bosman RJ, ttema-de Jonge ME, van Ogtrop ML, Zandstra DF, et al. Presence of tobramycin in blood and urine during selective decontamination of the digestive tract in critically ill patients, a prospective cohort study. Crit Care 2011 Oct 17;15(5):R240.

(15) Tantipong H, Morkchareonpong C, Jaiyindee S, Thamlikitkul V. Randomized controlled trial and meta-analysis of oral decontamination with 2% chlorhexidine solution for the prevention of ventilator-associated pneumonia. Infect Control Hosp Epidemiol 2008 Feb;29(2):131-6.

(16) Bleasdale SC, Trick WE, Gonzalez IM, Lyles RD, Hayden MK, Weinstein RA. Effectiveness of chlorhexidine bathing to reduce catheter-associated bloodstream infections in medical intensive care unit patients. Arch Intern Med 2007 Oct 22;167(19):2073-9.

(17) Climo MW, Sepkowitz KA, Zuccotti G, Fraser VJ, Warren DK, Perl TM, et al. The effect of daily bathing with chlorhexidine on the acquisition of methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus, and healthcare-associated bloodstream infections: results of a quasi-experimental multicenter trial. Crit Care Med 2009 Jun;37(6):1858-65.

(18) Vernon MO, Hayden MK, Trick WE, Hayes RA, Blom DW, Weinstein RA. Chlorhexidine gluconate to cleanse patients in a medical intensive care unit: the effectiveness of source control to reduce the bioburden of vancomycin-resistant enterococci. Arch Intern Med 2006 Feb 13;166(3):306-12.

(19) Lingnau W, Berger J, Javorsky F, Fille M, Allerberger F, Benzer H. Changing bacterial ecology during a five-year period of selective intestinal decontamination. J Hosp Infect 1998 Jul;39(3):195-206.

(20) Verwaest C, Verhaegen J, Ferdinande P, Schetz M, Van den BG, Verbist L, et al. Randomized, controlled trial of selective digestive decontamination in 600 mechanically ventilated patients in a multidisciplinary intensive care unit. Crit Care Med 1997 Jan;25(1):63-71.

(21) Oostdijk EA, de Smet AM, Blok HE, Thieme Groen ES, van Asselt GJ, Benus RF, et al. Ecological effects of selective decontamination on resistant gram-negative bacterial colonization. Am J Respir Crit Care Med 2010 Mar 1;181(5):452-7.

(22) Liberati A, D'Amico R, Pifferi S, Torri V, Brazzi L, Parmelli E. Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care. Cochrane Database Syst Rev 2009;(4):CD000022.

(23) Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ, et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. Am J Respir Crit Care Med 2002 Oct 15;166(8):1029-37.

(24) MRC/DH/MHRA Joint Project. Risk-adapted Approaches to the Management of Clinical Trials of Investigational Medicinal Products. Version: 10th October 2011

(25) Scottisch Intensive Care Society Audit Group, VAP Prevention Bundle - Guidance for Implementation, NHS National Services Scotland 2012

(26) Oostdijk EA, de Smet AM, Blok HE, Thieme Groen ES, van Asselt GJ, Benus RF, Bernards SA, Frenay IH, Jansz AR, de Jongh BM *et al*: Ecological effects of selective decontamination on resistant gram-negative bacterial colonization. *American journal of respiratory and critical care medicine* 2010, 181(5):452-457.

(27) Morris AC, Hay AW, Swann DG, Everingham K, McCulloch C, McNulty J, Brooks O, Laurenson IF, Cook B, Walsh TS: Reducing ventilator-associated pneumonia in intensive care: impact of implementing a care bundle. Critical care medicine 2011, 39(10):2218-2224.

Index of Appendices:

Appendix I: Definition of HRMO

Highly Resistant Micro Organisms (HRMO) comprises (multidrug) resistant gram negative bacteria (MDR-GNB) as well as other important resistant bacteria.

HRMO are defined as micro-organisms which:

1) are known to cause disease
2) have acquired an antimicrobial resistance pattern that hampers (empirical) therapy
3) have the potential to spread if – in addition to standard precautions – no transmission-based precautions are taken.

Three main groups of HRMO are distinguished:

- highly resistant **Enterobacteriaceae** (Table 1), including species of
  - ESBLs (all ESBL producing species)
  - All Carbapenemase - producing enterobacteriaceae
- highly resistant **gram-negative non-fermenters** (Table 2), including resistant strains of the following species:
  - Pseudomonas aeruginosa
  - Acinetobacter spp.
  - Stenotrophomonas maltophilia
- highly resistant **gram-positive bacteria** (Table 3), including
  - MRSA
  - Glycopeptide resistant Enterococci (GRE)

Definition of Highly Resistant Micro-Organisms[2]

Table 1. Definition of Highly Resistant *Enterobacteriaceae*

ESBL	Imipenem or Meropenem I/R	Colistin* I/R	Ciprofloxacin I/R	Gentamycin I/R	Amikacin I/R	Piperacillin I/R (if not an ESBL)	Cefotaxime or Ceftriaxone I/R (if not an ESBL)	Trimethoprim-Sulfamethoxazole I/R
A	A	A	B	B	B	B	B	B

A: resistance against an antibacterial agent from any one of the indicated groups in this category is sufficient to define the micro-organism as HRMO

B: resistance against antibacterial agents from at least three of the indicated groups of this category is required to define the micro-organism as HRMO

I: intermediate resistance; R: resistant; both I and R strains are considered resistant for the definition of HRMO's

* except intrinsically resistant Proteus, Providencia and Serratia spp.; these species are defined as a HRMO when they meet resistance criteria for the other antibiotics listed

[2] Modified from: A.P. Magiorakos et al., Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, Clinical Microbiology and Infection, April 2011

Table 2. Definition of highly resistant *Pseudomonas aeruginosa* or *Acinetobacter spp*.**

Imipenem or Meropenem or Doripenem I/R	Colistin I/R	Piperacillin or Pip/tazobactam or Ticarcillin I/R	Ceftazidime I/R	Gentamycin I/R	Amikacin I/R	Ciprofloxacine I/R
A***	A	B	B	B	B	B

A: resistance against an antibacterial agent from any one of the indicated groups of this category is sufficient to define the microorganism as highly resistant

B: resistance against antibacterial agents from at least three of the indicated groups in this category is required to define the micro-organism as HRMO

I: intermediate resistance; R: resistant; both I and R strains are considered resistant for the definition of HRMO's

** *Stenotrophomonas maltophilia* and *Burkholderia cepacia* are considered HRMO, whichever their resistance profile

*** For *Pseudomonas aeruginosa*, resistance to one of the other antibiotic groups is necessary to be defined as a HRMO

Table 3. Species that are considered HRMO, whichever their (additional) resistance profile

Vancomycin Resistant Enterococcus
Methicillin Resistant Stahpylcoccus Aureus
Stenotrophomonas maltophilia
Burkholderia cepacia
ESBL producing Enterobacteriaceae
Carbapenemase producing Enterobacteriaceae

Appendix II: Interruption of study medication in participants

Any eligible patient should be included in the study, and in principle receive study medication (except during the baseline period). However, in some situations, there may be a reason to interrupt or completely stop study medication in the participant. In such a situation, two things are important:

1. It should be documented in the ECRF
2. Data collection, including collection of surveillance samples and clinical culture data should continue **(i.e. withholding study medication is no reason to exclude an eligible patient)**

The R-GNOSIS study management advices to stop SOD or SDD in participants which have a clinical culture with:

- A micro-organisms that is only sensitive to colistin
- A micro-organism that is resistant to **both:**
    - carbapenems
    - **AND** tobramycin **OR** gentamycin **OR** colistin

Data collection on interruption of study medication

If application of study medication is interrupted or stopped in a patient for more than 24 hours or during 4 gifts or more, this should be reported in the case report form. This option is provided in the discharge form in Research Online, as shown in the textbox.

1. Was application of study medication interrupted for 24 hours or more (4 gifts or more) before extubation?

- Yes
- No

2. Which medication was interrupted?

- chlorhexidine 2% bodywash
- chlorhexidine 2% mouthwash
- SOD mouthpase
- SDD, only mouthpase was interrupted
- SDD, only enteric suspension was interrupted
- SDD, both mouthpaste and enteric suspension were interrupted

3. What was the reason for interrupting study medication in this patient?

- Patient has a clinical culture with a micro-organism that is only sensitive to colistin
- Patient has a clinical culture with a micro-organism that is resistant to carbapenems AND (tobramycin OR gentamicin OR colistin)
- Allergic reaction, please specify reaction: …
- Patient's (or proxy) decision, please specify reason: …
- Intolerance, please specify symptoms: …
- Gastro-enteral contra-indication:
  - Nil per os
  - Impaired bowel movements / gastric retention
  - No gastric tube in situ
  - Recent upper intestinal tract surgery
- Doctor's decision, please specify reason: …
- Other, please specify reason: …

4. What was the first day medication was interrupted?

dd-mm-yyy (calendar)

5. For how long has medication been interrupted?

– until extubation

Appendix III:

R-GNOSIS

Ecological Effects of Decolonisation Strategies in Intensive Care

Safety Committee Protocol

Part of

R-GNOSIS: Resistance in Gram-Negative Organisms: Studying Intervention Strategies

Feb 2014, version 2

UMC Utrecht

R-GNOSIS: Effect of decolonization strategies in Intensive Care	
Date	Dec 2013
Coordinating investigators	**B.H.J. (Bastiaan) Wittekamp,** MD, PhD Candidate Julius Center for Health Sciences and Primary Care University Medical Centre Utrecht Heidelberglaan 100 3584 CX Utrecht, The Netherlands Phone: +31 88 7555102 E-mail: b.h.j.wittekamp@umcutrecht.nl **N.L. (Nienke) Plantinga,** MD, PhD Candidate Julius Center for Health Sciences and Primary Care University Medical Centre Utrecht Heidelberglaan 100 3584 CX Utrecht, The Netherlands Phone: +31 88 7555102 E-mail: n.l.plantinga@umcutrecht.nl
Principle investigators	**Prof C. Brun-Buisson,** MD, PhD Director medical Intensive Care unit; head of Infection Control Hospital Henri Mondor, Créteil Assistance Publique – Hopitaux de Paris Université Paris-Est Créteil 51, Ave de Lattre de Tassigny, 94000 – Créteil, France Phone secr : + 33 1 4981 2391 [redacted] **Prof M.J.M. Bonten,** MD, PhD Professor of Molecular Epidemiology of Infectious Diseases University Medical Centre Utrecht Heidelberglaan 100 3584 CX Utrecht, The Netherlands Phone: +31 30 2507394

	Phone secr. +31 30 2523741 [redacted]

Introduction

This protocol describes the role of the Safety Committee during the R-GNOSIS “Ecological Effects of Decolonisation Strategies in Intensive Care” study, evaluating the effect of three decontamination strategies.
The objectives and details of the study are described in the study protocol. In summary the study will evaluate the efficacy of Selective Digestive tract Decontamination (SDD), Selective Oropharyngeal Decontamination (SOD) and Chlorhexidine Mouthwash (Oro-CHX) in ventilated ICU patients.

The safety committee consists of three external experts and has the objective to guard the ecological safety during the study. The SCom will have an active role during the intervention periods (SDD, SOD, Oro-chx). During these periods, the SCom will issue recommendations to continue or stop the study on a quarterly basis (three monthly), based on the results of monthly point prevalence cultures and input by participating ICUs. The committee will also receive an overview of SUSAR’s (suspected unexpected serious adverse reactions).

Amendments

This protocol version 2 from December 2013 replaces version 1 and includes the following amendments:

1. The ‘data-safety committee’ has been renamed ‘Safety Committee’, because they are not involved in the safety of data-collection itself, but rather in monitoring of the ecologic safety of the interventions (monitoring of resistance rates).
2. It has been decided to report point prevalence cultures to the SCom three monthly; the previous protocol left room for interpretation on this subject
3. A new member was added to the studyteam (Drs. N.L. Plantinga)
4. Prof. A.M.G.A. de Smet was appointed the chairman of the Safety Committee
5. The microbiology procedures have been adapted
    a. Point prevalence cultures will only be inoculated on MRSA chromogenic agar, VRE chromogenic agar and ESBL chromogenic agar (inoculation of carbapenemase chromagar media, colistin and tobramycin containing media has been abandoned)
    b. Once every three months, point prevalence samples will be inoculated on Mac Conkey media, to select three colonies for colistin resistance testing in the central laboratory at UMC Utrecht.
    c. Surveillance cultures will only be inoculated on ESBL chromogenic agar, after which colistin resistance testing is mandatory.
    d. Tobramycin resistance detection has become optional
6. The safety committee will also receive a three-monthly overview of SUSARs

SCom Members

The safety committee consists of three members. One of the members is the designated chairman of the SCom.

Prof. A.M.G.A. de Smet (chairman), MD, PhD, University Medical Center Groningen, University of Groningen, the Netherlands.

Professor de Smet is Anesthesiologist, Intensivist and Head of the Department of Critical Care Medicine at the University Medical Care Center of Groningen, the Netherlands. She has extensive experience in the field of decontamination strategies and coordinated the first large multi-center trial on SDD and SOD, published in 2009 (NEJM), amongst other publications around this theme.

Prof. A. Andremont, MD (University of Tours, France, 1976), PhD (University of Paris 11, France, 1986).

Doctor Andremont is certified in paediatrics, tropical medicine and medical microbiology. He is currently professor of microbiology at the University of Paris medical School in France, head of laboratory of Bacteriology at the 3BLM University Hospital Paris in France and head of the National Reference Center for bacterial resistance in commensal floras. Dr. Andremont has extensive experience on the impact of antibiotics on the commensal floras and expertise in infection control.

Prof. J. Chastre, MD (University of Medicine and Dentistry in Paris), PhD, University School of Medicine Paris 6, France.

Jean Chastre is Professor of Medicine at Paris 6 University School of Medicine and Director of the Medical Intensive Care Unit at Groupe Hospitalier Pitié–Salpêtrière in Paris, France. He is licensed and board certified in cardiology with a subspecialty in critical care. Dr. Chastre is a recognized expert in the performance of clinical outcomes research in the ICU setting. His clinical research has focused on the understanding, diagnosis, and treatment of pulmonary infections and the improved care of mechanically ventilated patients. He has published more than 190 peer-reviewed papers and authored or co-authored 18 book chapters.

The members are not involved in practical execution of the study or interpretation of data related to study outcome objectives. They have had no role in planning of the study and will play no role in publishing of data. The committee is therefore independent.

Primary *(added February 2015)* Objective: monitoring of antibiotic resistance

The primary objective of the safety committee is surveillance for emergence of resistant micro-organisms during the decolonisation strategies evaluated in the study. The intervention period is the time from start of the first regimen (after the baseline period) until the end of the last regimen. The intervention period does not include the baseline period, since no intervention other than standard care is planned in this period. However, monthly point prevalence cultures and surveillance cultures will be obtained during this period as well.

Emergence of resistance is measured by the prevalence of highly resistant micro-organisms (HRMO) in monthly point prevalence cultures obtained from all patients in the ICU. Also patients not receiving the decolonisation therapy will be cultured to determine the ecological effect.

HRMO's are defined according to the definition in appendix I of the Study Protocol and can also befound in the Safety Committee SOP and in the Microbiology laboratory manual. This definition is modified from an international expert proposal for interim standard definitions for acquired resistance.

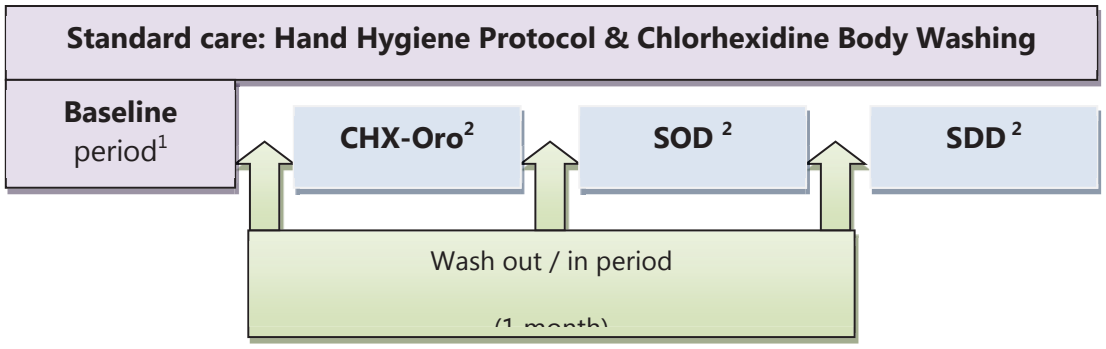
Prevalence of HRMO will be monitored on a monthly basis and reported to the Safety Commitee on a quarterly basis. Selective culture media will be used to screen for HRMO in point prevalence samples. The prevalence of HRMO and the prevalence of resistance mechanisms will be reported to the SCom. The high sensitivity of this method ensures adequate detection of resistant micro-organisms, although the lower specificity implicates false positive results. Positive cultures will be processed by the local laboratory to determine species and resistance pattern of the micro-organisms.

Point prevalence cultures: local testing Detection of resistant micro-organisms in all ICU patients	
	Respiratory and rectal
Monthly (e.g. 1st Monday of the month)	1. ESBL / Carbapenemase (rectal samples) 2. VRE (rectal samples) 3. MRSA (respiratory samples)

	4. Colistin (mandatory) and tobramycin (optional) resistance of isolates identified on ESBL chromogenic agar (rectal samples)

Study scheme *(adapted February 2015)*

RGNOSIS: ecological effects of decolonisation strategies in intensive care



[1] May include chlorhexidine mouthwashes if this was standard care before the study

[2] Randomised order per ICU

Clinical cultures

- Blood and respiratory samples obtained as part of routine standard patient care

Surveillance cultures

- Twice weekly in participants: peri-anal and respiratory culture
- Detect MDR-GNB and colistin resistance

Point prevalence cultures

- Monthly in all ICU patients: peri-anal and respiratory cultures
- Detection of MDR-GNB, colistin resistance, MRSA and VRE in all ICU patients.

**HRMO: Highly Resistant Micro Organism; MDR-GNB: multi-drug resistant gram negative bacteria, Per patient only 1st isolates of each HRMO from each body site will be stored and shipped*

Information provided to the safety committee

1. Results of point prevalence cultures obtained from all ICU patients during once monthly culture moments.

The results of the point prevalence cultures per ICU will be made available to the Safety Committee through a pre-defined format, which will summarise results per participating ICU. The SCom can obtain extra information from participating ICUs, if deemed necessary, by notifying the study coordinators, who will contact the specific ICU to provide extra data.

2. ICUs suspicion of increased resistance / outbreaks

When participating ICUs suspect an outbreak of resistant micro-organisms, the SCom should be notified. This suspicion can be based on any culture results available to the local sites, including twice weekly surveillance cultures of participants.

No patient specific data should be disclosed to the SCom and all data provided need to be free of patient identifiers.

3. Suspected Unexpected Serious Adverse Events.

The safety committee will also receive an overview of SUSAR's (suspected unexpected serious adverse reactions), and summary of intervention interruptions attributed to side-effects or intolerance to medications. *(added February 2015)*

Output by the Safety Commitee: Stop or go decision

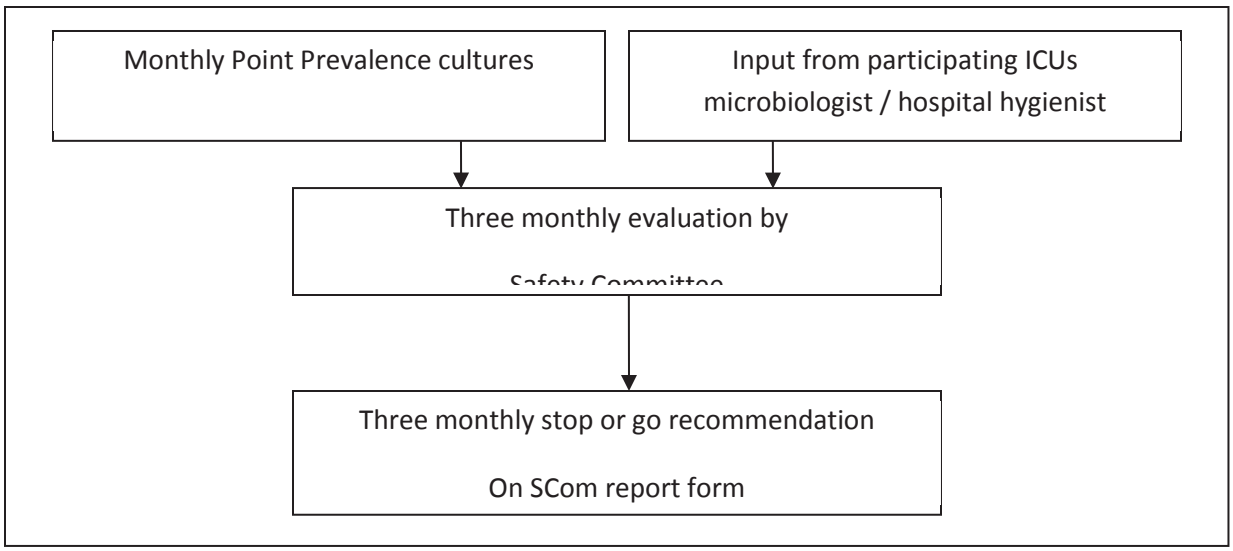
The members of the Safety committee decide on a three monthly basis whether there is a reason to stop or pause the study.

> *If the SCom deems that, based on screening results of point prevalence cultures or information provided by ICUs, there is a significant increase in the trend of the prevalence of a types of HRMO (VRE, MRSA or species producing carbapenemases / ESBL) the SCom will issue a recommendation to pause or stop the trial*

At least two of three members of the SCom should interpret culture results. The microbiologist or hospital hygienist can be consulted via the Study Management whether any observed increase in resistance prevalence could be caused by an outbreak or known trend in the hospital ecology, in other words if there are other circumstances that could explain the increase in antibiotic resistant organisms.

The stop or go recommendation should be issued three monthly by the chair of the safety committee. The form should be sent to the principle investigators.

The recommendation of the Safety Committee will be reported in the form of a SCom report form (see appendix). This form will be sent to the principle investigators (MB and CBB) and study coordinators (BW and NP) as soon as consensus on a recommendation has been reached by the SCom. Participating ICUs will only be notified when the SCom recommends to stop or pause the study.


Monthly Point Prevalence cultures
Input from participating ICUs
microbiologist / hospital hygienist
Three monthly evaluation by
Safety Committee
Three monthly stop or go recommendation
On SCom report form

Stopping or pausing the Trial

Pausing the study on a particular ICU will mean that on that ICU:

- No patients will be included in the study at the specific site.
- No patients will receive study related interventions unless this is deemed necessary by the local health care provider to control the outbreak.
- Data recording from included patients will not necessarily be stopped as this will not influence patient outcome, unless there is no intention to re-start the study.
- There are no limitations on infection prevention measures because of the study. All measures deemed necessary by the participating ICU to control an increase in antibiotic resistance are allowed.

Point prevalence cultures need to be continued during the interruption of the study in order to monitor the ward ecology and to determine when to restart the study.

Continuation of the trial

The trial can be continued if no outbreak is detected or cultures prove that the monthly prevalence of the HRMO reduces and is equal to or below the prevalence during the baseline period (standard care only).

Management of increased antibiotic resistance

Participating ICUs are responsible for management of an increase in prevalence of resistant micro-organisms. Management should be in accordance with local protocols according to good clinical practice. Consulting expertise from investigators is allowed and not regulated by this or any other protocol.

Summary of the Safety Committee protocol

Based on the results of monthly point prevalence cultures of all ICU patients the Safety Commitee will monitor ecological safety of the trial on a three monthly basis. Results of the point prevalence cultures will be presented to the SCom as: Prevalence of highly resistant micro-organisms according to the definition in the microbiology laboratory manual.
This will be specified in:

- Prevalence of highly resistant species of micro-organisms (e.g. prevalence of colistin resistant E.coli, MRSA or VRE)
- Prevalence of resistance patterns / resistance mechanism (e.g. prevalence of ESBL, colistin resistance)

In addition, participating ICUs should inform the SCom when an increase in prevalence of resistant micro-organisms is suspected.

On a three monthly basis the SCom will reach consensus on whether the trial can continue or should be paused:

If, based on results of point prevalence cultures, there is a significant increase in the trend of the prevalence of a class of HRMO (VRE, MRSA, carbapenemases / ESBL) the SCom should recommend pausing or stopping the trial.

The chair of the Safety Committee will report this recommendation to the principle investigators and study coordinator on the SCom report form (appendix).

More information on safety committee procedures can be found in the “Safety Committee Standard Operating Procedures”.

SCom report form: [*months and year*]

Date: ...-…-…

Based on the information provided, dated (…-…-…), the safety committee recommends

□ Continuation

□ Interruption*

of the "ecological effects of decolonisation strategies in intensive care" study.

* Motivation in case of interruption of the trial:

Could the (relative) increase of antibiotic resistant bacteria be caused by an ecological effect outside the study, according to the local investigator* / local microbiologist* / local infection control specialist*
□ Yes*
□ No*
□ Unknown

Please specify any contra-arguments for the recommendation (if applicable):

Chair of the Safety Committee:_____ **Signature:** _____

Send completed form to N.L.Plantinga@umcutrecht.nl
And the original to:

B.H.J. Wittekamp / N.L. Plantinga / M.C.Hopman, RGNOSIS WP6

Julius Center for Health Sciences and Primary Care

Stratenum 6.131

PO Box 85500

Appendix IV: Patient information leaflet

This information leaflet will be available during the study to all patients or representatives

Patient information RGNOSIS: effectiveness of preventive anti-bacterial treatment

A study is taking place on this ICU called: RGNOSIS: ecological effects of decolonisation strategies in Intensive care. The study is examining methods to reduce the emergence of resistant bacteria, which is a serious problem in intensive care patients. Previous research has shown that applying certain treatment interventions can reduce both the number of infections and the presence of resistant bacteria in the intensive care. These treatments have been used as standard care throughout the world for many years, but they have not been compared to each other yet.

Why this study? To evaluate the effect of three different treatments on the occurrence of bacteria, bacterial infections, resistance of bacteria to antibiotics and to establish which - if any - treatment is the best.

Who is participating in this study?

All adult patients undergoing mechanical ventilation (except from pregnant patients and patients with a known allergy to the treatment) are participating in this study and will receive treatment according to the study protocol.

How is the study performed?

Treatment which aims to reduce resistant bacteria will be given to each eligible patient. Each participant receives the same therapy during a certain period. Twice weekly, sputum and rectal samples will be obtained to measure the effect of therapy.

What are the treatments being used?

All patients will receive standard treatment during the entire study period. This treatment consists of: daily body washing with an antiseptic (chlorhexidine 2%) AND standard oral care AND a hand-hygiene program for health care workers as endorsed by the World Health Organisation.
According to 4 different study periods, each participant will receive one of the following extra treatments depending on his or her admission date:

Standard treatment only (no extra treatment) [period: DD-MM-YYYY until DD-MM-YYYY]

Oral care with a chlorhexidine gel, comparable to a toothpaste. The purpose is to kill bacteria in the mouth. [period: DD-MM-YYYY until DD-MM-YYYY]

Antibiotic mouthpaste will be given to patients four times daily to kill bacteria in the mouth.

[period: DD-MM-YYYY until DD-MM-YYYY]

Appendix V: SUSAR report form

The SUSAR report form can be found in the investigator site file. It should be filled in and sent within 24h to the coordinating investigators and project manager in Utrecht (addresses are included on the form)

Ecological effects of decolonisation strategies in intensive care

Serious Adverse Reaction (SUSAR) report form

version 2, Feb 2015

- ✓ Please see chapter 7.3 of the standard operating procedure to see what the criteria for reporting a serious adverse reaction are
- ✓ Please make sure you inform the coordinating investigator and project manager by mail or phone, within 24 hours(n.l.plantinga@umcutrecht.nl, b.h.j.wittekamp@umcutrecht.nl, m.c.hopman@umcutrecht.nl

Date of report:

RGNOSIS ID number of the participant:

Gender of the participant

- o Male
- o Female

Date of birth of the participant:

Date of serious adverse reaction:

Please describe the reaction in headlines (most important features/symptoms of the event, diagnosis if possible, detailed description can be given at the end of the form):

Is this event described in the available product information? (IMPD)

- o Yes
- o No

Name of the medication that is expected to have caused the SAR (active drug substances are described in brackets):

- o Chlorhexidine gel 1%

- SOD oropharyngeal paste (20 mg/g colistin sulphate, 46 mg/g nystatin (= 250.000 units/g) and 30.5 mg/g of tobramycin sulphate (= 20 mg/g tobramycin))
- SDD suspension (10 mg/ml colistin sulphate, 36.8 mg/ml nystatin (= 2 * 10^5 units/ml) and 12.2 mg/ml of tobramycin sulphate (= 8 mg/ml tobramycin))

Indication

Selective decontamination of the digestive tract to reduce colonisation with gram-negative bacteria.

Since when did the patient receive this medication (date and time)?

What dose did the patient receive?

……. mg/time and ……..… times/day

What was the route of administration of the drug?

- Nasogastric tube
- Oral
- Other: …

When did the patient receive the last medication (date and time)?

What is the batch number of the study medication:

How was administration of the study drug adjusted after the reaction?

- Stopped
- Dose lowered
- Dose increased
- No change
- Unknown
- Other: ……

Why was the event serious?

- Patient died
- Life threatening event
- Extension of hospital stay
- Resulted in persistent or significant disability or incapacity
- Other: ……..

Please describe the reaction that was noticed in this patient (nature of event, treatment given as reaction)

Please describe other relevant patient characteristics (reason of admission, relevant co-morbidity, relevant co-medication)
Please describe the possible relation between the event and the study drug
Did the patient use other drugs that are suspicious, and if so, describe which:
Did the patient recover?
o Yes o Is recovering o No o Recovered with sequelae o Patient died o Unknown
What was the date the patient recovered?
Were there other events (SUSAR's) in this patient?
o Yes (please add copy of the file of that report) o No
Does this reaction have consequences for the safety of other participants in this study?
o Yes, because: …… o No
According to you, what should be the consequences of this event for the study?

- Pause the study
- End the study
- Adapt patient information
- Adapt dose of the medication
- Adapt eligibility criteria for patients
- Other:

Additional remarks (Other information that could be relevant)

Contact details of the person reporting the SUSAR
Surname:
Title and initials:
Company / hospital
Address;
Telephone number:
Fax number:
e-mail address:

Information update (optional)

Date of most recent information update regarding this SUSAR (optional)
Most recent information update (optional)

Decontamination strategies in Intensive Care Units

Statistical analysis plan

Statistical analysis plan*

Analysis will determine the effect of each intervention on colonization rates, the occurrence of bacteraemia and the use of antibiotics. Statistical analysis of the primary objective and secondary objectives regarding mortality, antibiotic use and rate of colonisation and bacteraemia will include the use of random-effect logistic regression models to account for ICU-level clustering. Measured confounding factors will be fitted as covariates. Outcome will be presented as odds ratios.

All available data on patient colonization with MDR-GNB (both from screening and clinical cultures) will be used to determine, as carefully as possible, the ESBL-colonization status of each patient on every study day. Together with work package 8 (mathematical modelling, WP8), nosocomial transmission capacities (RA values) for different species of MDR-GNB during study regimens will be quantified. As a secondary aim species-specific RA values will be compared between wards. Available data will be used to quantify incidences of cross-transmission in both study periods, using sophisticated modelling approaches to be developed in WP8.

Decontamination strategies in Intensive Care Units

Statistical analysis plan (final version)

Available on Clinicaltrials.gov, identifier NCT02208154

Introduction

The aim of the analysis is to determine the effect of each intervention in the occurrence of bacteremia, patient survival, colonization rates, and the use of antibiotics. Statistical analysis of the primary objective and secondary objectives regarding mortality, bacteremia and ward-level colonization with antibiotic resistant bacteria will account for ICU-level clustering and the statistical methods used are described in detail in below. The use of antibiotics will be a descriptive statistic.

All available data on patient colonization with MDR-GNB (both from screening and clinical cultures) will be used to determine, as carefully as possible, the extended-spectrum beta-lactamase (ESBL) colonization status of each patient on every study day. Nosocomial transmission capacities (RA-values) for different species of MDR-GNB during study regimens will be quantified. As a secondary aim species-specific RA values will be compared between wards. Available data will be used to quantify incidences of cross-transmission in both study periods, using sophisticated modeling approaches.

Investigators and the R-GNOSIS staff will make every attempt to collect complete data from all subjects enrolled in the study. Where possible, automatic extraction of data from hospital information systems will be used (without disclosing patient identifiers). There will be regular contact between the study coordinating centre and study sites to track and retrieve missing data. Should culture results be inadvertently lost, those data will be treated as missing at random. All inferential analyses will be based on available data.

The detailed statistical analysis plan has been established prior to database lock and is divided into two parts.

Clinical outcomes (patient data)

The data

The data analysis will be performed on all patients included during the baseline period, the last 2 weeks of the wash-out/in periods and those included during one of the three intervention periods. Two ICU admissions of the same patient with less than 3 days in between will be merged and analyzed as one ICU admission.

The following cohorts will be made for analysis of the following clinical outcomes:

1) Cohort "ICU-admissions": ICU-acquired bacteremia, ICU survival.
2) Cohort "Hospital-admissions": Hospital survival
3) Cohort "first ICU-admissions", excluding re-admissions to the ICU within 30 days after prior ICU-discharge: 28-day survival

Missing data

Missing data will be retrieved where possible, after which a complete case analysis will be performed.

Statistical models

To adjust for potential selection bias in this cluster randomized trials with crossover (without blinding), the statistical analysis will be performed using doubly robust estimation. (1)

Propensity score model

The propensity score model will include the following a priori selected confounders:

- ✓ Age
- ✓ Gender
- ✓ Disease severity (either APACHE II or SAPS II score)
- ✓ Use of antibiotics upon ICU-admission
- ✓ Prior location before ICU-admission
- ✓ Admission type (medical/surgical/trauma)
- ✓ Charlson comorbidity score
- ✓ Hospital of recruitment (hospital)

As two different scoring methods , APACHE II or SAPS II, have been used to determine disease severity by different hospitals, two separate propensity score models will be fitted (one for hospitals that recorded APACHE II and one for hospitals that recorded SAPS II).

These propensity score models will be fitted in the cohort "ICU-admissions" using the R-package 'twang'. (2) This package uses generalized boosted models machine learning techniques to calculate weights for each patient. The resulting weights represent the inverse probability for a patient to be included in the baseline, CHX, SOD or SDD arm and will be used to weigh the data in the outcome models, creating pseudo-populations with an equal distribution of the specified covariates over treatment groups.

Outcome models

Separate models will be fitted per endpoint, as specified in table 1. All models will include the inverse probability weights, the confounders included in the propensity score model and the mean hand hygiene compliance per study period per hospital (hand hygiene compliance might differ per study period and act as a confounder on all outcomes) to obtain doubly robust estimators. A dummy variable indicating the measure of disease severity (APACHE II or SAPS II) will be included as an interaction with the standardized disease severity to overcome different hospital having registered different measures. In addition, two levels of clustering will be taken into account, as follows:

- Hospital of recruitment: fixed effect, acknowledging that the risk of the outcome differs per hospital
- Cluster period (i.e. periods 1-4 per hospital): random intercept, acknowledging that patients recruited in different periods within hospitals may be more alike with regard to the risk of the outcome.

Table 1. Outcome models per endpoint

Endpoint	**Model**	**Family**	**Link**	**Competing endpoints**	**Cohort**
ICU-acquired bacteremia	Cox proportional hazard	*NA*	*NA*	- ICU discharge - Death in ICU	"ICU-admissions"
ICU survival	Cox proportional hazard	*NA*	*NA*	- ICU discharge	"ICU-admissions"
Hospital survival	Cox proportional hazard	*NA*	*NA*	- Hospital discharge	"Hospital-admissions"
28-day survival	Generalized linear	(quasi)binomial	logit	*NA*	"First ICU-admissions"

Abbreviations: ICU, intensive care unit; NA, not applicable

Results will be presented as hazard ratios or odds ratios with 95%-CI.

R and STATA will be used to perform the analyses specified above.

Sensitivity analysis

As eligibility in this open cluster-randomized study was defined as "Expected length of MV >24h", selection bias may have occurred (as discussed under 'confounding adjustment'). To quantify this potential bias a sensitivity analysis will be performed in which patients who left the ICU within 2 days after study inclusion are excluded, as these patients could not reach the ICU-acquired bacteremia primary endpoint (which requires at least three days in ICU).

Exploratory analysis

As an exploratory analysis the treatment effect on 28-day survival and ICU-acquired bacteremia per ICU will be visualized in Forest plots, in which ICUs are ranked on the prevalence of bacteremia with highly resistant micro-organisms (HRMO) during the baseline period.

Antibiotic resistance (ward level data)

The data

The monthly point prevalence screenings on both included and non-included patients will be analyzed on two levels, each including outcomes for individual HRMO (e.g. carbapenem resistant GNB, MRSA, etc.) and the aggregate "any HRMO".

1) Cohort "respiratory tract"
2) Cohort "digestive tract"

Missing data

If a patient or tractus was not sampled, it will be excluded from the analysis (and the denominator). If an antibiotic susceptibility result was missing, the highest susceptibility result from the same species in the same tractus 7 days prior or after the point prevalence date was "imputed", if available. Completeness of susceptibility testing will be reported as a descriptive statistic.

Since monthly point prevalence measurements are taken on fixed days (i.e. first Monday, occasional exceptions accepted) on all patients present in the ward (both included and non-included) we do not expect bias due to selective inclusion per study period (as in the analysis of

the clinical outcomes).

Final models

We will perform logistic regression analyses with a log-link for each endpoint and include terms for underlying time-trend per hospital (months since study start * hospital) and time-trend per intervention (months since start study period * study period) and correct for repeated measurements on the same patient (corrected standard errors with sandwich estimator). Results will be presented as risk ratio's with 95%-CI.

References

1. Funk MJ, Westreich D, Wiesen C, Sturmer T, Brookhart MA, Davidian M. Doubly robust estimation of causal effects. American journal of epidemiology. 2011;173(7):761-7.

2. McCaffrey DF, Griffin BA, Almirall D, Slaughter ME, Ramchand R, Burgette LF. A tutorial on propensity score estimation for multiple treatments using generalized boosted models. Stat Med. 2013;32(19):3388-414.

Summary of changes to the statistical analysis plan

The original statistical analysis plan was included in the study protocol.

A more detailed description of this statistical analysis plan was posted on clinicaltrials.gov before analyses were performed (see page 119 of this document and onwards).