Study Protokoll of the Karmin II Study

Version 16.3.

Principal investigator: Prof. Dr. med. Petra Gastmeier

Co-Principal investigator: PD Dr. med. Rasmus Leistner

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1) Synopsis

Title of the Protocoll	Environmental cleaning strategies against hospital-acquired infections in non-intensive care wards
Intervention	The study will compare 3 non-ICU-level environmental cleaning agents applied to reduce hospital-acquired infections among adult medical and surgical patients. The three cleaning agents will be the following: A standard, commercially available soap product A standard, commercially available disinfecting product A standard, commercially available probiotic product All cleaning agents will be commercially available in already in use and approved for the purpose of environmental cleaning in German hospitals.
Objectives	Primary objective: Determine the additional effects of disinfection or probiotic cleaning in comparison with soap to reduce the incidence of hospital-acquired infections (HAI) Secondary objectives: Incidence densities of different types of HAIs Incidence densities of different HAI pathogens Incidence densities of different multidrug-resistant HAI pathogens
Hypotheses	Primary Hypothesis Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a reduction in the risk of hospital-acquired infections. Secondary Hypotheses Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a shift in distribution of pathogens in hospital-acquired infections. Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a reduction in the risk of hospital-acquired infections by multidrug-resistant pathogens Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a reduction in the different types of hospital-acquired infections.
Study design	The study is a single centre, cluster randomized, 3 period, 3 treatment, 3 sequence crossover trial.
Study population	Inclusion criteria Adult patients admitted to one of the designated non-ICU study wards. Exclusion criteria Intensive care units. Wards that do not comply the study's cleaning protocol.
Sample size	Assuming an average new acquisition of 0.36 HAIs /100 non-icu patients, design effect (DE) of 1.05 and a

	corresponding intracluster coefficient (ICC) of 0.0001, 18 wards with 15,630 patients (5,210 per study arm) will have a power of at least 80% to detect at least a reduction of 30% reduction of newly acquired HAIs in either of the two cleaning strategies (probiotic or disinfectant) compared to soap cleaning.
Randomization	Randomization into one of three arms will occur at the floor level containing 2 wards each. The block size will be 6. After the start cleaning protocol the subsequent cleaning agent will follow this order: Soap – Disinfection – Probiotitic-Soap-Disinfection.
Data Analyses	Descripitive, univariate analysis will be computed at the arm level. For the primary analysis incidence densities of HAIs will be computed on the arm level. For secondary analyses incidence densities of pathogens, multidrug resistant pathoges and typos of hospital-acquired infections. Further multivariable analyses will be computed enabling hazard ratios for hospital-acquired infections adjusted for the different cleaning strategies using soap as reference group and adjustment for further confounders will be calculated by Cox-proportional hazard regression analysis.

2) Persons/Institution involved in the study

a. Sponsor

Federal Ministry of Education and Research of Germany (03Z0818C).

b. Investigators

Pricipal investigator:

Prof. Dr. med. Petra Gastmeier, Institute of Hygiene, Charité

CoPrincipal investigator:

Dr. Rasmus Leistner, Institute of Hygiene, Charité

Coordinator of environmental cleaning:

Gregor Zakonsyk, Charité Facility Management

3) Background

Compared to other sources of infection, the significance of microbial contamination of inanimate indoor surfaces as a source of hospital-acquired infections (HAIs) has been insufficiently studied. [1] Even though, the number of studies on the association between environmental contamination and infection risk is growing, the causal connection between environmental cleaning and hospital-acquired infection often cannot be proven.[2-4]

Studies on the microbiome have shown that a reduced diversity of microorganisms can lead to an increased risk of infection.[5] Therefore, it was hypothesised that not only the presence of pathogens, but also the absence of a diverse composition of non-pathogenic environmental bacteria in the hospital environment may play a role in the development of nosocomial infections.[4,6,7] Based on this, studies were conducted on the influence of probiotic microorganisms on the hospital microbiome and its impact on nosocomial infections. Different predominantly lactobacillus species are used in probiotic cleaning agents.[8] Colonisation with probiotic bacteria can prevent the multiplication of pathogens by increasing competition for nutrients and space, and by secreting secondary metabolites that confer a survival advantage. For example, if microorganisms are removed from a surface by disinfection, diversity is disrupted. If a pathogen enters, it can thrive and colonise the space according to the exclusion principle of competition due to the lower competition. A recent study from Italy showed that probiotic cleaning may reduce pathogenic bacteria and provide relevant protection against hospital-acquired infections.[9]

Overall, there exist different environmental cleaning strategies such as the use of soap, disinfectant or even probiotics in medical facilities.[4,10] Nevertheless, there is a significant scarcity of high quality studies that assess at all the influence of different cleaning strategies like disinfection, soap or the recent option probiotic cleaners as means to control hospital-acquired infections.[11-13] To our knowledge, to date, there is no study that investigated the impact of different environmental cleaning strategies on the incidence of HAIs, using a state of the art study design such as randomized-controlled study.

4) Hypothesis

c. Primary Hypothesis

Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a reduction in the risk of hospital-acquired infections.

d. Secondary Hypotheses

Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a shift in distribution of pathogens in hospital-acquired infections.

Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a reduction in the risk of hospital-acquired infections by multidrug-resistant pathogens

Environmental cleaning with disinfection or probiotic agents in the hospital will not lead to a reduction in the different types of hospital-acquired infections.

5) Methods

a. Studiendesign:

The study is a single centre, cluster randomized, 3 period, 3 treatment, 3 sequence crossover trial. Core of the study are three environmental cleaning strategies with cross-over design, which will be performed on 18 wards of one medical centre. The three strategies will be compared for incidence of hospital-acquired infections in different wards for 4 months each. Because these strategies will be implemented as a ward-wide measure, all patients hospitalized on a study ward will be subjected to the allocated cleaning strategy of the ward (Figure 1). Wards will be assigned to the cleaning strategy in a random order and will then switch strategies after a wash-in period of one month in the 4 months. The strategy that alters the surface microbiome the least serves as reference.

Figure 1: Study design and

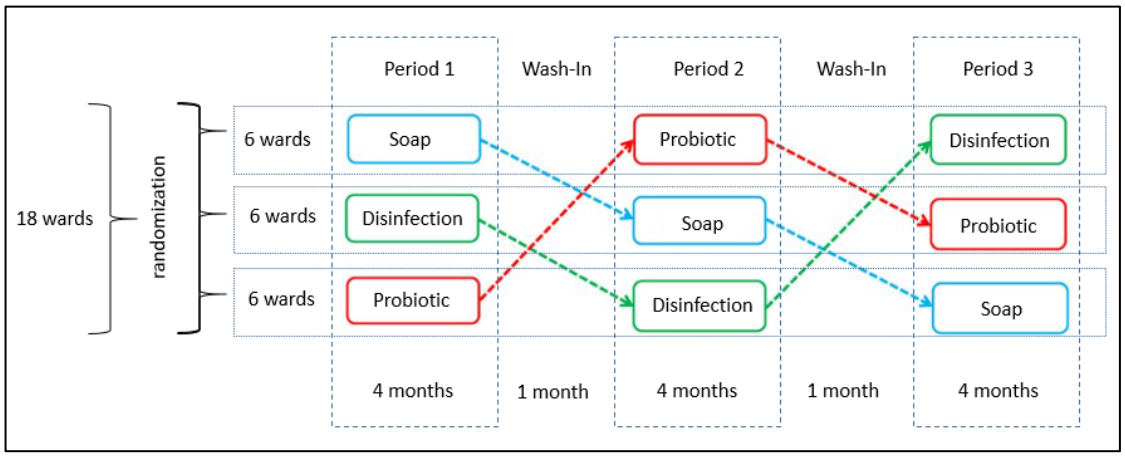
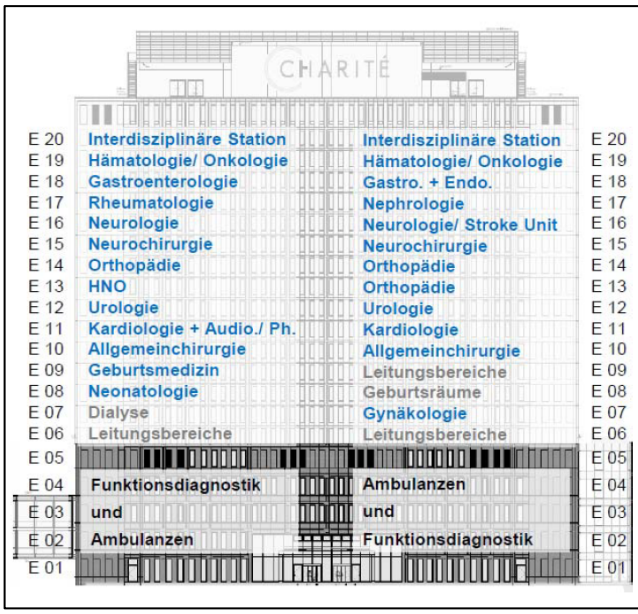


Figure 2: concreate planning at the study site



Station	Bemerkungen	06-10/2017	11/17-03/18	04-08/2018
M120A+B	Interdisziplinär	PB	R	D
M118A+B	Gastro	D	PB	R
M117A+B	Rheuma	PB	R	D
M115A+B	NCH	R	D	PB
M114A+B	Ortho	R	D	PB
M113A+B	HNO/Ortho	D	PB	R
M112A+B	Urologie	R	D	PB
M111A+B	Kardiologie	PB	R	D
M110A+B	Chirurgie	D	PB	R

R=Reinigungsmittel
D=Desinfektionsmittel
PB=Biotisches Reinigungsmittel

b. Standard of environmental cleaning

The Cleaning standard will be in line with the general cleaning standard of the entire study site (Charité Universitätsmedizin Berlin). Following rules apply for all cleaning personnel and procedures: After each room, discard all used wipes. After each room, all used wipe covers must be replaced and collected for wipe cover reprocessing. Hands must be disinfected before and after each room.

Maintenance cleaning is performed once a day in all patient rooms. This type of cleaning is divided into four types of, for teaching reasons, colour-coded surfaces (blue, yellow, pink and grey). To avoid cross-contamination from the wipes used, each of these surfaces is treated with a cleaning agent from separate color-coded buckets. In patient rooms, these are frequently touched surfaces such as door handles and handrails (blue). In wet rooms surfaces such as sinks and shower stalls (yellow) and toilet surfaces (pink). A fourth surface is the floor in patient rooms and wet rooms (grey).

Figure 03: Blue surfaces

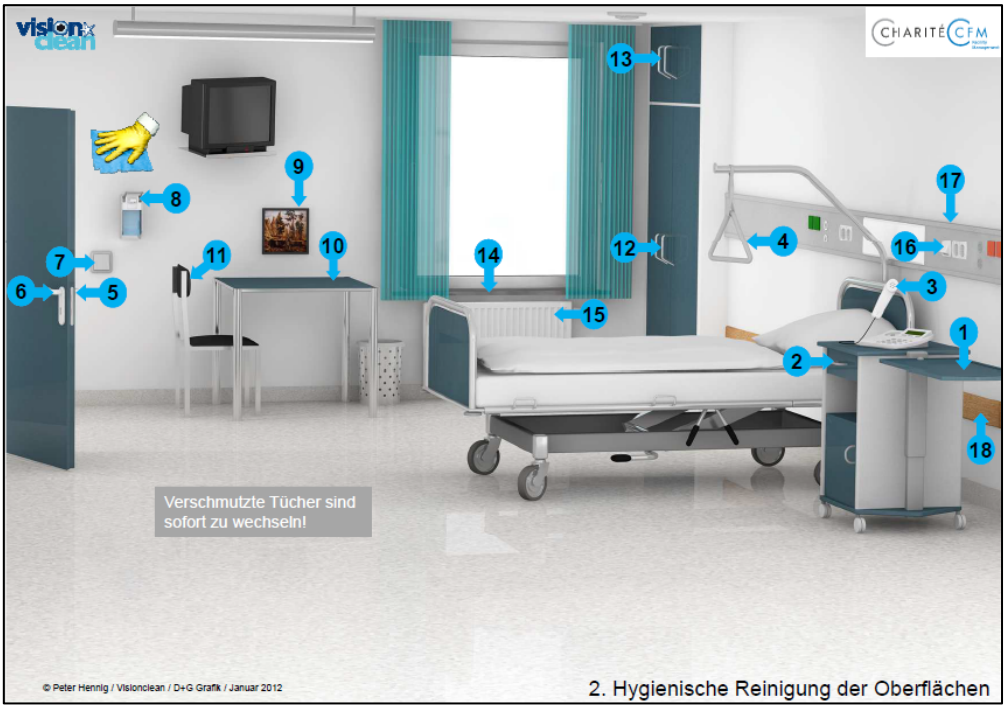


Figure 04: Yellow surfaces

Figure 05: Pink surfaces

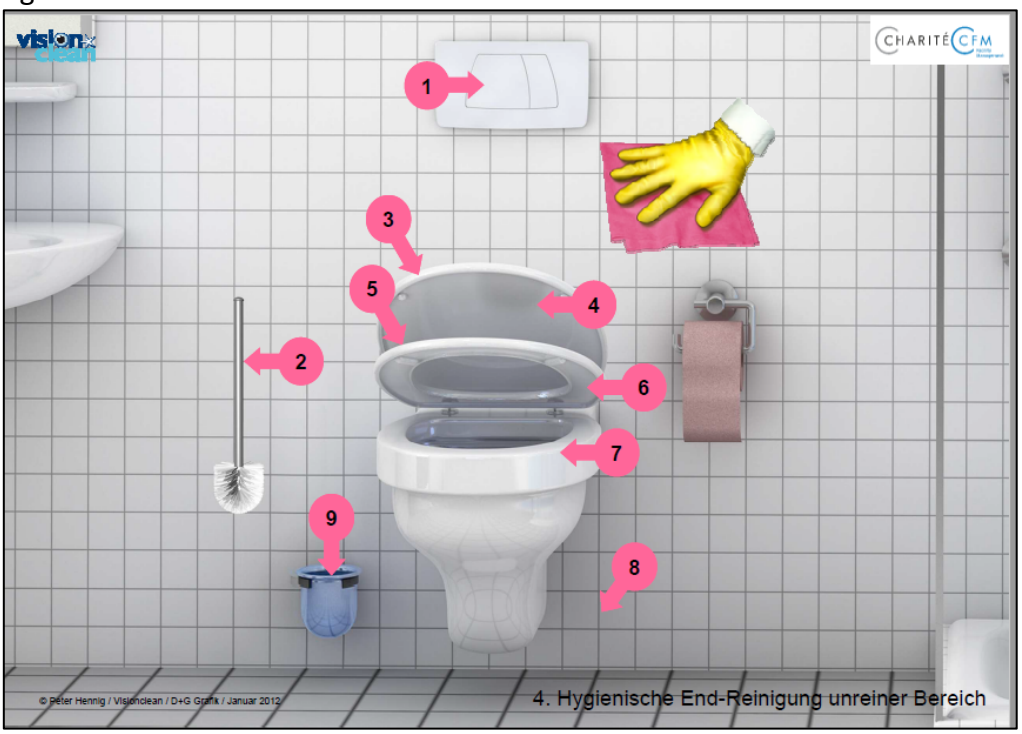


Figure 06: Grey surfaces

The cleaning of rooms with a potential risk of infection is defined as final cleaning. The cleaning of these rooms will only be carried out by personnel trained in this standard operating procedure. The following rooms will be subjected to targeted cleaning based

on potential infection risks after stays by patients who have had the following infections or colonization with multi-resistant pathogens, non-enveloped virus infections, measles open pulmonary tuberculosis infections.

c. Cleaning agents

- The soap product containes non-ionic surfactants, anionic surfactants, and fragrances (Limonene, Linalool, Butylphenyl Methylpropional, Citronellol, Coumarin) and Alkylethersulfat 2%, 2-(2-Butoxyethoxy)ethanol 2% in a total concentration of 1% (Brial Top®, Ecolab Inc.).
- The disinfectant product containes 2-phenoxyethanol (10%), 3-aminopropyldodecylamine (8%), benzalkonium chloride (7.5%) at a total concentration of 1%, with a contact time of 15 minutes (Incidin Pro®, Ecolab Inc.).
- The probiotic product containes a combination of bacteria: overall, $5x10^7$ CFUs/ml of Bacillus subtilis (ATCC6051), Bacillus megaterium (ATCC14581), Bacillus licheniformis (ATCC12713), Bacillus pumilus (ATCC14884), and Bacillus amyloliquefaciens (DSL13563-0) with a total concentration of 1% (PIP®, Chrisal NV).

d. Study periods and time line

The required study period is estimated based on the sample size calculation and the estimated time necessary to insure a sufficient number of patients included in the study (see there). To our knowledge, there is no study on to which extend different cleaning regimens influence each other. However, a study recently performed by our group showed that a steady state hospital microbiome is achieved after about 4 weeks of patient occupation (not published, preliminary data). Thus a wash-in period of 4 weeks will be established after the change of cleaning procedure. During this wash-in period no HAI analysis will occur. The analysis starts after the wash-in period. The analysis of incidence of hospital-acquired infections will then follow the 3 day rule. This means that only after day 3 of the analysis period HAIs can be detected.

e. Selection And Withdrawal Of Wards

The study will be realized in 18 wards in one hospital during a 15-month period from 07/2017 to 08/2018. Wards with adult medical and surgical wards will be selected for the study. Because cleaning strategies will be applied at the hospital level, the participation of the units requires the approval of the Charité head of infection control. To have equal conditions in all intervention periods average length of stay is expected to be no varying. The investigators may discontinue the participation of a study ward for the following reasons:

- The study ward does not follow the trial protocol with regard to the cleaning protocol
- The study ward does not implement the assigned strategy to satisfaction.
- The study ward has objections and rejects the participation in the study

In case the protocol is not sufficiently implemented, this issue should will be discussed among the investigators. They will decide whether replacement of the ward is

necessary. In the event of an unexpected change in antimicrobial resistance epidemiology in a certain ward (e.g. an outbreak), creating a situation in which adherence to protocol can no longer be recommended, the site will temporarily be withdrawn and alternative wards will be included. In the event of the withdrawal or discontinuation of a ward, the investigators will decide whether replacement of the ward is necessary. However, an ITT (intention to treat) analysis will be performed.

f. Randomization

Randomization will occur at the ward level. Groups of three wards will be randomized to one of the three arms. The investigators will be aware of the assigned cleaning strategy in order to monitor implementation of each strategy. Data analysts will be unaware of the IC strategy assignment.

g. Endpoints and Outcomes

Primary endpoints

The primary endpoint of the study is incidence density of hospital-acquired infections across the study period. The primary outcome is hospital-acquired infection (HAI) rate per 1,000 patient days. Hospital-acquired infection is defined as a newly acquired infection >3 days after hospital admission (admission day = day 1). If a patient leaves the hospital the surveillance for HAIs ends with the day of discharge. If a patient is transferred from one ward to another ward using the same environmental cleaning strategy, the patient stays in the analysis and the count of exposure days as well analysis for HAIs goes on in the respective study arm until the patient leaves the hospital or changes to a ward with another cleaning strategy. If the patient is transferred from one ward into another ward, using a different cleaning strategy the patients automatically switches the study arm. In this case a new count starts and the patient can contract a HAI only after three days on the second (and different cleaning strategy) ward. If in this case, during the first 3 days the patients is diagnosed with a HAI, it is counted for the former ward.

The definition of hospital-acquired infections will be in accordance with modified CDC definitions.[14] Most hospital-acquired infections require microbiological detection of a pathogen. Thus, analysis of HAIs will retrospectively be performed by analysis of microbiological results produced during routine clinical workup. The study personal is not allowed to be involved in this routine clinical workup. The HAI analysis will be patient based thus evaluating each pathogen detection for each patient individually for fulfilling of CDCs HAI surveillance criteria. [14] This analysis will be performed two times, each time by another study nurse trained in infection control. The results of both analyses will be compared and differences will be discussed together with the co-PI in order to achieve consensus. The analysis personal will be blinded concerning the cleaning procedure used during the time of individual patient stay.

Secondary Endpoints

- Distribution and incidence densities of different types of hospital-acquired infections such as urinary tract infections (UTI), hospital-acquired pneumonia

(HAP), blood stream infections (BSI), and surgical site infections (SSI) will be in accordance with modified CDC definitions. [14]

- Distribution and incidence densities of different pathogens found in the microbiological examination used as basis for the HAI criteria.
- Distribution and incidence densities of different multidrug-resistant pathogens found in the microbiological examination used as basis for the HAI criteria. The multidrug-resistant pathogens assessed will be methicillin-resistant S. aureus, Vancomycin-resistant Enterococcus spp. and multidrug-resistant Gram-negative pathogens (3. Generation cephalosporin-resistance plus fluoroquinolone-resistance)

6) Data collection

Data sheet for documentation of HAIs

Infektionserfassungsbogen für Normalpflegestationen Karmin

(Nur für Patienten mit einer unter Surveillance stehenden nosokomialen Infektionen auszufüllen. Nur eine Infektion je Bogen)

Krankenhaus:	CCM	Station:	
Patientenkennung:		Art der Station:	
Aufnahmedatum:	Geschlecht: w☐ m☐		Geburtsjahr:

INFEKTIONSDATEN

Infektionsdatum (Datum der ersten Symptome)**:**

Harnweginfektion: SYMP (D1)☐ ASYMP mit sek. Sepsis (D2)☐

Assoziation zu einem Harnwegkatheter: ja☐ nein☐

Labordiagnose (Erreger):

Infektion der unteren Atemwege: Pneumonie (C1a)☐ (C1b)☐ (C1c)☐ (C1d)☐

Bronchitis/Tracheobronchitis/Tracheitis (J1)☐

Assoziation zu einer maschinellen Beatmung:

Invasiv über Tubus/Tracheostoma (INV) ja☐ Nicht-invasiv (NIV) ja☐

Keine Assoziation zu einer maschinellen Beatmung ☐

Untersuchungsmaterial: Trachealsekret☐ BAL/PSB☐ Blut☐ Sonstiges☐

Labordiagnose (Erreger):

Gefäßkatheter-assoziierte Infektion

Primäre Sepsis: Labor bestätigt (B1)☐ klinisch diagnostiziert (B2) (nur Kinder ≤12 Monate)☐

Mukosa-Barrierestörung-assoziierte Sepsis (B3)☐ Arterien-/Veneninfektion: (F1) ☐

Assoziation zu einem PVK:	ja☐	nein☐
Assoziation zu einem konventionellen zentralen Gefäßkatheter (ZVK):	ja☐	nein☐
Assoziation zu einem teilimplantierten ZVK:	ja☐	nein☐
Assoziation zu einem Port:	ja☐	nein☐
totale parenterale Ernährung (innerhalb von 48h):	ja☐	nein☐

Untersuchungsmaterial: Blut☐ • intraOP entnommenes Material ☐ Katheterspitze☐

Labordiagnose (Erreger):

andere Infektionen:

Labordiagnose (Erreger):

KOMPLIKATIONEN

sekundäre Sepsis: ja☐ nein☐ **Tod:** ja☐ nein☐

7) MONITORING

a. Continuous Monitoring Of Compliance with planned cleaning strategy

The implementation of the cleaning strategy will be monitored once a week by local research personnel. The monitor will observe whether the planned cleaning agent is in use on each study ward. As the cleaning agent will not be labelled, the monitoring will be performed by check of colour and smell of the cleaning agent.

b. Observation Of Hand Hygiene And Use Of Protective Clothing

Infection control personal will conduct direct human observations of compliance with hand hygiene (HH). A minimum of 100 HH opportunities for each ward will be observed annually according to WHO methods. All health care workers in the ward at the time of the observations will be eligible for monitoring. Observations will be recorded anonymously for both the patient and the HCW.

c. Surveillance of environmental biological burden

In order to assess potential microbiological confounders and to assess the influence of the different cleaning strategies, we performed weekly quantitative microbiological environmental sampling. For this end, we use commercially available Rodac plates and assess the growth of common human-pathogenic bacteria from the Gram-positive and the Gram-negative spectrum. As most relevant pathogens concerning our primary outcome and known to have a undemanding growth, we chose *Enterococcus spp.* and *Escherichia coli*.

8) Data Management

Clinical and study personnel will collect data on paper forms and in a second step transfer it into digital form that will be stored on the secured server at the study site. Data will only be stored in the central database. No study participants or HCWs will be identified by name on any study documents or electronic data submissions.

9) Statistical analysis

a. Sample Size

18 wards of the one centre will participate in this study. Calculations are based upon the following assumptions:

- For this study, the average incidence of hospital-acquired infections is assumed to be at least 3.6 per 100 patients in Non-ICUs, alpha=0.025, and beta = 0.80.[15]

- Former studies showed exogenous pathogens (in contrast to endogenous pathogens) to be responsible for about 40% of hospital-acquire infections and earlier studies even showed a HAI reduction of 50% associated with probiotic cleaning. Therefore, the study is powered to detect a 30% reduction compared to the baseline-cleaning regimen with soap. [16]

- In order to determine the number of cases for a cluster-randomized study that correlates to the power of the number of cases at the individual level, this has to be multiplied with a design-effect factor: DE=1 + (n-1)*p. N is the number of individuals per cluster, and p is the intracluster correlation coefficient (ICC). The ICC is a measure

for the similarity of the data of the cluster. It describes the similarity of cluster data by comparing the variance within clusters with the variance between clusters. According to the authors' knowledge, there is no study, which describes inter- and intra-cluster variance in this setting. As we perform a crossover study, each cluster serves as their own control. We therefore set the ICC at 0.0001.

- As we apply a crossover trial, we assume a design effect (DE) of 1.05. This results in a necessary number of 15,630 patients, or 5,210 per study arm. With a DE of 1.05 in a cluster randomization, 18 clusters and 579 patients pro cluster, the corresponding ICC is 0.0001. This assumed ICC is justified because we assume only a small variance between clusters as they serve as their own controls.

- Assuming an average number of 35 beds per wards, an average length of stay of 7 days per patient, 1 ward will treat 140 patients per month, 1,680 per year. We assume an 80% occupation rate. We also assumed that 25% of patients would stay shorter than 3 days thus have to be excluded, as they cannot acquire a HAI per definition (requirement: stay longer than 3 days). Thus within one year, 1 ward would treat about 1008 patients. Thus 18 wards will have to be included in the study for a total duration of 12 months in order to approximately achieve the sample size mentioned above.

- Based on these preconditions, 18,144 patients will be observed in the trial, 6,048 in each intervention phase.

b. Descriptive Statistics

Description of parameters will be done as number and percentages for categorical parameters, as median and interquartile range for continuous parameters. Depending on the distribution of the parameters, differences will be tested using Fisher's Exact test, Chi-square test, T-test or Wilcoxon rank-sum test. Differences in incidence densities will be tested by Chi-square test for incidence densities.

c. Analyses Of Primary Outcomes

The primary outcome is the incidence of hospital-acquired infections. As hospital-acquired infections are time-dependent [17], incidence density will be the appropriate primary dimension in which the primary outcome will be measured. The incidence density of hospital-acquired infections will be compared between the three arms using soap cleaning as reference arm. Hazard ratios for hospital-acquired infections adjusted for the different cleaning strategies using soap as reference group and adjustment for further confounders will be calculated by Cox-proportional hazard regression analysis.

d. Analysis of Secondary Outcomes

Secondary outcomes are incidence-density of infections with multidrug-resistant pathogens, incidence-density of different types of hospital-acquired infections, incidence-density of different HAI-causing pathogens. The incidence densities will be compared between the three arms using soap cleaning as reference arm.

10) Ethics

This study will be conducted in agreement with the declaration of Helsinki and with the guidelines of Good Clinical Practice (ICH-GCP-Guidelines, CPMP/ICH/135/95) issued by the EMEA (European Medicines Agency).

To adequately determine the efficacy of the three strategies, they must be applied uniformly to all the patients in a unit, as though the strategy had become standard practice in that unit. Ward level rates of hospital-acquired infections will appropriately reflect non-independence of these events. Thus, the trial will request a waiver of written informed consent of individual patients in the participating wards. This waiver has been granted 2016 from the Charité University Hospital institutional review board (internal process number EA1/387/16). Different cleaning products are in use in hospitals all over the world. The three products that will be used in the study are commercially available and approved for environmental cleaning in hospitals. Moreover, the products would not be directly administered to patients but exploited for cleaning of hospital surfaces only.

A waiver will not adversely affect the rights and welfare of patients and the trial cannot practicably be carried out without a waiver. Given these considerations, a waiver of informed consent from patients in the participating wards is both important and appropriate for the proper conduct and analysis of this trial.

11) Publication of research findings

Manuscripts and abstracts prepared from the data collected during this trial will be prepared through the study investigators. Individual investigators will provide the principal investigator with publication or presentation materials in advance of publication/presentation to allow for review and comment as means of ensuring confidentiality, accuracy, and objectivity.

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