

BMJ Open Protocol for a prospective cohort study: Prevention of Transmissions by Effective Colonisation Tracking in Neonates (PROTECT-Neo)

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ABSTRACT

Introduction Transmissions of opportunistic bacterial pathogens between neonates increase the risk of infections with negative repercussions, including higher mortality, morbidity and permanent disabilities. The probability of transmissions between patients is contingent on a set of intrinsic (patient-related) and extrinsic (ward-related) risk factors that are not clearly quantified. It is the dual objective of the Prevention of Transmissions by Effective Colonisation Tracking-Neo study to determine the density of transmission events in a level III neonatal intensive care unit (NICU) and to identify risk factors that may be causally associated with transmission events.

Methods and analysis A full cohort of patients treated in a 17-bed level III NICU will be prospectively followed and transmission events between two or more patients will be documented. A transmission event occurs when isogenic isolates from two different patients can be identified. Isolates will be obtained by routine weekly screening. Isogenicity will be determined by whole-genome sequencing. During the study, relevant intrinsic and extrinsic risk factors will be recorded. Specimen and data will be collected for 1 year. We postulate that transmission density increases during episodes when demand for intensive care cannot be met by existing staff, and that threshold dynamics have a bearing on cohorting and hand hygiene performance. Poisson logistic regression, proportional hazard and multilevel competing risk models will be used to estimate the effect of explanatory variables.

Ethics and dissemination This study has been approved by the local ethics committee (study ID 287/18). The results will be published in peer-reviewed medical journals, communicated to participants, the general public and all relevant stakeholders.

Trial registration number The German Clinical Trials Registry (DRKS00017733); Pre-results.

INTRODUCTION

For various preventive purposes related either to maternal (in case of caesarean section) or child health (colonisation with group B streptococcus), neonates are exposed to antibiotics in up to 30% of cases, even in the

Strengths and limitations of this study

- Continuous screening of a complete level III neonatal intensive care unit (NICU) cohort, as well as whole-genome sequencing and typing of the most common nosocomial pathogens, will allow ascertainment of nosocomial transmission dynamics in neonates.
- Prospective enrolment of the total population at risk (all premature neonates), recording of risk factors/confounders and analysis using appropriate multilevel competing risk models will increase understanding of transmission dynamics in the NICU.
- The single-centre study design may limit the ability to generalise findings in other settings.
- The study results may form the basis for designing multicentre intervention trials for tailored measures to prevent transmission events.

absence of individual signs of infection. Moreover, preterm infants are frequently treated with antibiotics immediately after birth, since clinical signs of infection are non-specific and common in this patient group.¹ Neonate vulnerability to the acquisition of nosocomial, often antibiotic resistant, pathogens during treatment in neonatal intensive care units (NICU) is related to an immature immune system, because neonates and especially those delivered by caesarean section have no protective microbiome, and are hence only sparsely colonised by bacteria.¹⁻⁴

Accordingly, NICU house a unique cohort of exquisitely susceptible patients, who, being colonised by particular nascent microbiome communities, readily serve as recipients of opportunistic pathogens through transmission from others patients, healthcare workers or the hospital environment.^{1,5} Since the transmitted pathogens originate from nosocomial sources rather than from the maternal microbiome, they are likely more tenacious and

antibiotic resistant, and, consequently, also challenging to treat when causing infections. Hence, transmissions in NICUs often occur in clusters and cause outbreaks of infection, with dire consequences in terms of mortality, morbidity and permanent disabilities in patients, economic consequences, as well as possibly damaging the treating hospital's reputation.^{6,7} Data on colonisation dynamics in neonates are scarce and the impact of pathogens appearing in microbiological samples is difficult to assess. Whether colonisation with facultative pathogenic bacteria represents 'natural' microbiota assembled from contact with the parents, or whether they rather result from horizontal transmissions between NICU patients remains in most cases unresolved. However, bacteria transmitted horizontally—in contrast to bacteria transmitted vertically—that is, natural microbiome transfer from parents to offspring, are presumably more virulent, more frequently drug-resistant or multidrug-resistant and have a higher tenacity, since these bacteria often originate from hospital-adapted nosocomial lineages. The only way to shed more light on this issue is by rigorous molecular typing of all bacterial isolates within a defined patient cohort in an NICU setting.

Furthermore, other variables or risk factors for transmission of pathogens, in many cases, the prerequisite for infection, are poorly investigated in NICU patients. The impact of compliance with infection control measures, especially hand hygiene, also needs to be analysed. Other important factors that may influence transmission, such as bed occupancy rates, staffing levels (patient–nurse ratio per work shift) and the amount of nursing care a patient requires, must also be considered.

Aims and objectives

To discriminate between natural colonisation and transmissions, we have designed a study on the frequency of transmission of nosocomial pathogens in an NICU setting. During the study, we will screen for the most relevant nosocomial pathogens for NICU patients (*Enterobacteriales*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*), many of which are not only among the earliest colonising bacteria, but are also often associated with severe infections.

The main aim of the study is the identification of transmission events by (1) complete typing of all selected nosocomial pathogens using amplified fragment-length polymorphism (AFLP) and (2) whole-genome sequencing (WGS) of all strains that are not distinguishable by AFLP. Since we will use WGS, this will also allow for an identification of the so-called 'high-risk clones' and will allow analysis of evolution and transmission of circulating lineages and their resistance genes.⁸

The secondary aim of the study is the assessment of relevant risk factors that may influence transmission dynamics. Our primary hypothesis is that transmission events in the NICU occur more often when negative extrinsic risk factors accumulate (eg, during times of high bed occupancy, unfavourable nurse to patient ratio, neonates

with extreme intensive care demand and high colonisation pressure which means a considerable number of patients colonised with the same pathogen at the time of a transmission event). Our secondary hypothesis is that horizontal transmissions between patients lead to subsequent infections more frequently than vertical transmission. Therefore infection densities will be compared for patients who are part of one or more transmission events (cases) and those who are not involved in any transmission event (controls).

In an approach to control for confounding further patient-related variables will be collected and analysed. Thus distribution of variables such as date of birth, birth weight, gestational age as well as exposure to kangaroo care, procedures, diagnostic measures and consultation visits will be examined.

METHODS AND ANALYSIS

Study site

The single-centre prospective cohort study will be conducted at the NICU of the Medical Center—University of Freiburg, one of the largest tertiary care hospitals in Germany (with 2000 beds). The NICU has 17 beds, which consist of 8 fully equipped ICU beds and 9 intermediate care beds, and provides level III neonatal care (definition according to the American Academy of Pediatrics).⁹

Around 350 term and preterm newborns (20% of these with a birth weight of less than 1500 g), are admitted annually to the NICU. This unit covers the entire spectrum of diseases and congenital malformations in the preterm and term newborn infant. Accordingly, the NICU meets the highest standards of current neonatal care. It can, therefore, be assumed that patients at this NICU represent a cross-section of all the patients receiving care at maximum level neonatal units in Germany and possibly in other countries with highly developed healthcare systems, too.

Study population

Inclusion criteria: All patients admitted for ≥ 48 hours who have been screened at least once will be included in the study.

Exclusion criteria: All patients with a stay of < 48 hours, and all patients who have not been screened will be excluded.

Microbiological methods

Weekly screening of all NICU patients will be carried out in accordance with the national guidelines of the Robert-Koch Institute, Berlin, Germany.¹⁰ The screening regimen will include nasopharyngeal, rectal and, if applicable, wound or stoma swabs (eSwab, Copan, Brescia, Italy). Screening swabs and clinical samples will be inoculated on suitable media and incubated under aerobic conditions for 48 hours at 36°C and with CO₂ 5%. If growth on plates is detected, identification of microorganisms will be performed by matrix-assisted laser desorption

ionisation-time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany). Susceptibility testing will be performed using VITEK2 (Biomérieux, Nuertingen, Germany) or MIC test strips (Liofilchem, Piane Romano, Italy), respectively, and the results will be interpreted according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) clinical breakpoints. Presence of resistance genes will be confirmed by nucleic acid amplification tests for methicillin-resistant *S. aureus* and carbapenem-resistant Gram negatives.

Sample collection

Both wild-type and antimicrobial resistant variants of bacterial pathogens obtained from screening and clinical specimens will be collected and kept at -80°C for the duration of the study. The following indicator pathogens will be included: *S. aureus*, *Acinetobacter calcoaceticus*–*A. baumannii* complex and Enterobacterales.

Typing methods

The molecular genotyping procedure is stepwise. (I) Identification and susceptibility testing of isolated pathogens; (II) genotyping by AFLP will be applied as previously described.¹¹ *S. aureus* isolates will undergo *spa* typing as described elsewhere¹²; (III) clonal lines, defined as frequently occurring strains of the same species that cannot be differentiated by AFLP or *spa* typing will be further analysed using WGS to identify high-risk clones. WGS will be performed with the Illumina Nextera DNA Flex Kit library preparation and 2×150 bp paired-end reads. Incidence of transmissions (defined as transmission of a pathogen from one patient to the other as assessed by molecular typing) will be calculated per 1000 patient days.

Outcomes

Primary outcome

Transmission events: pathogens of the same genus/species in different patients which are indistinguishable after molecular typing.

Secondary outcomes

Incidence of multidrug-resistant and susceptible indicator pathogens: according to EUCAST clinical breakpoints; standard quantitative statistics.

Infection rates: density (infections/patient days×1000)

Collection of influencing risk factors, possible confounders and further variables

Throughout the study period the following risk factors, confounders and variables potentially influencing transmission dynamics will be extracted prospectively from patient charts or electronic systems (laboratory information system, clinical information system) by a study nurse and an infection control nurse (table 1):

To generate an estimate of compliance with hand hygiene throughout the study period regular observations of hand hygiene performance will be conducted on a monthly basis following an established protocol (measured as number of hand disinfections/number of valid indications×100).¹³

Statistical considerations

Data will be collected at individual patient as well as aggregated (monthly and unit) level. In a time series, analysis-approach time periods during which transmission events occur will be compared with those without transmission events in terms of the risk factors (namely bed occupancy rate, patient to nurse ratio, care efforts

Table 1 Risk factors, confounders and further variables

Risk factors	Measuring method/unit
Bed occupancy rate	Utilisation (occupancy days/possible occupancy days×100)
Patient to nurse ratio	Number of patients/day/number of nurses/day
Care efforts	INPULS (Intensivpflege und Leistungserfassungssystem) care categories ¹⁶
colonisation pressure	Number of patients colonised with the same pathogen concordant to place and time ¹⁷
Confounders/further variables	Measuring method/unit
Standard patient characteristics	Date of birth, birth weight, gestational weight
Localisation of the patient	Bed place; ID of the incubator
Kangaroo care	Received or not received for each patient
Antibiotic consumption	Application density (number of treatment days/number of patient days×1000 per antibiotic substance); overall consumption measured in DDDs (defined daily dose) per antibiotic substance/class
Device use	Number of catheter days/number of patient days×100 per catheter type
Ventilation days	Number of ventilation days/number of patients days×100 per non-invasive and invasive ventilation types, respectively
Surgical procedures	Type and number of procedure/patient
Invasive diagnostic measures	Type and number of measure/patient
Consultation visits	Type and number/patient

and colonisation pressure). Furthermore, patients who are part of a transmission event (donor or recipient of a bacterial pathogen) and patients who are not part of a transmission event will be compared and evaluation of possible confounding considering further variables will be performed. Analysis will include univariate and multivariate approaches. For univariate analysis, rate ratios (RRs), 95% CIs and p values will be determined for categorical exposure variables. Association of the dependent with continuous variables (eg, gestational age, body weight, days of relative staff deficit, etc) will be tested for significant differences by score test for trend. RRs will be reported as estimates for one unit increase in exposure. Poisson or negative binomial appropriate regression models will be used for multivariate analysis and precautions will be taken to avoid overfitting. Stringent criteria and stepwise entry into a forward approach will optimise then numbers of candidate variables. Effect modification will be tested by fitting interaction terms into the regression. All data will be collected into an MS Access database and analysed using the statistical software SAS 7.4, STATA and R. The primary analysis regarding the transmission events will be performed on monthly and unit level. We plan to include 100–140 patients. As 350 mostly preterm newborns are admitted annually to the NICU sample size will be achieved in the given time. All outcomes for the included patients and corresponding isolates will be analysed. We expect 50–70 transmissions per year (about five per month). A sample size calculation for the Poisson distribution¹⁴ yields that with an observation period of 12 months, we can detect a group difference between 3.5 and 6 transmissions per months. After examining the distribution (Poisson, negative binomial), appropriate regression models will be adapted to determine the transmission rate as a function of factors (bed occupancy rate, patient to nurse ratio, care efforts and colonisation pressure).¹⁵ If applicable, we will use the patient-individual level data for secondary analyses.

Patient and public involvement

No patients were involved in the design of the study nor its conduction. The public will be informed on publication of the study. Publication will be in an open-access manner.

DISCUSSION

We expect our results to form the foundation of informing infection prevention and control (IPC) strategies in the neonatal setting. We believe that effective transmission tracking and assessing relevant confounders as well as other possible influencing variables will improve patient safety. We aim to identify so-called control points that can be used in subsequent multicentre studies to implement randomised controlled IPC interventions.

ETHICS AND DISSEMINATION

No specific invasive measures will be taken or medication applied within the scope of our planned study. In other words, all the patients will receive standard medical care that is not influenced by the study. Furthermore, no patient will be denied therapy or preventive measures or additional diagnostics, whether included or excluded from the study. The results of the microbiological samples and the resistance patterns of isolated bacteria will be available to the treating physicians as usual, and will possibly influence patient care, for example, empiric antibiotic therapy. All additional patient data collected exclusively for this study will be kept inaccessible to third parties and analysed in anonymous form. No identifiable patient data will be published or permanently stored. Parental consent is not required as collection and analysis of the microbiological isolates is purely descriptive and corresponds to infection control quality criteria according to the German Infection Protection Act (IfSG) and the KRINKO (Kommission für Krankenhaushygiene und Infektionsprävention) commission as outlined in §23 section 1 IfSG. According to and in compliance with the WMA (World Medical Association) Declaration of Helsinki, Helsinki, Finland, June 1964, last revised in October 2013 in Fortaleza, Brazil, there are in our opinion no ethical concerns regarding the conduct of the study. The study was approved by the Ethics Committee, Medical Centre—University of Freiburg, on 28 August 2018 (registration number 287/18).

The results of the study should contribute to the development of targeted infection-preventive measures and adapted antibiotic therapy, thereby increasing patient safety for the extremely vulnerable study population of neonates. The results will be presented at national and international scientific meetings. Additionally, we aim to publish the results, preferably in open-access journals, to guarantee broad access for interested audiences. The sequencing data will be made available by uploading them to common online platforms.

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Contributors TG and NTM are the principal investigators of the study. They initiated, designed and drafted the protocol and coordinated all collaborations,

wrote the publication and reviewed the intellectual content of this paper. HG advised on designing the study. HG, DJ and SR advised on the sequencing methodology and on the phylogenetic analysis, and reviewed this paper for intellectual content. RH, PH, DK and SH provided clinical advice and made critical revisions. MW advised on the statistical design of the study and reviewed this paper for intellectual content. BB and GH advised on sampling strategies for microbiological samples and reviewed this paper for intellectual content. All listed authors have and will participate in the study, and have reviewed and approved the final manuscript.

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