Universitat de Girona
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Creating and implementing an evidence-based prevention bundle to reduce late-onset sepsis in extremely low birth weight newborns: a multicenter interrupted time series study

END OF TERM PROJECT

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I dedicate this project to my family, for their endless support.

1. ABSTRACT

Background: Late-onset sepsis results in significant mortality, morbidity and prolonged length of stay in already vulnerable extremely low birth weight newborns. Neonates admitted to the Neonatal Intensive Care Unit are at high risk of acquiring healthcare-associated infections because of the immaturity of their immune system and prolonged use of invasive medical procedures and devices. Bundles are a small set of evidence-based preventive strategies that when implemented simultaneously result in additional improvements in patient outcomes, than when implemented individually.

Hypotheses and Objective: This study aims to assess the efficacy of an infection prevention bundle in reducing late-onset sepsis incidence in extremely low birth weight newborns (<1.000g). Mortality and length-of-stay will also be evaluated.

Design and setting: We will perform a multicenter interrupted time series study among 8 participating Spanish Neonatal Intensive Care Units. Hospital Universitari Dr. Josep Trueta will be the reference center.

Intervention: All participating units will implement a self-designed prevention bundle focusing on: re-enforcement of hand hygiene, promotion of early enteral trophic feeding and specific antimicrobial stewardship strategies.

Methods: Extremely low birth weight neonates with ≥72 hours of life (n=547) will be included. Outcomes (late-onset sepsis incidence, mortality and related length of stay) will be collected monthly before (pre-intervention) and after (post-intervention) the implementation of our tailored bundle. Data will be analyzed by using statistical process control methods.

Expected results: We hypothesized that consistent implementation of the evidence-based bundle would result in a 20% reduction in late-onset sepsis.

Key words: late-onset sepsis · healthcare-associated infection · prevention · bundle · neonatal intensive care unit · extremely low birth weight · incidence · mortality rate · length-of-stay

2. ABBREVIATIONS

BSI Bloodstream infection

CBC Complete blood count

CLABSI Central-line associated bloodstream infections

CoNS Coagulase-negative Staphylococci

CPAP Continuous positive airway pressure

CRP C-reactive protein

CSF Cerebrospinal fluid

CVC Central vascular catheters

ELBW Extremely low birth weight

HAI Hospital acquired infection

LOS Late-onset sepsis

NICU Neonatal intensive care unit

PCR Polymerase chain reaction

PCT Procalcitonine

PVC Peripheral vascular catheters

VLBW Very low birth weight

3. INTRODUCTION

3.1. BACKGROUND

DEFINITION

Neonatal sepsis is generally referred as an infection, usually bacterial, occurring within the first 28 days of life for a term baby, and up to 4 weeks beyond the expected date of delivery in a preterm baby. Neonatal sepsis is often categorized as either early-onset (diagnosed during the first 72 hours of life) or late-onset sepsis (diagnosed after the first 72 hours of life). Early-onset sepsis is often due to transplacental or vertically ascending infections from the maternal genital tract to the newborn before or at the time of birth, and is most frequently caused by group B Streptococci or *Escherichia coli.*

Late-onset sepsis (LOS) is usually acquired from direct contact with healthcare providers or environmental sources (i.e. healthcare-associated or nosocomial infections) following prolonged admission to the Neonatal Intensive Care Unit (NICU) in preterm newborns [(1)]. Nosocomial infections are either primary bloodstream infections (BSI) not related to an infection at another site or secondary bloodstream infections caused by the spread of microorganisms in the blood from an infection at another body site (central-line associated bloodstream infection, ventilator-associated pneumonia, catheter associated urinary system infection…).

EPIDEMIOLOGY

According to EuroNeoNet Annual Report, the global late-onset sepsis rate for 2011 cohort was 23.8%, with a variation of 1.3% and 79.1% within units (33,7% were infants born in Spain).

Table 1: Late bacterial sepsis rates against birth weight. Adapted from EuroNeoNet Annual Report year 2011[(2)]

<501g		501-750g		751-1000g		1001-1250g		1251-1500g		>1500g		TOTAL	
N	%	N	%	N	%	N	%	N	%	N	%	N	%
34	39,1	320	44,3	501	36,2	350	22,6	296	14,8	54	6,8	1555	23,8

According to Spanish Neonatal Network (SEN1500) Annual Report of 2014, the global late-onset sepsis rate was 30,5%.

Table 2: Late bacterial sepsis rates against birth weight. Adapted from Informe Anual SEN1500[3]

<501g		501-750g		751-1000g		1001-1250g		1251-1500g		TOTAL	
N	%	N	%	N	%	N	%	N	%	N	%
20	60,0	313	61,8	602	45,3	673	29,3	1076	13,3	2684	30,5

Primary data collected as part of EuroNeoKissES surveillance project [4] conducted between 01.01.2014 and 31.12.2016 in Hospital Universitari Dr. Josep Trueta's NICU showed:

Table 3: No. of patients under surveillance, No. of LOS episodes, Total patient days, Cumulative incidence and Incidence density against birth weight. Adapted from EuroNeoKissESS. Unpublished data cited with permission.

Birth weigh (grams)	No. Of patients under surveillance	No. Of LOS episodes	Total patient days in the unit	Cumulative incidence	Incidence density
<500	1	0	29	0.00	0.00
500-999	46	12	1944	23.91	6.17
>999	88	11	2429	12.50	4.53
Total	135	23	4402	16.30	5.22

The global incidence density in all Spanish participating units was 18.97 for newborns <500g, 11.88 for newborns between 500-999g and 6.01 for newborns >999g.

Late-onset sepsis in preterm newborns is a major cause of morbidity and mortality. EuroNeoNet Report estimated that in 2011[2], the mortality rate for babies admitted to the NICU was of 10,9%. Among whom late onset sepsis was diagnosed in a 23,8%, representing the leading cause of mortality, followed by bronchopulmonary dysplasia (17,6%) and severe grade III-IV intraventricular hemorrhage (7,4%).

ORGANISMS ASSOCIATED WITH LATE-ONSET SEPSIS

In low birth weight newborns, Gram-positive bacteria caused the majority of LOS episodes, followed by Gram-negative. Certainly, since the late 1970s and early 1980s, Coagulase-negative Staphylococci (CoNS) particularly *Staphylococcus epidermidis,* has emerged as the most common pathogen causing LOS in newborns admitted to the NICU, accounting for approximately 50% of all infections.

Gram-negative organisms responsible for neonatal LOS mainly include *Escherichia Coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Not to forget fungi, especially *Candida albicans*, recounted to be one of the major pathogens causing LOS.

According to an epidemiological study from the "Grupo de Hospitales Castrillo" the most frequent causative pathogens of LOS in low birth weight newborns were:

Table 4: Pathogens causing LOS according to "Grupo de Hospitales Castrillo"[5]

Grams-positive organisms (55,5%)	*Staphylococcus Epidermidis* (42,3%) *Enterococus* (7,6%) *Staphylococcus Aureus* (3%)
Gram-negative organisms (28,5%)	*Escherichia Coli* (5,7%) *Klebsiella pneumonia* (7,6%) *Pseudomona aeruginosa* (5,7%) *Enterobacter cloacae* (2,2%) *Serratia marcescens* (2.4%)
Fungi	*Candida albicans* (15,4%)

The causative organism is an important predictor of overall outcome. Indeed, although less prevalent, Gram-negative organisms are associated with a more fulminant course of severe sepsis and/or septic shock, resulting in higher mortality rates.

RISK FACTORS FOR SEPSIS

Factors predisposing to late-onset sepsis include:

- Long-term use of invasive procedural devices (central or peripherally inserted vascular catheters, urinary catheters, endotracheal tubes, oral- or nasogastric feeding tubes,);
- Delay of enteral feeds (not receiving maternal breast milk) and thus prolonged duration of total parenteral nutrition;
- Excessive and prolonged use of broad-spectrum antibiotics, administration of steroids and histamine$_2$-receptor antagonists;
- Underlying respiratory and cardiovascular diseases
- Previous surgical procedures;

- Overcrowding and poor staffing ratios, insufficient knowledge and difficulties in the implementation of infection control practices (suboptimal hand hygiene);

In addition to those mentioned above, numerous host factors predispose the preterm newborns to sepsis, and involve immaturity of all levels of host-defense mechanisms, including both innate (barrier function) and adaptive immunity (cellular and humoral immunity). Indeed, the incidence of LOS is inversely associated with birth weight and gestational age[6].

CLINICAL PRESENTATION

Clinical presentation of nosocomial infections is often subtle and nonspecific, making early and accurate diagnosis a challenging task. Serial examination provides greater understating and any deviation from an infant's usual pattern of activity or feeding should be regarded as a possible warning sign of nosocomial bloodstream infection [1][7].

- Vital signs fluctuation: Hypotension or evidence of poor perfusion (recapillarization time >2s and cyanosis), tachycardia and bradychardia.
- Respiratory distress: apnea, tachypnea >60/min, grunting, nasal flaring, intercostal or sternal retractions, and rising ventilator requirements in the mechanically ventilated patient.
- Neurological abnormalities: lethargy, hypotonia, bulging fontanel, and seizures.
- Hypothermia, hyperthermia/fever and temperature instability;
- Pustules, vesicles, petechiae, erythema, and papules, jaundice;
- Gastrointestinal function: Feeding intolerance, abdominal distension, vomiting.

DIAGNOSIS

Because clinical signs and symptoms of neonatal sepsis are inconspicuous and nonspecific, a “suspected sepsis” diagnosis is made on the presence of identifiable risk factors and the neonatal symptoms or physical findings mentioned above.

Initial evaluation of the NICU patient with suspected infection should include: two blood cultures, a cerebrospinal fluid (CSF) culture (performed prior to starting antibiotics), a urine culture (if required), a complete blood count (CBC) with differentiation, C-reactive protein

(CRP) and procalcitonine (PCT) levels and study of molecular based techniques (polymerase chain reaction [PCR]).

Blood cultures remain the gold standard diagnostic tool for diagnosing sepsis, as the isolation of a pathogen from a blood culture is in fact the only method to confirm the diagnosis of neonatal sepsis. A lumbar puncture should be considered in all neonates for whom blood culture evaluation for sepsis is performed, because clinical signs suggesting meningitis can be lacking. The CSF obtained by lumbar puncture should be sent for culture, Gram stain, cell count with differential, and measurement of protein and glucose levels. A urine culture obtained by catheter or bladder tap should be included in the sepsis evaluation for infants >6 days of age. Additional cultures should be obtained from any other potential foci of infection (tracheal aspirates in mechanically ventilated newborns, sputum culture if intubated, peritoneal fluid,).

Blood samples for inflammatory markers should also be taken at onset of clinical manifestations of infection. A complete blood count with differential white blood cell count, including total number of neutrophils, and platelet count can be very useful in the evaluation of the NICU infant. Infection may result in leucokytes <5.000/mm^3 or >30.000/mm^3, neutropenia (<1500/mm^3) or neutrophilia (>15.000/mm^3) with an immature to total neutrophil ratio (I:T) ≥0,2 (immature circulating neutrophils appear in response to infection) and in thrombocytes <100.000/mm^3. C-Reactive protein levels > 1,0-2,0mg/dl and procalcitonine concentrations > 0,6ng/ml have proven to be cut-off values indicative of sepsis. Serial measurements of both acute phase reactants after the onset of clinical signs and symptoms have shown to increase its sensitivity and specificity[8]. Sequential determinations after starting antibiotic therapy may also be helpful in monitoring the response to treatment in infected newborns and thus may help clinicians guiding safe discontinuation of antibiotic therapy if levels remain persistently normal[9]. Proinflammatory cytokines such as IL-6, IL-8 and TNFα are also valuable biomarkers for early diagnosis of LOS[10]. The combination of these biomarkers holds promise to enable a quick and accurate diagnosis of LOS, reducing the number of newborns who receive antibiotic therapy needlessly[11].

Molecular pathogen detection methods are based on hybridization or amplification. Broad-range conventional Polymerase Chain Reaction (PCR) amplifies specific target conserved regions in the microbial genome and recently available real-time PCR monitors the microbial load and rapidly target specific microorganisms in clinical specimens. Microarrays uses hybridization of clinical samples to detect pathogens, microbial virulence and even the patient's immune response profile. Although molecular-based methods yield results with a high sensitivity and specificity (they are not affected by administration of antepartum antibiotics and have a low contamination rate like conventional cultures), less laboratory turnaround time and require much smaller volumes of blood, they cannot replace microbial cultures in the isolation of pathogens and the subsequent detection of antibiotic-sensitivity profile (antibiogram) and may only be performed as "add-on" tests[12].

Additional tests are usually obtained to differentiate sepsis from other conditions with similar presentations. Imaging studies (chest and abdominal X-ray, CT scan, MRI and abdomen and heart ultrasonography) can be critical in the evaluation of the newborn with suspected sepsis: chest radiography is mandatory in all patients with respiratory distress, apnea or ongoing oxygen requirements and can help rule out pneumonia, abdominal radiographs are essential in the diagnosis of necrotizing enterocolitis and echocardiography differentiates sepsis from congenital heart diseases.

Electrolytes can become imbalanced quickly in the infected neonate and should be rapidly recognized and corrected. Hypoglycemia and hyperglycemia are both associated with the stress of infection in the NICU patient. Metabolic screening should be performed to differentiate sepsis from Inborn Errors of Metabolism. By using capillary blood gasometry measures we can assess the respiratory and metabolic status ($PaCO_2$, PaO_2, bicarbonate and pH) of the patient.

TREATMENT

Since definite diagnosis of neonatal sepsis remains difficult, and potential negative outcomes may occur by deferred initiation of treatment, initial empiric broad-spectrum coverage antimicrobial therapy is started on presumed late-onset bloodstream infection.

An ideal choice of antimicrobial agents should be based on the likely organisms known to colonize each Neonatal Intensive Care Unit and its pattern of antibiotic susceptibility and resistance. Currently, CoNS are the most common cause of bloodstream infections in our NICU, followed by Gram-negative bacteria. Therefore, we use a combination of vancomycin and an aminoglycoside (amikacin) as first line initial antibiotic therapy while awaiting culture results. In addition, if meningitis is suspected, a third-generation cephalosporin, such as cefotaxime, should be added. If a Gram-negative infection is suspected or the course is fulminant, ceftazidime, another third-generation cephalosporin that possesses anti-*Pseudomonas* activity should be added (for dosage and administration please see **Annex 14.1**).

Duration of treatment is another important factor to consider in empirical antibiotic therapies. Duration of antibiotic therapy is usually from 10 to 14 days in most newborns with bloodstream infections, and up to 21 days in patients with meningitis. In well-appearing infants with negative cultures and persistently normal levels of sepsis biomarkers after 48 hours, antibiotic therapy should be discontinued, as they demonstrate a low likelihood for infection. If positive cultures are obtained and the causative organism is identified, empiric antimicrobial therapy should be replaced by organism-specific therapy. Early removal of catheters that may be the foci of bacterial infection is also compulsory, especially if there is no clinical improvement or cultures remain negative.

In addition to antibiotic therapy, the management of late-onset sepsis may require initial general supportive care measures such as optimal oxygenation (either supplemental oxygen administered via a nasal cannula or mechanical ventilator support with endotracheal intubation) and maintenance of adequate peripheral perfusion with intravenous fluid replacement therapy and even inotropic therapy (dopamine, dobutamine or adrenaline infusion) with constant monitoring of fluid and electrolyte balance[13].

PROGNOSIS

Hospital-acquired or nosocomial infections are probably the most important harmful condition not present at admission for adverse outcome. Not only associated with increased mortality and short-term morbidities (intraventricular hemorrhage, periventricular leukomalacia, chronic lung disease, necrotizing enterocolitis and retinopathy of prematurity), nosocomial infections and the associated systemic inflammatory response (with cytokine and free radical activation) also contribute to long-term disabilities in surviving ELBW infants including White Matter Injury (WMI) and poor neurodevelopmental and growth outcomes[14]. The presence of proinflammatory citokines in the central nervous system has been shown to inhibit proliferation of neuronal precursor cells, activate astrogliosis and microglial infiltration, and stimulate pre-myelinating oligodendrocytes cells death, increasing the risk of WMI, which seems to be the major lesion for the neurodevelopmental impairment observed in surviving infants.

Stoll BJ et al[15] proved that compared with uninfected infants, ELBW infants with LOS were significantly more likely to have adverse neurodevelopmental outcomes at the 18-month follow up assessment including increased risk of cerebral palsy, lower Bayley of Infant Development II scores (mental and psychomotor developmental index) and vision impairment, as well as impaired head growth (a know predictor of poor neurodevelopmental outcome). At school age, preterm children with history of late-onset sepsis had also worse outcomes when evaluating motor skills, intelligence (total IQ), verbal memory and attention compared to matched controls[16].

PREVENTION

Reducing the incidence of nosocomial infections requires a multifaceted approach. Certainly a number of well-established care strategies have been proved to reduce the risk of a newborn developing a nosocomial infection.

Hand hygiene remains one of the most effective method for preventing the spread of nosocomial infections[17]. Pessoa-Silva et al. showed that successful hand hygiene promotion was independently associated with infection risk reduction in VLBW neonates[18].

Capretti et al, demonstrated how the incidence of nosocomial infections in VLBW was significantly reduced after the introduction of a standardized hand washing protocol, saving approximately 10 hospital-acquired infection episodes/year, at a cost of $10.000 per episode[19]. In 2009, The World Health Organization published new evidence- and consensus-based recommendations for hand hygiene[20]. Recommendations for hand washing and hand antisepsis included among others:

- Use of an alcohol-based hand rub for routinely decontaminating hands in all clinical settings if hands are no visibly soiled (IA), or soap and water when dirty, contaminated or visibly soiled with blood or other body fluids (IB)[1].
- Perform hand hygiene before and after touching the patient (IB), before handling an invasive device for patient care, regardless of whether gloves are worn (IB), after contact with body fluids or excretions, mucous membranes, nonintact skin or wound dressings (IA), if moving from a contaminate body site to another body site during care of the same patient (IB), after contact with inanimate surfaces and objects in the immediate vicinity of the patient (IB) and after removing nonsterile gloves (IB).
- Conduct hand hygiene-promotion programs for health care workers were encourage improving sustained adherence (IA).

Unnecessary and frequent and/or prolonged empirical, broad-spectrum antibiotic therapy can be linked with antibiotic-resistant bacteria and increases the risk of fungal infections[21]. Judicious use of antibiotic therapy is strongly encouraged to reduce nosocomial infections in the NICU often caused by multiresistant bacteria and fungi, and include major strategies like accurately identifying patients who need antibiotic therapy (patients with high likelihood of infection), using local epidemiology to guide the selection of empiric therapy, avoiding therapy with overlapping activity, monitoring for toxicity, deescalating empirical treatment when a bacterial infection has not been proved and narrowing the spectrum of antibiotics when cultures are obtained and the causative pathogen is identified[22].

[1] Strength of recommendation I: Evidence from ≥1 properly randomized controlled trial.
Quality of evidence A: Good evidence to support a recommendation for use.
Quality of evidence B: Moderate evidence to support a recommendation for use.

The Infectious Disease Society of America and the Society for Healthcare Epidemiology of America have designed antimicrobial stewardships programs to improve clinical outcomes while improving control of antimicrobial resistance and include the following strategies: prescriber audit and feedback (IA), formulary restriction and prior authorization requirements for selected antimicrobial agents (IB), education (IB), guidelines and clinical pathways (IA), antimicrobial order forms (IB),.

Early enteral trophic nutrition has shown benefits in the prevention of late-onset sepsis without an increased risk of intestinal complications (necrotizing enterocolitis) in very low birth weight infants[23][24]. Defined as giving small volumes of milk (breast milk and/or formula), typically 10 to 15 ml/kg/day, starting within the first day of life and increased later when the patient's condition is considered stable, trophic feeding combines an attempt to overcome the lack of gastrointestinal stimulation during total parenteral nutrition with minimal stress to the ill infant. Theoretically, early trophic feedings prevent gastrointestinal atrophy, stimulate the growth of beneficial gut flora preventing intestinal bacterial contamination, and enhance gut mucosal immunity by providing large amounts of secretory maternal IgA antibodies, granulocytes, macrophages and lymphocytes[25], lactoferrin and prebiotics (bifidus factor). As mentioned above, earlier enteral feeding allows decreasing the use of total parenteral nutrition, that has been shown to have an immunosuppressive effect increasing the risk of systemic infection, and intravenous devices[24][26].

Lastly, ongoing microbiologic surveillance and reporting systems can contribute to the reduction of neonatal LOS by monitoring rates of infection and identifying emerging pathogens, both essential to understand the epidemiology and management of LOS and optimally allocate resources, reducing the burden of this disease[27] .

3.2. WHAT IS A BUNDLE?

The U.S. Institute for Healthcare Improvement (IHI) defined bundle as 'a small set of evidence-based interventions for a defined patient population and care setting that, when implemented together, will result in significantly better outcomes that when implemented individually'[28]. Hence, care bundles are a set of preventive measures built in continuous quality-improvement studies where all elements are well-established scientifically robust best practices that encourage multidisciplinary collaborative working while integrating the results of clinical research into daily clinical practice, providing behavioral changes and new insight on care processes.

Several Central-line Associated Bloodstream Infections (CLABSI) prevention bundles often concerning Central Venous Catheter (CVC) insertion and maintenance have already been created and implemented in worldwide NICUs. They often include a combination of the following interventions: proper hand hygiene and sterile contact barriers, proper CVC placement, proper hub care, daily evaluation need of CVC for removal, … [29].

Ventilator-associated pneumonia (VAP) in ventilated patients is also a serious hospital-acquired infection and thus "VAP prevention bundles" have also being design to reduce VAP rates in NICUs. "VAP prevention bundles" are usually composed of: head-of-bed elevation between 30 and 45 degrees, sterile suction of bronchopulmonary secretions from the airway, hand and oral hygiene and daily evaluation for readiness for extubation to nasal continuous airways pressure in order to decrease duration of mechanical ventilation whenever possible [30].

3.3. JUSTIFICATION

Late-onset sepsis results in significant mortality, morbidity and prolonged length of stay in already vulnerable preterm infants, rising substantial medical costs to an already overburdened healthcare system. Although advances in perinatal care have led to decreased mortality improving survival of extremely birth weight infants, the short and long-term potential complications of these extremely low birth weight newborns have not decreased. Indeed, in parallel with improvement in supportive care pushing the limits of viability as low as 23 weeks of gestation, the rate of nosocomial infections has upraised.

Life-sustaining invasive medical devices and procedures are often critical to preserve an infant's life and thus impossible to restraint making reducing the number of nosocomial infections a major challenge in the NICU. Up to now the best strategy to avoid neonatal late-onset sepsis in patients receiving health care lies in prevention, and therefore preventive interventions aimed at decreasing the infection rate need to be identify urgently and clinically proven.

There is growing evidence that care bundles may be effective to reduce rates of ventilator-associated pneumonia and central-line associated bloodstream infections in neonates [31][32]. Regrettably, this evidence is often limited to inconsistent and very specific preventive interventions only related to safe insertion and maintenance of catheters or ventilation devices, at a single-unit level. Additionally, data assessing the impact of bundles to reduce infection rates in extremely low birth weight neonates is specially lacking, reflecting the novelty of studying this particular population.

Therefore, this study aims to provide extended and new findings by creating and implementing an improved standardized and comprehensive infection prevention bundle composed of structured interventions for reducing all types of bloodstream infections using the best implementation strategies, in especially vulnerable extremely low birth weight newborns, on a nationwide scale, analyzing not only infection rates but also associated mortality and length of stay.

4. QUESTION, HYPOTHESIS AND OBJECTIVES

4.1. QUESTION

Does the implementation of an infection prevention bundle in Neonatal Intensive Care Units result in fewer cases, decreased mortality and length of stay due to late-onset sepsis in extremely low birth weight newborns?

4.2. HYPOTHESIS

PRIMARY HYPOTHESIS

By implementing an infection prevention bundle in Neonatal Intensive Care Units, the incidence of late-onset sepsis in extremely low birth weIght newborns will decrease.

SECONDARY HYPOTHESES

By implementing an infection prevention bundle in Neonatal Intensive Care Units, the mortality related to late-onset sepsis in extremely low birth weight newborns will decrease.

By implementing an infection prevention bundle in Neonatal Intensive Care Units, the hospital length of stay due to late-onset sepsis in extremely low birth weight newborns will decrease.

4.3. OBJECTIVES

PRIMARY OBJECTIVE

To assess the efficacy of the implementation of an infection prevention bundle in reducing late-onset sepsis incidence in extremely low birth weight newborns.

SECONDARY OBJECTIVES

To determine whether the implementation of an infection prevention bundle reduces the mortality of late-onset sepsis in extremely low birth weight newborns.

To determine whether the implementation of an infection prevention bundle reduces the hospital length of stay due to late-onset sepsis in extremely low birth weight newborns.

5. STUDY DESIGN AND METHODOLOGY

5.1. SETTING

This multicentric study will be conducted in national level IIIB or IIIC NICUs. Level IIIB units are defined as those providing sustained life support and comprehensive care for infants with extreme prematurity (28 weeks gestation or less) or extremely low birth weight or with severe and/or complex illness, including a full rage of respiratory support which may include conventional and/or high-frequency ventilation and inhaled nitric oxide. Level IIIB units also ensure prompt and readily available access to a full range of pediatric medical subspecialists and pediatric surgical specialists on site or at closely related institution. Additionally, level IIIC units can provide extracorporeal membrane oxygenation and on-site surgical repair of serious congenital cardiac malformations that require cardiopulmonary bypass[33].

5.2. DESIGN

A multicenter Interrupted Time Series (ITS) study will be performed to compare the rate of LOS, the mortality and the length of stay in NICU before and after the intervention.

ITS is a quasi-experimental research design used for evaluating the impact of a quality improvement program on the rate of an outcome collected at regularly spaced multiple time points (time series) before and after the "interruption" of our intervention, in a defined population of individuals[34].

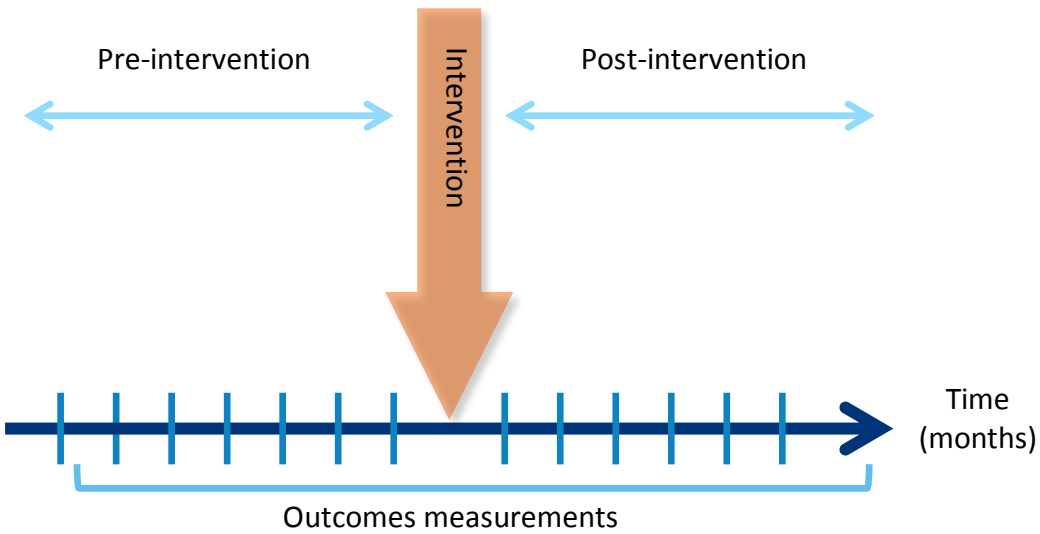


Figure 1: Study Design Schematic

In contrast to cross-sectional retrospective or before and after studies where there is only a single outcome measure before the intervention and a single outcome measure after, by making multiple assessments of the outcome variable both before and after the intervention, ITS allows us to estimate the dynamics of change in response to the intervention[35].

5.3. RESEARCH POPULATION

Participant inclusion criteria	Participant exclusion criteria
Any infant born at our hospital, and admitted to the NICU in the first 28 days of life, with the following features will be eligible: Extremely low birth weight newborns defined as birth weight of less than 1.000g More or at least 72 hours of life All **out born** infants admitted to the NICU within 28 days of birth should also be included if they meet the above criteria.	Early-onset sepsis Underlying hepatic or renal disease Congenital abnormality

5.4. SAMPLE

SAMPLE SIZE DETERMINATION

To determine the study's sample size we used online GRANMO® Calculator. We decided to compare levels of infection rate, mortality and NICU length of stay, during pre- and post-intervention periods. The proportion of late-onset sepsis during the pre-intervention period, assessed using Sen1500 Annual Report of 2014, was estimated of 45,3% for newborns between 751 and 1000g of birth weight (proportion in first-assessment). According to the results obtained in previous studies[32], we hypothesized that the implementation of our design bundle will result in an estimated drop in late-onset primary bloodstream infection rate of 20%. The anticipated dropout rate is of 20%. With an alpha risk set at 0.05 and a beta risk set at 0.2 in a two-tailed test, 547 subjects are necessary to be able to detect as statistically significant a difference consisting in an initial proportion of 0.45 and a final proportion of 0.36 in late-onset sepsis.

STRATEGIES FOR RECRUITMENT

According to data gathered from Spanish Statistical Office (www.ine.es) there were a total of 1.086 ELBW newborns in 2015. As illustrated in Figure 2, the Autonomous regions with higher numbers of ELBW newborns in 2015 were: Andalucía (180 ELBW births), Comunidad de Madrid (176 ELBW births), Cataluña (154 ELBW births), and Comunitat Valenciana (119 ELBW births) with an aggregate of 629 ELBW births in 2015.

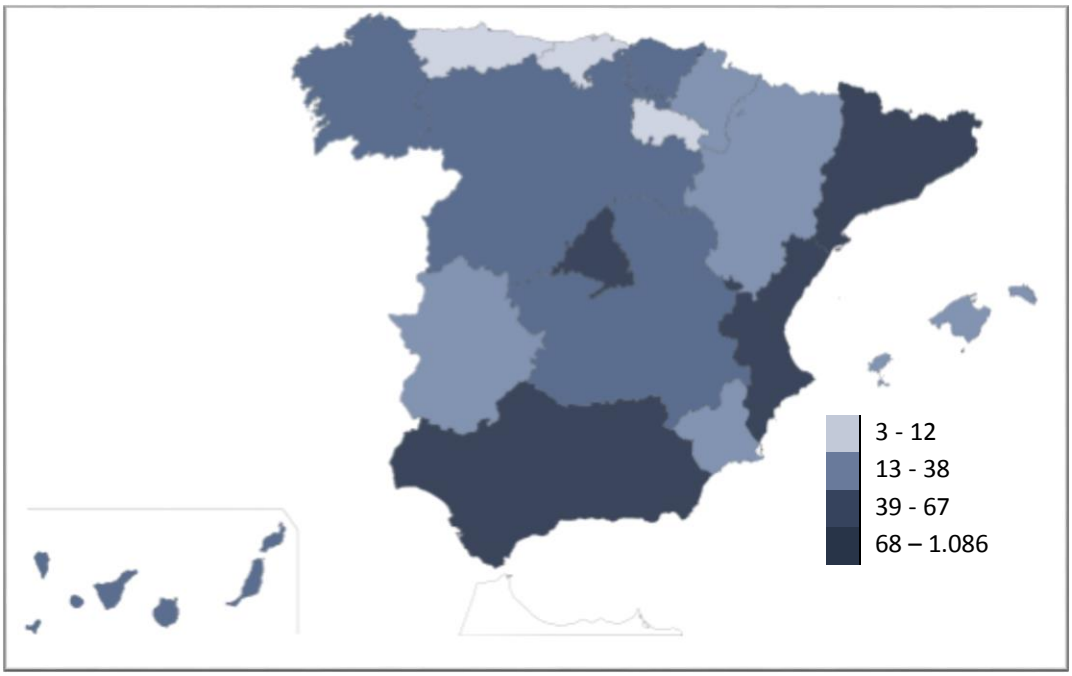


Figure 2: Number of ELBW neonates by Autonomous Region. Extracted from INEBase

Decided upon the sample size determination result, we designed a multicenter study with the participation of Neonatal Intensive Care Units from Andalucía, Comunidad de Madrid, Comunitat Valencia and Cataluña, inviting the following hospitals:

- Cataluña: Hospital Universitari Dr. Josep Trueta (reference center), Hospital Universitario Vall d'Hebron, Hospital Sant Joan de Déu;
- Comunidad de Madrid: Hospital Universitario La Paz, Hospital Universitario 12 de Octubre, Hospital General Universitario Gregorio Marañón;
- Andalucía: Hospital Universitario Virgen Macarena de Sevilla;
- Comunitat Valenciana: Hospital Universitari I Politècnic La Fe de Valencia;

5.5. VARIABLES

INDEPENDENT VARIABLE

The independent variable will be the implementation of our designed sepsis prevention bundle. Bundle compliance will be measured using an "all or none" approach [(36)], as a categorical dichotomous variable (yes/no).

PRIMARY DEPENDENT VARIABLE

The primary outcome variable will be the incidence of late-onset primary bloodstream infection in eligible infants diagnosed according EuroNeoKiss (European Neonatal Surveillance System) trial definitions[(37)]. These definitions were made based on the American National Nosocomial Infections Surveillance (NNIS) system of the Centers for Disease Control and Prevention (CDC) definitions[(38)] and rely on a combination of clinical symptoms, laboratory data and supportive data. We chose to include all forms of primary bloodstream infections: clinical sepsis, laboratory-confirmed bloodstream infection with a detected pathogen (but not CoNS) and laboratory-confirmed bloodstream infections with CoNS as the sole pathogen.

Clinical sepsis (infection without pathogen):

ALL the following criteria must be met:
Treating physician begins appropriate antimicrobial therapy for sepsis for ≥5 days
No pathogens detected in blood cultures or not tested
No apparent infection at another site
AND Two of the following clinical signs and symptoms:
Fever (>38°C) or unstable temperature or hypothermia (<36.5°C)
Tachycardia (>200/min) or new/increased bradycardia (<80/min)
Recapillarisation time >2s
New or increase apnea (>20s)
Unexplained metabolic acidosis (BE <-10mEq/L)
Other signs of BSI: skin color; increase oxygen requirement (intubation), unstable condition, and apathy.

Laboratory-confirmed bloodstream infection with proof of pathogen:

Non-CoNS pathogen isolated in blood culture or cerebrospinal fluid (pathogen not related to infection at another site)
AND Two of the following clinical signs and symptoms: Fever (>38°C) or unstable temperature or hypothermia (<36.5°C) Tachycardia (>200/min) or new/increased bradycardia (<80/min) Recapillarisation time >2s New or increase apnea (>20s) Unexplained metabolic acidosis (BE <-10mEq/L) Other signs of BSI: skin color; increase oxygen requirement (intubation), unstable condition, and apathy.

Laboratory-confirmed bloodstream infection with CoNS as sole pathogen:

CoNS isolated in blood culture or intravascular catheter as sole pathogen **AND ONE of the following criteria:**	
CRP >2.0 mg/dL / High interleukin	Neutrophil I/T ratio >0.2
Leukocytopenia <5/nl	Thrombocytopenia <100/nl
AND Two of the following clinical signs and symptoms: Fever (>38°C) or unstable temperature or hypothermia (<36.5°C) Tachycardia (>200/min) or new/increased bradycardia (<80/min) Recapillarisation time >2s New or increase apnea (>20s) Unexplained metabolic acidosis (BE <-10mEq/L) Other signs of BSI: skin color; increase oxygen requirement (intubation), unstable condition, and apathy.	

We decided to use these definitions and specifications for diagnosis of nosocomial infection since most participating NICUs already have processes in place to collect these data while participating in EuroNeoKiss surveillance project[39], ensuring uniformity and standardizing data collection. The incidence of late-onset primary bloodstream infection is also a categorical dichotomous variable (yes/no).

SECONDARY DEPENDENT VARIABLES

Neonatal Intensive Care Unit Mortality: defined as death after admission to the NICUs and prior to hospital discharge measured as categorical dichotomous variable (yes/no).

Neonatal Intensive Care Unit Length of stay: defined as the number of calendar days from the day of admission (counted as day 1) to the day of discharge (also counted as one day) measured as a continuous quantitative variable.

CONFOUNDING VARIABLES

Maternal and infant sociodemographic data

Gestational age: the time elapsed between the first day of the last normal menstrual period and the day of delivery, measured as discrete quantitative variable (weeks + days).

Small for gestational age: defined as infants with a birth weight below the 10^{th} percentile (for weight of all newborns at that gestational age) for gestational age, measured as a categorical dichotomous variable (yes/no).

Birth weight: child's weight at birth in grams (g) measured as a discrete quantitative variable (<500g, 501-750g, 751-1000g).

Ethnicity: measured as a categorical nominal variable (White, African American, Hispanic, Other).

Gender: measured as a categorical dichotomous variable (male/female)

Multiple Gestation: defined as any birth involving more than one infant, measured as a categorical dichotomous variable (yes/no).

Type of delivery: measured as a categorical dichotomous variable (vaginal delivery or caesarean section -elective or emergency-).

Prenatal care

Prenatal antibiotics administration: measured as a categorical dichotomous variable (yes/no).

Prenatal steroids administration: defined as administration of either a single course of betamethasone in two 12mg doses given intramuscularly (IM) 24 hours apart or dexamethasone in four 6mg IM doses at 12h intervals in women at risk of preterm delivery at 24-34 weeks of gestation and measured as a categorical dichotomous variable (yes/no).

Maternal drug abuse: measured as a categorical dichotomous variable (yes/no).

Clinics

5-minute Apgar score: The Apgar score is used immediately following the delivery of the baby to quickly evaluate the newborn's physical condition and assess if there is an immediate need for extra medical or emergency care (resuscitation in the delivery room). The Apgar rates Appearance (skin color), Pulse (heart rate), Grimace response (reflexes), Activity (muscle tone), and Respiration (breathing rate and effort). Each category is scored with a 0, 1, or 2, with 2 being the best score (please see **Annex 14.2**). Apgar scoring will be measured as a discrete quantitative variable (0-3, 4-6, 7-10).

Device utilization rate: describing the procedural percentage of patient days on which a certain device was used, calculated as the quotient of device days and the total number of a unit's patient days as a discrete quantitative variable:

Catheters[2] utilization rate =

(Number of days on which the patient had a catheter/Total patient days) x 100

Ventilation[3] utilization rate =

(Number of days on which the patient had a ventilation device/Total patient days) x 100

Antibiotic utilization rate: describing the proportion of patient days on which systemic antibiotics were used, calculated as the quotient of a unit's antibiotic days and total patient days as a discrete quantitative variable:

Antibiotic utilization rate = (Total antibiotics days/Total patient days) x 100

Surgery: measured as a categorical dichotomous variable (yes/no)

[2] Central vascular catheters, Peripheral vascular catheters and Umbilical artery/vein catheters are included in this sum.

[3] Continuous Positive Airway Pressure (CPAP) and endotracheal intubation devices are included in this sum.

Short and long-term complications

Intraventricular hemorrhage (IVH): typically initiated in the fragile periventricular germinal matrix, the majority of these newborns are asymptomatic and the diagnosis is based on screening cranial ultrasound.

- Grade 0: No subependymal or intraventricular hemorrhage.
- Grade 1: Subependymal germinal matrix hemorrhage only.
- Grade 2: Intraventricular blood, no ventricular dilation.
- Grade 3: Intraventricular blood, ventricular dilation.
- Grade 4: Intraparenchymal hemorrhage.

Measured as an ordinal qualitative variable.

Necrotizing enterocolitis (NEC): NEC is an acute inflammatory disease described as mucosal or deeper intestinal ischemia, necrosis and perforation, diagnosed clinically and radiographically using the following criteria:

- One or more of the following: bilious gastric aspirate or emesis, abdominal distension, occult or gross blood in stool AND,
- One or more of the following radiographic findings: *penumatosis intestinallis* (cystic or linear), hepato-biliary gas, *pneumoperitoneum*.

Measured as a categorical dichotomous variable (yes/no).

Bronchopulmonary dysplasia (BDP): also known as neonatal Chronic Lung Disease, is characteristically suspected when a ventilated patient is unable to wean from O_2 supplementation therapy, mechanical ventilation, or both, in the absence of underlying conditions including patent *ductus arteriosus* and ventilator-associated pneumonia. Diagnosis is made if the infant is still in hospital receiving any supplemental O_2 (of >21% FiO_2) on day 28. It will be measured as a categorical dichotomous variable (yes/no).

5.6. PROCEDURES, MEASUREMENTS AND STUDY INSTRUMENTS

Late-onset sepsis will be diagnosed by a neonatologist of the local research team and confirmed by an independent neonatologist using criteria mentioned above (see 5.5 Variables). In order to diagnose late-onset sepsis, the following diagnostic tests will be performed:

- Two blood cultures obtained from two different venipuncture or arterial puncture sites with at least a minimum volume of 1mL of blood and separated by at least 15 to 30 minutes, using strict aseptic techniques;
- A cerebrospinal culture obtained by lumbar puncture;
- A urine culture obtained by catheter or bladder tap for infants >6 days of age;
- Laboratory tests: Complete blood count, C-Reactive protein, procalcitonine and interleukins 6 and 8;
- Molecular techniques: Polymerase Chain Reaction.

5.7. STUDY INTERVENTION

A bundle of comprehensive preventive measures will be gradually implemented during the intervention period in participants NICUs. Our bundle was designed following IHI design guidelines suggesting that a bundle should have three to five elements, each one of them being already recommended and generally accepted as being safe and reliable care in national guidelines and by all clinicians participating. Each bundle element should also be relatively independent so that if not implemented (e.g.: because medically contraindicated) the remaining elements still could be applied and, descriptive rather than prescriptive, to allow individual customization. Hereafter, the intervention consists on creating a list of comprehensive evidence-based preventive measures, specifically designed for our chosen patient population (newborns <1.000gr of birth weight) to be applied simultaneously as a care bundle in our unit and in any other NICU participating.

Our designed bundle will be composed of: re-enforcement of hand hygiene practices, promotion of early enteral trophic feeding with breast milk, and specific antimicrobial stewardship strategies.

BUNDLE ELEMENT #1: RE-ENFORCEMENT OF HAND HYGIENE PRACTICES

All staff members will be instructed to perform hand hygiene according to the WHO Guidelines on hand hygiene in health care[20]:

- At the entry in the NICU, remove all rings, bracelets and watches and do not wear artificial fingernails or extenders. Wash hands with soap (4% Chlorhexidine Gluconate) and water when visibly dirty or soiled with blood or other body fluids, use an alcohol-based hand rub (Sterillium® or Instrunet®Aniosgel) placed near the infant for all routine antisepsis if the hands are not soiled.

Figure 3: Recommended soap and alcohol-based hand rubs for hand hygiene

- When washing your hands with an alcohol-based hand rub, apply a palmful of product and cover all surfaces of the hands and rub until dry. When using soap and water, wet hands with water and apply enough soap to cover all surfaces, rinse hands with water and dry thoroughly using single-use towel (posters and brochures on hand hygiene techniques will be distributed for further explanation and as remainders, please see **Annex 14.3**)
- Perform hand hygiene before and after touching the patient, before handling an invasive device for patient care, after contact with body fluids or excretions, mucous membranes, non intact skin, or wound dressings, if moving from a contaminated body site to another body site during care of the same patient, after contact with medical equipment in the immediate vicinity of the patient and after removing non sterile gloves.

BUNDLE ELEMENT #2: PROMOTION OF EARLY ENTERAL TROPHIC OR MINIMUM ENTERAL FEEDING

All units must initiate enteral trophic feeding practices as soon as possible (preferably within 48-72 hours of life) using the following feeding guidelines, unless contraindications to feeding are present: hemodynamic instability, abnormal abdomen (e.g.: bowel obstruction or ileus, necrotizing enterocolitis, acute abdominal distension,), perinatal depression, pulmonary instability and fluid and electrolyte instability.

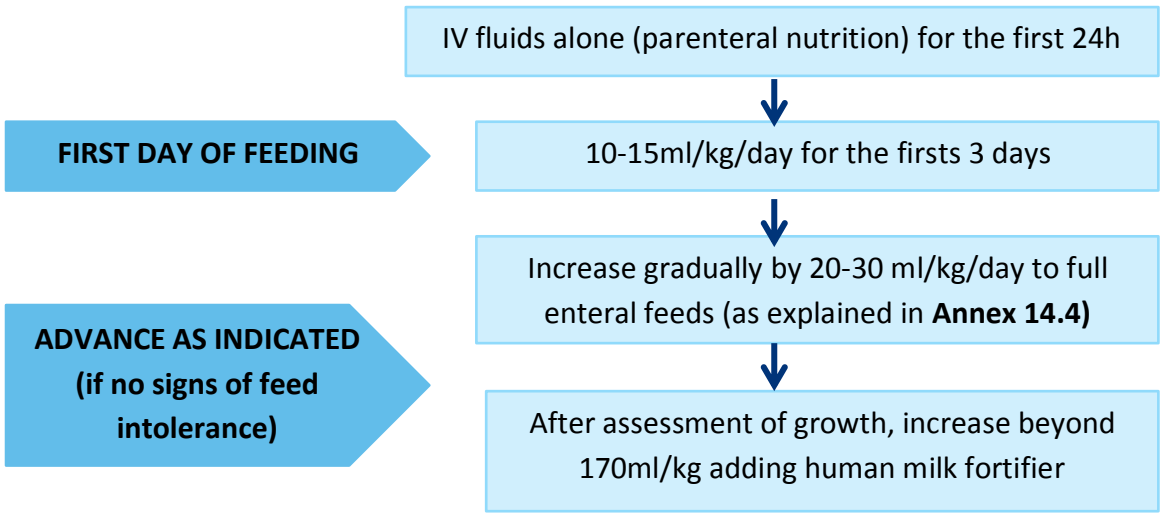


Figure 4: Algorithm for initiating and advancing enteral feedings in ELBW newborns

- Route of delivery and method of infusion: milk will be administered by gavage feeding through an oral- or nasogastric feeding tube (OG/NG) using a pump at a constant infusion rate of 1-2ml every 3-6 hours (cycle continuous infusion); If severe gastroesophageal reflux, persistent vomiting or risk of aspiration, consider using a nasojejunal (NJ) tube.
- Content of feeding: Maternal expressed breast milk (unfortified) or colostrum is the preferred milk; when unavailable or contraindicated, donor human breast milk from the milk bank may be used, after obtaining parental consent (please see **Annex 14.5)**. If refusal, preterm formulas (e.g. Nestlé® NAN Alprem) may then be used.

Monitor for signs of possible feed intolerance (including bloody or bilious gastric residuals or emesis, bloody stools, abdominal distension or discoloration,) and if present, reduce or discontinue feedings and assess the patient's status, tube placement, rate and method of delivery. Daily monitor serum glucose, urea, creatinine and electrolytes and adjust feeding by supplementing with parenteral nutrition.

BUNDLE ELEMENT #3: ANTIMICROBIAL STEWARDSHIP PROGRAM

The following strategies will be implemented as part as our antimicrobial stewardship program:

- Prescriber audit and feedback: the clinical pharmacist and the clinical microbiologist (members of the local research team) will review culture results and narrow antibiotic coverage promptly once sepsis is confirmed and provide feedback on toxicity and dose adjustments (e.g.: if deteriorated renal function….).
- Clinical and treatment guidelines/pathways: all neonatologists must following the ensuing algorithm to reduce inappropriate antimicrobial use,

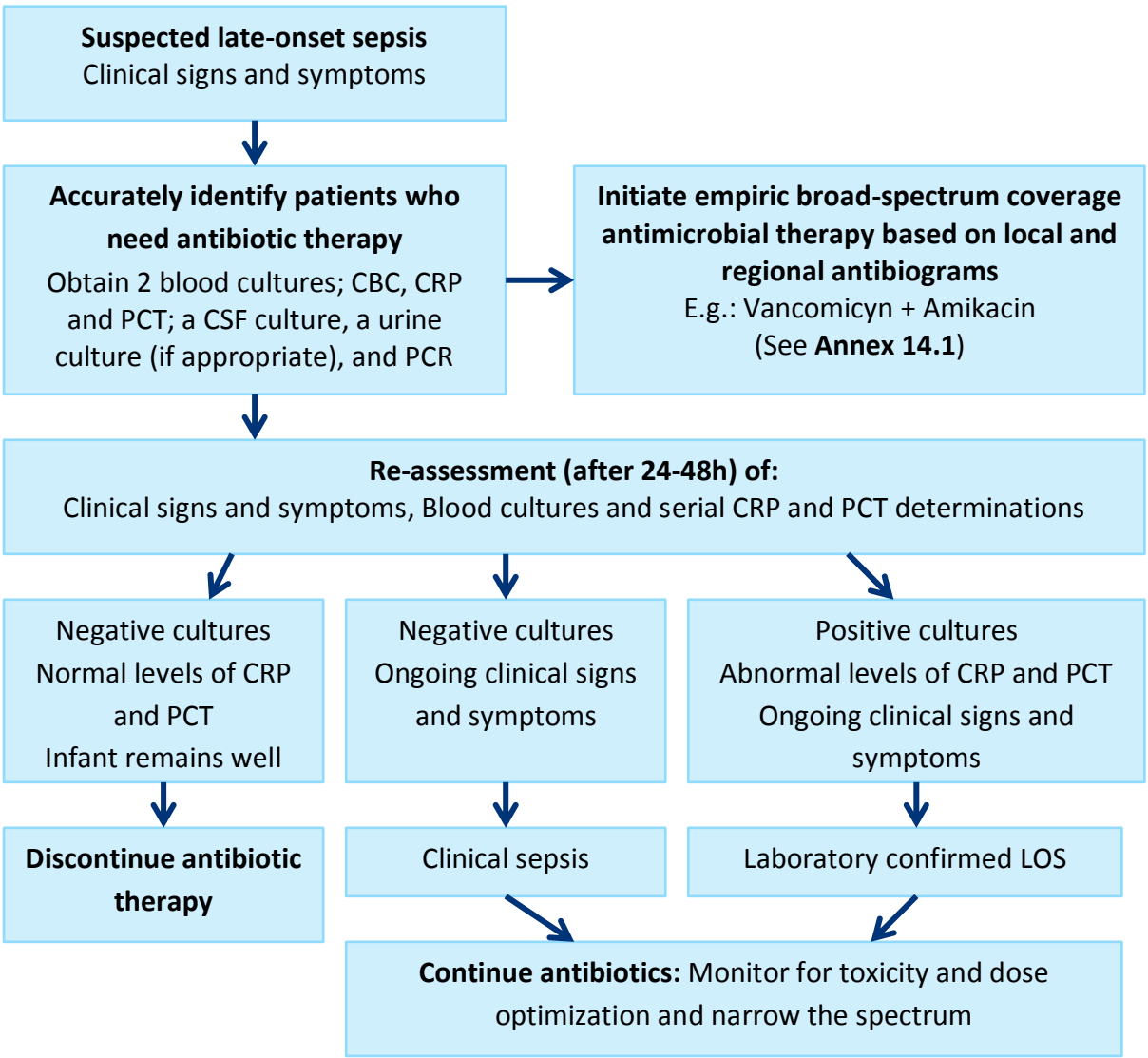


Figure 5: Antimicrobial clinical and treatment pathways

5.8. DATA COLLECTION

Baseline or pre-intervention data will be retrospectively collected from the unit's surveillance local reports. Following the intervention, a neonatologist of the local NICU research team will prospectively collect data on late-onset sepsis diagnosis and a purpose-trained research nurse will report adherence to our designed prevention bundle.

Data collection for compliance of each bundle element will be at random moments seven days a week and 24 hours a day (AM, PM or night) during usual daily care activities. A self-designed care bundle compliance report form (please see **14.6**) will be deployed in paper form to guide observation and must be fill out when unobtrusively observing staff.

Relevant data on late-onset sepsis (infection start day, isolated pathogen and type of primary bloodstream infection following diagnostic criteria) will be recorded in a specific Patient Progress Note (please see **Annex 14.7**) kept in the facility. Confounding variables (maternal and infant sociodemographic data, prenatal care, clinics and short and long-term complications) will also be reported in this Patient Progress Note.

Both charts will be distributed across participating units to simplify and standardize data collection and must be submitted monthly. If feasible, external auditing by a senior observer of source data is highly recommended to ascertain accuracy and minimize information bias in self-reporting.

6. DATA MANAGEMENT AND STATISTICAL ANALYSIS

UNIVARIATE ANALYSIS

A descriptive analysis of all variables will be performed. Categorical variables will be summarized as numbers (n) and percentages (%) while quantitative variables will be expressed as mean ± standard deviation (SD).

BIVARIATE ANALYSIS

The independent (bundle implementation) and both primary (incidence of late onset primary bloodstream infection) and first secondary (NICU mortality rate) dependent variables are all categorical variables and hence comparisons of pre- and post-intervention periods will be performed using Pearson's χ^2 test. Student's *t*-test will be used for analysis of difference while comparing the independent and second secondary dependent variable (length of stay), measured as a continuous quantitative variable.

MULTIVARIATE ANALYSIS

We will use segmented regression analysis to assess the impact of the intervention on the outcome measurements using a pre-intervention and a post-intervention segment. Segmented regression analysis uses statistical models to estimate the level and trend of the regression lines in the pre-intervention segment and changes in level (i.e., the immediate drop in rates rapidly after the launch of the care bundle) and trend (i.e., the gradual decline in rates over the follow-up period) after the intervention.

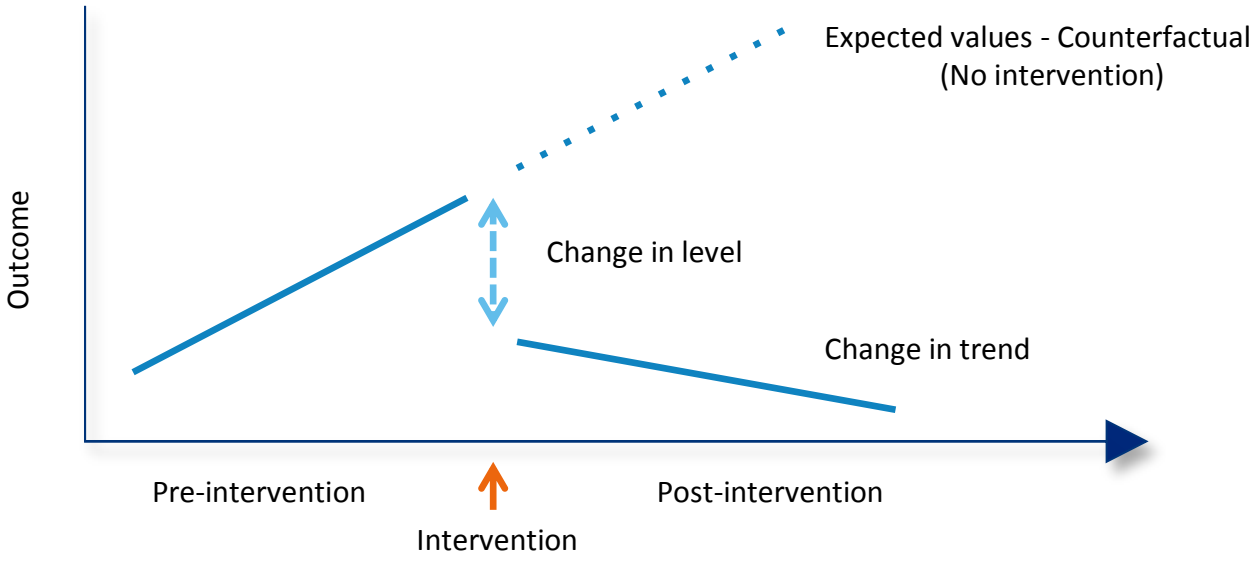


Figure 6: Segmented regression analysis of interrupted time series data

All observations made during for the 3-month intervention period are not enough to be included in the analysis as an independent segment and hence will be discarded.

Important measured confounders will be controlled by including them in the regression model. Data will be analyzed using IBM SPSS Statistics for Windows® predictive analytics software (IBM SPSS Statistics, Armonk, NY).

The level of statistical significance will be set at $p<0.05$, with a 95% confidence interval.

7. ETHICS APPROVAL

This research protocol will be submitted for approval to each Clinical Research Ethical Committee of all participants NICUs.

This study will be conducted in full conformance with the "Ethical principles for Medical research involving human subjects" of the World Medical Association Declaration of Helsinki (adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and lastly amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013).

This study will also be led within the following laws and regulations of Spain:

- *Ley Orgánica 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica.*
- *Ley Orgánica 14/2017, de 3 de julio, de Investigación biomédica (RD 1090/2015)*

Since this project involves newborns, signed informed consent of parents or legally authorized representatives will be obtained to be eligible for enrollment into the study, after providing comprehensive information (please see **Annexes 14.9 and 14.10**)

Subject's names will be kept on a password protected database and linked only with a study identification number to guarantee confidentiality according to Spanish's *Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal.*

All the investigators of this study declare that there are no conflicts of interest.

8. STRENGTHS AND LIMITATIONS

When designing a multicenter study conducted in multiple NICUs, different interpretations on how data is collected and measured may occur, leading to multiple potential information biases. To minimize information bias we used not only standardized definitions and diagnostic criteria and procedures, but also detailed report forms.

Furthermore, self-report by health-care workers within the same unit may lead to an specific observer bias causing discrepancy between actual and reported delivered care. In order to reduce the likelihood that NICUs may under- or over-report either bundle compliance or infection rates, we scheduled coordinating meetings and training sessions guaranteeing a complete understanding of the methodology of the study and even decided to conduct completing external audits.

We also acknowledge that the presence of an observer may induce better than usual behavior (Hawthorne effect), even when unobtrusively and discreetly observing the health-care workers to assess bundle compliance.

Since our study design does not involve randomization of the preventive intervention into an intervention group and a control group (ethically unacceptable), any interpretations regarding the causal effect of any association must be undertaken with caution (i.e. other events may have influenced the outcomes of interest and thus alternatives explanations for the changes in late-onset bloodstream infection incidence, mortality and related length of stay cannot be omitted). Nonetheless, the level and trend of the pre-intervention period may serve as control or counterfactual **(**please see **Figure 6)** for the post-intervention period, allowing us to still measure the impact of the intervention. Undeniably, a notable strength of interrupted time studies is that they provide intuitive graphical exposition of results, making it easy to identify when the intervention happened, what was happening before it, and what occurred immediately after it as well as in the follow-up period. Besides important measured confounders will be added to the multivariate analysis for adjusting the results.

Although care bundles have already proven to be effective in reducing harmful clinical outcomes, it is still a challenge to achieve 100% levels of bundle compliance. Nevertheless we used a validated implementation model (the Pronovost model, please see **Annex 14.8**) to guide implementation, expecting that there will be hardly any non-compliant units. Still, since we will be measuring compliance with each individual bundle element, we will be able to measure the percentage of bundle compliance and adjust for it when analyzing results, if necessary. By doing so we will also let participants see which elemetns of the care bundle are not being completed reliably in the unit, allowing them to identify their problematical areas to make specific changes to improve.

We also agree that bundles do not represent comprehensive care but adding more elements to the bundle in order to cover all individual needs will likely affect the consistency of the bundle, resulting in lower compliance.

9. WORK PLAN

RESEARCH TEAM MEMBERS:

- Principal investigator (PI) and study coordinator: he/she will supervise all aspects of the study (protocol design, recruitment, data collection, analysis, publication and dissemination). The PI will also be responsible for ensuring that all local research teams members have appropriate education and training.
- A medical student will assist the principal investigator.
- A research neonatologist, a research nurse, a clinical pharmacist with infectious disease training, and a clinical microbiologist for each participating NICU will form the local research team, responsible for protocol compliance and data collection.
- A qualified statistician will perform the statistical analysis.

Implementation will be guided by the Pronovost-model (please see **Annex 14.8**)

STAGE 1: FEBRUARY 2017 – JULY 2017

- Bibliographic research (February 2017) and study design (March 2017 – May 2017)
    - Elaborated by the PI (and study coordinator) and a medical student.
- Ethical evaluation (June 2017)
    - Submitted by the PI and assessed by the Clinical Research Ethical Committee of each participating NICU.

Besides the main objectives mentioned above, during stage 1, before implementing the care bundle, we should collect retrospective baseline data on the BSI incidence rate among the participating NICUs in order to compare progress post-implementation.

- Meeting 1 (July 2017): meetings between the PI and each unit will be scheduled to discuss the aims and methodology of the study. In order to guarantee adequate aggregated bundle compliance, the local teams will be educated through workshops, educational meetings, seminars and teaching sessions led by the PI. Posters, fact sheets and brochures will be distributed to reach a great number of staff ensuring collective understanding. Training campaigns will not only provide information on what needs to be done to implement the care bundle but also on how to observe, monitor and proceed to measure using the bundle compliance report form and the late-onset sepsis data collection sheet (Annexes 14.6 and 14.7).
- The PI will also encourage units to monitor current practice and address perceived barriers and potential resistance to successful implementation of the selected interventions, to identify motivators that could lead the implementation process started and coordination during stage 2, and to engage senior leadership and management support.

STAGE 2: AUGUST 2017 - FEBRUARY 2019

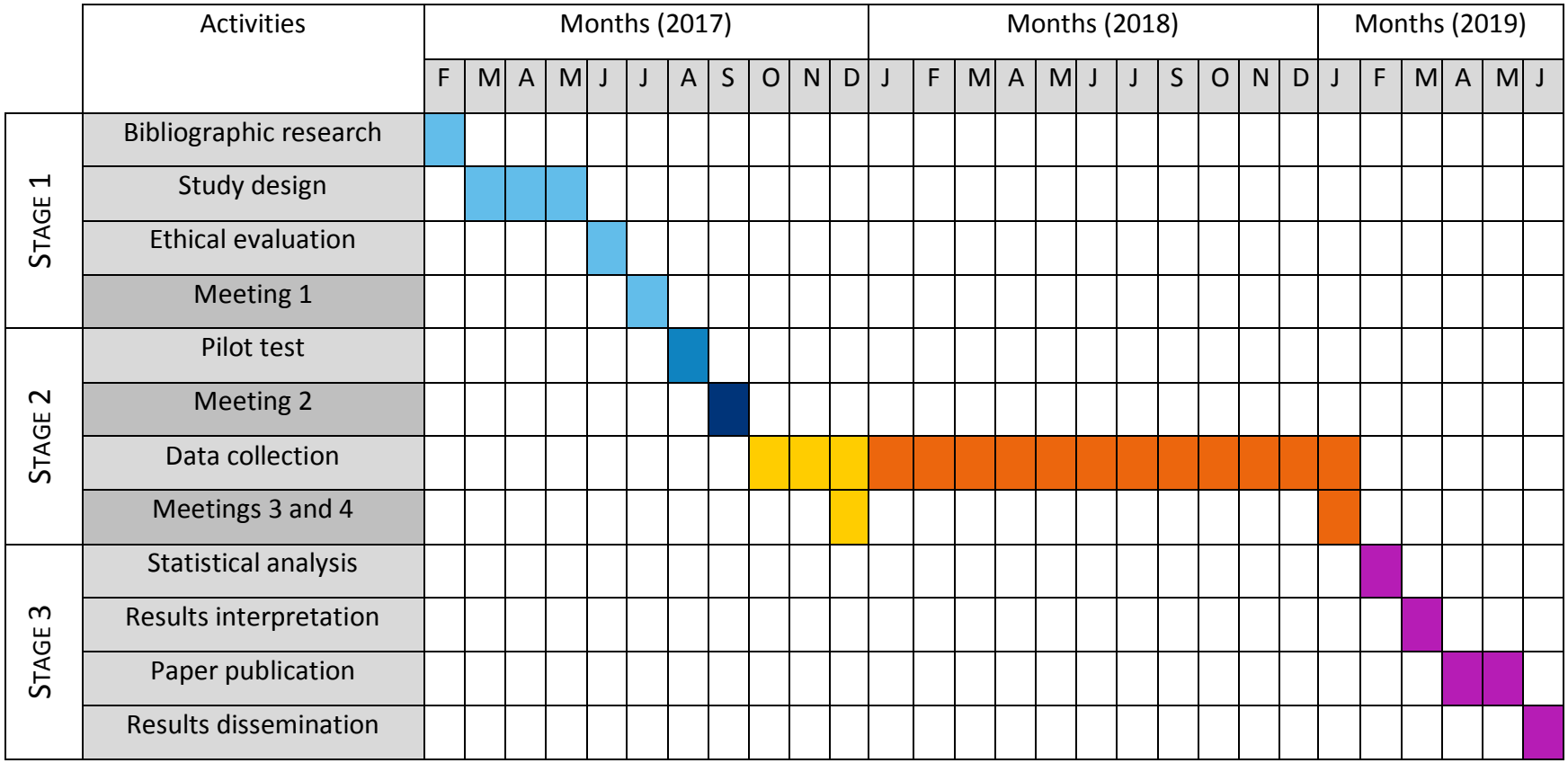
- Pilot test and meeting 2: throughout August 2017 a preliminary testing should be conducted in order to provide local feedback data on the resources required to carry out all bundle elements and to evaluate feasibility of implementation in different location hospitals to adapt and refine any step if needed.
    - Ideally, at least one unit of each Autonomous region participating in the study should perform a pilot test directed by the local research team.

- Data collection during the intervention period: We expect that at least 3 months will be needed until full implementation of the bundle by our health care providers is satisfactory accomplished. Therefore the bundle will be launched in all participating units from October 2017 to December 2017.
- Data collection during the post-intervention period: From January 2018 to January 2019, bundle compliance within each unit should reach at least 80 per cent. Continued regular audits of adherence with the care bundle on a going basis will be essential to ensure and maintain compliance. The local research team will submit monthly reports of the primary and secondary outcomes using the designed bundle compliance report form and the late-onset sepsis data collection sheet (as specified in 5.8 Data collection).
- Meeting 3 and 4: During all stages, data will be compared to the baseline measurement (meeting 3) and data collected in the previous period (meeting 4) and findings will be reported: the research team will individually report to the unit with audits and feedback on BSI rates and bundle compliance. Continual feedback on successful outcomes may encourage staff to be compliant with the care bundle and punctual feedback on negative outcomes may be an incentive to amend initial unwanted behavior without leading to discouragement.

STAGE 3: FEBRUARY 2019 – JUNE 2019

- Statistical analysis (February 2019)
    - Performed by a qualified statistician (as specified in 6. DATA MANAGEMENT AND STATISTICAL ANALYSIS).
- Results interpretation and discussion (March 2019) and paper publication (April 2019 – May 2019) written by the PI in collaboration with the local research teams, and supervised by the qualified statistician.
- Results dissemination (June 2019) by the PI (and study coordinator).

10. CHRONOGRAM



11. BUDGET

	Costs
STAFF	
Qualified Statistician: 35€/hour, 3 hours per day, 2 days/week per 4 weeks	840€
	Subtotal: 840€
MEETINGS AND EDUCATION	
Meetings: 50€/person. 9 persons (1 Study coordinator + 8 Research neonatologists) for a total of 4 meetings.	1.800€
Training: Workshops, educational meetings, seminars and teaching sessions	2.500€
	Subtotal: 4.300€
PUBLICATION	
Paper revision	500€
Paper publication	500€
	Subtotal: 1.000€
DISSEMINATION	
Travel and subsistence costs	
International Congress	
- Inscription fee	400€
- Travel costs	100€
- Accommodation and diets	150€
National Congress	
- Inscription fee	450€
- Travel costs	100€
- Accommodation and diets	85€
	Subtotal: 1.285€
	TOTAL: 7.425 €

Dissemination costs were estimated based on the *13th World Congress of Perinatal Medicine*, held on October 26-29, 2017 in Belgrade, Serbia[40] and the *XXVI Congreso de Neonatología y Medicina Perinatal*, held on September 27-29, 2017 in Zaragoza, Spain[41] inscription, travel and accommodation and diet fees.

12. FEASIBILITY

This study will be conducted in several Spanish NICUs, chosen because of their higher number of births of extremely low birth weight newborns, in order to ensure a largely available subject population, and thus adequate number of subjects to prove statically significant outcomes in the expected time frame of the study.

Since this study will be performed simultaneously at several centers, this protocol was elaborated using precise directions and definitions on how to discharge all elements of the bundle and measure all outcomes, avoiding any misunderstanding. Furthermore Pilot Tests would be carried through so as to assess further feasibility, in practice.

All materials and drugs (soap, alcohol-based hand rub, preterm formula, antibiotics…) needed to fulfill every element of the designed bundle that will be implemented during the study and all diagnostic procedures (cultures, laboratory tests, molecular techniques…) needed to measure the main and secondary outcomes are already available and performed routinely in all participating NICUs, requiring no additional special facilities, nor new equipment or special techniques. Additional personnel (except for a statistician) shall not be required. Thus, our cost budget includes mostly expenses due to meetings, education and publication and dissemination of our study, with an estimated total amount as few as 7.425€, making it financially feasible.

13. IMPACT OF THE STUDY TO THE NATIONAL HEALTH SYSTEM

Late-onset sepsis is a major health problem not only in terms of mortality and morbidity but also because of prolonged length of stay and thus increased hospital costs, particularly in extremely low birth weight infants (the most immature the infant, the higher the incidence of infectious complications). The financial burden of late-onset sepsis was evaluated in a comparative retrospective cohort study conducted at the Neonatal Intensive Care Unit of University Hospital of Antwerp, Belgium showing that the mean additional length of stay in newborns with late-onset sepsis (proven or suspected) was 24 days, and extra charges attributable to late-onset sepsis were estimated at 11.750€ per patient[(42)] .

By conducting this study, we aim to provide relevant scientific and clinical information on how to prevent late-onset sepsis using evidence-based and cost-effective measures that once proven “statistically significant” would result in a new preventive health policy that could be routinely implemented in all NICUs in order to reduce newborn mortality, morbidity and hospital expenditures due to late-onset sepsis.

Our expected positive results in preventing nosocomial infections by implementing care bundles may be relevant to future research including different preventive elements that the ones we chose, to reduce other types of infections, in other Intensive Care Units (Pediatric and Adult).

14. ANNEXES

14.1. DOSAGE AND ADMINISTRATION OF THE MOST COMMON USED ANTIBIOTICS[43]

AMIKACIN

DOSE AND ADMINISTRATION: IV infusion by syringe pump over 30 minutes

Gestational age (weeks)	**Postnatal Days**	**Dose** (mg/kg)	**Interval** (hours)
≤ 29	0-7	18	48
	8-28	15	36
	≥ 29	15	24
30-34	0-7	18	36
	> 8	15	24
≥35	All	15	24

VANCOMYCIN

DOSE AND ADMINISTRATION: IV infusion by syringe pump over 60 minutes

Gestational age (weeks)	**Postnatal Days**	**Dose** (mg/kg)	**Interval** (hours)
≤ 29	0-14	10	18
	>14	10	12
30-36	0-14	10	12
	> 14	10	8
37-44	0-7	10	12
	> 7	10	8
≥45	All	10	6

CEFOTAXIME

DOSE AND ADMINISTRATION: IV infusion by syringe pump over 30 minutes

Gestational age (weeks)	**Postnatal Days**	**Dose** (mg/kg)	**Interval** (hours)
≤ 29	0-28	50	12
	>28	50	8
30-36	0-14	50	12
	> 14	50	8
37-44	0-7	50	12
	> 7	50	8
≥45	All	50	6

CEFTAZIDIME

DOSE AND ADMINISTRATION: IV infusion by syringe pump over 20 minutes

Gestational age (weeks)	**Postnatal Days**	**Dose** (mg/kg)	**Interval** (hours)
≤ 29	0-28	30	12
	>28	30	8
30-36	0-14	30	12
	> 14	30	8
37-44	0-7	30	12
	> 7	30	8
≥45	All	30	6

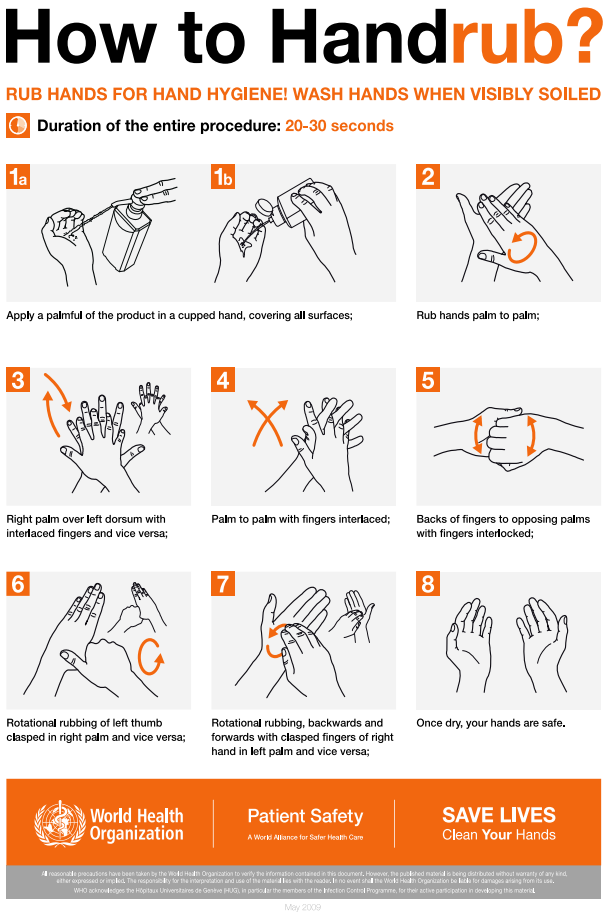
14.2. APGAR SCORING SYSTEM

	0 Points	1 Point	2 Point	Points totaled
Activity (Muscle tone)	Absent	Arms and legs flexed	Active movement	
Pulse	Absent	Below 100bpm	Over 100bpm	
Grimace (Reflex irritability)	Absent	Some flexion of extremities	Active motion (sneeze, cough pull away)	
Appearance	Absent	Body pink, Extremities blue	Completely pink	
Respiration	Absent	Slow, irregular	Vigorous cry	

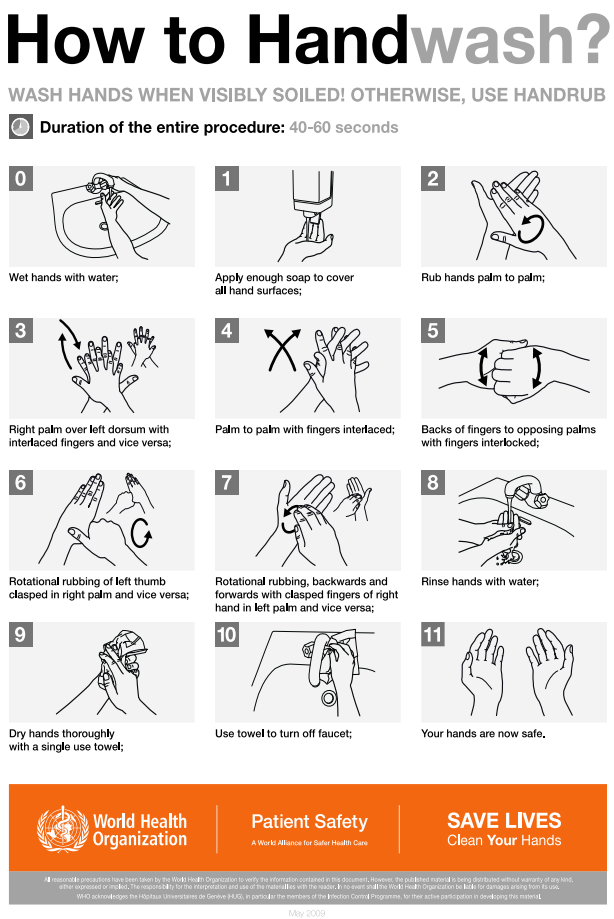
Severely depressed 0-3
Moderately depressed 4-6
Excellent condition 7-10

14.3. HAND HYGIENE TECHNIQUES

SOURCE: WHO SAVE LIVES - Clean your hands initiative ©World Health Organization 2009



http://www.who.int/gpsc/5may/How_To_HandRub_Poster.



http://www.who.int/gpsc/5may/How_To_HandWash_Poste

14.4. ENTERAL NUTRITION REFERENCE INTAKES FOR EXTREMELY LOW BIRTH WEIGHT NEWBORNS (UNIT/KG/DAY)

Day	Volume (ml)	Calories (Kcal)	Carbohydrates (g)	Amino Acids (g)	Lipids (g)	Sodium (mEq)	Potassium (mEq)	Calcium (mEq)	Phosphate (mEq)	Magnesium (mEq)
1	-	-	-	-	-	-	-	-	-	-
2	10	7	0.7	0.18	0.4	0.1	0.1	0.1	0.04	0.02
3	10	7	0.7	0.18	0.4	0.1	0.1	0.1	0.04	0.02
4	20	14	1.4	0.36	0.8	0.3	0.3	0.2	0.09	0.04
5	30	21	2.1	0.54	1.2	0.4	0.4	0.3	0.13	0.06
6	45	31.5	3.1	0.81	1.8	0.6	0.7	0.5	0.2	0.1
7	65	45.5	4.5	1.17	2.6	0.8	1	0.7	0.3	0.13
8	85	59.5	5.9	1.53	3.4	1.1	1.3	0.9	0.4	0.17
9	110	77	7.7	1.98	4.4	1.4	1.6	1.2	0.5	0.22
10	140	98	9.8	2.52	5.6	1.8	2.1	1.5	0.6	0.28
11	170*	138	15.4	4.4	6.8	4.2	3.3	6	2.3	0.9

** Breast milk + Almiron Fortifier™ 3.3*

14.5. DONOR MILK CONSENT FORM

1. DECLARO expresamente mi conformidad para la administración de leche humana de donante a mi hijo, así como a la inclusión de sus datos en un fichero automatizado de acuerdo a lo dispuesto en la Ley Orgánica 15/1999 de 13 de diciembre de Protección de datos de carácter personal.

2. He sido informado que leche humana procedente de donante debe ser administrada a mi hijo como parte del procedimiento terapéutico al que va a ser sometido. También he sido informado de las precauciones tomadas para evitar futuros riesgos.

FIRMA DEL PADRE/MADRE O PERSONA AUTORIZADA:

Nombre:……

Fecha:……

INFORMADO POR EL Dr.:……

14.6. LATE-ONSET SEPSIS PREVENTION BUNDLE COMPLIANCE REPORT FORM

LATE-ONSET SEPSIS PREVENTION CARE BUNDLE COMPLIANCE REPORT FORM

PATIENT ID:..... **DATE OF BIRTH** (dd/mm/yy):.....

DATA COLLECTION DETAILS

Hospital name:.....

Date of compliance check (dd/mm/yy):.....

Time of compliance check: ☐ AM SHIFT ☐ PM SHIFT ☐NIGHT SHIFT

Completed by (staff name):.....

Late-onset sepsis prevention bundle		
Hands have been washed following correct hand hygiene technique	YES	NO
Enteral trophic feeding has been started following the protocol	YES	NO
Antimicrobial prescriber audit and feedback has been fulfilled	YES	NO
Antimicrobial guidelines have been followed	YES	NO

☐ 100% compliance with the care bundle *(calculated by dividing the number of bundle elements completed, by the total number of bundle elements)*

Example | ~~YES~~ | NO |

Action plan (note any exceptions for not complying):

Comments:

NOTE: if a bundle element is considered medically contraindicated for a particular patient, the patient will be considered compliant with regard to that measure.

14.7. PATIENT PROGRESS NOTE

LATE-ONSET SEPSIS DATA COLLECTION SHEET (ADAPTED FROM NEO-KISS)

PATIENT ID:.....

Infection start date (dd/mm/yy):.....

Pathogen:.....

☐ **Two of the following clinical signs and symptoms**		
Fever (>38 °C) or unstable temperature or hypothermia (<36.5°C)	YES	NO
Tachycardia (>200/min) or new/increased bradycardia (<80/min)	YES	NO
Recapillarisation time >2s	YES	NO
New or increase apnea (>20s)	YES	NO
Unexplained metabolic acidosis (BE <-10mEq/L)	YES	NO
Other signs of BSI: skin color; increase oxygen requirement (intubation), unstable condition, and apathy.	YES	NO

☐ **Criteria for clinical sepsis (all of the following)**		
Treating physician begins appropriate antimicrobial therapy for sepsis for ≥5 days	YES	NO
No pathogens detected in blood cultures or not tested	YES	NO
No apparent infection at another site	YES	NO

☐ **Criteria for laboratory-confirmed BSI**		
Non-CoNS pathogen isolated in blood culture or cerebrospinal fluid (pathogen not related to infection at another site)	YES	NO

☐ **Criteria for laboratory-confirmed BSI with CNS as sole pathogen**		
CoNS isolated in blood culture or intravascular catheter as sole pathogen	YES	NO
And one of the following criteria		
CRP >2.0 mg/dL / High interleukin	YES	NO
Neutrophil I/T ratio >0.2	YES	NO
Leukocytopenia <5/nl	YES	NO
Thrombocytopenia <100/nl	YES	NO

Gestational age: …… (weeks + days)

Small for gestational age	☐ YES ☐ NO	**Gender**	☐ Male ☐ Female
Birth weight	☐ <500g ☐ 501-750g ☐ 751-1000g	**Multiple gestation**	☐ YES (No.: ……) ☐ NO
Race	☐ White ☐ African ☐ American ☐ Hispanic ☐ other	**Type of delivery**	☐ Vaginal delivery ☐ Caesarean section

Prenatal antibiotics administration	☐ YES ☐ NO
Prenatal steroid administration	☐ YES ☐ NO
Maternal drug abuse	☐ YES ☐ NO

5-minute Apgar score	☐ 0-3 ☐ 4-6 ☐ 7-10

Patient days: …… **CPAP + Intubation days:** ……

Antibiotics days: …… **CVC + PVC days**: ……

(Retrieve the total number of device utilization days using the "Device utilization" form annexed in the following page)

Surgery	☐ YES (Type:……) ☐ NO

IVH	☐ Grade 0 ☐ Grade 1 ☐ Grade 2 ☐ Grade 3 ☐ Grade 4	**NEC**	☐ YES ☐ NO	**BDP**	☐ YES ☐ NO

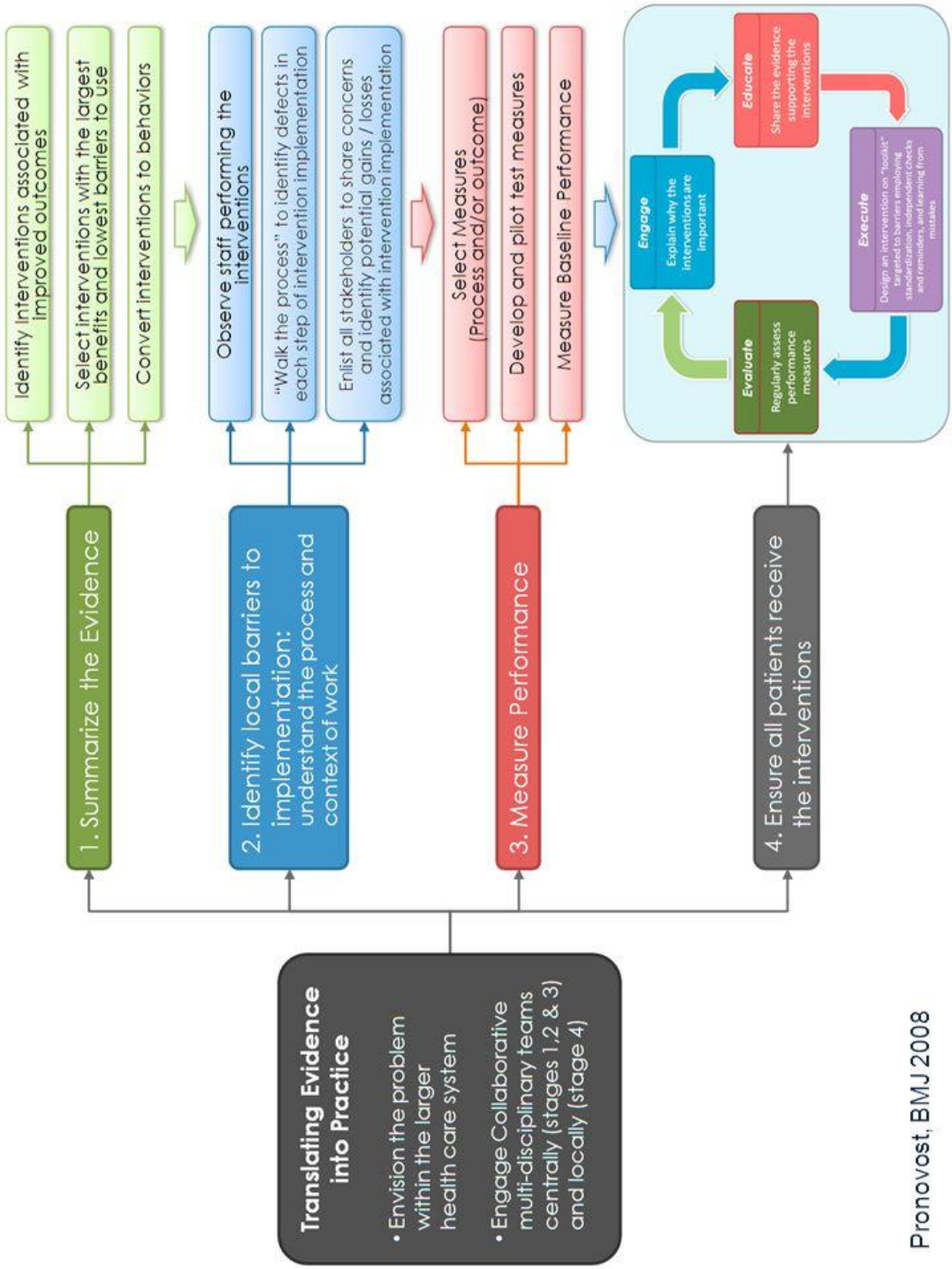
Example: ☑

DEVICE UTILIZATION FORM

	Month/Year: ……																													
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Σ*
CVC																																
PVC																																
CVC + PVC																																
CPAP																																
Intubation																																
CPAP + Intubation																																
Antibiotics																																

* Add up the figures at the end of the month. If 1500g achieved, transfer/discharge or death, add together all monthly totals and put the total on the Patient Progress Note.

14.8. THE PRONOVOST-MODEL



Overview of STOP-BSI Program (Text Version). December 2009.
Agency for Healthcare Research and Quality, Rockville, MD.
https://archive.ahrq.gov/news/events/conference/2009/pronovost/index.html

14.9. INFORMATION SHEET FOR PARENTS OR LEGAL TUTORS (SPANISH VERSION)

HOJA DE INFORMACIÓN AL FAMILIAR RESPONSABLE O REPRESENTANTE LEGAL

Hospital Universitari de Girona Doctor Josep Trueta

Replace with each participant institution logo

TÍTULO DEL ESTUDIO: IMPLEMENTACIÓN DE UN PAQUETE DE MEDIDAS PARA LA PREVENCIÓN DE LA SEPSIS NOSOCOMIAL EN RECIÉN NACIDOS DE EXTREMADO BAJO PESO AL NACER

CÓDIGO DEL ESTUDIO: ..... **PROMOTOR:**.....

INVESTIGADOR COORDINADOR: .....**CENTRO:**.....

Nos dirigimos a usted para informarle de que se esta llevando a cabo un estudio con el objetivo de evaluar la eficacia de una intervención preventiva que pretende reducir las infecciones que los recién nacidos de extremado bajo peso al nacer (menor o igual a 1.000gr) pueden adquirir en el hospital. Como padre o tutor legal del recién nacido, procedemos a informaros sobre este estudio, que ha sido previamente aprobado por el Comité Ético de Investigación Clínica del Hospital Universitari Dr. Josep Trueta (Centro coordinador), así como por el Comités de cada centro participante.

Participaran en estudio las Unidades de Curas Intensivas Neonatales (UCIN) de numerosos hospitales de Cataluña, Comunidad de Madrid, Comunidad Valenciana y Andalucía. Se espera incluir una muestra de al menos 547 recién nacidos de extremado bajo peso al nacer que hayan sido ingresados en las UCINs.

Este estudio recoge diariamente información sobre los procedimientos médicos realizados a su hijo/a (uso de catéteres, respiradores, antibióticos…) así como de los datos relacionados con el proceso infeccioso para conocer mejor su comportamiento y asi poderlo prevenir en el futuro. Con ese objetivo, se aplicarán una serie de medidas preventivas basadas en la mejor evidencia disponible, que buscan disminuir el numero de estas infecciones. Estas medidas preventivas se centran en practicar la higiene de manos apropiada, promover la nutrición enteral del recién nacido con leche materna o artificial en su defecto, y aplicar una estrategia lo mas efectiva y segura posible para la pauta de antibióticos dentro de la unidad.

Nuestro objetivo es valorar si estas medidas son eficaces para disminuir el numero infecciones adquiridas en el hospital, la mortalidad y la estancia en la UCIN. La realización de

este estudio no exige que se realicen pruebas clínicas o tratamientos adicionales (analíticas, radiografías, etc.) ni tampoco mas visitas de las que son estrictamente necesarias dentro de la practica clínica habitual. Su médico será la persona que acceda a la base de datos de la historia clínica y otros informes del paciente y quien recoja los datos necesarios para la posterior evaluación de resultados. Por lo tanto, no se prevé que la realización de este estudio comporte ningún riesgo adicional para la salud de su hijo/a.

CONFIDENCIALIDAD

De acuerdo con la Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal, los datos personales que se requieran (sociodemográficos, prenatales y clínicos), son solo los necesarios para cubrir los objetivos del estudio, y en ningún caso incluyen datos identificatorios (nombre, apellidos, numero de historia clínica). Los datos recogidos estarán asociados a un código relacionado con el estudio que en ningún caso permitirá identificar al menor. Solo su medico podrá conocer la asociación del código asignado con la identidad de su hijo/a. Ningún dato personal que permita la identificación del paciente será accesible a ninguna persona que no sea su medico, ni podrán ser difundidos por ningún medico, conservando en todo momento la confidencialidad medico-paciente. En la publicación de los resultados se conservara siempre la anonimidad de los pacientes. Además, con el fin de revisar los resultados del estudio, las autoridades sanitarias y organismos regulares podrán tener acceso a todos los datos. Todas las personas y entidades están sujetas y obligadas a mantener en secreto la identidad del paciente.

Delante de cualquier eventualidad que pueda surgir mientras se este participando en este estudio o por cualquier duda sobre el mismo que desee realizar una vez haya leído este documento, por favor diríjase a:

MEDICO RESPONSABLE: Dr/Dra.....

DIRECCIÓN: .....

TELÉFONO: .....

Se le entregará copia de esta hoja de información

14.10. INFORMED CONSENT FOR PARENTS OR LEGAL TUTORS (SPANISH VERSION)

CONSENTIMIENTO INFORMADO DEL FAMILIAR RESPONSABLE O REPRESENTANTE LEGAL

Hospital Universitari de Girona Doctor Josep Trueta

Replace with each participant institution logo

TÍTULO DEL ESTUDIO: IMPLEMENTACIÓN DE UN PAQUETE DE MEDIDAS PARA LA PREVENCIÓN DE LA SEPSIS NOSOCOMIAL EN RECIÉN NACIDOS DE EXTREMADO BAJO PESO AL NACER.

Yo (nombre y apellidos) ..... en calidad de ..... (relación con el participante) de ..... (nombre y apellidos del participante).

He leído la hoja de información que se me ha entregado.

He podido hacer preguntas sobre el estudio.

He recibido suficiente información sobre el estudio.

He hablado con: ..... (nombre del investigador)

Comprendo que la participación del paciente es voluntaria.

Comprendo que puede retirarse del estudio:

1º Cuando quiera

2º Sin tener que dar explicaciones.

3º Sin que esto repercuta en sus cuidados médicos.

Presto mi conformidad para que .....(nombre del participante) participe en este estudio y doy mi consentimiento para el acceso y utilización de los datos en las condiciones detalladas en la hoja de información.

FIRMA FAMILIAR O TESTIGO:	FIRMA DEL INVESTIGADOR:
Nombre:.....	Nombre:.....
Fecha:.....	Fecha:.....

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