Sepsis and antibiotic exposure in the neonatal period

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Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

This PhD project has brought together leading clinicians and scientists from Norway in a collaboration between partners with long-standing experience in neonatal and pediatric medicine. The work originated from the Department of Pediatric and Adolescent Medicine and the two Research groups; Pediatric and Adolescent Medicine led by Professor Knut Øymar, and the Safer Birth Bundle led by Professor Siren Rettedal, both at Stavanger University Hospital.

As a PhD candidate, I have been affiliated to the Department of Clinical Science, University of Bergen.
The main supervisor of this thesis was Siren Rettedal, MD, PhD and Professor II at the University of Stavanger.
Co-supervisors were; Knut Øymar, MD, PhD and Professor II at University of Bergen, Claus Klingenberg, MD, PhD, Professor at UiT-The Arctic University of Norway, and Arild Rønnestad, MD, PhD, associate professor at University of Oslo and leader of the Norwegian Neonatal Network (NNN).

Acknowledgements

This work had not been possible, without great contributions from my employer, my supervisors and the research groups, colleagues, family and friends. I am grateful for each and every one.

My research journey began shortly after I had started as a junior doctor at the Department of Pediatric and Adolescent Medicine at Stavanger university Hospital. My co-supervisor Knut Øymar came up with the idea of grouping clinical symptoms of early-onset sepsis in neonates to try to predict neonatal sepsis. We soon realized that this would be very challenging due to our small sample size, and abandoned this idea, however for me the seed to continue research was planted. My interest grew within the field of epidemiology. We continued to collect data on neonates with sepsis, and as the years went by- including almost two years as a junior doctor in the anesthetic department, working at the Rikshospitalet in Oslo, delivering and raising four children, living abroad, further education myself as a quality improver in Scotland- the numbers of included patients accumulated, and Knut Øymar was still patiently waiting for me to finish the initially planned "five-year study at Stavanger University Hospital". Eventually the study turned out to be a "23-year study at Stavanger University Hospital"! However, in retrospect, I would not have done anything differently! While living in China, I was fortunate to gain access to the strictly-locked-for-any-foreigners-gate at the Fudan University Hospital in Shanghai. The Neonatal Intensive Care Unit was designed to cover impressively 250 neonatal intensive care beds, not only covering the city's 23 million population, but also sick neonates transferred from other parts of China. While following their Canadian-Chinese educational program I met a wonderful and wise old neonatologist-who's name is hard to pronounce, spell and also to remember. I was lucky to learn from his experience and practice from a long neonatal career. Interestingly, he shared his experience that neonates with "C-reactive protein (CRP)-values around 150-180" did not necessarily have an infection, there could be other reasons for an elevated CRP. In poorer, rural areas of the world, due to lack of money or medication, some neonates with a significantly elevated CRP survived despite not being treated with antibiotics. This was new knowledge to me

being trained in the “era of antibiotics”, and inspired me to design and plan for an antibiotic stewardship program to be carried out in our unit after moving back home.

I have through my project been privileged to work with extremely talented and dedicated people.

My main supervisor Siren Rettedal, started out as my colleague at the Neonatal intensive care unit in Stavanger. Her excellent skills in research and her ability to see through the core issues is impressive. I am grateful for experiencing her enthusiastic engagement in research and neonatology, always searching for increased knowledge and improvement. Her warm and inspirational motivation has been of great contribution for me and for the project.

Knut Øymar was my co-supervisor throughout the project. With his huge knowledge within all aspects of research and infection, he has contributed with valuable ideas on planning the project, in the design and choice of methods and statistical analysis. He has inspired and motivated me with his enthusiasm for research: from the very start to the very end! I am deeply grateful for his support. Even more, he has inspired me, being a role model in research and in life in general! He has been a motivator for the Norwegian campaign of “Choosing wisely”, a valuable contribution in antibiotic stewardships.

Claus Klingenberg is internationally and nationally wide known for his great universal knowledge, enthusiasm and impressive skills in almost all aspects of neonatology and pediatric medicine. The impressive list of articles and research projects he has contributed to, shows there are no limits to this man’s knowledge! I feel very fortune to have learnt and benefitted from his ideas, experience and great communication skills. Claus’ generous support has been invaluable both in the three papers and to me personally.

Claus and Arild Rønnestad have served as my co-supervisors. Arild has an impressive knowledge in neonatology and epidemiology. During my time at Rikshospitalet, Arild inspired me with his clinical expertise. As head of the Norwegian Neonatal Network

(NNN), Arild has been heavily involved in research over many years, and contributed substantially in research of the Norwegian neonatal population. He has supported this project from the beginning, with great contribution to all aspects of the project.

None of my research would have been possible without the invaluable contribution by the statistical department. I am forever grateful for the assistance, support and patience (!) from Ingvild Dalen. I have also had great help and statistical assistance from Anastasia Ushakova. Both from the Department of Research, Section of Biostatistics.

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I would like to express my gratitude for all the support I have received from my colleagues at the NICU in Stavanger, many of whom have become close friends. Especially Nina Hapnes has contributed in the project, and she is also shared first author in paper 2. Her impressive skills for details and her eye for English grammar and vocabulary, has been of tremendous help. I have benefitted from her fluent English skills, but even more from her generosity and warm personality. Anne Marie Salte has from the start of the project been supportive and contributed greatly by inspiring me to never give up and keep pushing forward. Without her “jærske” believe and stayer-attitude I would not have been able to continue and make any project sustainable. I am thankful to the rest of my colleagues at the “extended 3D-family”,

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I am grateful for my dear family and friends for support and warm hearths. I am graced with a family that is the source of my love, inspiration and happiness. Their unremitting support, advice and understanding during this work has been crucial to me. My parents have always been my source of inspiration and have provided me with unconditional love and support. They are living examples of that the difference between success and failure, is action. My friends have always been my most important source of energy. Sonja has forever her special star, and I owe all my friends huge hugs. The love of my life, Gisle, has been of tremendous support. My four children Kristin, Nora, Eline and Camilla, the most important people in my life, will forever leave me in gratitude of being allowed to take part in their lives in so many ways. I express my deepest gratitude to my family.

Abstract in English

Background

The neonatal period (≤ 28 days of life) carries the highest lifetime risk for sepsis. Neonatal early-onset sepsis (EOS) (growth of a pathogen in blood culture obtained within the first three days of life) is associated with a high morbidity and mortality. Because of diagnostic challenges, antibiotics is the most frequently prescribed medication in neonatal intensive care units (NICUs). Controversies remain if early antibiotics in uninfected preterm neonates are associated with adverse outcome.

Objective/purpose

We aimed to increase knowledge on EOS and early antibiotic use in term and preterm neonates in order to optimise and improve future neonatal care and antibiotic stewardship programs. The specific aims for the three studies were; i) To describe the incidence of EOS, causative pathogens, antibiotic-resistance and antibiotic therapy over a 23-year period in a single NICU in South-West Norway. ii) To evaluate the associations between empiric antibiotic exposure within the first week of life and adverse clinical outcomes in an unselected population of uninfected very preterm neonates gestational age (GA < 32 weeks). iii) To evaluate if an approach using serial physical examinations (SPEs) could reduce the proportion of term neonates exposed to antibiotics for suspected EOS within the first 3 days of life, without affecting safety in a single NICU in South-West Norway.

Materials/methods

Paper 1 was a population-based single-centre longitudinal observational study at Stavanger University hospital (SUH) on EOS in all live born (LB) neonates born during 1996-2018 (23-years). Regression model was used to test for trends over time. **Paper 2** was a nationwide population-based observational study investigating associations between antibiotic exposure in the first week of life and short-term adverse outcomes to discharge, in neonates with GA < 32 weeks surviving seven days of age free of sepsis and necrotizing enterocolitis (NEC)/intestinal perforation born in Norway 2009 throughout 2018. Data from the Norwegian Neonatal Network was analysed by regression models, adjusted for confounders for mortality and morbidity. **Paper 3** was a single-centre, prospective population-based study where a new management strategy, SPE for suspected EOS, was developed, implemented and evaluated for improved diagnostic assessment and hence reduction of antibiotic exposure in term neonates born at SUH during the study period 2014-2018.

Results

There were101 out of 104 377 LB neonates, (incidence 0.97/1000/1000 LB) with culture-confirmed EOS **(paper 1)**. The incidence of Group B streptococcus (GBS) and *Escherichia coli* were 0.57/1000, and 0.11/1000, respectively. GBS was the most

common pathogen (59/93; 63%) in neonates with GA ≥ 28 weeks, and *E. coli* was most common (4/8; 50%) among extremely preterm infants (GA < 28 weeks). The incidence of EOS (overall), GBS and *E. coli* remained unchanged during the study period. The percentage of antibiotic-resistance in pathogens casing EOS was low (2/101; 2%).

Of 601 668 LB neonates, 5296 were GA < 32 weeks, of whom 4932 (93%) were included in the final analysis **(paper 2)**. Antibiotic exposure within the first week of life was strongly associated with higher adjusted odds ratios (aOR) of death (aOR 9.33; 95% confidence interval [CI] 1.10-79.5), severe morbidity (aOR 1.88; 95% CI 1.16-3.05), and severe bronchopulmonary dysplasia (BPD) (aOR 2.17; 95% CI 1.18-3.98) compared to those not exposed to antibiotics. Higher odds of severe NEC (aOR 2.27; 95% CI 1.02-5.06) was associated with antibiotics given ≥ 5 days. Most neonates were exposed to antibiotics the first week of life (3790/4932; 77%) despite a negative blood culture, but a decline in the proportion of neonates exposed to prolonged courses (≥ 5 days) from 55% to 24% was observed throughout the study period.

There were 17 242 term LB born neonates included in the baseline and the post-implementation period **(paper 3)**. After implementing SPE, the proportion of term neonates exposed to antibiotics was reduced from 2.9% in the baseline to 1.3% in the post-implementation period. The time from birth to start of treatment was reduced from median (IQR) 14 (5-28) to 7 (3-17) hours in infected neonates. The antibiotic exposure-days, the numbers of neonates diagnosed with culture-negative sepsis and numbers of blood samples taken were all reduced, from 320 to 129/1000 patient-days, from 11.8 to 6.7/1000 LB and from 332 to 223, respectively. The incidence of EOS remained unchanged, and there were no infection-attributable deaths/readmissions.

Conclusions

This project contributes with increased knowledge on EOS and early antibiotic use in term and preterm neonates. The incidence of EOS and common pathogens were in line with reports from other western networks, and was stable during the 23-year period at SUH. Antibiotic-resistance was low (**paper 1**). There was a strong association between early antibiotics the first week of life in uninfected very preterm neonates and severe NEC, BPD and death, regardless of duration of antibiotics (**paper 2**). Antibiotic stewardship with SPE reduced the percentage of term neonates exposed to early antibiotics, without affecting safety (**paper 3**). There is a continuous need for pathogens surveillance, and antibiotic stewardships are important as early antibiotic exposure is associated with adverse outcome in preterm neonates. This knowledge is important to optimise future neonatal care, but further studies are needed.

Abstract in Norwegian

Bakgrunn

Neonatalperioden (første 28 levedager) er den perioden i livet med høyest risiko for å utvikle sepsis. Tidlig nyfødt-sepsis (oppvekst i blodkultur innen første tre levedager) er assosiert med høy morbiditet og mortalitet. All antibiotika hos nyfødte startes empirisk før blodkultur foreligger da det finnes få diagnostiske hjelpemidler. Dette fører til at antibiotika er det mest brukte medikamentet i nyfødtintensivavdelinger. Flere observasjonsstudier har vist at antibiotikabehandling i første leveuke hos premature barn < 32 uker gestasjonsalder (GA) uten infeksjon er assosiert med alvorlig sykdom/død, mens noen få nyere studier har rapportert at antibiotika kan ha beskyttende effekt.

Formål

Øke kunnskap om tidlig nyfødt-sepsis og antibiotika hos terminfødte og premature nyfødte for å forbedre kvalitet innen nyfødtmedisin og antibiotika reduksjonsprosjekter ved å; i) beskrive forekomst av nyfødt-sepsis, hvilke bakterier som forårsaker dette, antibiotika-resistens og antibiotika-behandling de siste 23 årene i en nyfødtintensiv avdeling i sør-vest Norge, ii) evaluere mulig assosiasjon mellom antibiotika og alvorlig sykdom/død frem til utskrivelse/død i en uselektert populasjon av premature GA < 32 uker uten infeksjon, iii) evaluere om en diagnostisk metode med repeterte «Timesobservasjoner» trygt kan redusere bruk av unødvendig antibiotika de første tre levedøgn hos terminbarn.

Metode

Studie 1 var en populasjonsbasert longitudinal observasjonsstudie av alle levende fødte (LF) med tidlig nyfødt-sepsis født på Stavanger Universitets sjukehus (SUS) i perioden 1996 tom 2018. Regresjonsmodell ble brukt for å teste trender over tid.
Studie 2 var en nasjonal populasjonsbasert observasjonsstudie fra Norsk Nyfødt Kvalitetsregister og inkluderte alle LF barn GA < 32 uker uten infeksjon (overlevd syv dager uten sepsis eller NEC) i Norge fra 2009 tom 2018. Regresjonsmodell ble brukt for å evaluere assosiasjon mellom tidlig antibiotika og risiko for alvorlig NEC eller en sammensetning av alvorlig sykdom/død til utskrivelse/død etter første uke. Det ble justert for andre kjente årsaker til død og sykelighet.
Studie 3 var en populasjonsbasert prospektive observasjonsstudie fra SUS der en sammenlignet andel terminfødte barn behandlet med tidlig antibiotika før (22 måneder, april 2014 til februar 2016) og etter (23 måneder, januar 2017 til november 2018) at "Timesobservasjoner" var implementert i avdelingen.

Resultat

Vi fant at 101 av 104 377 LF hadde tidlig sepsis, (total forekomst 0.97/1000 LF) (**studie 1).** Forekomsten av Gruppe B streptokokker (GBS) og *Escherichia coli* var

henholdsvis 0.57/1000 LF og 0.11/1000 LF). GBS var vanligste årsaken til sepsis (59/93; 63%) hos nyfødte over GA ≥ 28 uker, mens *E. coli* var vanligst (4/8; 50%) blant GA < 28 uker. Forekomsten av nyfødt-sepsis var uendret i løpet av 23 år. Det var lav antibiotika-resistens blant mikrober som gir tidlig nyfødt-sepsis (2/101; 2%).
Blant 601 668 LF, var 5296 premature født før GA < 32 uker, inkludert 4932 (93%) uten infeksjon og inkludert i analysene **(studie 2)**. Det var 3790/4932 (77%) som fikk antibiotika i løpet av første leveuke. Andel premature som fikk lange antibiotikakurer (≥ 5 dager), sank i løpet av perioden fra 55% to 24%. Eksposisjon for antibiotika første leveuke var assosiert med høyere justert odds ratio (aOR) for død (aOR 9.33; 95% konfidens intervall [CI] 1.10-79.5), alvorlig morbiditet (aOR 1.88; 95% CI 1.16-3.05), og alvorlig lungesykdom (bronkopulmonal dysplasi, BPD) (aOR 2.17; 95% CI 1.18-3.98) sammenlignet med barn som ikke var eksponert for antibiotika første leveuke. Vi fant høyere aOR for alvorlig NEC (aOR 2.27; 95% CI 1.02-5.06) der antibiotikakurene varte ≥ 5 dager.
Blant 17 242 LF terminbarn som var født før og etter intervensjonen **(studie 3),** fant vi at etter at «Timesobservasjoner» var innført, refusertes andel terminbarn behandlet med antibiotika fra 2.9% til 1.3%, (57%). Tid fra fødsel til oppstart av antibiotika ble redusert fra median (IQR) 14 (5-28) til 7 (3-17) timer (50%), hos barn med infeksjon. Antall behandlingsdager falt fra 320 til 129/1000 pasientdager, og antall barn som fikk diagnosen klinisk sepsis, og antall CRP prøvetakninger ble redusert fra henholdsvis 11.8 til 6.7/1000 LF og fra 332 til 223. Forekomst av tidlig sepsis var uendret, og det var ingen nyfødte barn som døde eller ble re- innlagt med infeksjon.

Konklusjon

Dette PhD-prosjektet bidrar til økt kunnskap om tidlig nyfødtsepsis og korrekt antibiotikabruk hos nyfødte barn. Forekomsten av nyfødt-sepsis og hvilke mikrober som forårsaker dette ved SUS, er sammenlignbar med andre vestlige land, og holdt seg stabil siste 23 år. Det er lav forekomst av antibiotikaresistens (**studie 1**). Det var en klar assosiasjon mellom antibiotikabruk først leveuke hos premature barn < GA 32 uten infeksjon, og forekomst av alvorlig sykdom/død, på tross av justering for andre årsaker til dette (**studie 2**). Etter å ha innført «Timesobservasjoner» i avdelingen, reduserte vi andel terminfødte barn behandlet med antibiotika på en trygg måte (**studie 3**). Kontinuerlig overvåkning av hvilke bakterier som forårsaker nyfødt sepsis, og resistensmønster er viktig for å kunne gi optimal antibiotika, og flere studier er nødvendig. Antibiotika reduksjonsprosjekter kan bidra til å unngå unødvendig bruk av antibiotika til termin og premature barn da det er assosiasjon mellom antibiotika og alvorlig sykdom/død hos premature. Flere studier og kvalitets forbedringsprosjekter innen dette feltet av nyfødtmedisin kan dermed gi økt kunnskapen for videre optimalisering av behandling av premature og terminfødte barn.

List of Publications

Paper 1

Early-Onset Sepsis in Neonates - A Population-Based Study in South-West Norway from 1996 to 2018.

Vatne A, Klingenberg C, Rettedal S, Øymar K.

Front Pediatr. 2021; 9:634798. Published 2021 Mar 17. doi:10.3389/fped.2021.634798

Paper 2

Early Empirical Antibiotics and Adverse Clinical Outcomes in Very Preterm Infants – a Nationwide Study

Vatne A, Hapnes N, Stensvold HJ, Dalen I, Guthe HJ, Støen R, Brigtsen AK, Rønnestad A, Klingenberg C. (in manuscript)

Paper 3

Reduced Antibiotic Exposure by Serial Physical Examinations in Term Neonates at Risk of Early-onset Sepsis.

Vatne A, Klingenberg C, Øymar K, Rønnestad AE, Manzoni P, Rettedal S.

Pediatr Infect Dis J. 2020 May; 39(5):438-443.

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Abbreviations

AAP	**American Academy of Pediatrics**
AURC	**Area under the receiver operating curve**
BPD	**Bronchopulmonary dysplasia**
BW	**Birth weight**
CBC	**Complete blood cell**
CDC	**Center for Disease Control and Prevention**
CoNS	**Coagulase-negative *staphylococci***
CRIB2	**Clinical Risk Index for Babies 2**
CRP	**C-reactive protein**
E. coli	***Escherichia coli***
ELBW	**Extremely low birth weight**
ELGAN	**Extremely low gestational age neonate**
EOS	**Early-onset sepsis**
ESBL	**Extended-spectrum beta-lactamase producing Gram-negative bacteria**
GA	**Gestational age**
GBS	**Group B Streptococcus**
IPA	**Intrapartum antibiotics**
IVH	**Intraventricular hemorrhage**
LB	**Live-born**
LOS	**Late-onset sepsis**
MRSA	**Methicillin resistant strains of *Staphylococcus aureus***
MUSIQ	**The model for understanding success in quality framework**
MV	**Mechanical ventilation**
NEC	**Necrotizing enterocolitis**
NICE	**National Institute for Health and Care Excellence**
NICU	**Neonatal intensive care unit**
NNN	**Norwegian Neonatal Network**
OR	**Odds ratio**
PROM	**Premature rupture of the membranes**
PDSA	**Plan-Do-Study-Act**
PMA	**Postmenstrual age**

PCT	**Procalcitonin**
PVL	**Periventricular leukomalacia**
QI	**Quality Improvement**
RDS	**Respiratory distress syndrome**
ROP	**Retinopathy of prematurity**
SNAP II	**Simplified newborn illness severity and mortality risk scores**
SPE	**Serial physical examination**
The UK	***The United Kingdom***
The US	**The United States of America**
VGS	**Viridians group streptococci**
VLBW	**Very low birth weight**
VPN	**Very preterm neonate**
WHO	**The World Health Organization**

Preface

The ancient Greek god Thetis gave birth to Achilles. Unlike the other gods, Achilles was mortal. To make him immortal, his mother dipped the baby Achilles in the River Styx while holding him by his heels. Whilst his heels were untouched by the water, they became his only point of weakness [1].

In the neonatal intensive care unit (NICU), antibiotics have been prescribed to a large number of term and preterm neonates after delivery - given the risk of early-onset sepsis (EOS). For decades, a "better safe than sorry" approach to empiric antibiotics has become the commonplace [2].

Like Thetis dipping her baby Achilles into the river to secure immortality, neonatologists would routinely administer ampicillin and gentamicin to preterm neonates for days or even weeks, despite no evidence for infection. In 2008 in the United States of America (US), most (94-96%) extremely low birth weight (ELBW; < 1000 g) neonates received empiric antibiotic therapy after delivery [2-5], around half of them received prolonged courses ≥ 4-5 days [3, 4]. Moreover, 4-8% of all term neonates in the US and Europe were exposed to antibiotics during the first 72 hours of life [6, 7].

Unfortunately, as with Achilles, this strategy had a point of weakness. Since 2009, a number of publications from different centres and countries have reported that prolonged early antibiotic therapy (≥ 5 days) in the first week of life was associated with an increased risk for necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), late-onset sepsis (LOS) and death in very preterm neonates (VPN, gestational age (GA) < 32 weeks) or very low birth weight (VLBW; < 1500 grams) neonates [4, 8-10]. More than a decade after the first publications on this association, still most VLBW and ELBW neonates continue to be exposed to empiric antibiotics [4, 11-13], a third receive prolonged courses [9, 14, 15], and although some improvement have been seen in term neonates, still a high proportion of term neonates (2.3-8%) are exposed to antibiotics after birth [6, 7, 16].

This thesis addresses correct antibiotic use in neonates. To increase our knowledge, we sought a deeper epidemiological understanding of the burden of disease and antibiotic consumption, evaluated associations between antibiotics and adverse outcomes to discharge, and developed and implemented a diagnostic tool for reduction of antibiotic exposure. After the submission of the first paper in this thesis in January 2020, a number of new studies related to this topic have been published. This PhD project aimed to address research questions based on the current knowledge at that time, and consequently the Introduction section includes mainly articles published before our papers were published (January 2020, March 2021 and April 2022 for paper 3, 1 and 2, respectively). Recent publications are addressed in the Discussion section, in light of our results.

1.0 Introduction

1.1 Context in neonatal medicine

The neonatal period is defined as the first 28 days of life. Nearly half of deaths among children under 5 years of age in 2020 occurred during this vulnerable neonatal period. Neonatal mortality accounts for an increasing proportion of "under five-years" mortality, from 40% in 1990 to 47% in 2020 [17]. The United Nations Inter-Agency Group for Child Mortality Estimation, has estimated that 2.5 million deaths occur each year in the neonatal period [18-21]. The three most common causes of neonatal mortality are prematurity, intra-partum related complications such as birth asphyxia, and sepsis [22] (Figure 1).

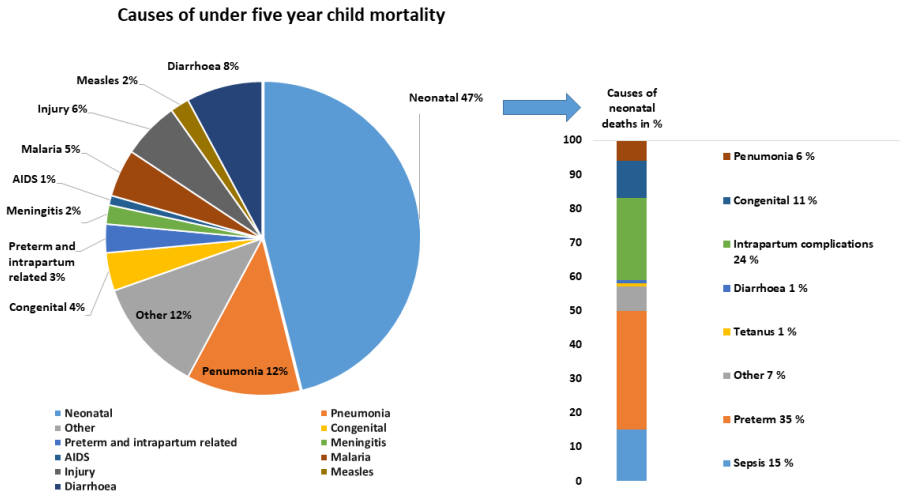


Figure 1 *Causes of deaths in children under five years and during the neonatal period. Modified from World Health Organization, Data from 2020. Reproduced with permission. Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions. Geneva: World Health Organization; 2020. Licensed: CC BY-NC-SA 3.0 IGO.*

Globally, there are enormous variations in access to neonatal care and the standard of care provided. However, the three main causes of deaths are the same in both high- and low-income countries, only differentiated by the scale of the cases [20, 23]. Infection is the third most common cause of death in the neonatal period, and represents 24% of global neonatal deaths (2018), including 15% caused exclusively by neonatal sepsis [20, 21]. The World Health Organization (WHO) estimates that 84% of all global neonatal deaths caused by infections could be prevented by timely diagnosis and appropriate antibiotic therapy [20]. Diagnostic challenges, fear of delayed treatment and antibiotic non-restrictive guidelines are among the daily challenges clinicians face [24], consequently, antibiotics are the most frequently prescribed drugs in the NICU [4, 14, 25]. Risk stratification programs to improve selection of neonates at risk of sepsis and identify the need of antibiotics treatment, have been increasingly demanded [24].

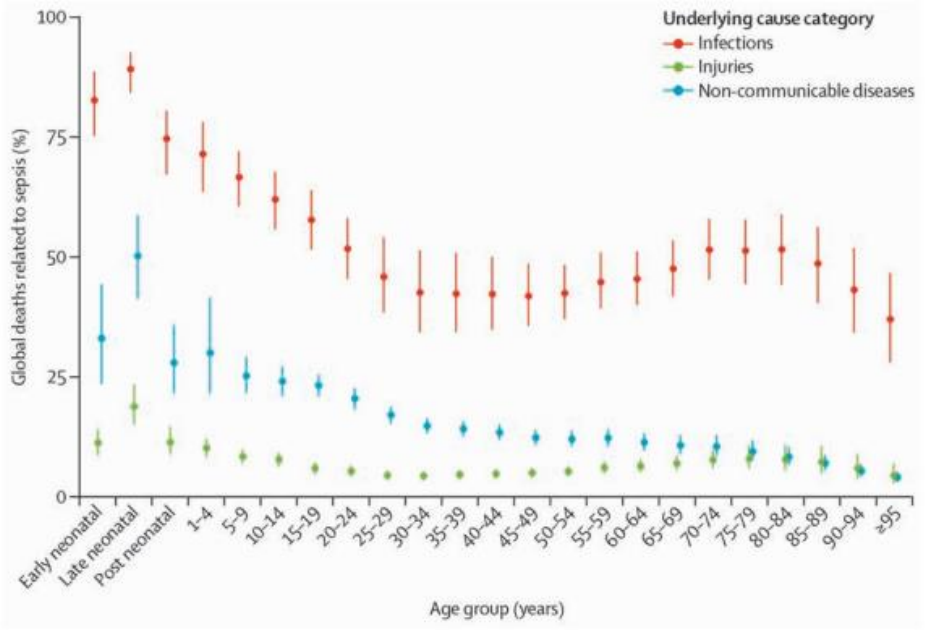
1.2 Epidemiology and burden of neonatal sepsis

1.2.1 Neonatal sepsis definition

In adults, the 2016 Sepsis-3 consensus defined sepsis as "a life-threatening condition caused by a dysregulated host response" to a bacterial infection that leads to potentially fatal organ dysfunction, including hypoxia, oxidative stress and high energy demand [26, 27]. Unlike in adults, an international consensus on specific criteria to define neonatal sepsis is still lacking [26, 28-31]. The hitherto most common definition of neonatal sepsis is a bacteraemia, defined as growth of a pathogen in a culture of blood or cerebrospinal fluid and antibiotic therapy for ≥ 5 days (or < 5 if death occurs) [32]. Growth of bacteria in blood culture obtained within the first three days of life (≤ 3 days) is commonly referred to as culture-positive EOS and a positive blood culture obtained after three days of life (< 3 days) is referred to as culture-positive LOS [32]. In epidemiological studies reporting exclusively on Group B Streptococcus (GBS), a positive blood culture up to seven days of age is often included in the definition of EOS [31, 33].

1.2.2 Global burden of neonatal sepsis

Globally, an estimated 3 million newborns are affected by sepsis in the first month of life [20]. The health expenses, maternal-neonatal separations and medical consequences of neonatal sepsis are imperative. Neonatal sepsis is a major cause of morbidity and mortality with potential life-long sequela [20, 32]. Both term (GA ≥ 37 weeks) and preterm (GA< 37 weeks) newborns are susceptible to infections as key parts of their immune systems are still developing and not fully functional [34]. The age-dependent maturation of the fetal immune system is ongoing during the pregnancy and after birth by exposure of antigens and environmental factors such as antibiotics and microbial colonization of the mucosal tissue [35, 36]. The immune system balances between immune tolerances and pro-inflammation in order to both prevent immune reaction between mother and foetus and to protect against infection [37]. In utero pathogen exposure is limited. Therefore, immunological memory function is lacking and the neonate's immune system is primarily dependent on innate immunity and trans-placental immunoglobulins [37]. Consequently, the highest lifetime risk for morbidity and mortality from sepsis than in any age group later in life, is in the neonatal period [38] (Figure 2).



Note: Bars represent 95% uncertainty intervals.

Figure 2 *The percentage of all global deaths (from any cause), related to sepsis in each underlying cause category in 2017, by age group and for both sexes. Reproduced from "Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions." Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.*

1.3 Early-onset sepsis – epidemiology, mortality and microbiology

1.3.1 Incidence in high-income countries

The incidence of EOS in high-income countries is inversely correlated with GA. Overall EOS incidence is approximately 0.7-1.08 per 1000 liveborn (LB) neonates. In term neonates, the incidence is 0.3-0.56 per 1000 LB, in moderately preterm (GA 28-36) 0.73-2.0 per 1000 LB and in extremely low gestational age neonates (ELGANs; GA < 28 weeks) 17.8 -18.5 per 1000 LB [16, 39-44]. Most LB neonates are born at term. In Norway, 5-6% of LB neonates are born preterm, including 0.3% born at GA < 28 [45]. Despite the incidence of EOS among ELGANs is up to 30-fold higher

compared to that observed among term neonates [46], the absolute number of neonates with EOS is higher in term or moderately preterm infants.

1.3.2 Mortality and EOS-attributable mortality rate

A dramatic decrease in overall mortality has been seen in neonates with EOS over the last 100 years, from 87% in 1928, after introduction of antibiotics and improved neonatal care [16, 47]. However, sepsis accounts globally for 15% of all deaths in the neonatal period [20, 21, 48], and EOS is still one of the most common causes of mortality among preterm neonates [49]. Mortality rate varies, depending on setting, causative microbes and GA. In high-income countries, mortality rate is 1-3 % in term neonates [16, 31]. In a Lancet review paper on neonatal sepsis, overall reported mortality rate among preterm neonates was 16%, including 12% at GA 29-33 weeks, 30% at GA 25-28 weeks, and 50-54% at GA 22-24 weeks [31]. The majority of neonates (75 %) who die from EOS is VLBWs [15, 31, 32, 44]. Mortality rate in neonates with GBS EOS is lower (5-9%) than in neonates with *Escherichia coli* EOS (13-33%), but when adjusted for GA the risk of death is similar [31].

1.3.3 Microbiology of EOS

In neonates with suspected EOS, empirical antibiotic therapy is initiated before blood culture results are available. Consequently, an epidemiological insight in microbes causing EOS in neonates is important to tailor optimal antibiotic treatment. The WHO calls for increased knowledge on local microbiological pattern to optimize antibiotic treatment [50].

Pathogens causing EOS are usually colonizers of the maternal genitourinary tract such as GBS and *E. coli* [51]. These pathogens can lead to contamination of the amniotic fluid, placenta, cervix or vaginal canal, and may ascend when the amniotic membranes rupture or even prior to the onset of labour [49, 51-53]. The pathogens can reach the neonate either in utero or intrapartum in a vertical mother-to-neonate transmission [31, 49, 52]. Maternal intake of contaminated food with *Listeria monocytogenes* can infect the neonate before labour and delivery, although rare [51].

Pathogens causing EOS include both Gram-positive and Gram-negative microbes [31, 32, 51]. While Gram-positive microbes are the most common cause of EOS in term and moderately preterm neonates, Gram-negative microbes are the most common among ELGANs [32]. Among Gram-positive microbes, GBS is the most frequent, and *E. coli* is the most frequent among Gram-negative microbes identified in blood cultures. Combined, GBS and *E. coli* in many studies account for approximately 70% of all EOS cases [42, 51, 54].

The remaining minority of EOS cases are accounted for by; other streptococci (most commonly viridians group streptococci (VGS) but also *Streptococcus pneumoniae*), *Staphylococcus aureus, Enterococcus species*, and Gram-negative enteric bacilli such as *Enterobacter species, Haemophilus influenza and L. monocytogenes* [51].

Different pathogenic organisms have different virulence factors. Virulence factors are cellular structures, biological molecules and regulatory systems that help the pathogens to grow and survive in their hosts, assist and promote host colonization and cause damage or death of the host if interventions are not instituted [55].

In the following paragraphs I will briefly elucidate on some of the most common or serious pathogens in neonatal sepsis.

Group B streptococcus (GBS; *Streptococcus agalactiae*) is a facultative Gram-positive diplococcus with several extracellular virulence factors including a polysaccharide capsule, beta-haemolysin, C5a peptidase, adhesions and immunogenic surface proteins [53, 56, 57]. Attachment to the epithelial surface via GBS surface proteins is a necessary component of colonization that is a prerequisite for an invasive infection. GBS invades cells by an active host cell process involving specific factors. A range of cell-damaging toxins, including beta-haemolysins and other haemolysins mediate the release of nutrients, on which GBS is dependent on during colonization. Ten type-specific polysaccharide capsular types have been identified, including serotypes Ia, II, III and V equally accounting for more than 95% of EOS-

cases, and serotype III accounting for the majority of LOS-cases and nearly all cases of neonatal meningitis [53].

In pregnancy, GBS may colonize the gastrointestinal and vaginal epithelium in asymptomatic women [53, 58]. Worldwide, approximately 18% (95% CI 17-19%) of women are colonized with GBS during pregnancy [59] with regional variation (11%-35%), and lower prevalence in Southern Asia (12.5% [95% CI, 10%-15%]) and Eastern Asia (11% [95% CI, 10%-12%]). In Norway and the United States (US), rates of maternal colonization are estimated to be 26%, whereas in United Kingdom (UK) and in Sweden the colonization rates are 29% and 25%, respectively [60-62]. The role of GBS in EOS is less clear in developing countries [63]. Colonization studies from developing countries have reported maternal GBS colonization rates 14-22%, lower than maternal GBS colonization rates in developed countries, proposing an explanation why Asian studies have reported a lower incidence of GBS [59]. On the other hand, southern Africa and Kenya report GBS as the predominate cause of neonatal sepsis [63], implicating that the GSB rates may be similar to that observed in Europe and the US.

Risk factors for maternal GBS colonization include chronic hypertension, tobacco use and maternal diabetes [58, 64, 65], possibly also maternal age < 20 years and ethnicity but this association is unclear [66, 67].

If intrapartum antibiotic prophylaxis (IPA) is not given, maternal GBS colonization results in colonization of the neonate in approximately 50% of cases, either intrapartum or through bacterial translocation despite intact membranes [59, 68]. An estimated 0.5-2% of colonized neonates develop EOS in the absence of IPA [59, 66, 68]. In colonized women, transplacental transmission of antibodies against GBS occur during the last trimester, explaining why premature neonates born prior to this has increased risk for GBS EOS [69]. In deliveries complicated by PROM and in cases of maternal intrapartum fever/chorioamnionitis, the increased risk for GBS EOS are due to the risk for ascending colonization, and the maternal inflammatory response to evolving intra-amniotic bacterial infection, respectively [64, 66, 70]. In some women,

transplacental transfer of protective GBS antibodies to the foetus is lacking or reduced [71]. Consequently, women who have previously given birth to neonates that acquired GBS EOS, have increased risk for GBS EOS in the next child [72].

Maternal colonization is a prerequisite for GBS EOS. The risk of EOS is especially increased if other risk factors are present including lower GA, maternal intrapartum fever/chorioamnionitis and a history of premature rupture of the membranes (PROM) [66, 70]. Interestingly, term neonates of GBS-colonised mothers are three times more likely to be admitted to a NICU compared to neonates of non–colonised mothers. A Norwegian study including 1694 neonates ≥ 37 weeks found that the majority of GBS-colonised neonates had respiratory distress and slightly increased C-reactive protein (CRP) levels, possibly because an immune response in the neonates, despite no invasion of bacteria [62] or a pulmonary hypertension induced by GBS-released phospholipids [62, 73].

In 3-5% of pregnancies, GBS is the cause of maternal asymptomatic bacteriuria or urinary tract infections, associated with increased risk of preterm delivery, PROM, chorioamnionitis and intrapartum fever [74], and indicates intrapartum vaginal colonization [75].

Around 85% of potential GBS EOS cases are now prevented by IPA [51], using either a screening-based or risk based strategy (refs). An important challenge is that GBS colonization may be either continuous, intermittent or transient in individual women [76].

Escherichia coli is the major species of the coliform group, a Gram-negative rod frequently colonizing the maternal vaginal canal. The pathogenesis of *E. coli* bacteraemia in neonates is complex and not well understood. One possible explanation may be bacterial translocation across the neonatal gut after the neonate has ingested maternal *E. coli* around the time of delivery, as demonstrated in animal models [77, 78]. The neonate often presents with bacteraemia with or without meningitis in the first days of life.

Some *E. coli* strains have virulence factors identified as being specifically important in neonatal sepsis, including properties to invade the intestinal epithelium, or express the K1 capsular antigen known to be responsible for 70-90% of neonatal *E. coli* meningitis [77]. The K1 capsular antigen is presently the best-described virulence factor, and neonates infected with K1 antigenic strains have increased morbidity and mortality compared to infants infected with other strains (59). Other virulence factors linked to neonatal sepsis include components that possible can alter its virulence after passage across the guts [77, 79].

E. coli is the second leading cause of EOS in neonates, accounting for 9-45% of all EOS episodes, and surpasses GBS causing the majority of EOS cases in ELGANs [32, 41, 43, 46, 67, 80]. When VLBW infants are considered alone, *E. coli* is the most frequent cause of EOS, accounting for 33-81% of episodes [31, 46]. Evidence is less clear whether the incidence of *E. coli* EOS in preterm neonates is increasing [42, 67, 80, 81]. There are ongoing speculations on a possible increase in *E. coli* EOS due to maternal IPA for GBS, or a shift towards greater survival of VLBW infants [39]. *E. coli* is often linked to high mortality and morbidity, but the increased mortality observed in *E. coli* EOS is partly due to the fact that *E. coli* more often affects very preterm neonates [31]. Another concern in *E. coli* EOS is the ability of this bacteria to develop resistance to commonly used antibiotics [67, 81, 82].

Enterobacterales is a large family of Gram-negative bacteria including, along with harmless intestinal bacteria, pathogens that can cause sepsis in neonates. Relevant Enterobacterales in neonates other than *E. coli* are *Serratia marcescens, Klebsiella pneumoniae* and *Enterobacter cloacae. K. pneumoniae* is a relatively uncommon cause of EOS in developed countries (0-3%) [42, 43, 46], although in developing countries, *K. pneumoniae is* reported to be the cause of 25% of community-acquired neonatal sepsis the first seven days of life in home-born neonates [83]. The main virulence factor of *K. pneumoniae*, the rmpA gene, is associated with the development of purulent meningitis, other virulence factors are associated its ability to develop

antibiotic resistance [84]. The other species in this group are mostly observed in LOS, rather than EOS, and will not be discussed further.

***Staphylococcus aureus* (*S. aureus*)** is a facultative Gram-positive cocci (sphere-shaped) that occurs in irregular clusters, in pairs or in single. The major criterion for identification is the ability to clot plasma. The pathogen is coagulase positive, and the presence of the enzyme coagulase separates the virulent pathogen *S. aureus* from the less virulent species belonging to the coagulase-negative staphylococci (CoNS). Typical *S. aureus* colonies are yellow in colour. *S. aureus* EOS usually originates from asymptomatic colonization of birth canal or breast milk. Infection is dependent on bacterial break of the host's protective epithelial of skin or mucus. *S. aureus* carry specific virulence factors such as capsule, teichoic acid, protein A and "clumping factor" in addition to producing toxins. The virulence factors are important for the pathogen's capacity to colonise a host, break the epithelial protection and invade the host including the production of toxins, mechanism that trigger phagocyte apoptosis (inhibition of component factors, agglutination and the formation of thrombi [85]. *S. aureus* show marked regional differences in clonal diversity and genotype distribution. [86].

The incidence of *S. aureus* EOS in industrialised countries has decreased over the last 20–30 years, and is largely surpassed by GBS and *E. coli*. The reported incidence of *S. aureus* EOS also depends on the definition of EOS used, as many cases occur on day 3-7 of life. In a study including all sepsis within the first seven days of life at two Scandinavian NICUs, *S. aureus* was the most frequent pathogen (11/33, 31%) [87]. One other study calculated the number of *S. aureus* EOS cases one could expect when using different definition of EOS in a single-centre unit of 5000 deliveries. If EOS was defined as ''within the first 72 hours of life'' versus "within the first 48 hours", there were nine cases of EOS *S. aureus* versus one case of EOS *S. aureus*, respectively every 30 months [88].

Although *S. aureus* is a rare cause of EOS in western countries, *S. aureus* EOS is associated with a high mortality, especially methicillin resistant strains of *S. aureus*

(MRSA) [88]. MRSA strains are resistant to all beta-lactam agents, but are mostly susceptible to vancomycin. The emerged resistance is as a result of a change in the penicillin binding protein (PBP2) encoded by the chromosomal *mecA* gene. *S. aureus* is still a common cause of EOS in developing countries and of LOS in western countries [51].

Viridians Group Streptococci (VGS) is a diverse group of streptococcal species. This heterogeneous group colonising the oropharynx and the gut is less well described, or even classified as unspecific or contamination [44] when found in blood cultures in neonates with EOS. Sepsis with VGS in adults are often associated with immunocompromised hosts [89]. VGS can form complex structures of biofilm, important for colonisation of the oral cavity. Additional properties include the ability to survive in an acidic environment and specific interaction with other microorganisms colonizing the same host [90].

Candida* species** and ***L. monocytogenes are described as rare causes of sepsis within the first week of life in extremely preterm infants [40]. Risk factors for fungal EOS are maternal fungal colonization and vaginal delivery, although Candida spp. are more frequently associated with LOS. Skin infection or invasive infection related to immature skin barriers are caused by heavy exposure to maternal colonization or intrauterine infection [51].

1.3.4 Changes over time in causative microbes and incidence of EOS

The Yale New Haven Hospital has reported ongoing surveillance of EOS pathogens since 1928 [47].There are also a few other longitudinal single- and multi-centre studies, networks' and some population-based studies with similar reports [40, 42, 80, 81, 91]. In the first 60 years of the Yale New Haven surveillance, *Streptococcus pneumonia* and Group A Streptococci (GAS), represented almost half of all EOS cases up to 1943, followed by a steady decline and almost no EOS cases caused by these microbes in the last two decades. Outbreaks of infections caused by *S. aureus* have been described in neonatal nurseries since 1889, with large epidemics in the 1920s, 1950s, and early 1970s [88, 92]. During the 1960-1970s, GBS emerged as the leading

cause of neonatal sepsis and replaced *S. aureus*, first in the US, and then confirmed in the rest of the developed world [47].

Over the past 30 years, the incidence of EOS has decreased substantially, most pronounced in GBS EOS. This is attributed to the implementation of IPA prophylaxis programs [93-95] where selected women are treated before delivery with intravenous benzyl penicillin or ampicillin in case of GBS colonization. In 1996, the Centre of Disease Control and Prevention (CDC) published a recommendation that all pregnant women should be universally rectovaginal screened for GBS at 35-37 weeks in pregnancy [94], as opposed to the risk-based IPA. The risk-based IPA, however, was advocated in the UK guidelines, and implemented also by Norwegian health authorities [96, 97]. The incidence of GBS EOS dropped after implementation of IPA [44, 47, 93-95, 98]. One randomized controlled trial (RCT) and several observational studies have shown the association between IPA and reduced risk of GBS EOS [68, 99, 100]. On the other hand, a 2014 Cochrane review including 500 women, found that IPA in colonised mothers appeared to reduce the incidence of GBS EOS (relative risk 0.14; 95% CI 0.04–0.74, but the numbers were too small to assess impact on mortality as the association might well be due to high risk of bias [101]. Despite IPA, GBS-EOS has not been eliminated [32, 46, 61, 80, 102]. This may be explained by the fact that vaginal colonization is a dynamic state with GBS colonization being either continuous, intermittent or transient in individual women, challenging screening programs as well as unclear reasons [103]. Among all women who screened negative for GBS early in the pregnancy, 7-8% will test positive at the time of delivery [104, 105]. In one study including term neonates with GBS EOS from 2003-2004, 116 cases of 189 (61.4%) GBS EOS cases occurred among neonates born to GBS-negative mothers [70]. Puopolo et al. showed in an 8-year study among term neonates, that 14/17 (82.8%) of GBS-EOS cases occurred among neonates whose mothers had screened negative for GBS [103]. Furthermore, Ascher et al. found that among 18/96 (18.4%) of GBS-EOS cases, proper IPA had been given, although these cases were identified as “failure of IPA” [106]. These studies indicate that despite adequate IPA, clinical examinations in symptomatic neonates are of paramount importance.

The IPA programs have not been implemented without expenses [97, 107]. There has been a massively increase in the antibiotic load on women in labour. Approximately 30-40% of all women in the US are exposed to IPA [44]. The main concern is the risk of a possible increased incidence of *E. coli* EOS especially with antibiotic-resistant strains when IPA contains broad-spectrum antibiotics, like amoxicillin. As *E. coli* EOS is the predominant cause of EOS in preterm infants [107], this is of special concern in preterm neonates [32, 46].

Screening-based programs may be associated with a slightly lower rate of GBS-EOS and a higher incidence of *E. coli* EOS when compared to risk-based programs. When comparing surveillance data from 2015–2017 to 2006–2009 in a screening-based program, the incidence of *E. coli* was increasing, a finding not described in populations where risk-based IPA was implemented [46, 54].

The conflicting reports on trends in microbiology responsible for EOS may be dependent on settings, populations, and on risk-based versus screening-based IPA. In the action plan to combat antibiotic resistance, the WHO calls for increased knowledge on local epidemiology [50]. Longitudinal surveillance for identification of changes in causative pathogens is important when tailoring optimal prevention IPA programs and empiric therapy strategies.

1.3.5 Clinical or culture-negative sepsis

Many neonates are diagnosed with clinical neonatal sepsis, or "possible" or "probable" sepsis in daily clinical work [27, 108]. These conditions are "presumed symptomatic infection but no bacterial cause identified", hence the term culture-negative or clinical sepsis. Many providers view sterile culture results with scepticism, especially if the neonate appears ill, a CRP is increased or small blood culture-volume was obtained. The lack of a uniform consensus in diagnosing neonatal sepsis in addition to scepticism of sterile blood cultures, are potential reasons why so many neonates are diagnosed with clinical sepsis. Culture-negative sepsis is lately being increasingly debated [27, 108]. The incidence of culture-negative sepsis is six to 16 times higher than the incidence of culture-confirmed EOS, and furthermore, its incidence varies

substantially between units and countries [27, 108]. Consequently, the antibiotic use varies widely, both within countries and globally, and up to 16 times more antibiotic is used for clinical sepsis than culture-confirmed EOS [14, 87, 109]. However, incidence and mortality rates of EOS do not show the same variation and mortality rate as incidence of clinical sepsis, suggesting that the variation in clinical sepsis is difficult to explain by medical reasons. Hence, some of the antibiotic use because of clinical sepsis may be unnecessary [16, 110, 111].

Most epidemiological studies exclude neonates diagnosed with clinical culture-negative sepsis, even though culture-negative sepsis contributes to a large number of neonates treated with antibiotics. Consequently, inclusion of this group is important in antibiotic stewardships programs and studies aiming to reduce unnecessary antibiotics [108].

1.3.6 Antibiotic resistance and EOS-isolates

The WHO has identified antibiotic resistance as one of the largest threats to global health [50]. Antibiotic resistance occurs naturally, but misuse of antibiotics accelerates this process. Neonates are at higher risk for prolonged carriage of multi-resistant bacteria when compared to adults [112], and they represent a reservoir for intra-household spread [113]. Worldwide, an estimate of three out of every ten deaths due to neonatal sepsis are caused by resistant pathogens [114].

GBS is susceptible to ampicillin/benzyl penicillin, but a substantial, and possibly increasing, rate of *E. coli* is resistant to common empirical antibiotics. Studies from the US have reported an ampicillin-resistance rate in *E. coli* between 64-85% [32, 44, 67, 79, 81]. In preterm neonates, a higher ampicillin-resistance rate has been found compared to term neonates [46, 115]. Some studies report an increasing ampicillin-resistance [79, 81] and a debate has been ongoing on whether neonates whose mothers have received IPA have higher risk of ampicillin-resistant *E. coli* EOS compared to neonates whose mothers have not been exposed to IPA [81, 107].

On the other hand, all the studies reporting increase incidence of ampicillin-resistant *E. coli* EOS were from around 2005 (2000-2010), and were mostly single-centres studies [67, 81, 82]. More recent studies, have found stable ampicillin-resistance rates [79, 115]. Some studies have indicated a slightly increased mortality among neonates with ampicillin-resistant *E. coli* infections despite appropriate empirical antibiotic therapy [82, 116]. These studies have been criticised for having multiple confounders, primarily small sample size and not adjusting for GA, and the findings are contradicted by others [44, 46, 115].

Gentamicin-resistance rates have been relatively low (0-14%) in *E. coli* isolates causing EOS [117], but there are reports of increasing rates [44, 81]. In a Spanish single-centre study, 28.6% of *E. coli* strains causing EOS in preterm neonates from 1994-2014, were gentamicin-resistant. The ampicillin-resistance in this study was 92.8%, and consequently, the authors called for a change in the empirical antibiotic regime [118]. A dual resistance to both antibiotics in current empirical antibiotic regime represents an important challenge. On the other hand, in large population-based study in the UK, the coverage of benzyl penicillin in combination with gentamicin for EOS-pathogens was 94-95% [102, 119, 120], but with a slightly reduced coverage in neonates GA < 32 weeks, and consequently, the slightly broader regimen amoxicillin and gentamicin for GA < 32 weeks was suggested by the authors [120].

Extended-spectrum beta-lactamase producing (ESBL) Gram-negative bacteria and MRSA have been described at 0-3% prevalence in 2010-2011 [102, 121, 122] in the UK and the US, although low rates have been reported from Norway [123].

1.4 Early-onset sepsis -- antibiotic exposure and adverse effects

Antibiotics are lifesaving in septic neonates and timely and appropriate antibiotic treatment affects morbidity and mortality [124]. Since reliable laboratory markers are unavailable at the time antibiotics are started, almost all antibiotics in neonates are started as empirical antibiotics.

1.4.1 Empirical antibiotics guidelines for EOS

Over the past two decades, there has not been any national guidelines for neonates at risk for EOS in Scandinavia. However, many clinicians have probably followed guidelines from the UK National Institute for Health and Care Excellence (NICE) [125] and/or the US (CDC) guidelines. The NICE guidelines were published in 2002, revised in 2012 and 2021 [125], and the CDC guidelines were published in 2002, revised in 2010 and then replaced by the American Academy of Pediatrics (AAP) recommendations in 2018 [66, 94].

Current empirical antibiotic regimen consists of benzyl penicillin (ampicillin is acceptable) in combination with an aminoglycoside [66]. Antibiotics should be initiated promptly and without any delay in neonates with suspected EOS [66]. According to NICE guidelines, suspected EOS in term neonates are neonates with "red flag" clinical instability and/or in neonates with "red flag" perinatal risk factors. According to AAP, there are three different approaches of stratifications of term neonates at risk for EOS; using categorical perinatal risk factors, the sepsis calculator or regular examination of clinical symptoms [66].

The categorical risk-based approach to identify any perinatal conditions (antenatal or neonatal) for prediction of EOS for prompt start of antibiotics after birth [44, 55], has been the fundamentals of earlier versions of the CDC and NICE guidelines [49, 94], and still is in one of three recommended approaches in the latest AAP recommendations. The second approach combines risk factors and clinical conditions in the Neonatal EOS calculator to calculate a neonate's individual risk of EOS. The predictive model is based on GA, highest maternal intrapartum temperature, maternal GBS colonization status, duration of rupture of the membranes and IPA [126]. Finally, the third recommended approach is based on serial physical examination (SPE) of the neonate's clinical conditions after birth and during the next 24-48 hours [66]. These approaches will be explained more in 1.6.1.

For preterm neonates, an approach based on structure of care according to local resources for stratification of neonates into low and high-risks according to perinatal

risk factors (infections/non-infection reasons for preterm birth), such as maternal preeclampsia, birth by Caesarean section and absence/present of labour and PROM, is suggested [66].

In former guidelines, inflammatory markers played a more substantial role in initiation of antibiotics. The 2012-AAP guidelines recommended to measure CRP and complete blood count 6-12 hours after birth in asymptomatic neonates at risk for EOS. This is now removed from the guidelines, and routine blood samples of inflammatory markers alone is not recommended to determine risk of EOS, both in term and in preterm low-risk neonates born by C-section without PROM.

These guidelines reflect the diagnostic challenges in EOS and that the "better-safe-than-sorry" approach that has been the common for almost two decades, may be changing.

1.4.2 Antibiotic exposure and overuse

Antibiotics are the most commonly prescribed medication in the NICU [4, 5]. Within the first week of life, due to the potential risk of EOS, 2.3-10% of all term neonates are exposed to antibiotics [6, 7, 16, 25], and an even higher proportion of preterm neonates. Over 75% of VLBW infants and over 90% of ELGANs receive empirical antibiotics in the first week of life [4, 11, 12, 14]. Many of these infants receive prolonged courses of antibiotics [9, 14, 15] despite the absence of positive blood cultures [4, 127] or clinical signs to support EOS. This occurs in settings where culture-positive EOS incidences are 0.5–0.8 (in term) to 10-30 (in preterm) /1000 LB. Thus, over 95% of neonates screened and treated for sepsis are uninfected and "unnecessarily" exposed to antibiotics.

The high rates of antibiotic exposure may not be surprising given the prevalence of clinical instability among preterm neonates [128], and recommendations that have advised the use of antibiotics for clinical instability and/or perinatal risk factors [14, 49, 94]. Especially among ELGANs, some degree of cardiorespiratory instability is almost invariable after birth, and also among healthy term neonates, mild and transient

symptoms may be present after birth [51, 128]. Clinical symptoms at birth are unspecific and can mimic a huge number of other neonatal conditions [20].

Despite an intention to prioritize treatment in high-risk neonates, most preterm births (2/3) occur in the setting of factors associated with risk of EOS (preterm labour, PROM or clinical chorioamnionitis), and to apply risk stratification strategies to preterm neonates in the same manner as for term neonates is even more difficult [15, 129]. The categorical risk-based approach recommended by NICE and APP has been increasingly challenged the last five years [2, 129]. The majority of studies that show association between risk factors and EOS, are performed before the introduction of IPA [130]. Consequently, many relatively low-risk neonates are exposed to empirical antibiotics [38, 66, 131]. Luck et al. reported that among 413 neonates with risk factors for EOS and all exposed to at least two days of empirical antibiotics, only 13 neonates (3.2%) had any clinical and laboratory evidence of EOS [132].

The lack of a uniform definition of neonatal sepsis has also contributed, in addition to guidelines and clinical insecurity in diagnosing sepsis [14], to different centres and regions having different clinical practices, hence a wide variation in antibiotic exposure and duration of antibiotics between sites [14, 111, 127, 133]. One study even observed a 40-fold variation in antibiotics use, independent of confirmed infection and infection-associated morbidities/mortality across 127 different NICUs [111]. Wide site variation may be associated with overuse of antibiotic, and likely reflects clinical uncertainty more than variation in EOS incidence and medical conditions across sites [14, 111].

A practice with overuse of antibiotics would be less problematic given the high mortality and morbidity associated with EOS, unless the effect of antibiotics in itself was not associated with adverse outcome.

1.4.3 Adverse effects of antibiotics – short and long-term

The dynamic non–resilient gut microbiota in term and preterm neonates is vulnerable to external factors, including antibiotic exposure [36, 134]. Disturbances in gut

microbiomes may lead to early gut dysbiosis and potentially increased risk for alteration in the neonate's immune system. The first 100 days of life seems to be a critical period of immune development [113, 135] and possible explains why there is an association between early antibiotics and adverse outcome at short [4, 8, 11, 133, 136] and long terms [137, 138].

In a seminal paper from 2009, Cotten et al. reported an association between empirical antibiotics during the first week of life and adverse outcome in uninfected neonates [4]. The research group reported adjusted odds ratio (aOR) for NEC or death to 1.39 (95% CI 1.10-1.54, $p < .001$), and aOR for death 1.46 (95%CI 1.19-1.78, $p < .001$) in 4039 VPNs < 32 weeks from 19 NICUs at the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network, when exposed to prolonged empirical antibiotics (≥ 5 days) during the first week of life. An association between early antibiotics and potentially harm, has later been shown by other networks and countries [139, 140], and includes not only prolonged duration of antibiotics [4, 8, 9, 140, 141], but also exposure to antibiotics per se [133, 139]. Early antibiotics and prolonged duration of antibiotics in uninfected neonates are associated with increased risk of NEC [4, 133, 139-141], death [8, 133], BPD [8, 10, 139, 142], severe cerebral lesions [139], LOS [9] and a composite outcome of severe morbidity and/or death [4, 9, 10, 133]. Some studies even report dose effect of antibiotics, each additional added day of antibiotics increasing the risk of adverse outcome [4, 8, 10]. However, some recent studies have challenged these findings, reporting neither association between prolonged early antibiotics and risk of death nor NEC [143], or even a protective effect against NEC when antibiotics are given the first days of life compared to no antibiotics [133, 144-146].

Observational studies reporting associations between antibiotics and adverse outcomes are mainly from selected populations [133]. These studies are prone to different types of bias with potential for confounding by indication; the sickest babies receive more antibiotics [8, 136, 147]. Flannery et al. found that when adjusting for important potential confounders like mechanical ventilation (MV), the association between

increased risk of early antibiotics and death/BPD was no longer present [13], although both Ting et al.[10] and Kuppola et al. [9] adjusted for mechanical ventilation in their analyses. Adjusting for CRIB2 score [8] and for Simplified newborn illness severity and mortality risk scores (SNAP II) >20 [10] as indicator of morbidity, have been used to reduce confounding by indication. A study from the Canadian Neonatal Network tried to overcome the risk of bias in their study on 2018 neonates GA < 29 weeks where each NEC case was matched with two controls for GA, BW and sex [141]. Randomized clinical trial (RCT) investigating the effects of early antibiotics versus no antibiotics are warranted, but high-quality, population-based studies are probably the best alternative to RCTs to elucidate on this complex topic.

Although the potential adverse effects of antibiotics in preterm neonates may not be directly relevant to the 2-8% of all term neonates receiving mostly short courses of antibiotics after birth, clearly there are other effects. Early antibiotics affecting the developing intestinal microbiomes have been linked to long-term health challenges [148]. Antibiotics given in the neonatal period or within the first year of life are associated with long term adverse outcome such as asthma, atopy, impaired early growth in childhood (in boys), overweight and juvenile arthritis later in life when compared to those who were not exposed to antibiotics during the first year of life [50, 114, 125, 135, 149, 150]. A study performed in Sweden on neonates exposed to antibiotics the first week of life found a strong associated increased risk of recurrent wheezing disorders by age 12 months in neonates born at GA 37. This increased risk persisted through 4.5 years of age [151].

Other aspects of adverse effects of unnecessary antibiotic are mother-infant separation and increased health expenditures, and antibiotics resistance [152]. These topics will not be covered in this thesis, but they underscore the importance of a restrictive antibiotic policy and the need of antibiotics stewardship. Antibiotics are administered to protect infected neonates, but treatment of uninfected neonates may become a burden for these children with increased risk of diseases later in life. Stoll and her group used the term “burden of GBS” to emphasize importance of action to reduce

GBS disease [32]. Consequently, the term “the burden of antibiotics” may be useful in emphasising the importance of reducing unnecessary antibiotics.

1.5 Early-onset sepsis – risk factors and diagnostic assessments

1.5.1 Perinatal risk factors

According to current CDC and the NICE guidelines for GBS treatment, all neonates at risk of EOS should have a thorough review of antenatal risk factors performed [153-155]. Such factors include documentation of maternal colonization status with GBS, GA, PROM, chorioamnionitis, younger maternal age, ethnicity, and previous delivery of an infant with invasive GBS diseases [38, 156].

GA is the strongest single predictor of EOS, with incidence of EOS inversely proportional to GA. Preterm neonates have higher risk of EOS compared to term neonates. A more than 10-fold higher risk of EOS is seen among VLBW neonates compared to the overall birth population [38, 39]. Even moderately low GA (34-36 GA) have two-three- fold higher risk of EOS when compared to neonates at GA 37-40 weeks [157, 158]. Even though a low GA and a low BW are strongly correlated, the increased risk of EOS is more strongly associated with low GA than with BW [156], because a poorly developed immune responses and lack of maternal transplacental pathogen-specific antibodies are dependent on GA rather than BW [34].

Mode of delivery and chorioamnionitis are also both strong predictors of EOS. Around 97% of preterm with GBS or *E. coli* EOS are born by vaginal or caesarean delivery after PROM or onset of labour; compared to 2.3% with EOS born by caesarean delivery in the absence of PROM or preterm labour [46, 66, 159]. Half of mothers of preterm neonates with GBS and *E. coli* EOS had clinical chorioamnionitis [159], and chorioamnionitis is associated with a slightly increased risk of and an almost three-fold increased risk of EOS in term and preterm neonates, respectively [160]. Maternal fever is often used as a surrogate for chorioamnionitis, the risk of EOS increases with maternal peak intrapartum temperature. Among over 18 000 neonates evaluated after

introduction of IPA, 1.9% had EOS when maternal fever was < 38℃ whereas 6.4% of the neonates had EOS when maternal fever was > 38.9℃ [131].

However, to identify neonates in need of antibiotics exclusively based on perinatal risk factors, has led to a high number of low-risk uninfected neonates being exposed to antibiotics.

1.5.2 Clinical signs and symptoms

Clinical signs and symptoms of EOS are initially often unspecific, and may vary by GA, severity of infection and microbial pathogen present. Symptoms can mimic a number of other common neonatal conditions and infection should be among initial differential diagnosis in any symptomatic neonate [20] (Figure 3 and Table1). Most neonates with EOS present with respiratory distress that initially cannot be differentiated from other diagnoses such as congenital heart disease, congenital diaphragmatic hernia, respiratory distress syndrome (RDS), pneumothorax or transitory tachypnoea of newborns, among others. Preterm neonates have often apnoea and/or bradycardia, orocyanosis (66%), lethargy (49%) and increased respiratory effort (43%) as the most common presenting features of sepsis [161].

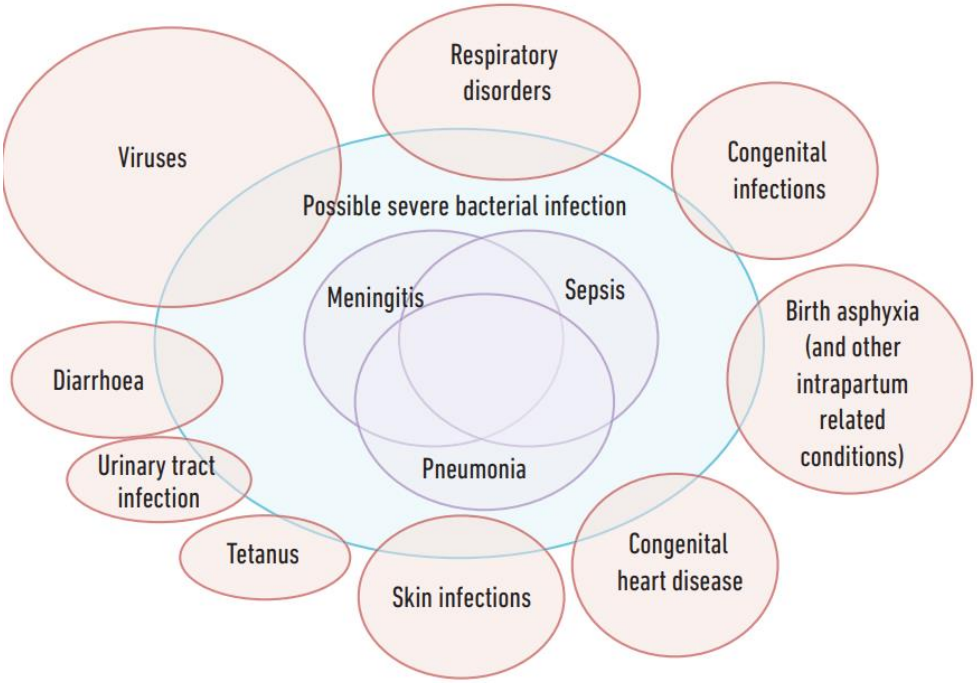


Figure 3 *Possible severe bacterial infection and overlap with other clinical syndromes. Reproduced from "Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions." Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.*

In general, symptoms are more severe with Gram-negative and fungal infections than with Gram-positive infections, and clinical presentations of EOS are more prone to be of multi-organ failure than what is the case for LOS [51].

Sepsis is often described as a dynamic condition, and subtle changes in respiratory status of newborns, temperature instability, or feeding problems can be the first signs of a life-threatening infection [27]. Frequent evaluation of the infant is critical in order to recognize the signs and symptoms of disease during the neonatal period, which can range from nonspecific to multi organ failure. Symptoms and signs may be classified according to WHO (Table 1).

Respiratory

- Tachypnea (respiratory rate > 60 for ≥ 1 hour)
- Apnea [2]
- Cyanosis with need of supplemental oxygen for > 1 hour
- Grunting
- Severe chest indrawings

Circulation

- Poor perfusion, capillary refill time > 3 seconds
- Mean arterial blood pressure < gestational age in weeks
- Anuria/oliguria
- Tachycardia (heart rate >160/min)
- Bradycardia (heart rate <100/min)

Gastrointestinal

- Poor feeding
- Abdominal distension
- Bilious emesis

General/skin/temperature

- Pallor, cold, clammy skin
- "Looking ill", not doing well
- Temperature instability (<35.5 °C or ≥ 37.7 °C)

Neurological

- Lethargy, drowsiness, decreased activity, hyporeflexia
- Irritability, tremor
- Seizures/convulsions, unconsciousness
- Bulging fontanel

Table 1 *Initial signs and symptoms of early-onset sepsis (EOS) used to define symptomatic neonates < 72 hours after birth. Modified from WHO: Pocket book of hospital care for children. Guidelines for the management of common childhood illnesses [162].* [2] *As defined by American Academy of Pediatrics [163]; " a cessation of breathing for 20 seconds or longer or a shorter pause accompanied by bradycardia (< 100 beats /minute), cyanosis or pallor".*

Most EOS cases (80-90%) present in the first 24 to 48 hours of life [31], and the majority develop symptoms before 6-12 hours of life [106].

1.5.3 Inflammatory biomarkers in blood

Common inflammatory biomarkers for use in neonates at risk for EOS are CRP, procalcitonin (PCT) and the complete blood cell (CBC) count. A sensitive diagnostic test may improve outcomes in EOS by [164] decreasing delay in treatment, while a test with high specificity may decrease "overuse" and exposure of unaffected neonates to antibiotics and invasive procedures by "ruling out sepsis [165].

At onset of symptoms of EOS, the predictive performance and sensitivity of common laboratory tests used in suspected EOS are poor. Maternal factors such as hypertension and preeclampsia, mode of delivery and factors in the early postnatal period trigger the same inflammatory reactions as with infection.

CRP and PCT are both nonspecific markers of inflammation. As CRP rises late in the course of infection, one single CRP test at birth lacks both sensitivity and specificity for EOS [166], although serial CRP tests with at least 8 hours intervals increase specificity and may decrease overuse of antibiotics [38, 167]. A study including 1678 neonates and 10 800 inflammatory-marker measurements found a high correlation between maximum values of CRP, PCT and sepsis within 36 hours after start of antibiotics. There was a 100% sensitivity in cut-off values at 16 mg/L and 2.8 ng/L for CRP and PCT, respectively between no EOS versus EOS, meaning that serial of both markers can rule out EOS in an excellent way [164].

The multi-centre NeoPINs study randomised neonates to PCT-guided decision-making in duration of antibiotic courses versus standard care, and found that PCT could safely be used when deciding the duration of antibiotic-courses in suspected EOS [168].

CBC components are in general, neither very sensitive nor specific for EOS. Used alone, CBS is inaccurate to diagnose EOS. A very low white blood cell count, absolute neutrophil counts and a high immature-to-total neutrophil ratio are on the other hand associated with increasing odds for EOS (OR, 5.38, 6.84 and 7.97 respectively) [165] despite a low sensitivity (0.3-54.5%). Thrombocytopenia has a low sensitivity and low positive predictive value as early onset thrombocytopenia is commonly associated with feto-maternal conditions rather than infection [165].

Despite a low positive predictive value of CRP, PCT or CBC, abnormal inflammatory markers in symptomatic neonates or in asymptomatic neonates with perinatal risk factors are still used by many clinicians. Both to start antibiotics and to prolong antibiotic therapy in neonates with a negative blood culture [27]. The updated guidelines from AAP and NICE are less focused on using inflammatory markers in decision-making to guide initiation of antibiotics [66, 125].

1.5.4 Blood culture

The gold standard for diagnosis of EOS is the growth of a pathogen in blood culture [54, 108, 169]. Blood cultures are limited by the time needed from collection to isolation of the infecting organism. Consequently, antibiotic exposure in neonates are started empirically and potentially resulting in a delay in appropriate antimicrobial therapy. However, 92-100% of all blood cultures in EOS obtain positive results within 24 hours [170]. In general, the sensitivity of blood culture is high, even if the neonate has very low levels of bacteraemia [169]. Blood culture sensitivity can be affected by the neonate's prior exposure antepartum and empirical antibiotics started before collection of the culture, but most importantly inadequate volumes. Preferably, 1 ml and minimum 0.5 ml are sufficient volumes and provides excellent sensitivity [169], although sensitivity decrease by 10-40% when the volume is 0.5 ml compared to 1 ml [169]. Consequently, adequate volume of cultures (> 0.5 ml for < 1 month of age) are more likely to yield positive culture results than inadequate volume [171].

1.6 Antibiotic stewardships and action to reduce antibiotics

A 2016 Lancet publication states:" *The global shortage of antibiotics that are available for use in neonates is of increasing concern, particularly given the increase of antimicrobial resistance and the marked paucity of research in this vulnerable population. Neonates should be more highly prioritized in research and development globally, including in the development of enhanced diagnostics that are practical for use in low-income and middle-income countries...* "[114]. The Norwegian Ministry of Health published an action plan in 2015 to reduce antibiotics in humans by 30% within 2020 to combat antibiotic resistance [172].

Antibiotic stewardship programs are designed to improve and measure the appropriate use of antibiotics by systematic interventions to select the correct initiation of antibiotics, duration of therapy, optimal antibiotic regimen, dose, and route of administration [173]. The substantial rates of antibiotic exposure in both term and

preterm neonates, and wide site variation in use of prolonged antibiotics in preterm neonates, most likely reflects clinical uncertainty in management and not differences in illness of EOS [14], and may all be potential aims for stewardships [174].

The opportunities for antibiotic stewardships in neonatal medicine are shown in Figure 4. I will focus on initiation and duration of antibiotics, and only briefly describe the other strategies.

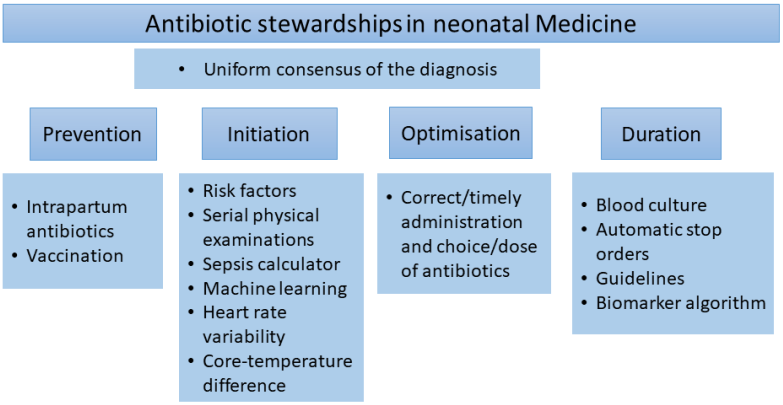


Figure 4 *The opportunities for antibiotic stewardships in neonatal medicine. Modified from; 1. Puopolo KM, et al. Management of Neonates Born at ≥35 0/7 Weeks' Gestation with Suspected or Proven Early-Onset Bacterial Sepsis. Pediatrics, (2018), and. 2. Keij FM, et al. Stratified Management for Bacterial Infections in Late Preterm and Term Neonates: Current Strategies and Future Opportunities Toward Precision Medicine. Front Pediatr, (2021). 9, p. 590969*

1.6.1 Initiation of antibiotics in suspected early-onset sepsis

Sepsis-score programs have shown to be effective in the adult population, but there are no scoring programs for high-risk neonates admitted to a NICU, only for neonates at the post-delivery units [175]. The different diagnostic approaches to identify neonates in need of antibiotic are according to AAP [66, 129]:

- ✓ Categorical risk-based stratification approach
- ✓ Serial physical examinations
- ✓ Multivariate risk assessment using the EOS calculator

The categorical risk-based stratification approach combined with clinical assessments at births, is mostly practiced for term and moderately term neonates the last two decades, and have led to a high proportion of neonates being exposed to antibiotics [14, 49, 52, 129]. One alternative recommended approach is serial physical examinations (SPE), based on a structured and repeated examination of neonates at risk for EOS where prompt empirical antibiotic is deliberately restricted to clearly symptomatic neonates [110, 129]. This approach is similar to WHO's recommendation to use clinical examination [22, 129]. Hitherto, only few and small studies have documented this approach, and more studies are needed [130, 176, 177]. Other approaches to improve initiation of antibiotics to the right neonates are using the sepsis calculator, machine learning and heart rate variability/ core-temperature differences [178]. Stewardships focusing on initiation of antibiotics, directly address the diagnostic challenges in neonatal sepsis [178], and "rapid, affordable and appropriate diagnostic tools are needed" [20].

1.6.2 Duration of antibiotics for suspected or confirmed early-onset sepsis

A study from Gothenburg, among 145 neonates born at ≤ GA 28 weeks, described a reduction in overall antibiotic days and treatment days after implementing an intervention with focus on short and standard treatment, in addition to more access to infection disease consultant advice [179]. Moreover, the use of meropenem was reduced from 69 to 44% [179]. In the SCOUT study, Cantey et al. performed a

thorough assessment of antibiotic use in one US NICU and subsequently performed an intervention with i) discontinue empirical antibiotic after 48 hours and ii) a maximum of five days of antibiotics in culture-negative sepsis and pneumonias; both by automatically ending the prescription in the electronic medical records [110]. Among the 1607 and 895 neonates in the baseline and intervention period, the antibiotics days were reduced by 27% (from 343 to 252 days per 1000 patient days) [110]. Similarly, results were reported from a Norwegian study were a simple targeted intervention to discontinue empirical antibiotics in neonates ≥ GA 34 weeks resulted in a reduced duration of antibiotics from 108 to 96 hours and in reduced proportion of neonates being exposed to antibiotics (from 2.5 to 1.8%) [109]. Furthermore, Zihlman-Ji et al. performed an intervention with PCT-guided algorithm in 35 642 neonates at risk for EOS [180]. The research group managed to reduce the duration of antibiotics therapy in 879 neonates from four to three calendar days from 2014 to 2018. These studies are stewardships based on adherence to the guidelines.

1.6.3 Other actions for correct use of antibiotics

An important strategy for prevention of EOS, is IPA. Other actions for correct antibiotics use may be a uniform consensus in diagnosing neonatal sepsis in addition to a clear definition of criteria for organ dysfunction are important, and should be prioritized [27, 178] Correct choice of antibiotics, based on local causative pathogens, and susceptibility pattern, correct dose and timely administration; are all important for optimal antibiotics use. However, a thorough discussion on this is beyond the scope of this thesis.

2.0 Aims of the thesis

The overall aim was to increase knowledge on EOS and early antibiotic use in term and preterm neonates in order to optimise and improve future neonatal care and antibiotic stewardship programs. The specific aims for the three studies were:

2.1 Paper 1: Burden of disease and antibiotic exposure

The primary aims were to describe the incidence of EOS, causative pathogens, antibiotic-resistance and antibiotic therapy over a 23-year period in a single NICU in South-West Norway between 1996 and 2018. **The secondary aim** was to assess possible trends in the incidence of EOS during the study period.

2.2 Paper 2: Antibiotic exposure and adverse effects

The primary aim was to describe associations between empiric antibiotic exposure within the first week of life and adverse outcomes (severe NEC and a composite outcome of severe morbidities/death) in an unselected population of very preterm neonates (GA < 32 weeks) born in Norway during 2009-2018. **The secondary aims** were individual components of the composite outcome, and to evaluate antibiotic exposure during the study period.

2.3 Paper 3: Diagnostic assessments and antibiotic stewardships

The primary aim was to develop, implement and evaluate if an approach using serial physical examinations (SPEs) could reduce the proportion of term neonates exposed to antibiotics for suspected EOS within the first three days of life, without affecting safety in a single NICU in South-West Norway during 2014-2018. **Safety measure** was time from birth to administration of antibiotics. **Secondary outcomes** were days of antibiotic exposure/1000 patient-days, incidence of EOS and culture-negative sepsis, and number of CRP blood sample test taken. **Safety measures** were incidence of infection-attributable deaths or infection-attributable readmissions within 14 days of life.

3.0 Subjects and methods

3.1 Study area and settings

In Norway, there are 20 NICUs across four health trust regions. Seven of the NICUs are at university hospitals. All NICUs have human donor milk available from breast milk banks, and virtually no VPNs receive formula prior to 33-34 weeks GA.

The Norwegian Neonatal Network (NNN) is a national population-based health registry where data on investigations, treatments and diagnoses on all neonates admitted to NICUs in Norway are registered daily by the attending physician [23, 181, 182].

NNN was founded in 2004 and is regulated according to the Norwegian Personal Health Data Filing Systems Act. All Norwegian NICUs enter clinical data on a daily basis. Data from NNN can include either the national database or the local branch covering data from each individual NICUs. Annual internal audits of NNN-data have shown NNN to be consistent and reliable [181].

SUH is serving a population of 370 000, with around 4500 annually births. SUH is the only hospital offering primary, secondary and tertiary obstetric and neonatal care for a well-defined population in South-West Norway, South-Rogaland. All premature neonates with GA ≤ 34-35 and all neonates receiving systemic antibiotic therapy are admitted to the NICU. In total, approximately 10% of newborns are admitted to the NICU. Data from SUH are presented in **paper 1 and 3**, whereas national data from all Norwegian NICUs are presented in **paper 2.**

3.2 Study design, participants, data collection, variables and outcome measures

3.2.1. Paper 1

Design

A population-based retrospective single-centre longitudinal observational study.

Participants

All LB neonates born at SUH during 1996-2018 (23-year period) with a positive blood culture obtained within the first three days of life were included, and all LB neonates born in the hospital's catchment area accounted for the total neonatal "background" population.

Variables, data collection and outcome measures

Data on clinical characteristics, causative pathogens and antibiotic susceptibility were registered on all included neonates. Detailed clinical information was extracted from the medical records including GA, BW, timing of first registered signs and symptoms of EOS, maximum value of CRP, antibiotics administered and number of days of antibiotic therapy, and infection-attributable mortality. The diagnosis of clinical chorioamnionitis was extracted from the mother's medical record. The aggregated number of LB neonates in the hospital's catchment area was extracted from the local birth registry. The study period was divided into two periods (1996-2006 and 2007-2018) for comparisons, to evaluate trends in the incidence of EOS.

Primary outcome measures:

1. The incidence of culture-confirmed EOS
2. EOS causative pathogens and their antibiotic-resistance patterns
3. Antibiotic therapy administered

Secondary outcome measures:

1. Trends in the incidence of EOS during the study period

3.2.2 Paper 2

Design

A nationwide population-based observational study.

Participants

All LB neonates born before 32 weeks gestation in Norway during 2009-2018 were eligible for inclusion. We excluded neonates with sepsis, death and severe NEC/intestinal perforation during the first week of life.

Variables, data collection and outcome measures

Neonates born in Norway during 2009-2018 with GA < 32 weeks were identified from the NNN. Dedicated members of the NNN at each site participated in quality control and supplementation of missing data for infants born between 2009-2014 in cases where infants were reported as dead, diagnosed with septicaemia and/or positive blood cultures but microbial pattern were missing or unknown, and reporting CRP values for cases identified with growth of CoNS in blood culture. NNN improved the daily registration in 2015 to include pop-up questions, to further improve the quality and consistency on the data collected and supplementation of data was consequently not performed for 2015-2018.

Background data including birth region, year of birth, mode of delivery, BW, BW Z-score [183], GA, clinical risk index for babies 2 (CRIB2) [184], severe intraventricular haemorrhage (IVH, grade 3 or 4) [185], Apgar score, plurality, gender and antenatal steroids (ANS) and clinical data including antibiotic use in calendar days, MV, growth velocity, common morbidities of prematurity, and mortality before discharge, were retrieved [45]. The total number of LB infants was obtained from the Medical birth registry of Norway.

Exposures:

We explored antibiotics both as a categorical and as a continuous measurement.

1. Categorical: Any exposure to antibiotics within the first three or seven days of life. Duration of antibiotics in the first seven days was subdivided into “short course” (1-3 days) and “prolonged courses” (4-7 days or ≥ 5 days).
2. Continuously: Numbers of days of antibiotic exposure in the first week of life.

Primary outcome measures:

1. Surgery for NEC after the first week of life.
2. A composite outcome of “Severe short-term morbidity” including any of the following outcomes diagnosed after the first week of life and until discharge from hospital; severe BPD, cystic periventricular leukomalacia (cPVL), severe retinopathy of prematurity (ROP) and/or death.

Secondary outcome measures:

1. The individual components of "severe morbidity” and /or death before discharge from hospital.

2. Annual proportions of antibiotic exposure and mean values of treatment duration.

3.2.3. Paper 3

Design

A single-centre, prospective population-based, quality improvement (QI) study.

Participants

All LB term neonates born at SUH during the study period 2014-2018 were included in the study.

Variables, data collection and outcome measures

Prospectively entered, registry data on number of neonates exposed to antibiotics, type of antibiotics, number of days of antibiotics and EOS diagnosis were collected from the local NNN database at SUH and then quality-checked against the neonates` electronic medical record. Time from birth to administration of antibiotic was collected from the neonates' medical record, and aggregated numbers of term LB neonates were collected from the hospital`s birth registry. The study period was divided into three: a baseline period, an educational period and a post-implementation period (Figure 5).

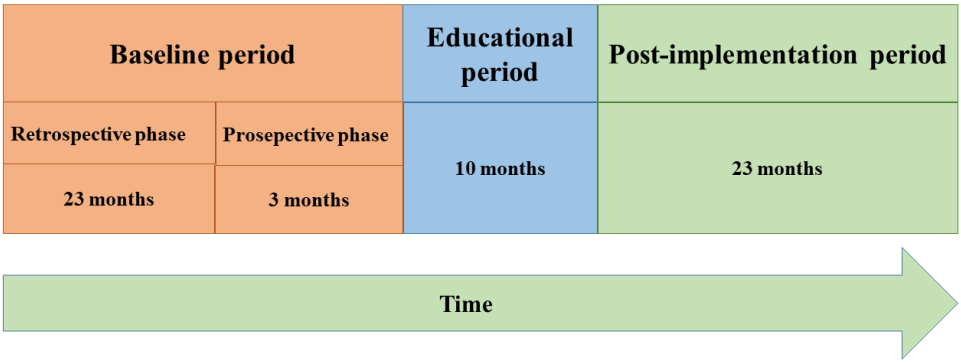


Figure 5 *Periods for implementation of Serial Physical Examination at Stavanger University Hospital*

A new management strategy was developed and implemented, during the 10-month educational period (March to December 2016). We evaluated and analysed the effects after implementing SPEs for suspected EOS in term neonates during a 23-month post-implementation period (January 2017 to November 2018), compared with a 26-month-baseline period (April 2014 to February 2016), consisting of a retrospective phase (23 months, 1.1.14-31.11.15) to control for observer effect, and a prospective phase (three months, 1.12.15-1.3.16).

Primary outcome measures:

1. Development and implementation of a risk assessment strategy with structured SPEs during the first 24-48 hours of life to identify EOS in term neonates.
2. The proportion of neonates exposed to antibiotics the first three days of life.
3. Safety measure: the time from birth to start of antibiotics in EOS and culture-negative sepsis.

Secondary outcome measures:

1. Total days of antibiotic therapy per 1000 patient-days.
2. The incidence of EOS and culture-negative sepsis.
3. Safety measures: the incidence of infection-attributable deaths or all infection-attributable readmissions within 14 days of life.
4. The numbers of CRP blood sample test taken.

3.3 Definitions

3.3.1 EOS and epidemiology

We defined neonatal sepsis as growth of a known pathogen in blood culture and antibiotic treatment of ≥ five days, or shorter duration if EOS-related death occurred in a neonate. Growth of bacteria in blood culture obtained ≤ 72 hours of life and > 72 hours of life, were classified as EOS and LOS respectively. Isolates of CoNS obtained during the first 72 hours were not regarded as EOS.

The incidence of EOS was reported as cases of EOS per 1000 LB neonates.

EOS-attributable mortality was death occurring within seven days after growth of pathogenic bacteria in blood culture and where sepsis was the assumed cause.

GA was based on prenatal ultrasound in week 17-19. Neonates were classified as; term (GA ≥ 37 weeks) or preterm (< GA 37 weeks). Preterm infants were further classified in moderately preterm (GA 32 to 36+6 weeks), very preterm (GA 28 to 31+6 weeks) and extremely preterm (< GA 28 weeks).

Hours from birth to start of antibiotics was defined as the time difference between the time antibiotics were started and the time of birth. The percentage of neonates receiving antibiotics was described as the number of term neonates receiving antibiotics divided by the total number of LB term neonates multiplied by 100.

In **paper 3**, we defined cases as culture-negative sepsis according to criteria suggested by the Norwegian paediatric association for ICD-10 P36.9: i) clinical signs of a possible infection, ii) minimum CRP level > 30 mg/L, iii) minimum duration of 5 days antibiotic therapy, and iv) other explanations for the clinical symptoms excluded. In **paper 1 and 2**, culture-negative sepsis was not included. Neonates were classified as symptomatic if they had signs of EOS according to the WHO (Table 1).

3.3.2 Blood culture medium, pathogens and grouping of bacteria

Blood cultures were obtained prior to the initiation of antibiotics using BacT/ALERT PF Plus Aerobic Pediatric culture bottles (BioMérieux, Inc., Durham, NC) at SUH (**paper 1 and 3**). For **paper 2**, blood culture systems at the different hospitals are not specified. Matrix-assisted laser desorption ionization - time-of-flight (MALDI-TOF) mass spectrometry was introduced in many hospitals in Norway around 2012-2013, gradually replacing traditional phenotypic species identification.

Traditional pathogens were classified in line with Bizzaro et al. [47]. Pathogens were grouped into Gram-positive and Gram-negative bacteria. In **paper 1**, VGS included *Streptococcus mitis* and *Streptococcus alactolyticus* and "Other streptococci" included *Streptococcus pyogenes* and *Streptococcus pneumoniae*.

3.3.3 Contaminants

Growth of *Micrococci, Propionibacteria, Corynebacteria, or Diphtheroids* in a single culture, and all cases of CoNS in blood cultures obtained < 3 days of life were

considered contaminants. When there was growth of more than one bacteria in the blood culture, and one of them was a known pathogen, this pathogen was included.

3.3.4 Antibiotic susceptibility

A blood culture pathogen was defined as susceptible to an antibiotic when the final interpretation report indicated "S" (susceptible) and nonsusceptible when the report indicated "R" (resistant) or "I" (intermediate). Antibiotic susceptibility testing followed the guidelines from the Norwegian working group for antibiotics, closely aligned with the EUCAST criteria [186]. After our study period was completed, the criteria was changed in 2019, to define "I" as a recommendations to increase the dose of antibiotics if treating pathogens defined as I [186].

3.3.5 Empirical antibiotic therapy

Empirical antibiotic was any antibiotics commenced before culture results were identified in a neonate at risk of EOS. We included all empirical antibiotics within the first week of life (=early empirical antibiotics), and all antibiotics within the first 72 hours, in **paper 2** and in **paper 1** and **3**, respectively. The local empirical antibiotic regimen for EOS at SUH consisted of ampicillin in combination with either tobramycin or gentamicin from 1996-2010, and benzyl penicillin and gentamicin from 2011-2018, as described in paper 1. There was no national guideline for empirical antibiotic therapy for EOS, but as described in **paper 2**, benzyl penicillin/ampicillin was combined with gentamicin in all empirical courses, and in **paper 3** benzyl penicillin was combined with gentamicin in all empirical courses. Probiotics were sporadically used for ELGANs in Norway between 2014 and 2017.

3.3.6 Other definitions

Clinical chorioamnionitis was prospectively diagnosed by the "on-call" obstetrician. Cases later diagnosed as histological chorioamnionitis were not included.

Severe ROP was defined as stage 3-5 and/or treated in either eye with laser/anti-vascular endothelial growth factor. Cystic periventricular leukomalacia (cPVL) was defined as cPVL ≥ stage 2 and determined by the most severe cranial ultra-sonogram

before hospital discharge or death [187]. Severe BPD was defined as receiving any respiratory support (non-invasive or mechanical ventilation), not only oxygen, at 36 weeks PMA. Severe NEC (defined as Bells stage ≥ 2b treated with laparotomy after the first week of life, in line with Battersby [188].

3.4 Statistics and power calculation

Data were analysed using IBM-SPSS version 24 statistical software (IBM, Armonk NY, USA) in paper 1 and 3. In paper 2, we used IBM SPSS Statistics Version 26 and Stata v. 17.0 for analyses. All tests were two-tailed. P-values of < .05 were considered statistically significant.

3.4.1 Paper 1

Results were expressed as mean with 95% confidence interval (CI) or median with interquartile range (IQR), as appropriate. Differences between groups and periods (1996-2006 versus 2007-2018) were analysed with t-test or Mann-Whitney test as appropriate for continuous data, and the chi-square test or Fisher-exact test for categorical data. Regression models were used to test for trends over time (linear) where year was the continuous predictor.

3.4.2 Paper 2

To calculate the power and sample size in paper 2 for an estimate of how many years to include in the study, we used one of our primary outcome; the incidence for NEC. We assumed that base-line risk for NEC was 2.0-2.5% in this population. Previous studies have shown odds ratios (OR) ranging from 1.4-7.1 for developing NEC after antibiotic exposure during the first week [4, 10, 133]. In order to show a significantly increased aOR (> 2) of NEC among neonates exposed to antibiotics during the first week of life compared to those receiving no antibiotic therapy, we needed a sample of minimum ~1000-1100 neonates in each group of antibiotic exposure. Around 600 LB very preterm infants (GA < 32 weeks) are born annually in Norway. Thus, a minimum

of four complete years would be needed for this sample provided equal number receiving antibiotics or not. However, as antibiotic administration will be uneven distributed among GA groups we included a 10-year cohort. Preliminary analyses indicated sufficient numbers in our sample to have a power of 80% at 5% significance level.

Descriptive results were expressed as counts and proportions for categorical variables, as means and standard deviations (SD) for continuous variables. Comparisons between groups of infants were performed with Chi square tests and analysis of variance (ANOVA)/Student's t tests, respectively. Annual proportions of antibiotic exposure and mean values of treatment duration were assessed and compared in regression models (logistic and linear), and presented with 95% CI allowing for clustering on siblings.

Unadjusted comparisons of antibiotic exposure groups would be biased due to confounding by indication. We adjusted for the following potential confounders and controlled for them in the analyses: GA (in days), sex, multiple births, mode of delivery, CRIB2, Apgar score at 5 min (Apgar'5), severe IVH, receiving ANS or not, MV within the first week of life, BW Z-score, year of birth and health trust region.

All confounders were used in the imputation models, along with all individual outcomes, the exposures number of days of antibiotics within the first week of life and antibiotics given the first three days of life (yes/no), and the auxiliary variables CRIB2 and growth velocity [189]. The variables GA, BW Z-score, year of birth, Apgar at5 minutes and days of antibiotics were all introduced into the imputation models as restricted cubic splines with three knots. Twenty imputed data sets were created. The main analyses were binary logistic regression with allowance for clustering on siblings. All potential confounders were included in the models. The effects of GA, BW Z-scores and year of birth were modelled non-linearly using restricted cubic splines with three knots. Missing values were imputed using multiple imputation by chained equations with logistic regression or predictive mean matching with five nearest neighbours for categorical and continuous variables, respectively. Results were

averaged over imputation samples by Rubin's rule, and are reported as aORs with 95% CIs. The set of confounders was highly predictive with an area under receiver operating curves (AURC) above 0.87 for both the composite outcome "severe morbidity" and mortality [190].

3.4.3 Paper 3

With a baseline rate of antibiotic treatment at 2.9% of all term infants, a sample size of 7792 term infants in the post-implementation period would be needed to detect a 20% reduction in antibiotic exposure with the power of 85% (α=0.05). At SUH around 4500 neonates were born every year when this study started, we would consequently need around 20 months in the baseline and 20 months in the post-implementation period.

R (version 3.5.1. R Foundation for Statistical Computing, Vienna, Austria) was used to perform an interrupted time series analysis, acknowledging time as a variable when assessing the intervention [191]. Results were expressed as mean with 95% CI or median with IQR, as appropriate. Differences between groups were analysed with t-test or Mann-Whitney test as appropriate for continuous data and the chi square test or Fisher-exact test for categorical data. All tests were two-tailed. P-values of $< .05$ were considered statistically significant.

3.5 Ethical considerations

3.5.1 Paper 1

The local institutional review board and data protection officer approved the paper as a local quality improvement project, and no further approval was needed.

3.5.2 Paper 2

This paper is based on data from NNN. This project has approval from the Ethical committee as part of a large ongoing project on outcomes after very preterm birth (REK Sør-Øst 2012/944, REK Sør-Øst 2012/944-7 for data collection on Group B

sepsis and REK Sør-Øst 2012/944-10 for collection on Gram-negative isolates). Access to data from NNN involves the release of de-identified personal data in accordance with the Medical Birth Registry of Norway and is regulated by the Privacy Act.

3.5.3 Paper 3

Data is based on aggregated data from NNN. Access to data from NNN involves the release of de-identified personal data in accordance with the Medical Birth Registry in Norway, Regulations and the Privacy Act. The paper was regarded as a quality improvement paper and approved by the institutional review board at SUH.

3.6 Funding

This research has been partly financed by internal scholarship at Stavanger University Hospital and by educational leave for Senior Consultants. The funder did not have any role in the paper design, data collection, interpretation nor the decision to submit the work for publication.

4.0 Summary of results

4.1 Paper 1

During the study period from 1996 to 2018, totally 104 377 LB neonates were born in the hospital's catchment area, on average 4538 (range from 4012 to 5050) each year. Among these 96 024 (92%) neonates born at term, and 8353 (8%) preterm < 37 weeks GA. There were 182 neonates with a positive blood culture obtained within the first three days of life, including101 culture-confirmed EOS cases and 81cases classified as a contaminated blood culture (Figure 6).

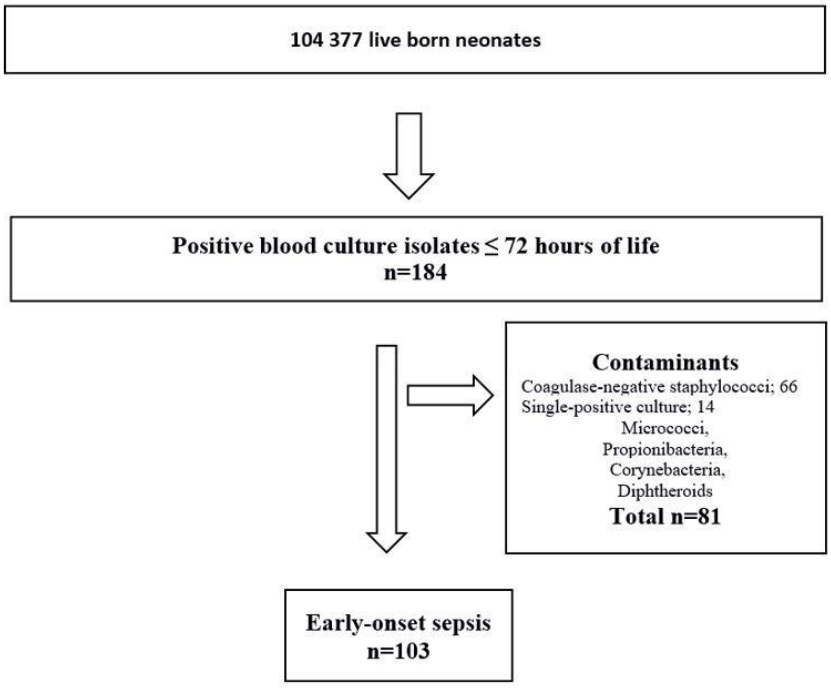


Figure 6 *Infants diagnosed with early-onset sepsis in a population-based observational study over 23 years at Stavanger University Hospital, Norway, 1996-2018.*

Of the 101 (incidence of 0.97/1000 LB neonates) culture-confirmed EOS cases, 89 were Gram-positive and 12 were Gram-negative bacteria. GBS was the most common pathogen (59/93; 63%) in neonates with GA ≥ 28 weeks, and *E. coli* was the most common pathogen (4/8; 50%) in ELGANs (GA < 28 weeks). VGS was the second largest group of pathogens with 10/101 cases (10%), predominantly occurring in term

neonates (9/10). Most cases of EOS were among term neonates, 71/101 (70%), although the incidence of EOS in moderately preterm (GA 28-36 weeks) and ELGANs neonates were 3.9 and 24-fold higher compared to term infants, respectively (Table 2).

	Incidence of EOS per 1000 LB			
	All EOS isolates	**GBS**	***Escherichia coli***	**VGS**
Infants	**Number infants**	**Number infants**	**Number infants**	**Number infants**
	Incidence (95% CI)	**Incidence (95% CI)**	**Incidence (95% CI)**	**Incidence (95% CI9**
All	N = 101	N = 60	N = 11	N = 10
N = 104 377	0.97 (0.71, 1.23)	0.57 (0.39, 0.75)	0.11 (0.03, 0.18)	0.10 (0.06, 0.19)
GA ≥ 37 weeks	N = 71	N = 45	N = 3	N = 9
N = 96 024	0.74 (0.52, 0.96)	0.47 (0.31,0.63)	0.03 (0.02, 0.09)	0.084 (0.014, 0.18)
GA 28-36 weeks	N = 22	N = 14	N = 4	N = 1
N = 7890	2.8 (1.43, 4.28)	1.8 (0.66, 3.0)	0.51 (0.01, 0.92)	0.13
GA < 28 weeks	N = 8	N = 1	N = 4	N = 0
N = 463	17.8 (6.2, 29.4)	2.2	8.2 (0.13, 16.3)	

Table 2 *Incidence of EOS per 1000 LB among infants born at Stavanger University Hospital during 1996-2018. Incidence; case per 1000 LB. GA; Gestational age, median (IQR), GBS; group B streptococci, VGS; viridians group streptococci, EOS; Early onset-sepsis, LB; Live births.*

The incidence of GBS and *E. coli* EOS were 0.57/1000, and 0.11/1000, respectively, and remained unchanged during the study period. The incidence of Gram-negative pathogens decreased by increasing weeks of GA (OR 0.79, 95% CI 0.69, 0.89, p < .001). There was no difference in the overall incidence of EOS between the two periods 1996-2006 and 2007-2018 (1.05/1000 versus 0.90/1000, p=.49) (Figure 7). However, for the period from 2000 through 2018 analysed separately, there was a mean decline in the incidence of EOS by 6% per year (95% CI 1%-10%) (p= .019). There was no change in the incidence of EOS during the study period for any of the GA groups when analysed separately.

EOS-attributable mortality was 6/101 (5.8%), including 7/71 (1.4%), 3/22 (13%) and 2/8 (25%) at GA ≥ 37 weeks, GA 28-32, and GA < 28 weeks, respectively. The EOS-attributable mortality was higher in neonates with GA < 28 weeks compared to term infants (p=.025). The median (IQR) GA in neonates who died was 34 (26-36) weeks.

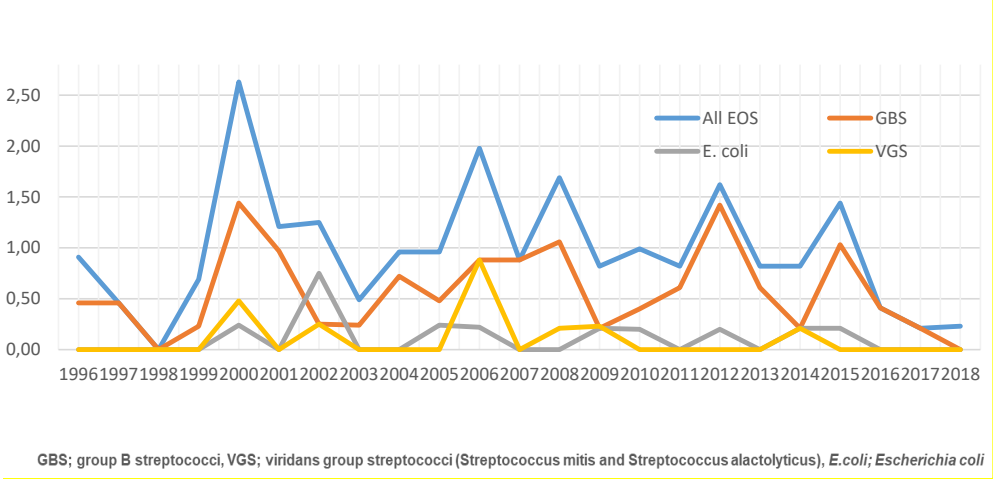


Figure 7 *Annual incidence of Early-Onset Sepsis per 1000 LB caused by all pathogens, GBS, E. coli and VGS during 1996-2018.*

In 2/101 (2%) isolates causing EOS, the pathogens were resistant to the empirical antibiotic regimen. All *E. coli* causing EOS in this study period were susceptible to aminoglycosides.

The initial empirical antibiotic regimen for EOS was in all cases a combination of benzyl penicillin or ampicillin and an aminoglycoside, but in 21/101 (21%) of cases a broad-spectrum antibiotic either was added to or substituted this regimen. The median (IQR) duration of antibiotic therapy for EOS declined from 14 (10-14) days in the period 1996-2006 to 8 (7-10) days in the period 2007-2018, (p < .013).

Overall, 95/101 (94%) neonates with EOS had onset of symptoms within the first 24 hours of life. Among neonates with EOS that were exposed to chorioamnionitis (14/101, 14%), all extremely preterm neonates (7/7, 100%) and 7/10 (70%) term neonates developed symptoms of EOS within the first six hours of life.

The median (IQR) time to start of EOS-symptoms was 3.0 (1.0, 13.0) hours. The proportion of neonates with symptoms at birth was higher among preterm 18/30 (60%) compared to term neonates 26/71 (37%), p = .047. The maximum CRP values were higher in neonates with Gram-positive compared to Gram-negative EOS, p= .003.

4.2 Paper 2

There were 601 668 LB neonates in Norway between 2009 and 2018. Of these, 5296 (0.88%) infants were born < 32 weeks: 3646 (69%) at 28-31 weeks and 1650 (31%) before 28 weeks. Within the first week of life, 239/5296 (4.5%) neonates died and 87/5296 (1.6%) had culture-proven EOS (Figure 8). There were 4932 neonates who survived the first seven days of life days without sepsis, intestinal perforation or NEC, and thus were included in the final analysis.

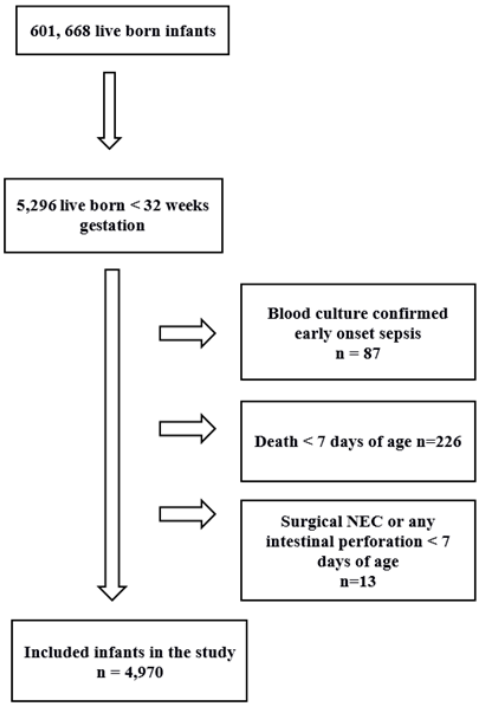


Figure 8 *Very preterm infants born in Norway during 2009-2018 and study inclusion. NEC; Necrotising Enterocolitis.*

A total of 1051 (21%) neonates had at least one severe morbidity and/or died (187; 3.8%) after seven days of life. The ELGANs accounted for 60% (624/1051) of neonates with severe morbidity and 84% (153/187) of all deaths. The severe NEC-rate among GA < 28 was 4.3% (60/1403), and 1.4% (71/4970) among all GA < 32 weeks.

There were marked differences in baseline characteristics between neonates receiving 0, 1-3 days, 4-7 or 5-7 days of antibiotics in the first week of life. Neonates receiving prolonged empirical antibiotics were smaller and more likely to have lower Apgar scores and higher CRIB2 scores.

Most neonates were exposed to antibiotics within the first three (3715/4932; 75%) and seven (3790/4932; 77%) days of life. During the 10-year study period, there was an overall decrease in exposure to empirical antibiotics

- A reduction in the overall proportion commenced on antibiotics from 82% in 2009 to 66% in 2018, $p < .001$.
- A reduction in the proportion of neonates exposed to prolonged antibiotics (5-7 days) in the absence of sepsis, from 55% in 2009 to 24% in 2018, $p < .001$.
- A reduction in duration of antibiotics of approximately 1.5 days among those receiving antibiotics, $p < .001$.

After adjusting for CRIB2 and relevant confounders, neonates exposed to antibiotics within the first week of life had increased aOR of death after first week of life (aOR 9.33, 95% CI 1.10-79.5), severe morbidity at discharge (aOR 1.83, 95% CI 1.18-2.83), severe morbidity or death after first week of life (aOR 2.07, 95% CI 1.30-3.30) and severe BPD (aOR 2.17, 95% CI 1.18-3.98), when compared to neonates not exposed to antibiotics in the first week of life (Table 3). Similar or even stronger associations between antibiotic exposures and morbidities and mortality were found when we adjusted for only CRIB2, or if we analysed infants exposed to antibiotics only within the first three days compared to no antibiotics in the first three days of life.

Antibiotic duration ≥ 5 days was associated with a more than two-fold increased adjusted odds of severe NEC, and with increased odds of severe BPD compared to

those exposed to antibiotics for 0-4 days. Compared to neonates not exposed to antibiotics, neonates exposed to 1-3 days or 4-7 days during the first week of life had higher adjusted odds of death and/or severe morbidity. Overall, each additional day of empirical antibiotics was associated with 14% higher aOR for death and/or severe morbidity. Finally, each additional day of empirical antibiotics increased the odds of severe BPD by 14%. For the other individual components (LOS, cPVL and ROP) of the composite outcome "severe morbidity" there were no differences in aORs in relation to antibiotic exposure.

Adjusted odds ratio (aOR) for adverse outcomes in relation to antibiotic (AB) exposure

Outcomes	aOR[a] (95% CI) Any AB vs no AB first 7 d[b]	aOR[a] (95% CI) 1–3 d vs. no AB[b]	aOR[a] (95% CI) 4–7 d vs. no AB[b]	aOR[a] (95% CI) 4–7 d vs. 1–3 d AB[c]	aOR[a] (95% CI) ≥ 5 d vs. 0-4 d AB[d]	aOR[a] (95% CI) Each additional day of AB
Severe NEC[e]	2.25 (0.28-18.4)	2.02 (0.22-18.3)	2.37 (0.28-19.8)	1.17 (0.46-2.98)	**2.27 (1.02-5.06)**	1.18 (0.97-1.43)
Death after first week of life	**9.33 (1.10-79.5)**	**10.2 (1.14-91)**	**8.9 (1.03-77)**	0.87 (0.43-1.79)	1.27 (0.70-2.31)	1.16 (0.99-1.35)
Severe morbidity[f] or death after first week of life	**2.07 (1.30-3.30)**	**1.87 (1.11-3.13)**	**2.22 (1.35-3.64)**	1.19 (0.81-1.74)	**1.59 (1.15-2.20)**	**1.14 (1.05-1.22)**
Severe morbidity at discharge[f,g]	**1.88 (1.16-3.05)**	1.69 (0.99-2.88)	**2.03 (1.22-3.38)**	1.20 (0.80-1.80)	**1.58 (1.12-2.21)**	**1.12 (1.04-1.21)**
LOS after first week of life [h]	0.92 (0.56-1.50)	0.84 (0.48-1.46)	0.97 (0.58-1.62)	1.16 (0.77-1.74)	1.21 (0.88-1.68)	1.01 (0.93-1.09)
Severe BPD (36 weeks) [i]	**2.17 (1.18-3.98)**	1.89 (0.97-3.71)	**2.36 (1.25-4.47)**	1.25 (0.77-2.03)	**1.61 (1.08-2.39)**	**1.14 (1.04, 1.25)**
Severe ROP (treated) [j]	0.68 (0.23-2.03)	0.46 (0.12-1.71)	0.81 (0.26-2.51)	1.76 (0.70-4.44)	1.55 (0.81, 2.96)	1.07 (0.92, 1.25)
cPVL, N (%)[k]	1.35 (0.57-3.16)	1.68 (0.67-4.20)	1.10 (0.45-2.72)	0.66 (0.34-1.28)	0.83 (0.46-1.48)	0.96 (0.84, 1.10)

Table 3 *Adjusted odds ratio for Mortality and Morbidities among very preterm infants (n=4932) and early empirical antibiotic exposure*

[a] *Adjusted for GA (non-linear), sex, multiple births, mode of delivery, AB, antibiotics; CRIB2, Apgar score at 5 min, ANS, mechanical ventilation within the first week of life, BW Z score (non-linear), IVH grade 3-4, year of birth (non-linear) and birth region.*
[b] *Reference is 0 days.*
[c] *Reference is 1-3 days.*
[d] *Reference is 0-4 days.*
[e] *Severe NEC; necrotizing enterocolitis, defined as Bells stage 2 or 3 and diagnosed after the first week of life requiring severe intervention/review.*
[f] *Severe morbidity; any severe BPD, cPVL, severe NEC after the first week of life or severe ROP in either eye during the hospital stay.*
[g] *n=4745, deaths excluded from analysis*
[h] *LOS; Culture positive sepsis after first week.*
[i] *Severe BPD; severe bronchopulmonary dysplasia, receiving any respiratory support (not solely oxygen) at 36 weeks postmenstrual age.*
[j] *ROP; severe retinopathy of prematurity, defined as stage 3-5 and/or any treated ROP in either eye.*
[k] *cPVL; cystic periventricular leukomalacia.*

4.3 Paper 3

Overall, 17 242 term neonates were LB during the baseline and the post-implementation periods and were included in the analyses (Figure 9).

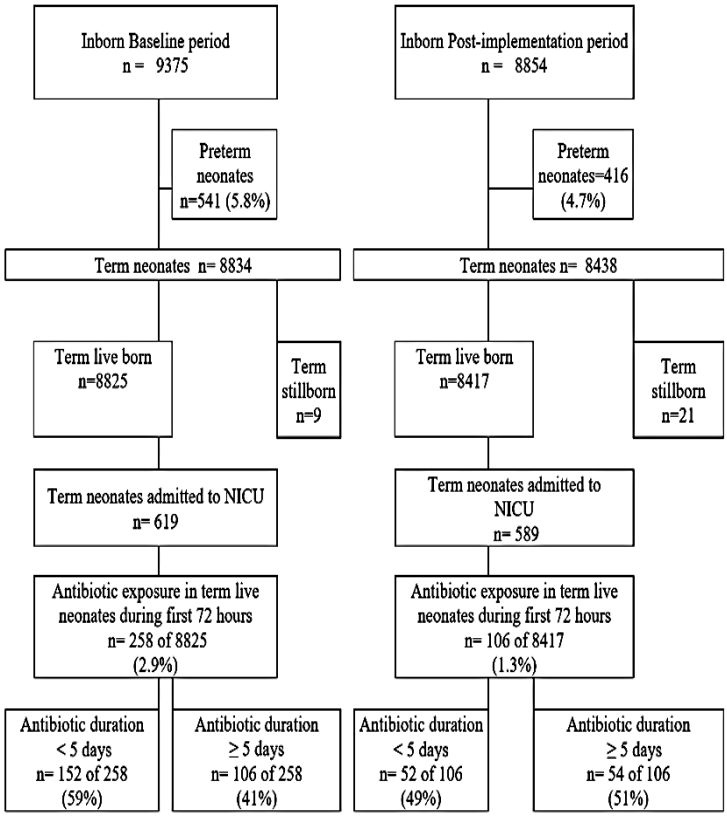


Figure 9 *Flowchart – All neonates born at Stavanger University Hospital in the baseline and the post-implementation period, admissions to the neonatal unit and antibiotic therapy.*

After implementing the new SPE-strategy there was a 57% relative reduction in the proportion of neonates exposed to antibiotics during the first three days of life, declining from 2.9% to 1.3%. The interrupted time series analysis (Figure 10) showed a sharp decline in term neonates exposed to antibiotics after the implementing of the

new strategy, and then a subsequent relatively stable rate of neonates exposed to antibiotics.

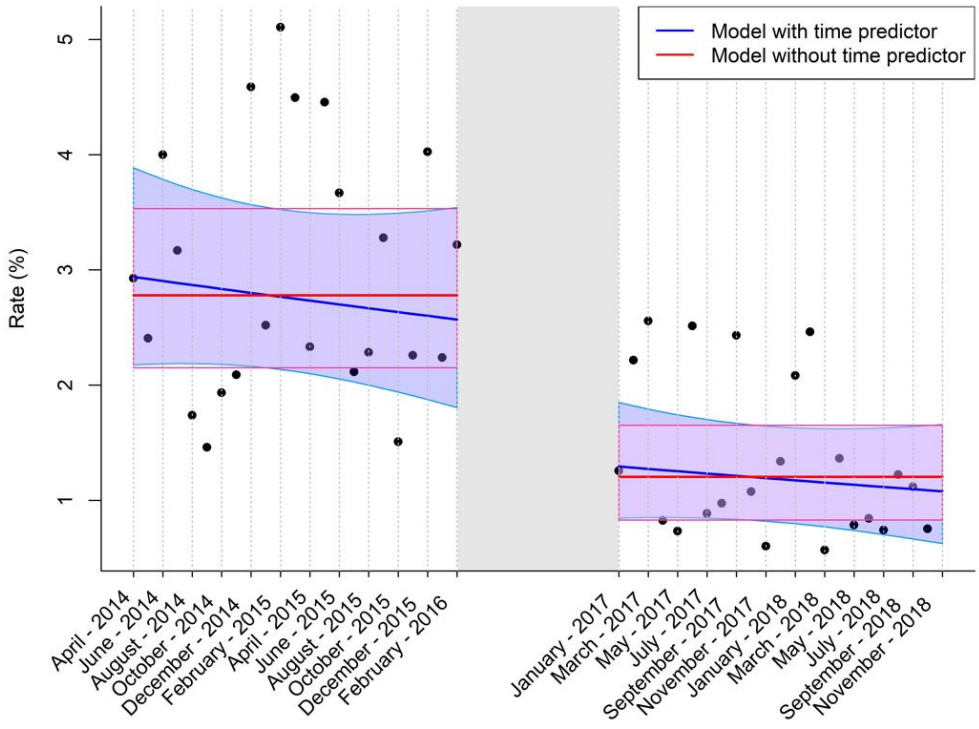


Figure 10 *Interrupted time series analysis - Proportion of all LB term infants (≥37 weeks gestation) receiving antibiotic during the first 72 hours of life in the baseline and the post-implementation period. LB; live-born.*

There was a 50% relative reduction (14 versus 7 hours) in the time to initiate antibiotics in infected neonates (culture-positive and culture-negative EOS). There were no readmissions for infection within 14 days of birth or cases of infection-attributable deaths; all deaths were related to perinatal asphyxia or to major congenital birth defects or disorders.

Moreover, we observed a 60% relative reduction in antibiotic therapy-days per 1000 patient-days (Table 4). The incidence of culture-positive EOS did not differ between the two periods, however, the incidence of cases labelled as culture-negative EOS

declined from the baseline period to the post-implementation period. The number of CRP blood samples taken before and after implementation of SPE were reduced from 332 to 223.

None of the culture-positive EOS-cases were related to chorioamnionitis, and in the post-implementation period, all neonates with culture-positive or culture-negative EOS presented with clinical symptoms within 24 hours of life. The overall median duration of treatment was the same. (Table 4).

There was no increase in the proportion of term neonates admitted to the NICU and staffing levels remained unchanged.

	Baseline	Post-implementation	*P* value
Term live-born neonates; n	8825	8417	0.16*
Term neonates admitted to the NICU; n (%)	619 (7.0)	589 (7.0)	0.97*
Culture-positive EOS; n	5	1	0.22**
Incidence, all pathogens/1000 term live-born	0.57	0.12	0.22**
Incidence, GBS/1000 term live-born	0.44	0.12	0.37**
Culture-negative EOS; n	111	55	< 0.001*
Incidence, per 1000 term live-born	11.8	6.7	< 0.001*
Term neonates exposed to antibiotics; n (%)	258 (2.9)	106 (1.3)	< 0.001*
Admitted within 4 hours[1], n (%)	135 (1.5)	71 (0.8)	<0.001*
Admitted due to symptoms between 4-72 hours, n (%)	123 (1.4)	35 (0.4)	<0.001*
Days of antibiotic exposure per 1000 patients days; mean (95% CI)	320 (304-337)	129 (118-141)	< 0.001***
Duration of antibiotic therapy (days); median (IQR)	4.25 (2-5)	5 (2-5)	0.12****
Hours from birth until administration of antibiotics in all culture-positive and -negative EOS; median (IQR)	14 (5-28)	7 (3-17)	0.003****
Number of CRP analyses per month; mean (95% CI)	332 (307-357)	223 (194-252)	<0.001***
All-cause mortality; n	4	4	
Infection-attributable mortality; n	0	0	
Antibiotic therapy reinstituted within 14 days; n	0	0	
Meningitis; n	1	0	

Table 4 *Main results from the baseline and the post-implementation period of a quality improvement project with serial physical examinations of neonates at risk for early-onset sepsis (EOS).[1] Neonates with risk factors and early symptoms. CI; confidence interval, CRP; C-reactive protein, GBS, Group B streptococci, NICU; Neonatal Intensive Care Unit, IQR; Interquartile range. *Chi square test **Fisher's Excact test ***Students t-test ****Mann-Whitney test*

5.0 Discussion

5.1 Methodological considerations

All clinical studies are at risk of systematic errors. This may lead to results that differ from the true value and represents important challenges to the quality of the research. STROBE checklist is an international initiative developed, to "STrengthen the Reporting of OBservational studies in Epidemiology [192]. We did not use STROBE in the planning of our studies, but when finalizing the manuscripts, we actively used the checklist. We will discuss relevant items from the checklist in the following sections in relation to our papers.

5.1.1 Paper 1

Study design

In this paper, we aimed to describe epidemiology and antibiotic use in neonatal EOS. We clearly defined criteria for EOS and clinical risk factors. We classified causative pathogens in line with Bizzaro et al. and Stoll et al., and had clear criteria for exclusion of contaminants [32, 47].

A retrospective observational design is suitable to analyse microbial trends over a relative long period. To be able to describe incidence of a disease in a selected population, the design of the study needed to be strictly population-based. SUH is the only hospital in the area, and the catchment area of the hospital is well-defined. However, Flekkefjord hospital is a small maternity unit where the parents for a short period were able to choose which hospital to be transferred to when their newborn were ill; SUH or the Hospital of Southern Norway, Kristiansand. This could have influenced the numerator and denominator in the calculation of incidence (per 1000 LB). To be able to reduce this bias, we used postal code to find the correct number of LB and number of cases of EOS that belonged to SUH's catchment area.

We used a consistent definition of sepsis [32]. The data was collected by one researcher. Coding and clinical predictors were clearly defined and registered uniformly throughout the study period. All these factors may reduce bias.

One of the key strengths of the paper is the long duration of retrospective data collection. SUH has a relatively large number of annual deliveries (4000-5000) and the long study period allowed us to study low-frequency events like EOS. The population was unselected. The number of neonates included in our study roughly equals the total number of births in Norway over a two-year period. We have complete data sets on all neonates with positive blood cultures for the entire study period, including antibiotic susceptibility patterns, antibiotic use, and neonatal and maternal data for the entire period. The study includes detailed maternal and neonatal information. We only included culture-confirmed episodes regarded as the "gold standard" for the definition of neonatal sepsis in this study.

There are clearly also limitations. This study is based on data from a single-centre NICU. In other settings, potentially both the epidemical pattern and the antibiotic resistance rates can vary from our findings. Higher antibiotic consumption can drive higher antibiotic resistance rates. Consequently, our findings may not be generalizable to other countries, settings with higher burden of neonatal sepsis, or settings with limited resources and weak healthcare systems. Another important limitation is the low rate of ELGANs included. However, this is representative for the Norwegian populations. Albeit in line with other regions of Norway, it may be lower when comparing with countries outside Scandinavia.

Finally, there are always inherent limitations with retrospective data. The data is collected for another purpose than for the specific study, and may be incomplete and inaccurately registered.

Microbiology

One important bias in this study could be the volume collected for blood cultures. Our population was identified by neonates that had a positive blood culture within the first

three days of life. Cultures with adequate volumes (> 0.5 ml for <1 month of age) are more likely to result in growth than cultures with inadequate volume [171]. It is possible that clinicians obtained to small volumes and consequently the number of positive blood culture was lower than if a higher volume had been taken. However, the EOS incidence reported is in the higher range of currently reported incidences in high-income countries and thus we believe that our numbers are fairly reliable. Blood culture sensitivity can be also be affected by the neonate's prior exposure to IPA which could potentially reduce the number of neonates identified with culture-positive EOS. On rare occasions, blood cultures may have been drawn after antibiotics were started, possibly resulting in false negative cultures. However, the incidence of EOS in our study was comparable to studies both in Scandinavia and Europe, which make this less likely.

Generalizability

Our NICU is the size of an average NICU in many western countries and offers tertiary care, except neonatal surgery, for severe medical conditions. For NICUs in Norway, and in other countries with a good health care system and relatively low rates of antibiotic resistance in these causative pathogens, our findings may be generalizable.

5.1.2. Paper 2

Study design

Register-based studies are well suited to answer research questions when a large number of neonates is required, in particular when population-based data on relatively rare conditions like EOS and NEC are needed.

This study has numerous strengths due to its nationwide, decade-long approach. The population-based design, including all uninfected live born VPNs surviving seven days of age free of sepsis and NEC/intestinal perforation, is a major strength of this paper and avoids selection bias. This design enables us to explore associations between early antibiotics and adverse outcome when corrected for confounders. Daily prospective

recording in the registry NNN ensures almost complete data sets. However, the quality of registry data depends on the quality produced by a large number of clinicians. During 2009-2014, we regarded the quality of the some of the data in NNN as suboptimal. We therefore performed quality check and adding data that were missing, as described in the method section. From 2015, the quality of data was improved, with pop-up questions, which enabled us to use the data without any supplementation for 2015-2018.

We were unable to report data on chorioamnionitis or intrapartum antibiotics. This is a limitation as the neonate's total exposure to antibiotics may represent one reason why studies show conflicting results on this topic.

To be able to answer our objectives, we had to ensure a clean population without overlapping populations and hence our inclusion criteria needed to be narrow, in line with other relevant studies on this topic [4, 8, 13, 140, 143]. First, neonates with a culture-confirmed sepsis the first week of life had to be excluded from the paper as in these neonates antibiotic therapy was considered clinically necessary. Second, if there is a causality between antibiotic exposures and adverse outcomes related to microbiome disruption we aimed to assess all outcomes after, and not during, the antibiotic exposure. Consequently, the exposition variables were defined as events during the first week of life, and outcome variables were defined as events occurring after the first week. This meant that we had to exclude events in the first week of life that also could appear as an outcome variable after the first week, identified as death or NEC (surgical or medically treated)/intestinal perforation during the first week.

In the power calculation, to estimate how many years we needed to include in the study, we used severe NEC as the primary outcome (as presented in the Methods section). We used severe NEC because this has lower incidence than the composite outcome and also than the individual components in the composite outcome (the other primary outcome). However, the incidence of severe NEC in ELGANs (4.3%) was comparable to that reported in other studies (4.3%-5.4%) [23, 193], but the overall rate of severe NEC was lower (1.4%) than we had anticipated in our power calculation

(2.5%), influencing the power of our study. We included a 10-year cohort with almost 5000 VPNs. Larger studies with inclusion of more ELGANs may be needed for robust estimates on possible harmful effects of different durations of antibiotics on low-incidence outcomes like NEC. We included common neonatal morbidities in the composite outcome in alignment with other studies. Ting et al. included IVH grade 3 or 4, NEC, BPD, severe ROP, death and LOS [10]. Kuppola et al. included NEC, LOS or death [9]. Finally, Cotten et al. included a composite of NEC or death [4].

Definitions and categorical groupings were chosen to align with other relevant studies [4, 8-10, 13, 143, 144, 194]. We used a strict definition for NEC, as we believe this is important when exploring association with antibiotics that are potentially lifesaving. NEC definitions vary in the literature [193, 195], and consequently, this complicates comparisons between studies. The "severe NEC" definition we used, is in line with the one used by the UK neonatal network [188]. We excluded NEC-cases diagnosed in the first week of life for three reasons. First, if there is a causality between antibiotic exposure and adverse outcomes related to gut microbiome disruption we aimed to assess all outcomes after, and not during exposure. Second, "preterm NEC" usually occurs after the first week of life [196]. Third, spontaneous intestinal perforation is often misclassified as NEC but usually occurs earlier, thus by excluding "NEC-cases" in the first week of life we pragmatically reduce misclassifications [196-198]. Using a strict NEC definition is important, focusing on those infants that may benefit most from interventions or prophylactic strategies [23, 188, 193].

Confounding by indication

The main limitation of this paper is the possibility of confounding by indication; the sickest babies receive more antibiotics and that is the reason why they have more adverse outcomes, not the antibiotic exposure per se. It is impossible to adjust and control for absolutely all potential confounders [8]. As expected, baseline characteristics showed marked differences between infants receiving 0, 1-3 days, 4-7 or 5-7 days of antibiotics in the first week of life; neonates receiving prolonged

empirical antibiotics were smaller and more likely to have lower Apgar scores and higher CRIB2 scores.

We carefully adjusted for a set of confounders, established predictors for mortality and morbidity, showing high predictive values for morbidity and mortality and thereby reducing risk of confounding [190]. However, as expected, there was a correlation between our set of confounders and antibiotic exposure, and to a lower degree for antibiotic duration in days, consequently reducing the likelihood of confounding by indication for these analyses. For BPD, we added mechanical ventilation during the first week as an important confounder.

We performed risk analyses both with a set of confounders and with exclusively CRIB2, and the results were almost similar.

In all analyses for death, we note the wide CIs. This indicating uncertainty with effect estimates and risk of residual confounding when evaluating mortality in observational trials [190]. We can only speculate if our results would have turned out differently regarding our wide CI if a higher number of neonates had been included. On the other hand, this result (increased odds of death after early antibiotic exposure) was observed with different statistical models when adjusting for the set of confounders, only adjusted for CRIB2 score and only adjusted for GA.

Generalizability

In Norway, all preterm neonates receive human milk in the first several weeks after birth. Our findings may not be generalizable to settings with different nutritional practices [199, 200].

5.1.3 Paper 3

Study design

In this paper, we present a large cohort of neonates that in the post-implementation period was managed by a clinical risk-based assessment using SPEs in selected neonates at risk for EOS. The population-based design was well suited to answer our research questions. The study was designed within a QI framework, shown to be an efficient method for rapid implementation of new knowledge into clinical practice [201]. During the baseline period, the EOS management strategy was based on the 2010-NICE guidelines [155]. Neonates exposed to any perinatal risk factors (PROM >18 hours, chorioamnionitis, maternal GBS colonization or bacteriuria, previous sibling with GBS infection) and with clinical symptoms suggestive of EOS or an elevated CRP level received antibiotic therapy. Antibiotic therapy was discontinued if no signs of clinical instability persisted and blood cultures remained sterile for up to 36-48 hours. We included only term neonates in the study because we predicted that most clinicians would have a lower threshold for starting antibiotics in preterm neonates.

During the educational period, a multidisciplinary NICU team developed a management strategy with SPEs for suspected EOS in term neonates. We used the "model for understanding success in quality framework" (MUSIQ) [202] to reveal any contextual factors that could affect the success of the intervention, and QI methodology with Plan-Do-Study-Act (PDSA) [201]. Case-discussions, simulations, clinical audits, and regular multi-disciplinary NICU meetings were used to improve knowledge and reduce inter-colleague variability in clinical assessment.

The new management strategy was implemented in January 2017. "At-risk" neonates, including neonates exposed to maternal chorioamnionitis or a previous sibling with a GBS sepsis, and neonates who during the first 72 hours of life developed clinical symptoms indicating a possible sepsis, were routinely admitted to the NICU for management with SPEs for identification of neonates with EOS [38, 155]. As part of

our intervention, we decided to admit neonates with symptoms and/or exposure to chorioamnionitis in accordance with previous studies showing that these are at increased risk of EOS. Escobar et al. showed that in an era of IPA: there were infection rates of 6.1% and 10.3% in neonates with maternal fever > 38.9 degrees and in neonates that were critically ill, respectively [131]. Thus, the more severe maternal signs of chorioamnionitis and the more neonatal clinical symptoms and signs, the higher likelihood of an infection [131]. We were able to confirm, through post-discharge follow-up in all infants, that there were no infection-related readmissions.

The intervention was based on important clinical principles. First, most neonates do not have EOS despite perinatal risk factors [131, 203]. Secondly, mild transient symptoms are more likely to be caused by other neonatal conditions than EOS [20, 51, 132]. Mild transient symptoms were accepted under close monitoring. Third, early and prompt administration of antibiotics is essential if clinically strong suspicion of EOS [42]. If vital signs did not improve/aggravate over time despite corrective actions such as pain management after vacuum, or if the neonate was/became clinically ill, antibiotic was started [20]. Inflammatory markers were limited to be taken only on indication, not as a screening for infection [166]. Finally, the decision to initiate antibiotics was still made by the attending physician, but first after discussion and in agreement with a consultant neonatologist.

Neonatal and obstetric management were unchanged throughout the entire study period, which allowed us to isolate the effect of the intervention.

Intervention bias

We did not have the resources needed to perform a RCT; hence, we designed the study as a QI project with a "before-and-after" design with inherent limitation compared to RCT [204]. In a RCT, the two groups would have been distinct separated with comparable factors, both measurable and unmeasurable. In contrast, when comparing two groups before and after an intervention, the outcome may be influenced by other unknown variables in the NICU, as with all QI projects [205]. Our results could

potentially be caused by natural variation in antibiotic exposure [204]. To reduce this bias, we performed our analyses also with Statistical Process Charts (SPS), and got the same results. We acknowledged the Hawthorne effect that any outcome may be changed by the moment staff start to focus on an intervention [206]. However, we reduced this bias by dividing the study period into three periods, and we did not change any other major guidelines in the NICU during the study period. Hence, we consider that the markedly reduction in antibiotic exposure was a result of the implemented SPE-based strategy.

Generalizability

This QI-project can be regarded as an establishment of a new antibiotic stewardship program. The program is based on a structured and repetitive examination of selected neonates the first days of life. This approach clearly requires intensive individual medical assessments in a well-staffed setting, and consequently may not be generalizable to settings unable to provide this level of care [178].In settings with low incidence of EOS, the actual number of neonates at risk is low. In our study, the numbers of neonates being assessed hourly with SPE was lower than the number of neonates treated with antibiotics due to suspected EOS; hence, the NICU workload was not increased. The majority of neonates with EOS develop symptoms within the first 6-12 hours, emphasising that the neonate's risk decreases with increasing hours after birth. This project is also in keeping with the precision medicine medical model; where focus is on customization of healthcare and a «holistic» way of stratifying neonates instead of the «old fashioned» one size fits all approach [178].

5.2 Discussion of results

Epidemiology and burden of EOS

Although there are many studies reporting on population-based microbial pattern as causative agents in EOS, **paper 1** is the only Norwegian paper including the entire birth population over more than two decades including more than 100 isolates. Rønnestad et al. and Fjalstad et al. both reported on selected neonatal populations, ELGANs and term neonates, respectively [16, 40], whereas Størdal et al. reported on all GAs, but over a ten year period from 2010 to 2019 in a single-centre NICU with only 30 cases of EOS [207].

The overall incidence of EOS at SUH during 1996-2018, was 0.97/1000 LB (**paper 1**). This is comparable to other reports from Norway (0.54-1.1/1000 LB, in term and in all GA, respectively) [16, 207], Sweden (0.9/1000 LB) [80], UK (0.7/1000 LB) [42] and the US (0.77-1.08/1000 LB) [32, 46, 67], but higher than reported from France (0.43/1000 LB) [208].

During 1996-2018, we found a 24-fold higher incidence of EOS in ELGANs (17.8/1000 LB) compared to term neonates (**paper 1**), similar to the 30-fold higher EOS rate reported by Stoll et al. [46]. In **paper 2** we also show that the incidence of EOS in ELGANs (45; 27/1000 LBs) was higher than in GA 28-32 weeks (42; 11/1000 LBs), $p < .001$, during 2009-2018 in Norway. The high incidence of EOS in ELGANs reflects the vulnerability of preterm neonates. This is emphasising the importance of clinicians' caution in care of preterm neonates as EOS is adding extra on the burden of prematurity.

Our key findings, are that GBS is the most common causative pathogen in EOS (**paper 1 and 3**), but *E. coli* dominates in ELGANs (**paper 1**). These findings are similar to other study groups and networks [40-42, 46, 67]. We found a higher proportion of GBS cases (59%) than reported from the US (30-38%) [41, 46, 67], but comparable to other Norwegian (58%) [16], Swedish (40%) [80] and UK studies (43%) [42]. On the other hand, the proportion of *E. coli* was lower in our study, in line

with other studies [46, 67]. Interestingly, the proportion of *E. coli* were lower than a recently published Norwegian study (11% versus 20%) [207], possible because the median GA in our study was slightly higher than the median GA in this study (39 versus 35 weeks). We found a low proportion of *S. aureus* (8.9%), in line with previous reports. VGS accounted for 10% of all EOS cases (**paper 1**). Other studies often classify VGS as "other Gram positive" or "unspecific", a proportion that accounts for 1-16% of all EOS cases [41, 46, 207]. We noted that in all VGS-EOS, the neonates had clinical symptoms, increased CRP > 10 mg/L, were regarded as septic by the consultants, and consequently treated with five or more days of antibiotics. The role of this group of possible pathogens needs further investigations.

The overall incidence of EOS remained stable for the entire study period in **paper 1**. However, for the period from 2000 through 2018, there was a mean decline in the incidence of EOS by 6% per year (95% CI 1%-10%) (p= .019). We note that this was not an a priori planned analysis, but this result is in line with important multi-centre studies in settings comparable to our setting [42, 43] and one Swedish study [80], all showing a significant reduction in EOS incidence over the last two decades. On the other hand, Yale New Haven reported an overall stable EOS incidence around 0.9/1000 from 2004 to 2013.

The stable incidence of *E. coli* EOS and GBS EOS during the 23 years (**paper 1**) is in line with other European studies [42, 80], although our numbers of *E. coli* cases may be too small to detect any changes. Studies from the US and Australia consistently reports higher rates of *E. coli* EOS [32, 43, 46, 107, 209]. Surveillance data from multicentre US NICUs including 235 EOS cases identified from 217480 neonates born during 2015-2017 and 2006-2009, found an increasing incidence of *E. coli* EOS in ELGANs [46], with incidence rates of 8.9/1000 LB compared to previous 5.1/1000 LB (p=0.008). Risk-based IPA program, based on UK guidelines, were implemented in our NICU in 2008 [96, 97]. The US' incidence of GBS EOS declined from 1.8/1000 LB in 1990 to 0.25/1000 LB in 2018, after implementation of a screening-based IPA program [94, 122, 153]. Limitations in the GBS IPA strategies have been

demonstrated both in risk-based and in screening-based programs [46, 210], but screening based programs may be associated with a slightly lower rate of GBS EOS and a higher rate of *E. coli* EOS. Some European studies report lower incidence of *E. coli* EOS than in US and Australia, a finding usually accompanied by a higher incidence rate of GBS, as also described in our paper [42, 80]. Yet, the incidence of GBS in our paper is well comparable to other studies [16, 32, 46].

The maximum CRP levels in neonates with EOS at SUH were relatively low. This was the case both in Gram-positive and Gram-negative bacteria, in line with other studies [87, 211]. This is an important reminder for clinical evaluation of neonates at risk of EOS and the importance of not using CRP as a "diagnostic tool" to identifying neonates with EOS [129, 212].

The EOS-attributable mortality among GA < 37 (17% in **paper 1**), was slightly lower than Stoll et al. (29%) [128], but among GA < 32, the mortality was higher in **paper 1** compared to **paper 2** (17% in **paper 1** and 8% in **paper 2).**

Antibiotic resistance

We demonstrated a low level of antibiotic resistance **(paper 1**); the majority (98%) of EOS isolates were susceptible to the current empirical antibiotic regimen; benzyl penicillin and gentamicin, and the combination of these two antibiotics continues to have an excellent coverage for EOS in our setting. In some settings, gentamicin-resistance among *E. coli* strains have been reported to be over 16%. This implicates that in some settings almost two in 10 *E. coli* infected neonates will be infected with isolates resistant to the combination of penicillin/ampicillin and gentamicin [122].

Although numbers in our study are small, the Gram-negative antibiotic-resistance rate remained unchanged. This important finding is comparable to the findings from a US study where they described a stable *E. coli* antibiotic-resistance rate during 2009-2017 [213]. On the other hand, antibiotic-resistance rates in this US study were substantially higher than in our study. Our antibiotic-resistance rates correlate well with a UK study reporting a 7% antibiotic-resistance rate to the empirical antibiotic regimen of benzyl

penicillin and gentamicin among 328 EOS isolates [119]. Furthermore, in this study there was in fact a decrease in antibiotic-resistance rates from 2005-2009 to 2010-2014, and also a lower antibiotic-resistance rate among Gram-negative isolates causing EOS versus LOS [119]. This correlates well with the findings of Flannery et al., that pathogens causing EOS in the US are not affected by the increasingly extreme antibiotic-resistance seen globally [122, 213], where 3 out of 10 deaths in neonatal sepsis are caused by antibiotic-resistant pathogens [20]. The newly published GARDP study reports on 3200 cases of neonatal sepsis across 11 low-, middle- and high-income countries (in Europe; Greece and Italy). In 2019, there were 140 000 neonatal deaths caused by antibiotic-resistant pathogens, an increasing number [214, 215].

Antibiotic-resistance rates are generally low in the whole population in Norway due to a restrictive antibiotic policy, although gentamicin-resistance of *E. coli*, ESBL and MRSA are found in the neonatal population [123]. Consequently, continuously surveillance of resistance pattern in pathogens causing EOS is important [15, 122, 213]. A potentially increasing challenge in the future is the fine balance between potentially ineffective empiric antibiotics with associated morbidity and mortality, and the side effects of routine use of broad-spectrum empiric antibiotics [216]. Caile et al. suggests that despite a slightly broader spectrum regime including amoxicillin and gentamicin would provide a better cover in EOS among neonates GA < 32 (amoxicillin/gentamicin versus benzyl penicillin/gentamicin: 93% vs 86%, p=0.211) there should be reluctance in changing the first line regime as the majority of neonates receiving empirical antibiotics are among term and near-term neonates [119]. None of the *E. coli* isolates in **paper 1** was resistant to gentamicin. This is in line with Størdal et al. (0% gentamicin-resistance rate) [207] and Stoll et al. [46]. A Norwegian study from a single-centre unit in 1989 throughout 1994, identified 27 *E. coli* isolates, both EOS (39%) and LOS, and here none of the isolates were resistant to the aminoglycoside netilmicin [217].

In 2011, our NICU changed from ampicillin to benzyl penicillin as the beta-lactam backbone for empiric EOS therapy. Ampicillin use is associated with a higher *K.*

pneumoniae gut colonization, including ampicillin-resistant strains [218]. Our results do not support the need to change empirical regime to ampicillin instead of benzyl penicillin.

Antibiotic use and exposure

In our three papers, we reported on antibiotic use in these periods; 1996-2018 (**paper 1**), 2009-2018 (**paper 2**) and 2014-2018 (**paper 3**). Although there was no uniform national guideline on antibiotic recommendation for EOS, in all cases a combination of benzyl penicillin or ampicillin and an aminoglycoside were used. This is in line with both a large European multi-centre survey among 38 countries where the most frequently used antibiotics were ampicillin and gentamicin (54.6%) [219], and a US multi-centre study were Flannery et al. report that among 297 hospitals ampicillin and gentamicin accounted for 74% and 68%, of administered antibiotics, respectively [14]

We observed an overall decrease in exposure and duration of empirical antibiotics locally in Stavanger from 1996-2018 (**paper 1**) and nationally in VPN from 2009-2018 (**paper 2**). We believe this is a result of an increasing focus on antibiotic overuse in neonates over the last decade in Norway, primarily from 2015 and after publication of the first Norwegian paper on antibiotic use in neonates and regional variation [16]. In **paper 3** we describe an intervention to reduce antibiotics exposure among term neonates that more than halved the antibiotic exposure among term neonates at our hospital. After the study period, we have disseminated this intervention program to six other Norwegian NICUs and will report on the results shortly. The antibiotic stewardship SPE intervention described in **paper 3** together with another initiative in Norway have both contributed to reduced exposure of antibiotics [109]. These initiatives coincided with a major campaign in Norway on antibiotic stewardship [172], Mundal et al. found in a Norwegian study a reduction in exposure to antibiotics in term and near term infants during 2015-2019, and the reduction was significantly higher in NICUs with ongoing local antibiotic stewardship programs [182]. Our study, among others, have shown it feasible and safe to reduce unnecessary antibiotics both in term [109, 182, 212], and preterm neonates [176, 220-222].

The median duration of antibiotic therapy for EOS declined markedly from the period 1996-2006 to the period 2007-2018 (**paper 1**). In VPN we also found a clear reduction in proportion of neonates exposed to prolonged courses (≥ 5 days) of empirical antibiotics during 2009-2018 (**paper 2**). Flannery et al. reported no change in antibiotic exposure among VLBW from 2009-2015 in a multi-centre study (297 NICUs) [14], although a slight decrease in rates of prolonged antibiotics was found [14]. Reduced rates of prolonged courses (from 49% to 35%) was also reported in a US cohort among VLBW neonates [143].

Associations between antibiotics and adverse outcome

The last decade, the associations between early empirical antibiotics and severe morbidity/death have been explored in many observational studies [4, 9, 110, 139, 140, 144]. **Paper 2**, with an unselected sample of all Norwegian VPNs surviving seven days of life free of NEC, intestinal perforation and sepsis contributes to this discussion. Any empirical antibiotic exposure within the first three or seven days of life, independent of duration, was associated with increased odds of severe morbidity and/or death, and severe BPD after adjusting for established indicators of illness severity and potential confounders [2, 223].

Neonates exposed to antibiotics during first week of life had an approximately nine-fold increased aOR of death compared to no exposure. This finding was tested with various statistical models, and suggest that any empirical antibiotics within the first week of life is associated with increased risk of death in non-infected infants, in line with other studies [8, 133]. Any antibiotic exposure was also associated with increased aOR of severe morbidity, an effect mainly caused by the increased odds of severe BPD and NEC. There was a dose-effect with increasing days of exposure associated with increasingly aOR of severe morbidities.

We found neither a protective nor a harmful effect of shorter antibiotic exposure initiated within the first days of life in relation to severe NEC (**paper 2**). Our data contradicts a suggested protective effect of early antibiotics against NEC. A protective

effect against NEC was proposed in recent studies applying a wider NEC-definition, including studies with a majority of medical NEC-cases [133, 144-146]. Cotten et al. first reported an association between prolonged empirical antibiotics and NEC [4], and this has been subsequently reported by many others [133, 139, 140, 142]. However, a large paper from the US Neonatal Research Network revealed no association between prolonged antibiotics and NEC/death [143]. In contrast, a Canadian paper showed that prolonged early antibiotic exposure in VLBW-infants was associated with increased odds of the composite outcome mortality or any severe morbidity (severe neurologic injury, ROP, NEC, BPD, or hospital-acquired infection) [10].

We also found that antibiotic courses ≥ 5 days in uninfected VPNs was strongly associated with increased odds of severe NEC. Our data supports a policy of cautious use of empirical antibiotics in the first week of life [27, 108].

We found a more than two-fold increase in aOR of severe BPD in infants exposed to antibiotics during the first week of life, and a dose-effect with increasing days of exposure (**paper 2**). In our statistical model, we adjusted for number of MV days in the first week of life as an important measure of early respiratory disease severity [13], in addition to the other confounders. The association between early antibiotics and BPD has been explored in other studies [8, 10, 13, 139, 142]. One paper found no association between early antibiotics and BPD, after adjusting for early respiratory support [13]. This was in contrast to three other large North-American studies reporting associations between duration of antibiotics and increased risk of and/or severity of BPD [8, 10, 142], and a French population-based paper among 648 low-risk ELGANs reporting a 2.3 increased aOR of moderate to severe BPD after early antibiotic exposure [139]. Respiratory tract dysbiosis has been linked to onset, progression and severity of BPD [224-227]. A few studies on biological data may suggest a link between antibiotic exposure and BPD risk, via the gut-lung axis [228]. It has been speculated that gut dysbiosis may trigger inflammation, and hence influence BPD development [229, 230]. The role of the intestinal microbiota in BPD is far from fully evaluated [226].

Diagnostic assessments and antibiotic stewardship

In **paper 3** we describe how we designed and implemented SPEs to guide decision-making on initiation of antibiotics in term neonates at risk of EOS. We developed a new management strategy were the key elements were hourly, structured SPEs of vital signs (Table 1) for at least 24-48 hours and performed by nurses in the NICU. Well-appearing at-risk neonates who had not developed clinical signs compatible with EOS by 24 hours of life or 24 hours after onset of subtle symptoms were discharged to the maternity ward or home. Our baseline level of antibiotic exposed neonates at SUH was 2.9%, higher than the average 2.3% level in Norway in 2009-2011 [16]. The new strategy more than halved the antibiotic exposure in the first three days of life to 1.3% in the post-implementation period, a significant lower level than at the baseline period.

Berardi et al. found 1.6% antibiotic exposure after implementing SPE in an Italian neonatal unit [130], and Joshi et al. reduced antibiotic exposure in 277 chorioamnionitis exposed neonates > 34 weeks from 100% to 11.6% [177] by using SPE. Finally, Cantoni et al. managed 7628 term neonates with a new strategy including SPE, and reduced antibiotics exposure from 1.2% to 0.5% [176]. However, we included only term neonates in our study, as opposed to both Berardi et al. and Joshi et al. who included neonates from GA 34 and 35 weeks, respectively [130, 177]. On the other hand, despite that the incidence of EOS are higher in near-term neonates (> 34-35 weeks) than in term neonates, the actually number of term neonates clearly represent the majority of EOS cases.

Different versions of the categorical risk-based approach have been recommended for risk assessment for neonates since around 2000 [66]. In Norway the recommended standard of care was an approach combining risk factors and clinical conditions at birth, although there were no national guidelines and the practice varied between sites Most started empirical antibiotics in neonates with symptoms at birth, some started antibiotics in neonates with chorioamnionitis, with or without symptoms, in preterm neonates with additional risk factors such as PROM/GBS-colonised mothers, GBS-infected mothers and finally, in all preterm neonates < 28 weeks. In 2010, CDC

guidelines was revised and a new algorithm was presented taking in to account that that IPA-exposed neonates were at lower risk than non-exposed neonates were. Moreover, and important for our study (**paper 3**), these 2010 guidelines recommended a 48-hours assessment of non-exposed neonates without clinical symptoms. This reflected, "a new wind was blowing". Still, the recommendation was to measure inflammatory biomarkers in blood and to obtain a blood culture in non-IPA exposed neonates GA < 37 weeks or PROM > 18 hours. In this setting, we designed and developed a tool to identify neonates with EOS in selected neonates at risk for EOS (symptomatic or chorioamnionitis-exposed), to reduce unnecessary use of antibiotics in neonates. Later, in 2018 AAP revised and replaced the 2010 CDC guidelines [66], recommending three different approaches [129]. The clinical risk assessment with SPEs was based on publications by Cantoni et al., Berardi et al. and Joshi et al. published in 2013, 2015/2016 and 2018 respectively [130, 176, 177, 221, 222]. Our project was designed and implemented before the 2018-AAP guidelines [129] and the reports of Berardi et al. [130] and Joshi et al. [177], but our study follows a similar pathway. We used and still use a different name of the repeated observations, "timesobservasjoner" hourly observation, but changed our English name to SPE to support this approach. We tested an assessment approach where diagnostic blood test and prompt treatment were replaced by repeated clinical examination in selected neonates, while ensuring timely start of antibiotics in infected neonates. The fact that the AAP 2018 recommendation is based on few studies indicates that our finding is an important contribution to this recommendation [129, 130, 176]. SPEs are performed by slightly different approaches. Cantoni et al. managed at-risk neonates with clinical examinations without laboratory test, the following hours after birth; 1, 2, 4, 8, 12, 16, 20, 24, 30, 36, 42 and 48 [176]. Berardi et al. monitored at-risk neonates, including neonates who received inadequate or no IPA, with vital signs the following hours after birth; 3, 6, 12, 18, 36 and 48 [130]. Joshi et al. performed SPEs with vital monitoring on well-appearing at-risk neonates ≥ 34 weeks every 30 minutes the first two hours, then every four hour for 24 hours [177]. However, all studies acknowledge an important characteristic of EOS that this is a dynamic condition developing within the

first hours to days in a neonate's life. At Lucile Packard Children's Hospital, Stanford US they have now safely managed more than 20 000 neonates with SPE [231]. A recent French study implemented initial clinical monitoring and limited blood tests in 1152 neonates GA > 36 weeks, and subsequently managed to reduce initiation of antibiotics from 1.4 to 0.8%. A recent study from Spain compared 417 neonates GA ≥ 35 weeks assessed with SPE and limited laboratory evaluation to 381 neonates managed by standard care, and they impressively report that antibiotic exposure was reduced from 6.1% to 0.7%, without any delay in diagnosis [232]. McDaniel et al. published in "Top articles in Pediatric Hospital Medicine July 2019 to June 2020" that SPE could safely reduce antibiotics in neonates at risk of EOS [233].

The 2018-AAP recommendation still recommend assessment of risk factors as one of three approaches when deciding initiation of antibiotics in term neonates, and the latest NICE guidelines divide risk factors into "red-flag" categories. Both recommend risk-based approach when deciding initiation of antibiotics in preterm neonates [66, 125]. Chorioamnionitis is considered a "risk factor" for EOS, and Joshi et al. showed that chorioamnionitis is a major driver of antibiotics [177]. Among 277 well-appearing chorioamnionitis-exposed neonates ≥ 35 weeks, antibiotic exposure declined from 100% to 11.6% by changing from a risk-based approach to a clinical assessment approach. None of the 277 neonates had EOS [177]. Already in 2012, Flidel-Rimon et al. evaluated the positive predictive value of risk factors for EOS. The research group found that prematurity was the only risk factor with increased OR and significant absolute incidence of EOS among 22 215 neonates of all GA [234]. This implicates that chorioamnionitis should not be assessed as a risk factor for EOS [234].

Algorithm based solely on risk factors may expose too many neonates to antibiotics, and fail to identify neonates in-need of antibiotics [130]. In a large population-based study, 531 asymptomatic neonates with risk factors needed to be exposed to antibiotics for each neonates who subsequently developed EOS [235]. A multi-centre study from Wales reported that 16% of all neonates > 34 weeks received antibiotics when the 2014 NICE guidelines were followed [236]. A research group in the Netherlands evaluated the Dutch guidelines, adapted from NICE guidelines, and found that one-

third (31.5%) of neonates started on antibiotics received longer courses than three days [237]. Furthermore, they found that there was low adherence to the guidelines meaning that neonates did not receive antibiotics as recommended and called for revised guidelines [237]. Most preterm births are in settings were "risk-factors" are present. The large numbers of studies reporting adverse outcome after early exposure and prolonged courses of antibiotic in uninfected preterm neonates - advocate that identification of preterm neonates in need of antibiotics potentially should be done differently than stratification by risk-factors [14, 66].

A feasible and safe approach to reduce empirical antibiotics in neonates, may be the use of the integrated sepsis calculator integrating risk factors and clinical presentation the first 4 hours of life [129, 238, 239], as recommended by AAP and NICE guidelines. A meta-analysis of 13 studies including 175 752 neonates, showed that the sepsis calculator could almost halve antibiotic use compared with a categorical risk-based approach [238]. In a single-centre study of 2076 neonates ≥ 35 weeks, the proportion of neonates exposed to antibiotics for suspected EOS was reduced from 4.8% to 2.7% after the calculator was implemented [240]. Goel et al. compared the sepsis calculator with the NICE guidelines, and found that the sepsis calculator could potentially reduce empirical antibiotics in three out of four neonates [236]. On the other hand, Hershkovich-Shporen et al. retrospectively compared the number of EOS cases that would have been predicted by the sepsis calculator and by the CDC guidelines [241]. They found that CDC would have identified more asymptomatic neonates with EOS than the calculator. By using the sepsis calculator Kuznewiez et al. managed to reduce antibiotics exposure from 5% to 2.6% the first 24 hours of life in a study including more than 200 000 neonates ≥ 35 weeks [242]. On the other hand, clinical symptoms the first four hours after birth has low positive predictive value, as this may mimic other neonatal condition, potentially leading to overuse of antibiotics. EOS is also a dynamic condition. Some neonates with EOS are well-appearing at birth and develop clinical signs after the first 4-6 hours of life. When evaluating the EOS calculator, there were six cases of neonates with EOS that had been well-appearing at birth and with low EOS calculator risk scores, including five neonates presenting with

symptoms ≥ 5 hours of life among 56 261 neonates [243]. Interestingly, in Hershkovich-Shporen's study, 19 of 50 neonates with EOS (38%) would not have been identified with neither the calculator nor the CDC guidelines [241]. These neonates did not have any risk factors nor any symptoms four hours after birth, and most of them (12/19) had GBS EOS [241]. Almost similar finding to this research group, Goel et al. found that three out of seven EOS cases (43%) were not identified by neither the calculator nor the NICE guidelines because they were asymptomatic at birth and without any risk factors [236]. Finally, Snoek et al. managed to compare both the NICE and Dutch guidelines with the calculator, and found that the sensitivity of the calculator was lower than both the Dutch and the NICE guidelines [244]. Consequently, the calculator results in less antibiotics in uninfected neonates at the cost of treatment delay in initially asymptomatic EOS neonates, or in other words, the calculator "undertreat" while the categorical risk-based approach "overtreat" [244].

In clinical practice, this would mean to identify neonates with EOS despite a low estimated risk and initially reassuring clinical condition. In a study were the three approaches recommended by the AAP were retrospectively compared, Beck et al. found that among 7396 neonates, including 5.4% with inadequate IPA, none of the neonates developed EOS [245]. On the other hand, Escobar calculated that these cases occur at rates of approximately 1 in 10 000 LB among term and moderately preterm neonates [24]. In Norway, this means around six neonates with EOS would annually be missed if evaluated only by the calculator.

Despite a low positive predictive value of CRP and CBC, these inflammatory markers are commonly used in neonates at risk of EOS for decision making in initiation of antibiotics. Duvosin et al. found that clinical examinations (at 8 and 12 hours of life) and a limited use of inflammatory markers in neonates with risk-factors, reduced the delay in antibiotic initiation in infected neonates [246]. Overuse of diagnostic tests are linked to over-treatment of antibiotics, and both Cantoni et al. and Berardi et al. limited the use of inflammatory blood samples and relied on repeated clinical examination to identify neonates in need of antibiotics [130, 176]. These important

findings are supported in **paper 3**, where the number of CRPs taken on asymptomatic neonates was reduced, and the delay in initiation of antibiotics in infected neonates was halved. The importance of these results is underscored by the fact that one hour delay in initiation of antibiotics in a septic adult, is associated with increased risk of mortality and morbidity [247, 248], although this association is never shown in neonates or children. On the other hand, serial CRP and PCT measurements can be used for decision-making and support to reduce prolonged duration of antibiotics in neonates already started on antibiotics [164, 249].

Safety is a mandatory aspect in all antibiotic stewardships. Mortality and morbidity is a challenging measure of safety because the low incidence of case-related mortality/morbidity in EOS. Consequently, the safety measure "time between birth and start of antibiotics in infants with culture-positive or culture-negative EOS" was chosen a predictor potentially important for patients' outcomes (**paper 3**). Berardi et al. used "complication or a worse outcome" [191] and Kuzniewiz used readmission for EOS and adverse clinical outcome including need for inotropes, mechanical ventilation, meningitis and death [243] as safety measures. However, even though we did not identify safety concerns with our approach (**paper 3**), much larger and preferably population-based studies with a sufficient power to assess direct safety measure such as also the presence of meningitis, or sepsis-related mortality in addition to studies on long-term outcomes are needed for both the sepsis calculator and for SPE [244, 245].

The Norwegian government's national aim was to reduce all use of antibiotics with 30%, and the use of broad-spectrum antibiotics within hospitals with 30% from 2015 to 2022 [172], but there has not been any specific focus on reducing initiation of antibiotic. From 2012, in our unit, we have had increased focus on ending empirical antibiotics after 36-48 hours in neonate without any signs of sepsis, and reducing prolonged antibiotic courses. The Norwegian Paediatric association has recommended a uniform definition of clinical sepsis in order to reduce overuse of diagnose and consequently prolonged antibiotics courses. Dretvik et al. in a QI study from 2016-

2017 showed that overall duration of antibiotic therapy in neonates without infection was around 48 hours [109]. This was markedly lower than the median of 4 days exposure in uninfected term neonates in a Norwegian study from 2009-2011 [16, 109].

Mundal et al. reported that despite a decrease in antibiotic exposure among term neonates in Norway, a significant higher decrease is seen in NICUs with ongoing QI projects [182]. This emphasise the importance of ongoing antibiotic stewardships. The lowest proportion of exposed neonates across Norway was within the West health trust, where our QI project was ongoing.

5.3 Clinical and diagnostic implications for patient care

This thesis addresses optimal antibiotic use in neonates. To increase our knowledge, we sought a deeper epidemiological understanding of the burden of disease and antibiotics, evaluated associations between antibiotics and adverse outcomes to discharge, and finally, we developed and implemented a diagnostic tool for reduction of antibiotic exposure. Our finding may have important clinical and diagnostic implication for improved clinical care.

5.3.1 Epidemiological understanding of the burden of disease and antibiotics

Local surveillance data is important as all antibiotics in neonates are started as empirical antibiotics. A deep epidemiological knowledge of pathogens and their susceptibility patterns is consequently essential [20]. Empirical antibiotics should be tailored towards local epidemiology to reduce risk of treatment failure in infected neonates.

NICE guidelines and WHO recommendations suggest benzyl penicillin combined with gentamicin as the recommend empirical antibiotics regime in neonates at risk of EOS in all GA, unless local surveillance data of pathogens indicates a resistance-pattern that needs more broad-spectrum antibiotics [20, 125].

Our data has clinical implication for neonates of all GA. In our setting, benzyl penicillin combined with gentamicin is an excellent coverage for relevant pathogens across all GA. There was a stable incidence of GBS and *E. coli* EOS during the last 23-years, contrary to some reports showing increases incidence of *E. coli* EOS.

Results from **paper 1** is important in the fact that there is no indication of increased antibiotic resistance during the last 23 years. Norway has traditionally been an antibiotic restrictive country, and this finding correlated well with our expectations. Neonates are at higher risk for prolonged carriage of multi-resistant bacteria when compared to adults, and they represent a reservoir for intra-household spread [112]. This highlights the public health benefit of limiting antibiotic resistance in this population.

Broad-spectrum antibiotics may drive antibiotic resistance, and a restrictive antibiotic policy may ensure that we still have efficient antibiotics in the future. However, we acknowledge that benzyl penicillin and gentamicin may not be the best cover in other settings with higher antibiotics resistance rates [250].

The duration of treatment in EOS-cases was observed to decline from 14 days to 8 days during the first and the last period in paper 1. Long courses are shown to drive antibiotic-resistance in Gram-negative intestinal pathogens and may have direct importance for patient care [113].

5.3.2 Antibiotic exposure and associated effects

Increased knowledge of association between short-term effects of antibiotics in uninfected VPNs is important, as a major proportion of the preterm neonatal population is exposed to empirical antibiotics during the first week of life [4, 11, 12, 14]. Observational studies reporting associated adverse outcomes in uninfected neonates exposed to early antibiotics are mainly performed in multi-centre or network studies from Canada and the US [4, 8-10]. This is important, as the total antibiotic load a neonate is exposed to, also prior to the birth, may be important. In the US, 30-40% of all women in labour are exposed to antibiotics, and some studies even report as high

numbers as 50-69% of women exposed to IPA [44, 46]. Population-based studies from countries were risk-based IPA is performed, could add important knowledge as different types of IPA-programs could potentially bias the results. Our study contributes with important knowledge in this topic. A few multi-centre studies have claimed that short courses of early antibiotics the first three days of life, have a protective effect for NEC [144, 145]. **Paper 2** does not support this finding. Nor the finding that some other studies have reported, that early antibiotics does not have any effect on adverse outcome to discharge/death [13, 143].

In **paper 2** we showed that early antibiotic exposure and prolonged courses in uninfected VPN are associated with BPD, NEC and death compared to neonates not exposed to antibiotics. Possible harmfulness of early antibiotics has major clinical implications for this vulnerable patient group, as unrestricted use of antibiotics in this population potentially adds an extra burden to being premature [251].

The many publication reporting on association between early antibiotics and adverse outcome, including **paper 2**, in addition to the low incidence of EOS have made a situation where a RCT can potentially evaluate on causality [2]. The NANO study randomizes preterm neonates to early empirical antibiotics or no antibiotics, a design that long was not seen feasible, but unethical [252]. However, a better understanding of how to stratify risk for EOS, both in term and preterm neonates have developed the last decades [159]. The results from the NANO study, however, will earliest be expected in 2024.

Findings from **paper 2** may inform on empirical antibiotic guidelines and recommendations for early antibiotics in preterm neonates. A reluctant policy to initiate early empirical antibiotics in low-risk preterm neonates could possible improve the short-term outcome of this vulnerable group of patients. The risk of EOS in a preterm neonate born after Caesarean-section without PROM, is potentially lower than the risk of being exposed to antibiotics, and consequently the threshold for starting antibiotics should be high [66]. Prolonged courses of antibiotics should be limited to infected neonates with positive blood cultures.

5.3.3 Diagnostic assessments and Antibiotics stewardships.

The unique antibiotic paradox is to limit antibiotic exposure in uninfected neonates and enable early detection and access to effective antibiotics in infected neonates [114, 174]. Although antibiotics are administered to protect neonates, multiple recent studies suggest that antibiotics have potential risks in preterm neonates and possible also in term neonates, as well as benefits in infected neonates [14]. “No antibiotic treatment” is not the same as “no care”, and the balance between overuse of antibiotics and under treatment of EOS, is essential.

A better understanding on how to stratify risk for EOS, both in term and preterm neonates have developed over the last decades [159], but clinicians still need improved diagnostic tools to help in decision-making whether to initiate antibiotic or not. We implemented our antibiotic stewardship program with SPE (**paper 3**) for improved identifications of neonates with EOS and for reduction of initiation of antibiotics. After introduction of the SPE, we have learned that SPE has direct impact on patients’ safety and improved clinical care. A strategy for better identification of EOS may reduce treatment-delay and hence improve patient safety. No strategy of risk assessment for EOS, neither the categorical risk assessment, the sepsis calculator nor SPE, can immediately identify all neonates who will develop EOS within the first days of life [66]. Both the multivariate risk assessment using the EOS calculator and the primarily clinical risk-based assessment using SPEs have a documented potential to reduce empirical antibiotics in term or moderately preterm (> GA 34) neonates during the first days of life [238]. One important finding in our study was that there were no increase in the overall proportion of term neonates who were admitted to the NICU after implementing the new management strategy, as antibiotic stewardships often require changes in workflow in most obstetric and/or neonatal units. Implementing only SPEs may be an alternative in units where the EOS calculator is difficult to implement.

Exposure to IPA is of important knowledge to the clinicians, as Berardi et al. found that neonates exposed to IPA, had a lower median time of start of symptoms (one

versus six hours after birth) compared to neonates unexposed to IPA [235]. This can potentially be implemented when further developing SPEs.

SPE may contribute to a reduction in emergence of antibiotic resistance and adverse outcomes in neonates. Furthermore, SPE may reduce separation between mother and neonate, parental anxiety and health expenditures, as a reduced number of neonates need to be treated with antibiotics.

There are no studies on preterm neonates < 34- 35 GA managed by SPE. Stratification into low-risk and high-risk groups by using delivery characteristics, is one approach recommended [66, 125]. In low-risk neonates, empiric therapies may not be required [253].

Despite the main focus of our stewardship was reduction of initiation of antibiotics by SPE, we also experienced a reduction in duration of antibiotics days. One effect of QI projects is standardisation by adherence to guidelines, and this result is most probably caused by standardisation of adherence to guidelines among staff.

6.0 Conclusions

Based on the results from the studies and considering the methodological limitations, the specific aims of the theses can be answered as follows:

Burden of EOS and antibiotic exposure

- ✓ In an unselected population including all neonates at SUH, the overall incidence of EOS was 0.97/1000 LB, and the incidence of GBS and *E. coli* were 0.57/1000, and 0.11/1000, respectively
- ✓ GBS was the most common pathogens causing EOS in neonates GA ≥ 28 weeks, and *E. coli* was the prominent pathogens in extremely preterm neonates GA < 28 weeks.
- ✓ There was no significant difference in the overall incidence of EOS between the two periods 1996-2006 and 2007-2018 (1.05/1000 versus 0.90/1000).
- ✓ The percentage of antibiotic-resistant pathogens causing EOS was low (2/101; 2%).

Antibiotic exposure and adverse outcome

- ✓ In an unselected nationwide population including uninfected neonates GA < 32 weeks, there was a strong association between early antibiotics in the first week of life and NEC, BPD and death after the first week of life, regardless of duration of antibiotics.
- ✓ Prolonged antibiotic courses for more than four days was associated with increased risk of severe NEC after the first week of life.
- ✓ Most VPNs neonates were exposed to antibiotics during the first three (75%) or seven (77%) days of life, despite a negative blood culture.
  There was a reduction in the proportion of VPNs exposed to prolonged antibiotic courses (five days or longer) in the absence of sepsis from 55% to 24% during the study period

Diagnostic assessments and antibiotics stewardship

- ✓ In an unselected population including neonates ≥ GA 37 weeks at SUH, we developed and implemented a tool for identification of neonates at risk of EOS by using SPE.
- ✓ With this initiative, we observed a significant reduction in proportion of term neonates exposed to early antibiotics, from 2.6% to 1.3%
- ✓ The time from birth to administration of antibiotics in infected neonates (culture-positive EOS and culture-negative sepsis) was reduced from 14 to seven hours after the intervention.
- ✓ The number of neonates diagnosed with culture-negative sepsis was reduced from 11.8 to 6.7/1000 LB, and the average number of CRP blood samples per month taken in the NICU were reduced from 332 to 223. Days of antibiotic exposure declined from 320 to 129/ 1000 patientdays.
- ✓ There were no infection-attributable deaths or readmissions within 14 days of life, and the incidence of EOS after implementing SPE remained the same as before the intervention.

7.0 Future perspectives

Burden of EOS and antibiotic exposure

Novel efforts for prevention of EOS, including strategies to decrease incidence of chorioamnionitis and preterm birth, are needed [46] as the incidence of EOS among preterm neonates are up to 30-fold higher than among term neonates.

There is a continuously need to monitor pathogens and their resistance pattern to optimize and ensure efficient antibiotic us. Large population-based studies including resistance pattern and trends over time on *E. coli* isolates causing EOS in preterm neonates, are needed. There is still controversy regarding the incidence of *E. coli* in the era of IPA. Although we did not find any change in incidence of *E. coli* in our study, a larger national study would be able to answer this question.

Vaccination against GBS show potentially optimistic results although this has been technically demanding [254]. A GBS vaccine can potentially change both the incidence of GBS EOS and overall neonatal EOS, and consequently type of empirical antibiotic required.

Antibiotic exposure and adverse outcome

Large population-based studies to evaluate association between early antibiotics and adverse outcome are still needed. The last years' reduction of antibiotic exposure enables more studies on preterm neonates not exposed to antibiotics, and hence studies including other design and statistics such as propensity score matching can be performed [147].

Diagnostic assessments and antibiotics stewardship

Low-cost antibiotic stewardships can be performed in single or multicentre NICUs. The majority of blood cultures in EOS are positive within 24 hours. Stewardship programs can be targeted to aim on specific goals, such as to stop antibiotics after 24 hours in negative cultures of adequate volumes. A restrictive antibiotic policy is important to optimise future neonatal care. Future guidelines and recommendation

promoting a more restricted antibiotics policy is important as well as effective antibiotic stewardships to reduce exposure and duration of antibiotics.

We need studies on novel tools for integration of digital clinical observation, such as use of infrared thermography and computer learning for improved diagnostic assessments in neonates at risk.

Rapid molecular detection of pathogens and identification antibiotic resistance has shown potential benefits in adults, but more studies are needed also in newborns [255].

Despite our SPE antibiotic stewardship was efficient and safe, large multi-centre studies and possible international studies, are needed to ensure enough neonates with culture-positive EOS for safety measures.

8.0 Source of data

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Early-Onset Sepsis in Neonates - A Population-Based Study in South-West Norway From 1996 to 2018

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Background: The epidemiology of early-onset sepsis (EOS) may change over time. Longitudinal surveillance of causative pathogens, antibiotic susceptibility patterns and antibiotic therapy is important for optimal therapy strategies.

Objectives: To describe the incidence of culture-confirmed EOS, causative pathogens, antibiotic susceptibility patterns and antibiotic therapy over a 23-year period.

Methods: Retrospective population-based study from a single-center neonatal intensive care unit at Stavanger University Hospital, Norway, covering a population in South-West Norway, during the 23-year period 1996–2018.

Results: Of 104,377 live born infants, 101 infants (0.97/1,000) had culture-confirmed EOS; 89 with Gram positive and 12 with Gram-negative bacteria. The EOS-attributable mortality was 6/101 (5.8%). For the three most prevalent pathogens the incidences were; Group B streptococcus (GBS) 0.57/1,000, *Escherichia coli* 0.11/1,000 and viridans group streptococci (VGS) 0.10/1,000. GBS was the most common pathogen (59/93; 63%) in infants with gestational age (GA) $\geq$ 28 weeks. In contrast, among extremely preterm infants (GA <28 weeks) the incidence of *E. coli* infection was higher than for GBS infection. The second most common bacterial pathogens causing EOS among term infants were VGS. There was no change in the incidence of EOS for the entire study period, but from 2000 to 2018 there was a mean decline in EOS by 6% per year (95% CI 1%−10%) ($p = 0.019$). The incidences of GBS and *E. coli* did not change during the study period. The initial empirical antibiotic regimen for EOS was in all cases a combination of benzylpenicillin or ampicillin and an aminoglycoside, but in 21/101 (21%) of cases a broad-spectrum antibiotic was either added or substituted this regimen. In 2/101 (2%) EOS cases, the pathogens were nonsusceptible to the empirical antibiotic regimen. All *E. coli* isolates were susceptible to aminoglycosides.

Conclusion: GBS was the most common causative pathogens in EOS, but *E. coli* dominated in infants with GA <28 weeks. There was no change in the incidence of EOS during the entire study period. The current empiric regimen with benzylpenicillin and gentamicin provides a very high coverage for EOS in our setting.

Keywords: infection, early-onset sepsis, neonatal sepsis, antibiotic therapy, antibiotic resistance, antibiotic susceptibility

INTRODUCTION

Early-onset sepsis (EOS) remains a major contributor to neonatal morbidity and mortality (1). Although most EOS cases occur in term infants, incidence and infection-attributable mortality is higher in preterm infants, inversely related to gestational age (GA) (2). In many countries and regions the incidence of EOS has decreased in the past decades, in particular after implementing effective intrapartum antibiotic prophylaxis (1, 3–6). Among term (GA ≥ 37 weeks) and moderately preterm infants (GA 28–36 weeks) with EOS, group B streptococci (GBS) are the dominant pathogens identified in blood cultures (5–7). In contrast, among extremely preterm infants (GA < 28 weeks), *Escherichia coli* is often the most frequently detected pathogen (5). Patterns of other bacterial pathogens causing EOS are less well-described.

In infants with suspected EOS, empirical antibiotic therapy is commenced before blood culture results are available. Longitudinal surveillance for identification of changes in causative pathogens, clinical outcomes including mortality, and antibiotic susceptibility is important when tailoring optimal prevention and empiric therapy strategies (5, 8, 9). However, data on antibiotic susceptibility is often not reported in epidemiological studies. There is growing concern about increasing antibiotic nonsusceptibility among pathogens causing EOS, especially in Gram-negative pathogens where often few therapeutic options are available (10). Increasing nonsusceptibility rates, could potentially threaten the effectiveness of standard empiric regimens (5, 10–12). In the action plan to combat antibiotic resistance, the World Health Organization calls for increased knowledge on local epidemiology and antibiotic susceptibility patterns (9).

In this population-based study including more than 100,000 live born (LB) infants, we aimed to describe the incidence of culture-confirmed EOS, causative pathogens, antibiotic susceptibility patterns and antibiotic therapy over 23 years in South-West Norway.

MATERIALS AND METHODS

Setting

Stavanger University Hospital in South-West Norway is the only hospital for a well-defined population of around 370,000 inhabitants, offering primary, secondary and tertiary obstetric and neonatal intensive care. There have been 4,000–5,000 annual deliveries during the last decades. All infants born in the catchment area and who receive intravenous antibiotic therapy for EOS have been admitted to the neonatal intensive care unit (NICU) in Stavanger.

Study Design, Participants, and Data Collection

This is a single-center, population-based retrospective study over a 23-year period from January 1996 to December 2018. The annual number of live births with a GA of ≥22 weeks during the study period were obtained from the maternity unit. Newborn infants with positive blood cultures obtained during the first 72 h of life were identified by the local microbiology laboratory blood culture registry, and causative pathogens and antibiotic susceptibility were registered. Detailed clinical information was extracted from the medical records for all infants with culture-confirmed EOS, including GA, birthweight (BW), symptoms and signs of EOS, infection-attributable mortality, maximum value of C-reactive protein (CRP), choice of antibiotics and duration of therapy. The diagnosis of clinical chorioamnionitis was extracted from the mother's medical record.

Definitions, Microbiology, and Antibiotic Therapy

EOS was defined as growth of pathogenic bacteria in a blood culture obtained ≤72 h of life, and treatment with antibiotics ≥5 or <5 days if death occurred (1). Culture-negative EOS is a controversial diagnosis (13), and we did not include cases coined as culture-negative sepsis also due to lack of a uniform consensus definition. EOS-attributable mortality was defined as death occurring within 7 days after growth of pathogenic bacteria in blood culture where sepsis was the assumed cause. The incidence of EOS was defined as cases of EOS per 1,000 LB infants. Clinical chorioamnionitis was prospectively diagnosed by the responsible obstetrician, and for this study the clinical diagnosis was obtained from the medical records. Cases later diagnosed as histological chorioamnionitis were not included. Infants were classified as symptomatic if they had signs of EOS, and the time from birth to onset of symptoms was registered.

Blood cultures were obtained prior to initiation of antibiotic therapy using BacT/ALERT PF Plus Aerobic Pediatric culture bottles (BioMérieux, Inc., Durham, NC) throughout the study period. Matrix-assisted laser desorption ionization - time-of-flight (MALDI-TOF) mass spectrometry was introduced in 2012, gradually replacing traditional phenotypic species identification. Micrococci, propionibacteria, corynebacteria, or diphtheroids grown alone in a single culture, growth of more than one bacteria, and all coagulase-negative staphylococci (CoNS) were considered contaminants (1). Pathogens were grouped into

Gram-positive and Gram-negative bacteria. Viridans group streptococci (VGS) include *Streptococcus mitis* and *Streptococcus alactolyticus* and "Other streptococci" in this report include *Streptococcus pyogenes* and *Streptococcus pneumoniae.* A blood culture pathogen was defined as susceptible to an antibiotic when the final interpretation report indicated S (susceptible) and nonsusceptible when the report indicated R (resistant) or I (intermediate). Antibiotic susceptibility testing followed the guidelines from the Norwegian working group for antibiotics (14), closely aligned with the EUCAST criteria (15).

The local empirical antibiotic regimen for EOS consisted of ampicillin in combination with an aminoglycoside (tobramycin or gentamicin) from 1996 to 2010, and benzylpenicillin and gentamicin from 2011 to 2018.

Ethical Considerations

The local institutional review board and data protection officer approved the study as a local quality improvement project that did not need approval by the regional ethics committee.

Statistical Analyses

Data were analyzed using IBM-SPSS version 24 statistical software (IBM, Armonk NY, USA). Results are expressed as mean with 95% confidence interval (CI) or median with interquartile range (IQR), as appropriate. Differences between groups and time periods (1996–2006 vs. 2007–2018) were analyzed with *t*-test or Mann-Whitney test as appropriate for continuous data, and the chi-square test or Fisher-exact test for categorical data. Regression models were used to test for trends over time (linear) where year was the continuous predictor. All tests were two-tailed. *P*-values of <0.05 were considered statistically significant.

RESULTS

Incidence and Causative Pathogens

During the 23-year study period, 104,377 infants were LB. Of these; 96,024 (92%) infants were born at term, and 8,353 (8%) infants were born preterm before 37 weeks gestation. Among the preterm infants, 7,890 (7.6%) had GA between 28–36 weeks and 463 (0.4%) GA <28 weeks. There were 101 infants with culture-confirmed EOS (**Figure 1**). The overall incidence of EOS, incidence by causative pathogens and incidence for different groups of GA are presented in **Table 1** and with subgroups in **Figure 2**. Most cases of EOS were among term infants; 71/101 (70%), but the incidence was higher among preterm infants (**Table 1**). Compared to term infants, the incidence of EOS in moderately preterm (GA 28–36 weeks) and extremely preterm (GA < 28 weeks) infants were 3.9 and 24-fold higher, respectively.

Among term and moderately preterm infants, GBS was a more frequent cause of EOS than *E. coli*. In extremely preterm infants however, there were four cases of *E. coli* infection and only one GBS case (**Table 1**). The incidence of Gram-negative pathogens decreased by increasing weeks of GA (OR 0.79, 95% CI 0.69–0.89, $p < 0.001$). VGS was the second largest group of pathogens with 10/101 cases (10%), predominantly occurring in term infants (9/10).

The yearly incidence of EOS for different pathogens are shown in **Figure 3**. There was no difference in the total incidence of EOS between the two periods 1996–2006 and 2007–2018 (1.05/1,000 vs. 0.90/1,000, $p = 0.49$). There was no change in the incidence during the study period for all infants with EOS or for EOS caused by different pathogens (data not shown). However, for the period from 2000 through 2018 analyzed separately, there was a mean decline in the incidence of EOS by 6% per year (95% CI 1–10%) ($p = 0.019$). There was no change in the incidence of EOS during the study period for any of the GA groups when analyzed separately (data not shown).

Clinical Characteristics and Mortality

In total, 14/101 infants with EOS had been exposed to chorioamnionitis; 10/71 (14%) term, no moderately preterm, and 4/8 (50%) extremely preterm infants. Among infants with EOS exposed to chorioamnionitis, all extremely preterm infants and 7/10 term infants developed symptoms of EOS within the first 6 h of life. Overall, 95/101 (94%) infants with EOS had onset of symptoms within the first 24 h of life. The median (IQR) time to start of EOS-symptoms was 3.0 h (1.0, 13.0). The proportion of infants with symptoms at birth was higher among preterm 18/30 (60%) compared to term infants 26/71 (37%), $p = 0.047$.

The mortality and maximum value of CRP for different groups of GA with EOS are shown in **Table 2**. Six infants with EOS died; five of these were born preterm. The median (IQR) GA in infants who died was 34 (26–36) weeks. The EOS- attributable mortality was higher in infants with GA <28 weeks compared to term infants ($p = 0.025$). The maximum CRP values were higher in infants with Gram-positive compared to Gram-negative EOS, $p = 0.003$.

Antibiotic Therapy and Susceptibility

The antibiotic regimen for EOS was in all cases a combination of benzylpenicillin or ampicillin and an aminoglycoside. In 21/101 (21%) of cases, a broad-spectrum antibiotic was either added or substituted later the empiric regimen. There was no change in the number of infants given broad-spectrum antibiotics between the periods 1996–2006 and 2007–2018.

The median (IQR) duration of antibiotic therapy for EOS declined from 14 (10–14) days in the period 1996–2006 compared to 8 (7–10) days in the period 2007–2018 ($p < 0.013$).

All GBS isolates were susceptible to benzylpenicillin, and all *E. coli* isolates were susceptible to both gentamicin and a third-generation cephalosporin. No Gram-negative isolates produced extended-spectrum beta-lactamase (ESBL). 10/11 (91%) of *E. coli* isolates were nonsusceptible to ampicillin (**Table 3**).

Among the nine *Staphylococcus aureus* EOS isolates, one isolates was nonsusceptible to both benzylpenicillin and aminoglycosides; the combination empiric regimen in use. Eight of nine isolates were susceptible to an aminoglycoside. One isolate was methicillin resistant (MRSA), but susceptible to an aminoglycoside. Six out of nine *S. aureus* EOS isolates were nonsusceptible to benzylpenicillin. All other Gram-positive EOS isolates in

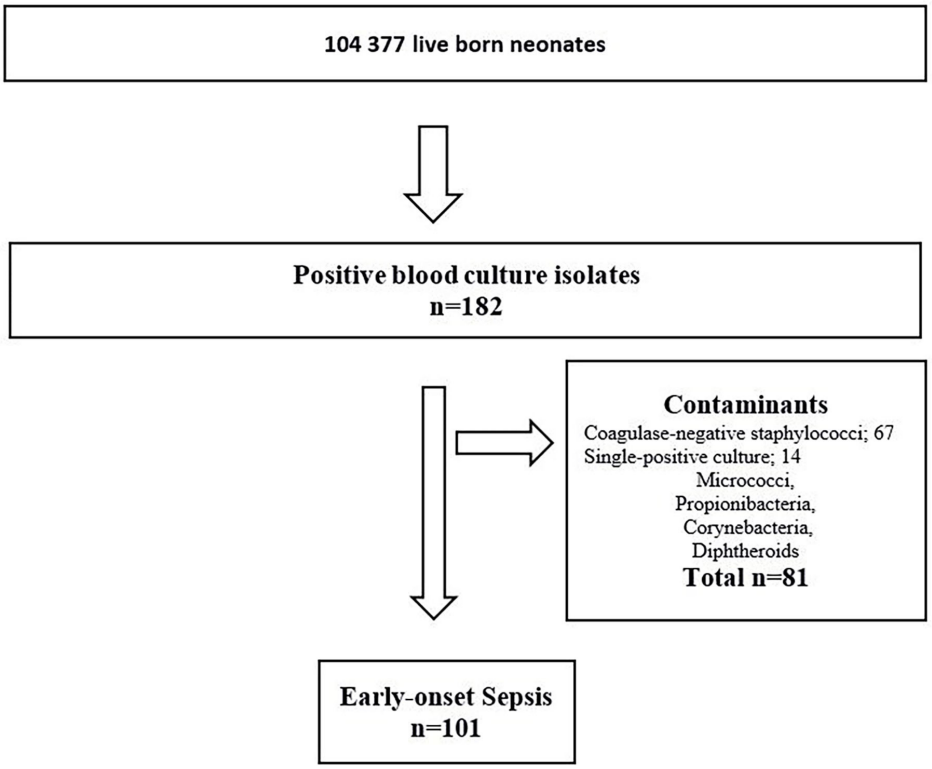

FIGURE 1 | Flowchart – Infants diagnosed with early-onset sepsis in a population-based observational study at Stavanger University Hospital, Norway, 1996–2018.

TABLE 1 | Incidence of early-onset sepsis (EOS) per 1000 live births among infants born at Stavanger University Hospital during 1996–2018.

	All EOS isolates	GBS	*Escherichia coli*	VGS
Infants	**Number infants incidence (95% CI)**	**Number infants incidence (95% CI**	**Number infants incidence (95% CI**	**Number infants incidence (95% CI**
All	*N* = 101	*N* = 60	*N* = 11	*N* = 10
N = 104,377	0.97 (0.71, 1.23)	0.57 (0.39, 0.75)	0.11 (0.03, 0.18)	0.10 (0.06, 0.19)
GA ≥ 37 weeks	*N* = 71	*N* = 45	*N* = 3	*N* = 9
N = 96,024	0.74 (0.52, 0.96)	0.47 (0.31, 0.63)	0.03 (0.02, 0.09)	0.084 (0.014, 0.18)
GA 28–36 weeks	*N* = 22	*N* = 14	*N* = 4	*N* = 1
N = 7,890	2.8 (1.43, 4.28)	1.8 (0.66, 3.0)	0.51 (0.01, 0.92)	0.13
GA < 28 weeks	*N* = 8	*N* = 1	*N* = 4	*N* = 0
N = 463	17.8 (6.2, 29.4)	2.2	8.2 (0.13, 16.3)	

Incidence; case per 1,000 live births.
GA, Gestational age, median (IQR); GBS, group B streptococci; VGS, viridans group streptococci.

this study were uniformly susceptible to benzylpenicillin. The overall non-susceptibility rate to the current empirical regimen benzylpenicillin and gentamicin was 2/101 (2%) (**Table 3**).

DISCUSSION

This study reports the epidemiology of EOS and antibiotic susceptibility over more than two decades in a well-defined

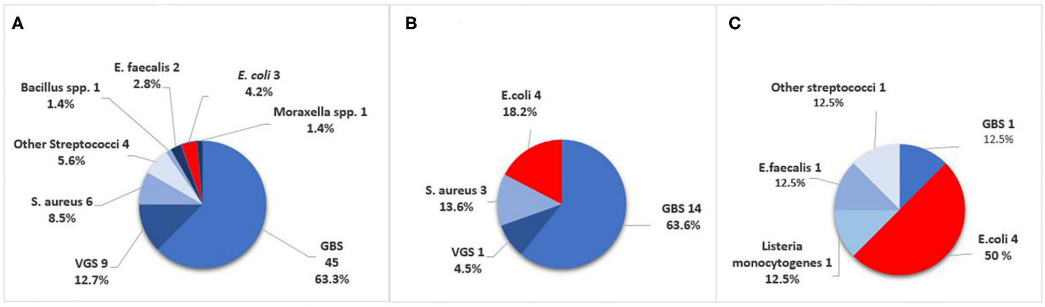

FIGURE 2 | Causative pathogens in culture-confirmed early-onset sepsis (EOS) in infants born at Stavanger University Hospital, 1996–2018. **(A)** GA ≥ 37 weeks. **(B)** GA 28–36 weeks. **(C)** GA < 28 weeks. GBS, Group B Streptococci; VGS, viridans group streptococci.

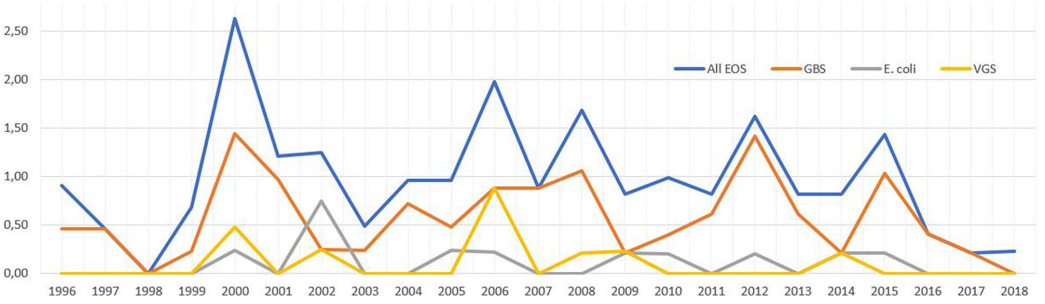

FIGURE 3 | Annual incidence of early-onset sepsis (EOS) per 1,000 live born caused by all pathogens, Group B streptococci (GBS), *Escherichia coli* and viridans group streptococci (VGS) during 1996-2018. GBS, Group B Streptococci; VGS, viridans group streptococci.

population in South-West Norway. Key findings are that GBS is the most common causative pathogen in EOS, but *E. coli* dominates in infants with GA <28 weeks. The incidence of EOS remained stable for the entire study period, but with a possible decline in incidence for the period 2000–2018 analyzed separately. Finally, we demonstrated a low level of antibiotic nonsusceptibility, and the current recommended empirical antibiotic regimen (benzylpenicillin and gentamicin) continues to have an excellent coverage for EOS in our area.

The overall incidence of EOS was 0.97/1000 LB, comparable to reports from other epidemiological studies from Sweden (0.9) (3), United Kingdom (0.7) (16) and the United States (0.77–1.08) (1, 5, 17). We found a 24-fold higher incidence of EOS in extremely preterm infants compared to term infants, a slightly lower ratio than the 30-fold higher EOS rate reported by Stoll et al. (5). The high rate of EOS cases in extremely preterm infants is a reminder of the vulnerability of preterm infants, emphasizing the importance of clinicians' high vigilance in care of these infants.

VGS, along with *E. coli*, was interestingly the second largest group of pathogens that caused EOS, predominantly in term infants. All infants with VGS-EOS had clinical symptoms suggestive of sepsis, CRP levels above 10 mg/L and received ≥5 days of antibiotics, indicating a clinically relevant sepsis episode. In many other studies, VGS are excluded as "unspecific" (3), complicating the comparisons between studies. Stoll et al. reported that 7/235 (3.0%) infants with EOS had growth of VGS (5), whereas 10/862 (1.2%) infants in the 1989–2003 data from Yale-New Haven had VGS sepsis (8). In the Yale-New Haven data, commensal organisms such as VGS increased during 1979–1988, possible because the simultaneously rise in preterm patient population with longer duration of NICU hospitalization and central vascular catheters. In our study though, VGS were almost exclusively found among term infants. VGS have also been reported to be the cause of EOS among infants exposed to chorioamnionitis and being asymptomatic within 6 h of age (18).

We found no change in the total incidence of EOS for the entire 23-year study period, nor in the incidence of Gram-positive or Gram-negative EOS. Data from Yale-New Haven Hospital showed a decrease in EOS from 1979 to 2004, and from 2004 to 2013 a stable incidence of around 0.9/1,000 (6). In contrast, a recent population-based study from Sweden showed a significant reduction in EOS incidence over the last two decades (3). However, both studies had a baseline incidence that were

TABLE 2 | Early-onset sepsis (EOS) attributable mortality and maximum C-reactive protein (CRP) levels among infants of different gestational age (GA) born at Stavanger University Hospital, 1996–2018.

	All cases EOS		Gram-positive EOS		Gram-negative EOS	
	Deaths/Cases (%)	CRP* (mg/L)	Deaths/Cases (%)	CRP* (mg/L)	Deaths/Cases (%)	CRP* (mg/L)
All infants	6/101 (5.8%)	65 (39–110)	3/89 (3.3%)	67 (44–120)	3/12 (25%)	30 (11–62)
GA ≥ 37 weeks	1/71 (1.4%)	65 (43–122)	1/67 (1.5%)	67 (47–126)	0/4 (0%)	22 (5–39)
GA 28–36 weeks	3/22 (13%)	71 (22–99)	2/18 (11%)	65 (10–96)	1/4 (25%)	55 (17–114)
GA < 28 weeks	2/8 (25%)	45 (13–112)	0/4 (0)	92 (20–167)	2/4 (50%)	30 (10–60)

IQR, interquartile range; EOS, Early-onset sepsis; GA, gestational age.
**C-reactive protein (CRP) values are the highest values reported during the sepsis episode and presented as median and interquartile range (mg/L).*

higher compared to our study. We found a significant reduction in the total EOS incidence with a mean 6% decline per year for the period 2000–2018. This was however not an a priori planned analysis, and should be interpreted with caution. On the other hand, it concurs with two multicenter studies from 2005–2014 in UK and 2002–2012 in Australian and New Zealand reporting a decreasing trend in the incidence of EOS to 0.7/1,000 and 0.83/1,000, respectively (16, 19).

E. coli, associated with high mortality rates, caused a substantial proportion of EOS in extremely preterm infants. We found a stable incidence of *E. coli* EOS during the 23 years, in line with other European studies (3, 16), but numbers may be too small to detect significant changes. In contrast, US and Australian studies (1, 5, 19–21) consistently reports higher rates of *E. coli* EOS. Stoll et al. reported an increasing incidence of *E. coli* sepsis when comparing surveillance data from 2015–2017 to 2006–2009 (5), with overall incidence rates of 0.4/1000 LBs and 12.1/1000 LB in infants with GA <28 weeks. The lower incidence of *E. coli* EOS in European studies are usually accompanied by a higher incidence rate of GBS, as in our study (3, 16). Yet, the incidence of GBS in our study is comparable to other reports (1, 5, 7). We did not find any change in incidence during these years. Risk-based intrapartum antibiotic preventive strategies against GBS, based on UK guidelines, were implemented in our unit in 2008 (22, 23). Limitations in the GBS prevention strategies have been demonstrated both in risk-based and in screening-based programs (5, 24), but the latter may be associated with a slightly lower rate of EOS caused by GBS.

We found that the maximum CRP level in both Gram-positive and Gram-negative bacteria were relatively low. This is in line with other studies reporting on elevation of inflammatory markers in EOS (25, 26), and is an important reminder for clinical evaluation of infants at risk of EOS within a structured strategy (4, 27).

One of the key findings in this study was that the vast majority (98%) of EOS isolates were susceptible to the current empirical antibiotic regimen; benzylpenicillin and gentamicin. Although numbers are small, the Gram-negative antibiotic nonsusceptibility rate, remained unchanged. This is comparable to a recent study, from the USA where they found a stable *E. coli* nonsusceptibility rate during 2009–2017 (10). The nonsusceptibility rates were on the other hand substantially higher than in our study. Our nonsusceptibility rates correlate well-with a UK study reporting a 7% nonsusceptibility to the empirical antibiotic regimen of benzylpenicillin and gentamicin among 328 EOS isolates (11). Furthermore, Cailes et al. reported a decreasing nonsusceptibility from 2005–2009 to 2010–2014, and also a lower nonsusceptibility rate among Gram-negative isolates causing EOS vs. late-onset sepsis. Nonsusceptibility rates are low in Norway due to a restrictive antibiotic policy, although increasing gentamicin nonsusceptibility of *E. coli*, extended-spectrum beta-lactamase producing Gram-negative bacteria and MRSA are increasingly found (28). This, correlates well-with Flannery et al. findings that pathogens causing EOS in the USA are not affected by the increasing extreme drug resistance seen globally (10, 29). In 2011, our NICU changed from ampicillin to benzylpenicillin as the beta-lactam backbone for empiric EOS therapy. Ampicillin use is associated with a higher *Klebsiella pneumoniae* gut colonization, including ampicillin nonsusceptible strains (30). Our results do not support the need for empirical use of ampicillin instead of benzylpenicillin.

TABLE 3 | Antibiotic nonsusceptibility rates in cases with early-onset sepsis at Stavanger University Hospital, 1996–2018.

	Nonsusceptible
***Escherichia coli* (*n* = 11)**	
• Ampicillin	10/11 (91%)
• Gentamicin	0/11 (0%)
• Third generation cephalosporin	0/11 (0%)
***Staphylococcus aureus* (*n* = 9)**	
• Gentamicin	1/9 (11%)
• Oxacillin (methicillin-resistant -MRSA)	1/9 (11%)
All early-onset sepsis isolates (*n* = 101)	
• Benzylpenicillin + gentamicin (combined)	2/101 (2%)

All group B streptococci (N = 60) and all viridans group streptococci (n = 10) were susceptible to benzylpenicillin.

Key strengths of this study are the 23-year long study period and the strictly population-based design. The number of infants included in our study roughly equals the total number of births in Norway over a 2-year period. We have complete data sets on all infants with positive blood cultures, including antibiotic susceptibility patterns, antibiotic use, and neonatal and maternal data for the entire study period. The data were collected by a single researcher. The study includes detailed maternal and neonatal information.

We only included culture-confirmed episodes regarded as the "gold standard" for the definition of neonatal sepsis. There are also limitations. The data were collected from a single-center and the findings may not be generalizable to other countries with high antibiotic consumption driving high antibiotic nonsusceptibility rates, settings with higher burden of neonatal sepsis, or settings with limited resources and weak healthcare systems. Another important limitation is the low rate of extremely preterm infants. Albeit being in line with other regions of Norway, it may be lower compared to countries outside Scandinavia. Finally, there are inherent limitations with retrospective data.

CONCLUSION

In this population-based study of EOS over 23 years, we found that that GBS was the most common causative pathogens in EOS, but among extremely preterm infants *E. coli* dominated. We found no change in the incidence of EOS during the whole 23-year period, but with a possible decrease in incidence during 2000–2018. Empirical benzylpenicillin and gentamicin in combination provides a very high coverage for EOS pathogens in our setting.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AV conceptualized and designed the project, collected and analyzed data, wrote the first version of the manuscript, and revised the manuscript. She had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. KØ conceptualized and designed the project, directed and organized all phases of the project, analyzed data, contributed with statistical analyses and supervised AV during all the phases of the project. CK and SR supervised AV during final phases of the project, analyzed data, revised the manuscript for intellectual content and approved the final manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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