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EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS OF PAEDIATRIC BLOODSTREAM INFECTIONS 1998-2018 IN STOCKHOLM

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Cover picture *Staphylococcus aureus* bacteria (purple) being engulfed by neutrophils (blue), which are a type of human white blood cell.

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EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS OF PAEDIATRIC BLOODSTREAM INFECTIONS 1998-2018 IN STOCKHOLM

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”Utan tvivel är man inte riktigt klok”

Tage Danielsson

ABSTRACT

Bloodstream infections (BSI) constitute a common, serious, and potentially preventable cause of morbidity and mortality in children. Good knowledge of epidemiology, aetiology and the occurrence of antimicrobial resistance is essential for early effective empiric antibiotic therapy. Also, to identify areas for improved antimicrobial therapy and possible preventive measurements for BSI in different risk groups.

The general aims of this thesis were to describe the aetiology, risk factors, and occurrence of bacteria resistant to antimicrobial therapy in children aged 0–17 years, with BSI under 20 years.

In papers **I**, **II** and **IV**, we found a crude BSI incidence rate of 25.5/100,000 children, aged 0-17 years, over the study period, with differences between different ages and risk groups. From 15/100,000 in children, aged 3–17 years to 180/100,000 live births at neonatal wards (children 0–2 months), but 40/100,000 for 0–2 month-old children warded outside neonatal wards. Over the study period different strategies to prevent BSI attributed to a decreased risk for BSI. Introduction of vaccine against *Streptococcus pneumoniae* declined the incidence of BSI with 30% in children aged between 3 months and 2 years. Implementation of a a risk-based intrapartal antibiotic prophylax program against early onset *Streptococcus agalactiae* BSI had most impact to reduce the incidende in new-borns. BSI in children without underlying co-morbidities has become rare and are caused by a limited numer of pathogens. In children with cancer, underlying co-morbidities and neonates warded at the neonatal wards the aetioloogy is much more diversed. *S. aureus* was the most prevalent pathogen.

In **paper III**, we studied the antibiotic prescription, and concluded a changing pattern in the prescription of antimicrobial therapy, with a proportional decrease in the treatment of community-acquired infections and an increase in prophylactic therapy to specific risk groups of patients.

In **paper V**, we found acquired antimicrobial resistance (AMR) in 9.2% of all invasive isolates. The trend for AMR increase for both Gram-positives and Gram-negative bacteria. The proportion of Enterobacterales producing extended-spectrum beta-lactamases (ESBL) increased from 1.6% to 14.1%. A high proportion (64.7%) of ESBL-producing strains was multidrug-resistant. During the last period, 6% of *S. aureus* were MRSA (methicillin-resistant Staphylococcus aureus). The oncology patient group had the highest proportion of ESBL-producing Enterobacterales. A history of travel in the past six months to a non-Nordic country by the child or a household member was identified as a risk factor.

In conclusion, the thesis adds knowledge about the aetiology and epidemiology of BSI in children from a Swedish perspective. The findings are highly important for designing empiric antibiotic therapy regimes and for planning targeted measurements to improve therapy and prevent BSI.

LIST OF SCIENTIFIC PAPERS

1. Joachim Luthander, Rutger Bennet, Christian G Giske, Anna Nilsson, Margareta Eriksson. Age and risk factors influence the microbial aetiology of bloodstream infections in children. Acta Paediatrica 2013; 102: 182-186.

2. Joachim Luthander, Rutger Bennet, Christian G Giske, Anna Nilsson, Margareta Eriksson. The aetiology of paediatric bloodstream infections changes after pneumococcal vaccination and group B streptococcus prophylaxis. Acta Paediatrica 2015;104: 933-939

3. Joachim Luthander, Rutger Bennet, Anna Nilsson, Margaretha Eriksson. Antimicrobial Use in a Swedish Pediatric Hospital. Results From Eight Point-prevalence Surveys Over a 15-Year period (2003-2017). Pediatr Infect Dis J 2019; 38:929-933

4. Joachim Luthander, Rutger Bennet, Christian G Giske, Margareta Eriksson, Anna Nilsson. Trends of Pediatric Bloodstream Infections in Stockholm, Sweden: A 20-year retrospective study. Pediatr Infect Dis J 2020 Aug 5. doi: 10.1097/INF.0000000000002850.

5. Joachim Luthander, Rutger Bennet, Per Nydert, Margareta Eriksson Christian G Giske, Anna Nilsson. Antimicrobial resistance in children with bloodstream infections – a population-based study in Stockholm, Sweden. In manuscript

CONTENTS

LIST OF ABBREVIATIONS

ABR	Antibacterial Resistance
AMR	Antimicrobial Resistance
BSI	Bloodstream Infections
CAI	Community-Acquired Infections
CFU	Colony Forming Units
CRP	C-reactive protein
EOS	Early-onset Sepsis
ESBL	Extended Spectrum β-lactamase
FiO_2	Fraction of inspired O2
GAS	Group A streptococcus
GBS	Group B streptococcus
HAI	Hospital-Acquired Infection
ICU	Intensive Care Units
IPD	Invasive pneumococcal disease
LOS	Late-Onset Sepsis
MDR	Multi-Drug Resistance
PBPs	Penicillin Binding Proteins
PCT	Procalcitonin
PCV	Pneumococcal conjugate vaccine
PICU	Paediatric Intensive Care Unit
SAB	Staphylococcus aureus Bloodstream infections
SBI	Serious Bloodstream Infections
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment score
qSOFA	Quick SOFA
UTI	Urinary tract infection
VRE	Vancomycin-Resistant Enterococcus faecium
WBC	White Blood cells Counts

1 INTRODUCTION

Childhood mortality has decreased globally during the past decades. Financial and political efforts to increase access to child-care services, an increase in maternal education, implementations of vaccines, HIV/AIDS, malaria, and tuberculosis programs are some crucial factors contributing to decreased mortality. Still, in total, 6.2 million children under 15 years of age died in 2018, a child died every fifth second. More than half of those deaths were preventable, and infectious diseases caused approximately 60% of these deaths. (1-4) Among infectious disease, sepsis is a significant contributor to global morbidity and mortality in children. (5) In addition to mortality, infection contributes substantially to both paediatric emergency departments visits and hospital admissions, (6)

Epidemiological studies of severe paediatric sepsis indicate that bacteraemia contributes substantially to severe sepsis and high mortality rates in children. (7-9) Bloodstream infections are, therefore, a significant and potentially preventable cause of death.
Most of the global child mortality occur in low-income countries; although, even in high-income countries, infectious diseases make a significant contribution to deaths in children. In the U.K., infection-associated deaths constitute 20% of all childhood death. (10) Early recognition, supportive therapy, and adequate antimicrobial therapy are cornerstones for a successful outcome. (11) Normal physiological, hemodynamic, and immunologic response and aetiology differ between adults and children. Therefore, it is not feasible to adapt adult data on the paediatric population. (12-16) Effective empirical antimicrobial therapy depends on good knowledge of the aetiology and local resistance pattern in bloodstream infections. (17, 18) Antimicrobial resistance is increasing globally and threatening paediatric health. National population-based data on the epidemiology of bloodstream infection are sparse and therefore motivates studies on the aetiology of paediatric bloodstream infection in Sweden.

2 BACKGROUND

2.1 SERIOUS INFECTIONS IN CHILDREN

Epidemiological studies of the paediatric infectious diseases reveal an enormous burden of disease. Every single child will, at some point during childhood, get infected. A new-born is, in some respects, immunological naive, with a need for further maturation of the immunological system for acquiring effective resistance and resilience against microbiological agents. Contact with the environment is essential for the development of an effective immune system, and at the same time, it is potentially life-threatening. Bacterial colonisation early in life is universal for all children but comes with a risk for infections. There are several well-known risk factors for susceptibility to infections, but the question is why some infants only get colonised by bacteria. At the same time, some develop a severe infection and need hospitalisation and antimicrobial and supportive therapy to avoid a dismal outcome. Most infections are of viral origin, self-limiting and do not require antimicrobial therapy.

Serious infections are defined as illnesses with a fever, that could cause disability or death if diagnosis and treatment are delayed. The conditions usually considered as serious infections are sepsis, bacteraemia, pneumonia, pyelonephritis, osteomyelitis, septic arthritis, meningitis, severe bacterial infections like mastoiditis, acute sinusitis, encephalitis, and also viral bronchiolitis and some viral infection, like, e.g. enterovirus, early in life. The overall risk for a serious infection in the child population varies, with studies showing less than 1% risk in a primary care setting, (19-21) but 10–15% risk during visits to the emergency department will suffer for a serious bacterial infections requiring antimicrobial and supportive therapy. (19, 21, 22) In a European prospective multicentre study evaluating the role of severe paediatric infectious diseases in paediatric hospitals, children with suspected sepsis or a severe focal infection were included. Sepsis was found in 43.2% of all cases and had a worse outcome compared to severe focal infection. Both severity and mortality were higher in children with an identified causative pathogen than those with no causative pathogen. The mortality was 1–3% in previously healthy children and 7–10% in chronically ill children. (23) The mortality rate also differs amongst countries. Based on register data, children in the U.K. had a risk ratio of 1.84 to die of infectious diseases compared to Sweden. The mortality rates due to infections were 63.9/100,000 children under five years of age, and 10.5/100,000 children between 5 and 15 years of age in the U.K., compared to 34.6/100,000 children under five years, and 7.85/100,000 children between 5 and 15 years of age in Sweden. (24, 25) Several factors probably explain these differences, but dissimilarities in the distributions of pathogens, e.g. *N meningitidis*, is one important factor. The distribution of some highly virulent bacteria differs amongst different areas and countries and affects disease severity and mortality rates.

Data from Sweden Statistics on registered diagnosis for infectious diseases among hospital-admitted children show the incidence of serious infections in Sweden (Figure 1a and 1 b). Pneumonia and urinary tract infection are the most prevalent infections, with 191 hospital admissions per 100,000 inhabitants for pneumonia, 159 per 100,000 for urinary tract infection, and 27/100,000 for sepsis in children aged 0–4 years as in 2018. (26) It is important

to identify the child at risk of developing a life-threatening infection among other children who present with a benign, usually viral, infection with near to zero mortality.

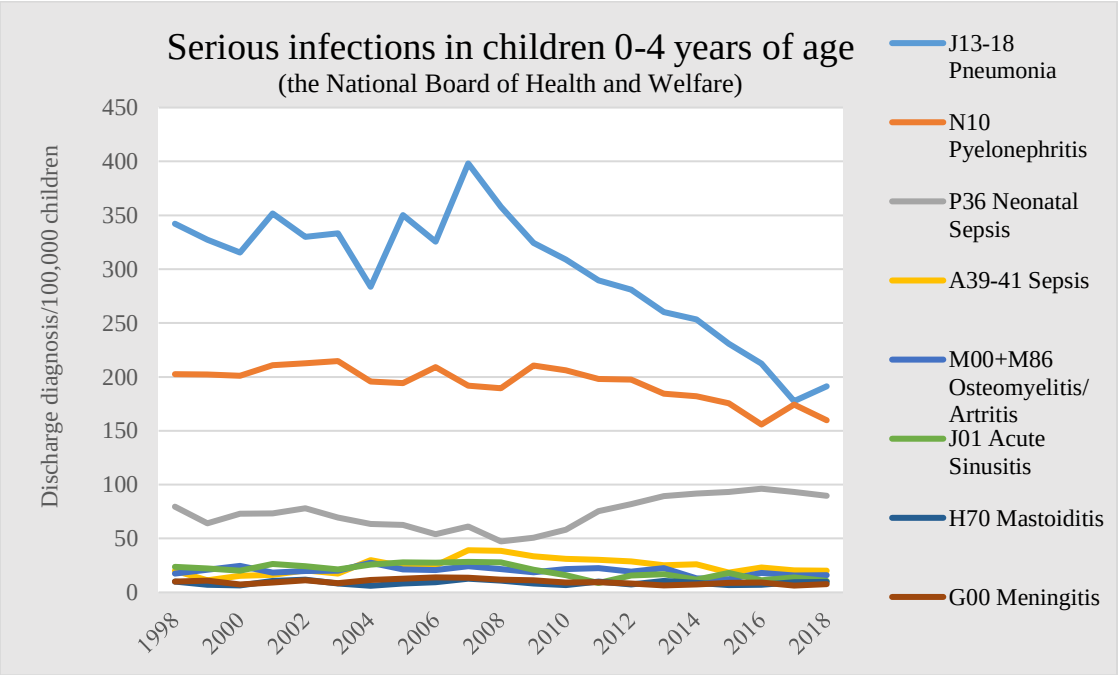

Figure 1a Discharge diagnoses in children 0-4 years of age admitted to hospital.

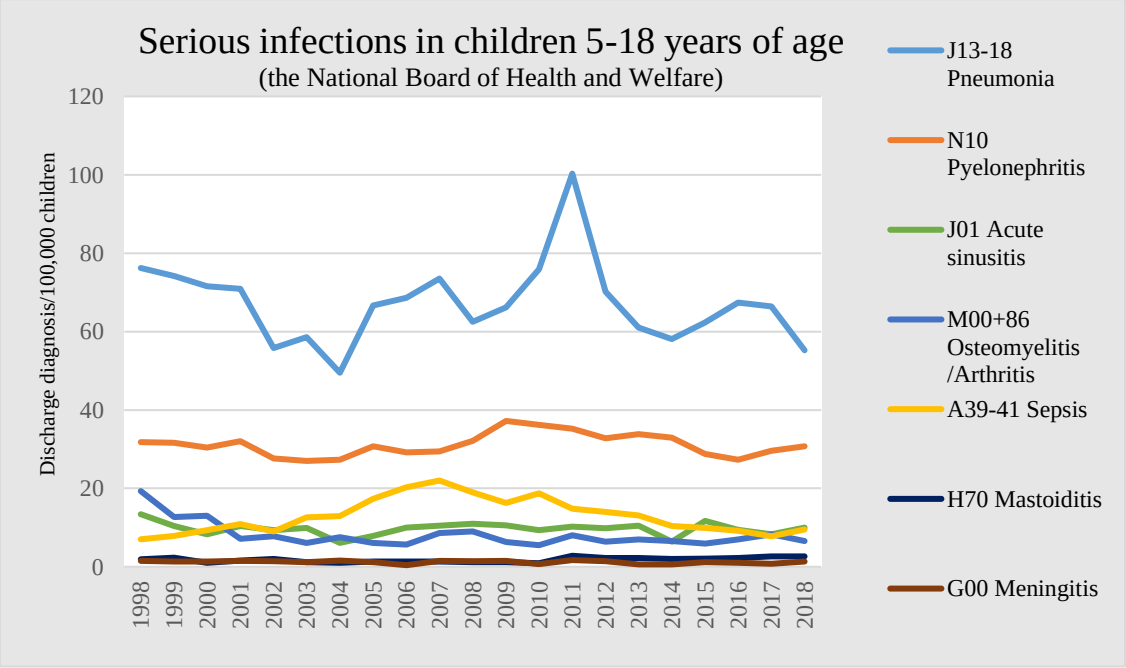

Figure 1b. Discharge diagnoses in children 5-18 years of age admitted to hospital.

2.2 SEPSIS

2.2.1 Current and past definitions of sepsis

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection, according to the 3rd International Consensus Definition for sepsis and septic shock. (27) Earlier definitions established at the first sepsis conference in 1991, and updated in 2001, focused on the host's response to infection [systemic inflammatory response syndrome (SIRS)]. In 2005 a paediatric, age-specific definition was established at the International Paediatric Sepsis Consensus Conference. (28) The SIRS criteria did not necessarily indicate a dysregulated or life-threatening response. Furthermore, SIRS is present in many hospitalised patients, including those who never develop infection and never incur adverse outcome. The SIRS-based definition lacked specificity to identify children with a higher risk for mortality and yet missed 10% of children warded at intensive care unit (ICU) with infection and organ dysfunction. (29) Still, a definition is important for early identification, to promote adequate initial management and therapy, and to facilitate diagnostic registration for later evaluations. The current 3rd definition was established in 2016 in response to the awareness that the previous definition of sepsis performed poorly, both in identifying early infections and in selecting patients in need for early, aggressive intervention and therapy in both adults and children.

The sepsis-3 task force working with the new sepsis definition recognised that sepsis is a syndrome with no validated criterion standard diagnostic test. The task force evaluated the clinical criteria that best predicted in-hospital mortality, ICU mortality, and/or ICU admission > three days. The task force compared three different scoring systems: The sequential organ failure assessment (SOFA) score (initially the sepsis-related organ failure assessment), the Logistic Organ Dysfunction System (LODS), and SIRS. The SOFA-score and the LODS system were superior to SIRS in predicting mortality in ICU but similar to SIRS for patients outside the ICU with suspected infections. The sepsis-3 task force regarded SOFA as more well-known and more straightforward than the LODS system and therefore recommended the use of SOFA score.

The SOFA score is a mortality prediction score based on the degree of dysfunction in six organ systems; the fraction of inspired O_2 (FiO_2), platelets counts, Glasgow Coma Scale, bilirubin, mean arterial pressure and creatinine, and is intended for patients admitted to ICU. A SOFA score of 2 or greater identifies a 3 to 11-fold increase of dying compared to SOFA score less than 2. (27, 30) However, there is, at present, no consensus on how to define organ dysfunction in children and thus the sepsis 3-task force excluded children in their analysis. There are several scoring systems for organ dysfunction in children, albeit all slightly different than SOFA. Validation of age-adjusted SOFA, the p-SOFA score has demonstrated excellent discrimination for in-hospital PICU mortality due to sepsis. (29, 31) P-SOFA is, therefore, a feasible scoring system to identify patients with a high probability to die in the PICU. However, p-SOFA (or SOFA) is not developed to help identify patients at risk for sepsis in the emergency department. In adults, quick-SOFA(q-SOFA), with an evaluation of the presence of altered mental status, systolic blood pressure below 100

mmHg and respiratory rate of 22/minute are used as criteria for early identification of patients with suspected sepsis to prompt clinicians to further investigate for organ dysfunction. Low blood pressure develops late in paediatric sepsis, and tachypnoea occurs early among febrile children with infections with near-zero mortality, as in bronchiolitis. Q-SOFA has performed moderately in paediatric patients, with the area under the curve (AUROC) of <0.7, thereby interpreted as sub-optimal performance (29, 32). Evaluation of the paediatric early warning score systems (PEWS) revealed both higher sensitivity and specificity compare has qSOFA and with an AUROC of >0.8 implying good performance to identify children with sepsis (32, 33)

To summarise, the definition of sepsis in adults is also adapted for children, but the paediatric criteria are undefined, and the optimal screening tool required for rapid recognition is still unclear. In the "Surviving sepsis" international guidelines, the authors recommended the implementation of a screening system for timely recognition of sepsis for the management of septic shock and sepsis-associated organ dysfunction in children. (11) A uniform paediatric sepsis definition is challenging. Pathophysiology, clinical presentation, and therapeutic approaches are under the influence of the maturation process and highly affected by age. It is important to be aware of the key differences in aetiology, presentation and resuscitation in children compare to adults. (12)

2.2.2 Epidemiology of sepsis

The risk for paediatric sepsis is not easy to establish. In a meta-analysis, based on paediatric population-based observational studies, the aggregated estimated incidence of sepsis was 48 (95% CI 27–86) cases per 100,000 person-years for sepsis, 22 (95% CI 14–33) cases per 100,000 person-years for severe sepsis, and 2202 (95% CI, 1099–4360) cases per 100,000 live births for neonatal sepsis. The crude mortality rate was 9–20% and varied from 1–5% for sepsis, 9–20% for severe sepsis to 11–19% for neonatal sepsis. (5) The burden of severe sepsis in children admitted to paediatric intensive care units (PICU) was evaluated in a global point prevalence survey conducted on five days throughout 2013–2014 in 26 countries (the SPROUT study). The prevalence of children with severe sepsis was 8.2% (95% CI, 7.6–8.9%), and the mortality was 25%. In 40% of the sepsis cases, the primary site of infection was the respiratory tract, and 26% of patients had a positive blood culture. (7) Similar prevalence rates are observed in other studies, but also are both lower and higher rates of children with sepsis.

The prevalence rates differ significantly depending on what inclusion criteria are adopted; inclusion of patients with a combination of infection and organ dysfunction, or inclusion based on diagnostic codes for severe sepsis and septic shock. A tenfold difference in the prevalence rates and mortality can be attributed to the criteria used. Among hospitalised children in the U.S. the prevalence was 3.1%–7.0% when using combination code compare to 0.45%–0.84% using the severe sepsis ICD-9-CM codes. (8, 34, 35) Similar differences are reported for the mortality rates. The 28 days mortality for combination code patients were 4.4%–8.2% and 15.4–21.2% in children with codes for severe sepsis. (6,30)

In Sweden, the annual incidence rate for severe sepsis was reported to be 687/100,000 adults with in-hospital mortality of 19.8%, (36) giving an estimation of 40,000 cases per year and 6000 deaths. The incidence of paediatric sepsis in Sweden is not known. According to diagnose codes for children 0–4 years of age discharged from hospitals, the incidence for sepsis was 118/100,000 children in 2018. (26)

2.2.3 Increasing incidence and decreasing mortality

Retrospective register studies based on children warded at hospitals with diagnosing codes according to the International Classification of Diseases (ICD) codes for severe sepsis or septic shock 995.95 or 785.52 respectively, reveal increasing incidences of sepsis. In the US, the estimated incidences increased from 0.56/1,000 children in 1995 to 0.89/1,000 children in 2005 (35), and from 0.93/1,000 children in 2006, to 1.59/1,000 children in 2012. (37) A prospective study from Australia and New Zealand reported an average yearly increase of 0.08–0.09 cases per 100,000 children in paediatric sepsis at ICUs between 2002–2013. (38) Also, the prevalence of children hospitalised for sepsis increased in the U.S. In studies including patients with infection, in combination with a diagnose code for organ dysfunction, an increase from 3.7% to 4.4% and from 0.4% to 0.7% for the patient diagnosed ICD-9-CM codes for severe sepsis codes respectively (34) At the same time, sepsis mortality rates declined between 2004 and 2012 in all categories. (9, 34)

Table 1 Summary of studies on paediatric sepsis

Author	Study year	Inclusion	Incidence	Prevalence	Mortality
Hartman, [35] U.S.	1995–2005	ICD-9-CM-codes	0.56–0.89/1,000		
Schuller, [37] U.S	2006–2012	ICD-9-CM-codes	0.93–1.59/1,000		
Balamuth, [34] U.S.	2004–2012	• Combination codes • ICD-9-CM codes		3.7–4.4% 0.4–0.7%	10.6–6.8% 27.8–16.9%
Ruth,[9] U.S.	2004–2012	• Inf.+organdysfunct. • ICD-9-CM codes		6.2–7.7% 3.1%	18.9–12.0%

Bloodstream infections (BSIs) are identified as one of the most severe causes of sepsis. Following a similar trend, the decline in mortality from BSI in children correlates with the decline in mortality from high virulent pathogens, concomitant with increase in pathogens more commonly found in healthcare-associated infections. (39-41)

2.3 BLOODSTREAM INFECTIONS

2.3.1 Definition of bloodstream infections

Bloodstream infection (BSI) and septicaemia are sometimes interchangeably used. Septicaemia is like a BSI, bacteria in blood in combination with signs of an infection, but usually refers to severe symptom fulfilling diagnostic criteria for severe sepsis. BSI is an infection with a high potential for developing into sepsis, severe sepsis, and septic shock. BSI is identified as isolation of viable bacteria or a fungal pathogen in a blood culture bottle, bacteraemia (or fungaemia) in combination with clinical signs of infection. Bacteraemia could occur, either as a result of spread from an initial focal infection reflecting a deterioration of the disease, or during daily activities like toothbrushing, or when a natural physical barrier, such as skin, is compromised. In the case of toothbrushing, bacteraemia is usually transient and a clinically benign condition where the host immune system eliminates the bacteria from the blood. In the circumstances with impaired host immunity, the presence of foreign material, or anatomical lesions could lead to bacteraemia, and the usually benign situation could result in clinical infection, a BSI, or focal infections, such as osteomyelitis, with a risk for substantial morbidity, sequelae and mortality, (Figure 2).

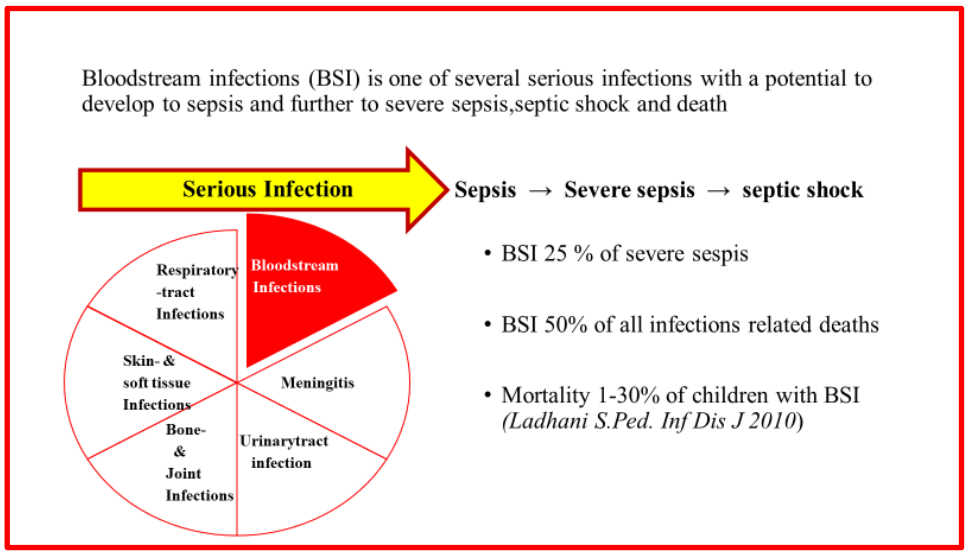

Figure 2 Serious infection with the potential to develop to sepsis

2.3.2 Detection of bacteria in the blood

There are several laboratory methods to identify microorganisms. The different methods for detecting bacteria in blood are microbial cultures, detection of bacterial 16S rDNA, targeted PCR, detection of specific bacterial structures, and detection of antibody developed against a specific pathogen. Blood culture is the gold standard to detect bacteria in the blood. An isolated organism in the culture is considered a positive blood culture. However, a culture requires viable bacteria, and the method is surrounded by some problems, i.e. sampling volume, contamination, pre-analytic time, up to 48 hours turnover time before a result. In children, a small blood volume is considered to contribute a significant problem for the opportunity to catch a viable bacterium. The higher the blood volume cultured, the higher yield. Modelling data and clinical studies on adults with BSI reveal that the bacterial concentration ranges from 0.01–1 CFU/mL in 50% of BSI cases. The bacterial load is

positively correlated to the severity of the infection and could exceed 100 CFU/mL for severe clinical infections with a high bacterial load. The bacterial concentration is concluded to be comparable between children and adults. (42) The sensitivity for blood cultures has been estimated to be 95% when 3 CFU are sampled, which implies that a total volume of at least 30 ml is required for detecting most BSI low-level bacteraemia (<10 CFU/mL) or very low-level of bacteraemia <1 CFU/mL. (43) The majority of neonatal children (64%) and of children two months to 13 years of age (60%) has been found to have low-level bacteraemia with <10 CFU/mL and a substantial proportion of neonates and children >2 months had very low-level bacteraemia with <1 CFU/mL. (44) The sensitivity for paediatric blood cultures has been estimated to be 40% when collecting 2 ml blood and 75% when collecting 6 ml. (45) The current recommendation for the correct blood volume in paediatric blood cultures is based on the assumption that it is safe to collect 4.0–4.5% of the child's total blood volume which is required to detect low concentrations of pathogens in the blood. The recommended volume varies between centres, approximately; 1–1.5 mL for children weighing less than 4 kg, 3 ml for children 4–7.9 kg, 6 ml for children 8–11 kg, and 7 ml for >11 kg. (46)

Table 2 Blood sampling for culture in children

Bacterial concentration		**Infections**	**Required blood volume**
0.01–1 CFU/mL	Very-low level	• 23% of children with BSI >2 months of age • 42% of neonates	
<10 CFU/mL	Low level	• 64% of neonate • 60% of children	30 ml are required to achieve a positive result with 95% sensitivity
>100 CFU/mL	High lever	Severe infections	3 ml

The blood volume in children				70–80 ml/kg
Accepted as safe sampling blood volume				4–4.5%
Recommended volume	<4 kg; 1–1,5 mL	4–7.9 kg; 3 mL	8–11 kg; 6 mL	>11 kg; 7 mL

Studies on the amount of blood that was actually retrieved in paediatric blood cultures found that 46% of the submitted blood culture bottles contained adequate blood volume and blood cultures with adequate blood volume were more likely than those with an inadequate blood volume to yield positive blood culture results. (47)

In most studies regarding BSI, about 85% of all collected samples are negative and with varying degree of pathogens that could be considered as possible contamination of commensal skin microbiota. Low positive rates have raised the question of the utility to obtain a blood culture. For children admitted to hospital due to community-acquired pneumonia, the rate of a positive blood culture is under 5% on average, but significantly higher in children with complicated pneumoniae. In pneumonia, complicated with pleural effusion, lung abscess, necrosis and drainage, or coincidence of distal site of infection had up to 75% positive blood culture rates. (48, 49) Children with positive culture had a longer duration of fever before admission and significantly higher CRP levels than blood culture-negative children. There was no difference between the bacteriaemic and nonbacteremic

groups for WBC counts or for the other more common (severe) infections. In children admitted to hospital with suspicion for pyelonephritis/UTI blood culture is also a part of the diagnostic evaluation. For UTI, there is an inverse correlation between age and positive blood culture, elevated creatinine, and pathological ultrasonography are also reported as a risk factor for positive blood culture in children (50, 51).

Given the problems for blood cultures with low sensitivity and up to 48 hours to present a preliminary result, there is a need for other diagnostic tools for BSI and sepsis. During the last two decades, molecular methods to detect bacterial DNA has been developed. Bacteria are a prokaryote organism and lack an enveloped nucleus. The protein-producing, ribosomes, in all prokaryote cells have different subunits. The smallest subunit, 16S, is found in all bacteria and a highly conserved part similar in all bacteria that could be detected by PCR, and another hypervariable, species-specific, region. With 16S rDNA sequencing, bacteria present in a sample can, in a culture sequencing manner, be identified to the species level. The 16SrDNA sequencing for normally sterile fluids like cerebrospinal fluid, synovial fluid and pleural effusions are already in clinical use. The use of 16S rDNA sequencing in blood has proven not to work so well due to low bacterial concentration, and no method has been established for clinical use even if some have shown promising results. (52) (53)

2.3.3 Epidemiology of BSI

In Table 3, available data on BSI are summarized. Since there are major differences in study populations and primary endpoints in these studies it is difficult to draw general conclusions. There are some similar observations reported such as an decrease in incidence over the past years (54-56) and an increase of children with underlying co-morbidity leading to a change in the spectrum of pathogens associated to BSI (38,39.56).

Table 3 Summary of available studies on BSI in the paediatric population

		Sepsis in PICU	with BSI	mortality
Weiss, [7]	The SPROUT-study	8.2%	26%	(20–40%)
Ruth, [9]	49,153 PICU admitted	7.7%	67%	14.4%
Boeddha, [57]	the EUCLID study	n=795	42%	6%
Spaulding, [54]	BSI in 162 U.S paediatric hospital		0,3%	5%
Ruiz-Ancona, [58]	Incidence of BSI in Spain		224/100,000	
Skogberg, [59]	Incidence of BSI in Finland	<1 year	514/100,000	18%
		1–13 years	33/100,000	0,6%
Agyeman,[17]	Incidence of BSI in Switzerland	0–17 years of age	25/100,000	7.2%
		0–28 days	146/100,000	11%
Greenhow, [19, 55]	Incidence of BSI in U.S.	7–59 days of age.	0.57/1,000	
		3–to 36 months	21/100,000	
Laupland, [56]	Inc. of BSI in Canada	0–17 years of age	53/100,000	5%
Henderson, (60)	Incidence of BSI in the U. K	1–11 month of age	362/100,000	
		5–15 years of age	36/100,000	

It is also evident that the epidemiology is distinctly age-related. The prevalence for infants, <1 year of age, is considerably higher than for children over one year of age. The epidemiology is, besides from the occurrence of co-morbidities, distinctly age-related. The prevalence for infants, <1 year of age, is considerably higher than for children over one year of age.

2.3.4 Aetiology

The aetiology of paediatric BSI has changed considerably during the last three decades. The introduction of vaccines against previously dominant pathogens has been effective. (61) Improvement in public health and access to healthcare facilities and antimicrobial therapy has reduced community-acquired infections. However, progress in the treatment of prematurely born children, oncology patients, cardiovascular patients, and other groups of children with severe co-morbidities has led to a patient category that requires more frequent, and longer hospital-stays, with a risk for health-care-associated infections. (62, 63)

The classical, highly virulent paediatric pathogens that cause community-acquired infections in otherwise healthy children is declining, and paediatric BSIs are partly replaced by other bacteria in more vulnerable patients, and more often cause hospital-acquired infections.

The most frequently isolated pathogens in paediatric BSI are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus* spp, *Streptococcus agalactiae*, coagulase-negatives staphylococci (CoNS), *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* spp. and, *Pseudomonas aeruginosa* (19, 39-41, 64-68). The aetiology is related to age, patients' characteristics, and specific hospital settings. (19, 40, 41, 67, 69)

2.3.4.1 Staphylococcus aureus

Staphylococcus aureus is one of the most frequently isolated pathogens in paediatric BSI after implementation of HiB-vaccines and PCV vaccines. *S. aureus* is a skin coloniser also found in anterior nares. A wounded skin or a depleted mucosal barrier causes the bacteria to leave its equilibrium state of a commensal and become infective. *S. aureus* infections vary from mild skin infections to severe life-threatening infections with complications of bacteraemia, such as endocarditis. Neutrophils play an essential role in the human defence against *S. aureus*, and premature infants with immature neutrophil or other patients with impaired neutrophil function are at greater risk for infections. (15*) S. aureus* also have the capacity to produce several toxins involved in more severe conditions. (70, 71)

- PVL: Necrotising pneumonia with Panton-Valentine Leucocidin (PVL) toxin-producing *S. aureus*.
- Exfoliation of skin: Staphylococcal scalded skin syndrome (SSSS) mediated by exfoliating toxins A and B, that cleave desmosome junction in the epidermis, causing painful blisters and exfoliation of the skin.
- TSS-1: Toxic shock syndrome with a rapid onset of massive production and release of cytokines. The *S.aureus* toxin TSS-1 acts as a superantigen and activates a large proportion of T-cells that release a cascade of pro-inflammatory cytokines.

Healthcare-associated *S. aureus* BSI (SAB) is commonly related to indwelling catheters. Complications occur in 30% of cases, including thrombosis, endocarditis, and recurrence of infections. The risk for a complication is related to the time of bacteraemia. (72) The possibility of saving a central line of patients in SAB, but without signs of complication, has been discussed. Salvaging of the central line is feasible in the majority of children, and attempts to clear the bacteraemia can be considered for 48 (73) to 72 hours without increased risk for complication. (74)

2.3.4.2 Streptococcus pneumoniae

Streptococcus pneumoniae (pneumococcus) is a pathogen that colonises the mucus membrane in the nasopharyngeal tract in children and their close contacts. A viral upper respiratory tract infection often precedes an invasive pneumococcal infection with pneumonia, bacteraemia, sinusitis, other ear-nose-throat infections, and meningitis. The species has over 90 different serotypes with different pathogenic capacities and geographical distribution. (70, 71) Efforts to include serotypes found in invasive infections for preparing vaccines have been successful. Pneumococcal conjugate vaccine (PCV) targeting the most prevalent serotypes has decreased the incidence of invasive pneumococcal diseases significantly. However, *Streptococcus pneumoniae* has a substantial aetiology in paediatric sepsis. (75)

2.3.4.3 Streptococcus pyogenes

Streptococcus pyogenes are usually called Group A Streptococcus (GAS) following the Lancefield classification of beta haemolysing streptococci. The bacteria are transmitted through droplets from one person to another and cause pharyngotonsillitis, mild skin infections to more severe local infections, and life-threatening invasive infections. GAS can produce toxins and cause scarlatina and life-threatening streptococcal toxic shock syndrome. After an acute GAS infection, immune-complex reaction following a risk of developing glomerulonephritis and rheumatic fever, which is rare. (70, 71)

2.3.4.4 Streptococcus agalactiae

Streptococcus agalactiae is often classified as beta-haemolytic Group B streptococcus (GBS). It is a part of the commensal intestinal microbiome in 30% individuals and contracts the vaginal flora in those who are colonised. Vaginal GBS colonisation confers a risk of 1.1% for early-onset (EOS) GBS infections after vaginal delivery. (76) Colonisation is also associated with preterm birth, especially when there is evidence of maternal ascending infection (bacteriuria). (77) GBS is the most common neonatal infection, with a case fatality rate of 9.6%. (78) Late-Onset GBS infection is associated with maternal colonisation but is also evident in cases without colonisation, indicating that horizontal acquisition from non-maternal caregivers may also be a part of pathogenesis. GBS can be isolated from 25% of milk samples of colonised, breastfeeding mothers, though milk also contains protective antibodies (unspecific secretory-IgA), and protective human oligosaccharides against invasive GBS infection. (79, 80) There is no recommendation of withholding breastfeeding in GBS-colonised mothers. (81)

2.3.4.5 Enterococcus species

Enterococcus faecalis and *Enterococcus faecium* are a part of the intestinal microbiome and are much less virulent than *S. aureus*, *S. pneumoniae*, GAS and GBS. They appear as opportunistic infections, and appear in polymicrobial cultures, where the significance of their presence could be challenging. Children with comorbidities and extended hospitalisation with indwelling medical devices are at a higher risk for infection. Prolonged bacteraemia could cause endocarditis. Urinary tract infection occurs in children with underlying urinary abnormalities or urine catheters, and a normal urinary tract. (71)

2.3.4.6 Coagulase-negative staphylococci

Coagulase-negative staphylococci (CoNS) is a group of over 40 bacteria, the most noteworthy are *Staphylococcus epidermidis*, *S. saprophyticus*, *S. haemolyticus*, and *S. lugdunensis*. *Staphylococcus epidermidis* is the most common species and is a part of normal skin commensal flora. It produces a biofilm on foreign material, and like *S. aureus*, the host defence depends on neutrophilic phagocytosis (bacterial killing). Infections in children with indwelling catheters, particularly in premature neonates and children with neutropenia, puts them at risk for CoNS infections. CoNS contribute to 50% of neonatal BSI. In otherwise healthy children, isolated CoNS are usually considered contaminants. (71)

2.3.4.7 Enterobacterales

Escherichia coli, *Klebsiella* spp., *Salmonella* spp., *Enterobacter* spp., *Serratia*, *Proteus* spp., *Citrobacter* spp., *Shigella* spp., *Hafnia* and *Morganella* are all members of the family of *Enterobacterales* colonising the intestinal tract. Members of this family vary from highly virulent pathogens to low virulent commensals that can still be considered significant in patients with underlying co-morbidities contracting infections during hospital care.

E. coli is the most commonly isolated species of the *Enterobacterales* and compromise >90 of all urinary tract infections. E. coli has become the most common pathogen among neonates and the second most common in children after the neonatal period in the U.S (54)

2.3.4.8 Haemophilus Influenzae

Haemophilus influenzae is a gram-negative bacterium that is either capsulated, with the outer lipopolysaccharide layer of the cell wall surrounded by a polysaccharides capsule, or non-capsulated. As for other capsulated bacteria, differences of the polysaccharides composition constitute a base for its identification together with its ability to escape or activate the host immune defence. Encapsulated *H. influenzae* isolates are classified into six serotypes, a, b, c, d, e, and f. Non-encapsulated *H. influenzae* colonises the nasopharyngeal mucus membrane in up to 80% of the population, and the capsulated form colonized 5–10% of the population before the HiB vaccine era. Type B was the most common and highly virulent serotype. Similar to the infection from other encapsulated bacteria, children aged between 6 months and 2 years, without protective maternal antibodies and with a lower ability for opsonisation and phagocytosis, are the largest risk groups for invasive infection. Individuals with asplenia, sickle cell anaemia, complement deficiency, and immunodeficiency are other risk groups for invasive infections. (68(82, 83)) Invasive infections could occur in individuals with risk or in children with a preceding viral upper respiratory tract infection. HiB infection with

meningitis, bacteraemia, epiglottitis, cellulitis, pneumonia, and osteoarthritis contributed to considerable morbidity and mortality before the introduction of the Hib vaccine.

The highly effective and safe protein-polysaccharide conjugate Hib vaccines have been used for 30 years and have almost eliminated Hib disease. Most Haemophilus infections are now due to the non-capsulated form, the non-typeable *Haemophilus influenzae*. (84))

2.3.4.9 Neisseria meningitidis

N. meningitidis colonises the nasopharyngeal mucus membrane in 20–25% of the adult population. Transmission occurs from an infected person to the person by close direct or indirect contact. Most contaminated individuals develop no or mild symptoms (sore throat and upper respiratory tract infection) in a few days, before developing protective antibodies and becoming asymptomatic carriers for some months. In up to 1–3 % of infected individuals, the bacterial acquisition leads to a more severe infection with septicaemia (25%), meningitis (15%), a combination of both symptoms (60%), or other focal infections like conjunctivitis. The lipopolysaccharide layer of the bacterial cell-wall has an endotoxin that causes immune activation.(71) However, it is also surrounded by a polysaccharides capsule that causes invasive infection. There are twelve encapsulated serotypes, 6 of which, A, B, C, W135, W and Y, cause the majority of invasive infections. The distribution of serotypes varies by geographical region . Serogroup A dominates the African meningitis belt, whereas serogroup B and C dominate Europe.

For an unknown reason, *N. meningitidis* is rare in Sweden but still life-threatening for those contracted with invasive infection. The crude incidence for invasive *N. Neisseria* infection is 0.6/100,000 individuals, which correspond to approximately 50–60 cases per year in Sweden, of whom 16% are 15–19 years old, and 11% are 0–4 years old. (85) Serogroup MenW is the most frequent isolated serotype.(86) Worldwide, serogroups MenB and MenC dominate. High endemic areas with >10 cases/100,000 individuals and epidemics with 1000/100,000 individuals occur. In countries with higher endemic rates, the mortality is reported to be 10%(87). Our low incidence of invasive *N. meningitides* implies our crude mortality rates of BSI

2.3.5 Age-dependent incidence

The overall incidence for BSI is approximately 140–160/100,000 individuals, based on relatively limited number of population-based studies, mostly in high-income countries and sometimes without calculating age-adjusted differences in incidence rates. (88) Studies in low-income countries reveal a far higher risk for BSI in children, with 8–38% with BSI of all hospital admission, but figures for incidence are not easily accessible. (84) For children in comparable settings the incidence is reported as follow: 3–4/1,000 live births, 0–28 days of age in Sweden (89), 2.3/1,000 for late-onset sepsis (LOS) in Italy (90), and 1.42/1,000 in France (91) The Swiss Paediatric Sepsis Study reports 1.43/1,000, 0–28 days of age, but only 0.28/1,000 for community-acquired LOS (92). The author concluded major differences in epidemiology, host characteristics, and patient-centred outcomes between EOS, hospital-acquired LOS, and community-acquired LOS. After the neonatal period, the incidence dropped to 0.21–0.57/1,000 children. (19, 40, 55, 56)

2.3.6 Community-acquired, Hospital-acquired or Healthcare-associated infections BSI in children

Infections identified from samples taken more than 48 hours after hospitalization, has since the early 70s, been categorized as hospital-acquired or nosocomial infections (HAI), and infections identified in samples within 48 hours after admissions as community-acquired infections (CAI). The definitions were implemented for surveillance of nosocomial infection. (93) The rationale for the distinction was evidence of a different aetiology between the two categories, with implication on empirical antimicrobial therapy, and an aim to find measures to prevent nosocomial infection, increase the quality of hospital care and improve treatment outcome (94, 95). CAIs were predominantly due to organism with virulent pathogens capable of infecting otherwise healthy persons. HAI was associated with exposure to invasive devices and procedures with staphylococcus and gram negatives as the dominant flora, table 4. (95)

Table II. Frequency of isolation of pathogenic organisms from hospital-acquired infections

Organisms	*No. of isolates*	*Site of infection (%)*			
		Wound	*Urinary tract*	*Respiratory tract*	*Skin*
Mixed organisms		16	6	7	3
No organisms		8	—	52	32
S. aureus	117	48	4	13	29
S. epidermidis	12	—	—	—	—
Group A streptococcus	7	—	—	7	—
Enterococcus	8	1	4	—	2
Pneumococcus	6	—	—	5	—
H. influenzae	9	—	—	5	2
E. coli	108	20	43	4	7
Klebsiella-Enterobacter	84	17	20	7	3
Proteus sp.	32	8	14	1	7
Serratia marascen	14	1	7	1	2
Salmonella sp.	13	—	—	—	—
P. aeruginosa	50	9	8	8	12
C. albicans	27	2	6	3	10
Other	9	2†	—	1†	2‡

*Nine peritonitis, 1 mediastinitis, 10 eye infections, 31 thrush.
†Represents 1 *Herellea vaginicola*, 2 streptococcus (not Group A or D).
‡Represents 1 Flavobacteria.
‖Represents 3 Erwinia sp., 3 Flavobacteria, 1 streptococcus (not Group A or D).

Table 4 Distribution of pathogens in hospital acquired infection. Garner et al. The Journal of pediatric 1972

Children at higher risk for HAI include those with invasive devices and underlying co-morbidities requiring hospital care, those with a breached host defence, or those who are immunocompromised. These groups of patients have increased over time, and the health care organisation has developed, resulting in lengthy hospital stays and increased outpatient and home treatment of complex conditions involving invasive devices. This change has resulted in typical HAI infections presenting as a CAI, and the CAI/HAI dichotomy does not reflect the most plausible aetiology in BSI. (96) Health care-associated infections (HCA or HCAI) have, therefore, been introduced. HCAI are infections that occur because of health care. Still, there is inconsistent use of these criteria, and there is a lack of evidence to distinguish between CAI, HCA and HAI BSI in children. (96)

2.4 ANTIMICROBIAL RESISTANCE

2.4.1 Definitions

Antimicrobials are a group of drugs that antagonise the growth of living microorganism, including bacteria (antibiotics), viruses (antivirals), fungi (antifungals) and parasites (antiparasitals). Antimicrobial resistance (AMR) is the ability of a microorganism to resist the action of an antimicrobial agent. The microorganism tries to adapt to its environment to survive. In the case of bacteria, antibiotic resistance (ABR) is the ability of bacteria to resist the action of an antibiotic. There are several classes of antibiotics that act on different targets and are designed for certain bacteria. Some bacteria are intrinsically resistant to certain antibiotics. The global threat of AMR is when susceptible bacteria acquire resistance through genetic changes because of their adaption under the antibiotic stress. Heavy use of antibiotics acts as a driver for the development of acquired antimicrobial resistance. Once the microorganism becomes resistant, it can spread between humans, animals, and the environment. If a bacterium is resistant to three or more classes of antibiotics, it is called multidrug-resistant (MDR). ABR occurred soon after antibiotics were discovered. An overview of antibiotics and the following resistance is shown in Figure 3.

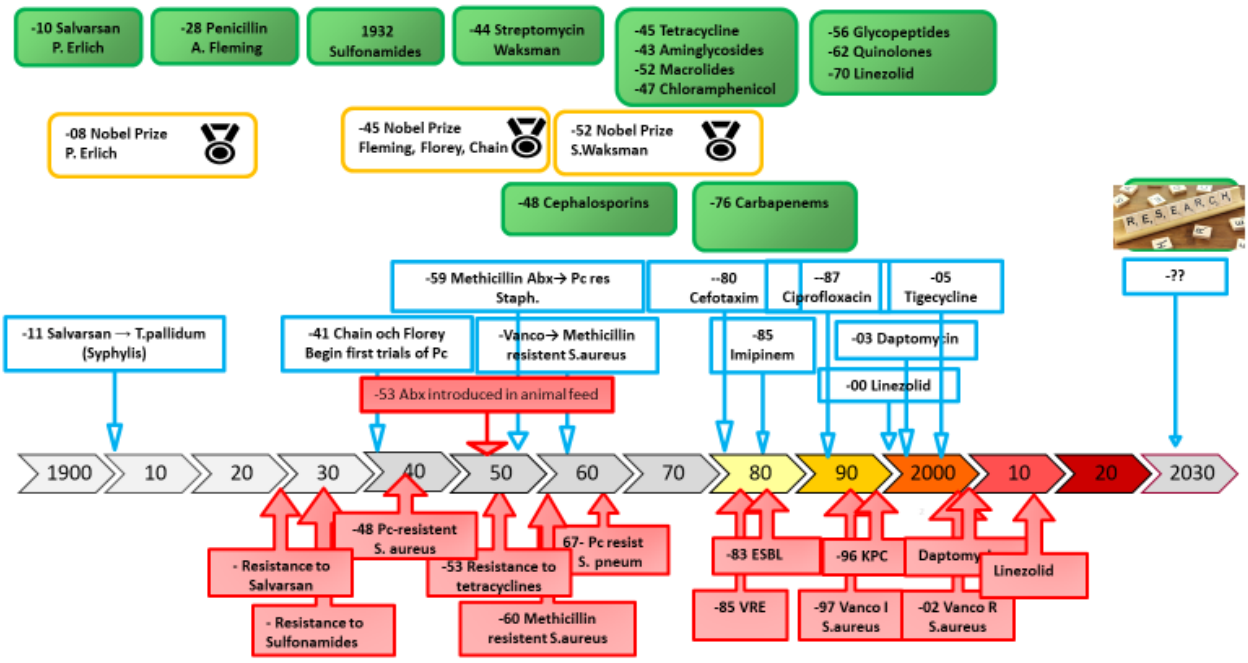

Figure 3 A summarised overview of the introduction of antibiotics and subsequent resistance

2.4.2 The burden of antimicrobial resistance

AMR challenges effective therapy against infections, and reports reveal an enormous global burden. National reports from five out of six WHO regions report *E.coli* and *Klebsiella pneumoniae* are 50% resistant against third-generation cephalosporins, fluoroquinolones, whereas *S. aureus* is resistant against methicillin. (97) Globally, 700,000 people are estimated to die because of antimicrobial-resistant infections every year, with 50,000 deaths occurring in Europe and the U.S. (98) The estimation of the global burden of AMR is, in general, based on studies involving low sample size, and patients with severe infections, (97). The estimated

burden of AMR is partly extrapolated from European data. Such extrapolation may lead to uncertain results, emphasising the need for more comprehensive antimicrobial surveillance data at a local level for both community-acquired infections and healthcare-associated infections. (99, 100) To tackle the threat of AMR and the need for surveillance data, the WHO launched a global antimicrobial resistance surveillance system (GLASS), providing a platform for collection, analysis, and sharing of standardised AMR data. (101) The European data on AMR is retrieved from hospitals across Europe. These are mostly tertiary hospitals, and the data regarding invasive infections diagnosed in the hospital are reported to the European Antimicrobial Resistance Surveillance Network (EARS-net). The European Centre for Disease Prevention and Control (ECDC) publishes this data. The ECDC estimated that 670,000 infections occur in the EU/EEA due to bacterial resistance to antibiotics, and approximately 33,000 people die annually, as a direct consequence of AMR infections. Many of these infections (63%) were considered hospital-acquired infections (HAIs). AMR infections have increased in all countries reporting to EARS-Net. (102) From a European point prevalence survey in 2016–2017, 8.9 million HAIs in hospitals and long-term facilities were identified. A microorganism was isolated in 53% of hospital-admitted patient, one-third of the bacteria associated with HAI were resistant to antibiotics, and more than half of the HAIs were considered preventable. (103)

2.4.3 Development of resistance

The emergence of AMR is, as mentioned, a natural evolutionary response to antimicrobial exposure. Antibiotic overuse and inappropriate antibiotic use are the main reasons for the increase in AMR. Studies over the antibiotic use in several European countries report that up to 50% of the prescribed antibiotics could be considered unnecessary or inappropriate(104-107).

In countries with high AMR occurrence, there is also a high antibiotic consumption. In the WHO report on surveillance of antibiotic consumption 2016–2018, the total antibiotics consumption in humans ranged from 4.4 to 64.4 Daily Delivered Doses (DDD)/1000 inhabitants. Third-generation cephalosporins, quinolones and carbapenems, categorised as "Watch antibiotic" due to their high potential for AMR development, accounted from less than 20% to over 50% of antibiotics consumed. (108). In Europe, the average total consumption of antimicrobials in 2018 was 20.1 DDD/1000 inhabitants with a country range of 9.7–34.0 DDD. (109) The total consumption in Sweden was 11.1 DDD/1000 inhabitants in 2019 with a decreasing trend since 2011; a trend also evident in children. (110) The differences in antibiotic consumption between countries are substantial when it comes to consumption in the community. The consumption in hospital settings varies little. In 2018, the EU/EEA population-weighted, mean consumption of antibacterials for systemic use in the hospital sector was 1.8 DDD/1000 inhabitants per day, ranging from 0.8 in the Netherlands to 2.5 in the U.K., and 1.65 in Sweden. (111) Paediatric antimicrobial use is declining in Sweden (110). Antimicrobial consumption globally remained relatively constant in high middle-income countries between 2011 and 2015. (112) Still, the prevalence of AMR increased.

Antimicrobial usage in animals is a key contributor to total antimicrobial consumption. Sweden prohibited the administration of growth-promoting antibiotics to healthy animals in 1986. The sales of antibiotics for administration to animals in Sweden were 9.5 tonnes, or 12

mg/kg animal in 2019 (compare to 61 tonnes or 90 mg/kg in humans). (110) The median sale in European countries was 61.9 mg/kg, range 3.1–423 mg/kg. The countries with the highest sales for animal use also had the highest occurrence of AMR. (113) Statistical modelling estimated a global average of 365 mg/kg antibiotics used in 2010, with a projected 67% rise until 2030. (114) A study comparing data of veterinary antimicrobial use with national reports of AMR prevalence found a strong positive correlation between AMR and antibiotic use in animals, also suggesting that antimicrobial use in animals is capable of explaining variations amongst AMR status between nations (115). The causality has been debated but has also been supported (116-118) and considered to be established. (119)

2.4.4 Interactions between antimicrobials and antibiotic

Antimicrobial agents, antibiotics, all have a mutual aim to kill bacteria. The killing is achieved through a variety of mechanisms of action, target sites, chemical structures, and bacteriostatic (inhibiting) or bactericidal (killing) effects. Bacteriostatic antibiotics need a functional immune system to achieve sufficient efficacy or a complementary antibiotic. There are five main target sites for antimicrobial action; (120)

- Cell wall synthesis
- Protein synthesis
- Nuclein acid synthesis
    - DNA or RNA
- Metabolic pathways
- Cell/plasma membrane function

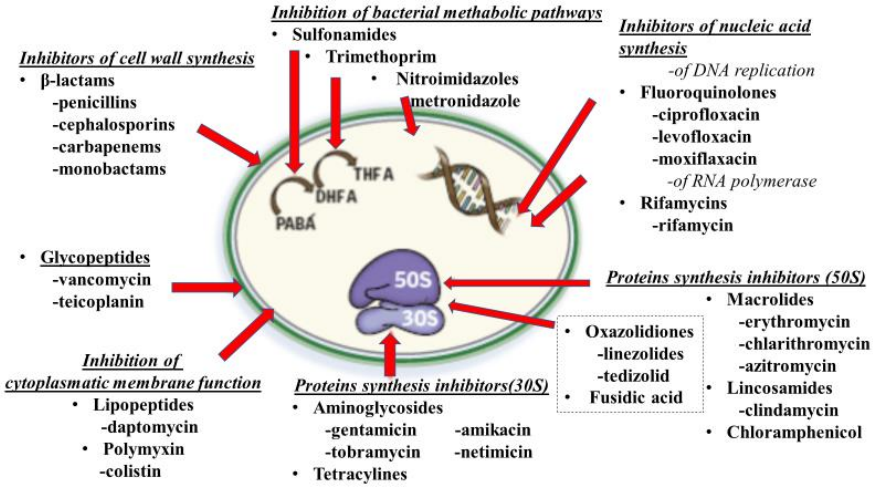

Figure 4 Target sites for antimicrobial actions

Bacteria are prokaryotic organisms. In contrast to the eukaryotic organism, prokaryotes do not have a cell nucleus. Bacterial DNA is formed in a single circular chromosome, and additional DNA is carried in plasmids. The plasma membrane is covered by a protective cell wall. The primary and unique component of the bacterial cell wall is peptidoglycan. In gram-positive bacteria, the peptidoglycan forms a thick layer surrounding an inner plasma membrane. In gram-negative bacteria, the peptidoglycan layer is thin and overlaid by lipopolysaccharide and lipoprotein layer. The thick hydrophilic cell wall in gram-positive bacteria retains the violet colour while gram-negative bacteria lose the violet colour and appear pink under a microscope, following gram staining. External to the cell wall, some bacteria have a protective polysaccharide capsule. The pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae* type B, *Neisseria meningitidis* are examples of bacteria with a capsule that protects against phagocytosis by host cells. Those layers are important for the characteristics of the bacteria. The cell walls and their reproductive process are also the targets for antimicrobial agents. (120)

2.4.4.1 Inhibitors of cell wall synthesis

Synthesis of the bacterial cell wall includes several steps, including the production of cell wall subunits, transport through the plasma membrane and insertions into the wall. The different stages of synthesis are potential targets for antimicrobial actions. Inhibitors of cell wall synthesis are active against bacteria that are in their growth phase. The most important antimicrobials are listed below.

• β-lactams - penicillins - cephalosporins - carbapenems - monobactams	➢ Bind to Penicillin Binding Proteins (PBPs) in the cell wall ➢ Gram-negatives; β-lactams enter the outer membrane through porin channels and reach the cell wall. ➢ Gram-positives lack outer membrane binds directly to PBP	➢ Lysis of the cell wall
• Glycopeptides - vancomycin - teicoplanin	➢ Binds to terminal D-ala-D-ala in cell wall subunits	➢ Prevents incorporation of subunit and inhibit the synthesis of cell wall

2.4.4.2 Protein synthesis inhibitors

Several antimicrobials inhibit different steps in the synthesis of essential bacterial components, from chromosomal material to proteins. They are separated into two groups, those acting on the ribosomal subunit 30S, or 50S, of the bacterial ribosome complex. Aminoglycosides inhibit bacterial RNA in two ways, leading to their bactericidal effect. Other protein synthesis inhibitors have a bacteriostatic effect.

30S	• Aminioglycosides - gentamicin - tobramycin - amikacin - netilmicin • Tertracyclines	50S	• Macrolides • Lincosamides - clindamycin • Chloramphenicol
			Interferes with the formation of the initiation complex between 50S and 30S subunits
			• Oxazolidiones - linezolid - tedizolid
			Binds to elongation factors
			• Fusidic acid

2.4.4.3 Inhibitor of nucleic acid synthesis

This mechanism of action includes inhibition of DNA replication or RNA polymerase activity. Fluoroquinolones interfere with DNA synthesis by blocking DNA gyrase (more effective against gram-negative bacteria) and/or topoisomerase IV (more effective against gram-positive bacteria). Rifampicin, another nucleic acid synthesis inhibitor, binds to RNA polymerase. In both cases, the cell dies, and the effect is bactericidal.

➢ Inhibition of DNA replication	➢ Inhibition of RNA polymerase
• Fluoroquinolones - Ciprofloxacin - Levofloxacin - Moxifloxacin	• Rifamycins - rifampicin

2.4.4.4 Inhibition of bacterial metabolic pathways

Both sulfonamides and trimethoprim inhibit precursors in nucleic acid production and have a bacteriostatic or bactericidal effect. Metronidazole is a prodrug that activates in anaerobic cells leading to reactive compounds that interact with nucleic acid and proteins and have a bactericidal effect.

• Sulfonamides	➢ Blocks enzymatic activation required for the synthesis for purines and pyrimidines and thereby the synthesis of nucleic acid synthesis
• Trimethoprim	➢ Same as sulfonamides but downstream synthesis pathways
• Nitroimidazoles - metronidazole	➢ interaction and breakdown with bacterial DNA

2.4.4.5 Inhibition of cytoplasmatic membrane function

• Lipopeptides - daptomycin	➢ Depolarised and disrupting the cytoplasmatic membrane in gram-positive bacteria, with a bactericidal effect
• Polymyxin - colistin	➢ Disrupt the phospholipid structure in gram-negative cell membranes, with a bactericidal effect

2.4.5 Different mechanism of AMR

In the context of AMR, bacteria have three fundamental ways to respond to antibiotics; they are either susceptible or naturally resistant, or they acquire resistance. Natural or intrinsic resistance restricts antimicrobial drug activity for all members of a specific species. Acquired resistance is the bacteria's way to escape from the threat of antibiotics and survive. Bacteria develop resistance as an adaption to survive antibiotic pressure; favouring those with the ability to resist the antibiotics. Resistance arises from bacterial chromosomal mutations. These mutations result in the synthesis of altered proteins that increase the bacterial ability to resist antibiotic killing or inhibition. Bacteria can develop resistance in different ways.

- Production of enzymes
  - β-lactamases, including ESBL, are enzymes that inactivate β-lactam antibiotics by hydrolysing their β-lactam ring, inhibiting their ability to bind to PBP in the cell wall.
  - Aminoglycoside modifying enzymes
  - Macrolide- and lincosamide inactivating enzymes
  - Chloramphenicol acetyltransferases
  - Quinolone inactivating enzymes

- Alteration of outer-membrane permeability in gram-negative bacteria resulting in inhibited passage of antibiotic molecule into the cell.
- Alteration of target sites
  - PBPs, mutations in the synthesis of PBP alter proteins and impair binding of β-lactam antibiotic to PBP in the cell wall.
  - Methylation of ribosomal RNA confers resistance to macrolides and lincosamides
  - Mutations in the chromosomal genes for DNA gyrase confer resistance to fluoroquinolones

- Efflux pumps, mediated by transmembrane protein channels that actively export antimicrobial agents out of the cell. This is the main mechanism for resisting tetracyclines, and also a mechanism for resisting macrolides and fluoroquinolones.

- Alteration of metabolic pathways, caused by mutations in the antimicrobial chromosome, leading to an alternative way for synthesis a step inhibit by a particular antibiotic—one way of resistance to sulfonamides and trimethoprim.

These resistance mechanisms affect the antibiotic action in three main ways; 1; The target site may be altered, resulting in a lower affinity for the antibiotic; alternatively, an additional target may be synthesized. 2; Altered uptake with decreased permeability of the cell wall or pumping out the drug with efflux mechanism. 3; Drug inactivation where enzymes modify or destroy the antibacterial agent, table 5. (120)

Table 5 Mechanism of resistance of commonly prescribed antibiotics.

Antibiotic, examples	Mechanism of resistance		
	Altering target	Altered uptake	Drug inactivation
Beta-lactams	+	+	+
Glycopeptides	+		
Aminoglycosides	+	+	+
Macrolides	+	+	+
Tetracyclines	+	+	
Lincosamides	+		
Sulfonamides/Trimethoprim	+	+	
Fluorquinolones	+		+

2.4.6 Transmissions of resistance between bacteria

Once a bacterium has acquired a resistance mechanism, its next generation will inherit the genes. The resistance can also be transmitted to other bacteria. The new genetic material carrying the resistance mechanism is exchanged between bacteria through three main routes, summarized in Figure. 5.

Bacterial transformation includes uptake of free genetic material from the environment and its incorporation into the bacterial chromosome. During transduction bacteriophages (viruses that infect bacteria) infect a resistant bacterium and incorporate bacterial genetic material in new phages that are then able to transfer the resistance genes to another bacterium. Finally, conjugation implies that two bacteria conjugate and resistance genetic material, encapsulated in plasmids, are transferred from one bacterium to the other.

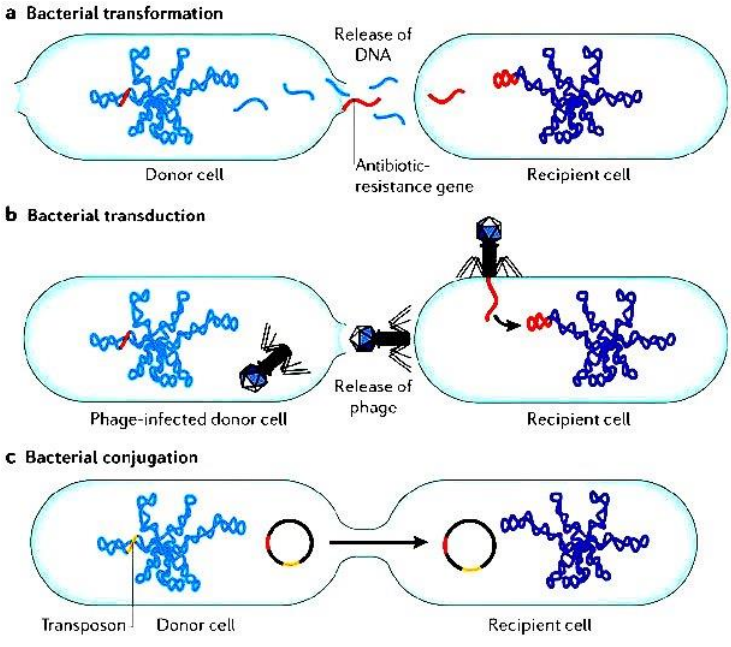

Figure 5 Exchange mechanism of genetic material from one bacterium to the other. Reprint with permission provided by Springer Nature and Copyright Clearance Center

2.4.7 Resistance of clinical importance

Every bacterium is resistant to some antibiotics. A group of bacteria causing BSI has been identified, requiring special attention: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp., ESKAPE-pathogens. The ESKAPE pathogens are a group of infectious bacteria that have garnered particular attention for their ability to escape or evade standard therapies through AMR, and their increasing prevalence in BSI. In the U.S, the ESKAPE pathogens contribute to 42.2% of bacterial isolates in BSI, and infected patients have an increased length of hospital stays and worse outcomes, compared to non-ESKAPE pathogens. (121) These bacteria are of particular significance in hospital-acquired or healthcare-associated infections. From a paediatric point of view, AMR in *Escherichia coli*, *Streptococcus pneumonia* and *Enterococcus faecalis* are also important. (102)

2.4.7.1 MRSA

After the discovery of penicillin in 1928, penicillin G was available for general use in the U.S in the 1940s. In 1948 *Staphylococcus aureus* started to show resistance to penicillin due to production of β-lactamases. In 1959, methicillin was discovered as one of several semi-synthetic penicillin molecules active against β-lactamase/penicillinase-producing *Staphylococcus aureus*. Shortly after methicillin was introduced, strains of *S. aureus* acquired a gene, *mecA*-gene, encoding for a penicillin-binding protein (PBP2a). PBP2a have a low affinity to penicillin, which abolished antibiotic incorporation in the cell wall. (122) The *mecA* gene is regulated by several factors; one is the presence of methicillin, and other is penicillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) was sporadic in children until the 1990s, when endemic community-acquired staphylococcal infections first were noticed(123). *Staphylococcus aureus* is the most frequently isolated gram-positive bacteria causing BSI in both adults and children, with the highest incidence rate in infants < 1-year-old, and older individuals. The incidences are relatively similar from different regions, but the proportion of MRSA varies with regions and country (64, 124). In adults the mortality rate of MRSA BSI is described to be higher than Methicillin susceptible *S. aureus* (MSSA). (84) In children. mortality in both MSSA and MRSA BSI is low, and MRSA is not a risk factor for increased mortality. (125, 126) Endocarditis as a complication of *Staphylococcus aureus* bloodstream infections (SAB) is rare in infants, compared to adults, but together with pneumonia is associated with higher mortality. (125, 126)

	Global	**US**	**Europe**	**Sweden**
MRSA			16.4%[127]	1,9%[127]
Paediatric data	15%[125], Israel 13% [126], Australia, NZ 3.1%[128], Gambia	19-38%[129, 130]	16.4% [18] 7.6% [131]	-

2.4.7.2 Penicillin-non-susceptible Streptococcus pneumoniae

Streptococcus pneumoniae has been one of the dominant pathogens in paediatric bacteraemia worldwide, with considerable morbidity and mortality. Since the introduction of the Pneumococcal conjugate vaccine (PCV) targeting the most prevalent serotypes, the incidences of invasive pneumococcal diseases have decreased significantly. However, *S. pneumoniae* still has a substantial burden in paediatric sepsis (75) After the introduction of the vaccine, non-vaccine serotypes, rather than vaccine-types cause invasive pneumococcal diseases. Whole-genome sequencing studies have revealed how *Streptococcus pneumoniae*, adapted to its environment with changes in virulence beyond serotypes, resulting in the invasive capacity of serotypes that were noninvasive before PCV introduction. Antibiotic resistance increased in non-vaccine serotypes, causing invasive pneumococcal diseases against both erythromycin and penicillin. (132). The mechanism of penicillin resistance consists of alterations in the PBPs resulting in a reduced affinity to penicillin and a variable spectrum of other β-lactams. The term non-susceptible refers to both susceptible with increased exposure (higher dose), and resistant. (127)

	US	**Europe**	**Sweden**
PNSP		0.1-40%[127]	5.2%[18, 127]
Paediatric data	20%[133]	13.4% [18]	

2.4.7.3 VRE

Enterococcus spp. BSIs are closely related to healthcare-associated infections, with catheter-associated UTI, central line associated-BSI, intra-abdominal infections in patients with underlying disease, and immunosuppression constituting up to 10% of HAI BSI (134, 135) Vancomycin-Resistant Enterococcus faecium (VRE) is associated with an increased risk for in-hospital mortality and length of stay. (136) The risk for VRE correlates with exposure to vancomycin, fluoroquinolones, and meropenem(137) In children *Enterococcus* BSIs are caused by vancomycin susceptible *Enterococcus*, and neither mortality nor length of stay increases, but risk factors for VRE are similar. (138)

	US	**Europe**	**Sweden**
VRE	30% [139]	17.3%[127]	1.4% [18, 127]
Paediatric data		9.0% [18]	

2.4.7.4 ESBL

Extended-spectrum β-lactamase (ESBL) is a group of enzymes conferring resistance to β-lactams; penicillin, cephalosporins and monobactam, but not carbapenems. Most ESBLs are inhibited by a β-lactamase inhibitor (clavulanic acid, tazobactam and sulbactam). β-lactamase genes are mostly carried in plasmids, mobile genetic element, with a high ability for transmission through bacterial conjugation. The first ESBL-producing strain was identified in 1983 in Germany and had since then effectively spread all over the world. They are most

commonly found in important clinical pathogens, *Escherichia coli* and *Klebsiella pneumoniae*, but are also found in other gram-negative bacteria, mostly in other Enterobacterales *(Proteus, Enterobacter* spp., *Citrobacter Serratia, Salmonella, Shigella, Morganella* and other Enterobacteriaceae) and also in *Pseudomonas* and *Acinetobacter*. There are several groups of ESBL enzymes with a difference in activity against different pathogens. The different types also have different geographic distribution. The main classes of ESBL are listed below. All classes have numerous variants. In clinical microbiology, the screening breakpoint to suspect ESBL production is a minimal inhibitory concentration (MIC) of >1 mg/L for two third-generation cephalosporins. (122)

- $ESBL_A$.
    - CTX-M (cefotaximase)
    - SHV (Sulfhydryl variable)
    - TEM (Temoneira, =index petient)

- $ESBL_M$
    - Plasmid-AmpC
- $ESBL_{CARBA}$, (listed below)

The proportion of ESBL in Enterobacterales in children is increasing, but with a geographical difference in prevalence. Invasive isolates associated with ESBL have a higher multidrug resistance compared to screening isolates. (140, 141) Prior hospitalization, long hospital stay, indwelling catheters, and antibiotic use have been described as risk factors in adults. In children, the risk factors include a recent visit to a healthcare centre, underlying comorbidity, NICU understaffing and poor low hygiene standards. (142, 143)

	Global	**U.S.**	**Europe**	**Sweden**
ESBL, in invasive isolate			15.1% [127]	8.3% [127]
Paediatric data	29% [144] South Africa 66% [145] Neonate, Iran 67.% [146] Neonates, Taiwan 87% [147] Neonates, India	12% [142]	12.9% [18]	-

2.4.7.5 Carbapenemase-producing Enterobacterales (CPE)

Due to the spread of ESBL-containing bacteria, the use of carbapenems has increased, with a subsequent increase in carbapenem-resistant Enterobacterales. Carbapenemases are β-lactamases with the ability to hydrolyse the β-lactam ring in penicillin, most cephalosporins, and to varying degrees in carbapenems and monobactams. They were first found in the 90s and are mostly found in invasive *Klebsiella pneumoniae* infections. With high rates of incidence in some European countries, Greece reported 63.9% of all invasive Klebsiella isolates as *Klebsiella pneumoniae* carbapenemases (KPC) in 2018, but considerably lower in most other European countries (127). There are several classes of carbapenemases,(122)

- $ESBL_{CARBA}$
    - Metallo β-lactamases (MBL)
    - *Klebsiella pneumoniae* carbapenemases (KPC)
    - OXA-48

Geographic trends in the distribution of carbapenemases in children are described to be similar in adult and paediatric population(148).

	Global	**US**	**Europe**	**Sweden**
E-coli	<1%[149]	<0,1%[150]	0.1%[18]	<0.1% [127]
Klebsiella	4%[149]	0.6%[150]	7.5.5% [127]	0.2%[127]

Patient-level risk factor for CPE in children is, as for ESBL, including underlying chronic medical condition, invasive medical devices, frequent or prolonged hospitalisations, prior antibiotic exposure and travel from endemic regions. (148)

2.5 EMPIRIC ANTIMICROBIAL THERAPY

2.5.1.1 Antibiotic

Empiric antibiotic indicates the antimicrobial therapy chosen without knowledge of the causing organism. Well-chosen empiric antibiotics should provide a good cover for the most suspected pathogens, and consider the local pattern of antimicrobial resistance, and still be as narrow in the antibacterial spectrum as possible.

Of equal importance are pharmacokinetic and pharmacodynamic (PK-PD) consideration. What does the body do with the drug (PK)? What does the drug do to body (PD)? Pharmacokinetics (PK) is the relation between an administrated dose and the achieved concentration at the site of infections and comprises absorption, distribution, metabolism, and excretion of the drug. PK differ with age; infants have large distribution volume, low protein binding and immature renal excretion and metabolism. PK also includes the drug's ability to passage over membranes and barriers to the site of the infection, together PK properties affect the amount and administrations interval of dosing.

Pharmacodynamics (PD) describes the relationship between antimicrobial concentration and its killing effect. It includes the drug's mechanism of action. Only the free fraction, nonprotein bounded, part of the drug has an antibacterial effect. The effect varies in three principal different ways. (71)

- T>MIC
  - Duration of when a drug concentration remains above the minimum inhibitory concentration (MIC).
  - To achieve a killing effect, time over MIC should be >40% of the time.
  - Higher concentration does not improve the killing effect.
  - β-lactams, macrolides and lincosamides are examples of antibiotics for this time-dependent killing.
- C_{max}/MIC
  - Maximal drug concentration over MIC
  - The higher peak concentration, the higher killing effect but also increased risk for toxicity.
  - To achieve killing the concentration should be 2-4 times MIC for effective killing.
  - Aminoglycosides, have this concentration-dependent killing.
- AUC/MIC
  - Area under the serum concentration-time Curve.
  - A combination of time and concentration dependent killing.
  - The killing is correlated to time during 24 hours that remains over MIC.
  - Glycopetides, quinolones, linezolid belong to this group

2.5.1.2 Early aggressive vs Watchful waiting

Febrile infants less than 3 months of age have the highest risk for serious bacterial infection and BSI. The risk is inversely correlated with age, with up to 20% of those < 1 month of age with fever without an identified clinical source, have a serious bacterial infection. (151). One important question is if all children below a certain age ought to undergo a full septic workup and be admitted with early aggressive therapy for a possible severe infection, or if there is an opportunity for select low-risk cases in the high-risk population. Strategies for identification of those with high risk for a possible severe infection has been scrutinised in several studies, and different decision trees have been validated. (152-155) It seems that with a thorough clinical examination, a blood test with one, or a combination of the inflammatory parameters CRP and PCT, urine analysis, the probability to exclude a severe infection is high.

A low probability for a severe infection could promote a watchful waiting strategy and a possibility to withhold antibiotic therapy instead of early aggressive empiric therapy. To adapt a watchful waiting strategy, it is critical to facilitate serial clinical and laboratory assessment among well-appearing infants, also infants < 1 month of age. A watchful waiting strategy could reduce unnecessary exposure to broad-spectrum antibiotics. (156, 157)

2.6 PREVENTION OF BLOODSTREAM INFECTIONS

2.6.1.1 Vaccination

Immunisation against pathogens that cause the most prevalent and severe infections is an effective way to decrease morbidity and mortality. Before the introduction of Haemophilus influenzae type B (HiB) vaccine, the global burden of HiB infections was enormous. Severe HiB-infections (i.e. meningitis, bacteraemia, pneumonia, epiglottitis, cellulitis, and osteoarthritis) contracted approximately 2.2 million children 0–4 years of age yearly, with up to 23% mortality depending on the type of infection. The global incidence of HiB-infections was approximately 370/100,000 children 0–4 years of age and 31/100,000 HiB meningitis; 500,000 children died every year just because of HiB infections. (83) The vaccine effectiveness was 95%, and in countries with high coverage, the declining effect on the burden was magnificent. From a Scandinavian perspective, it reduces HiB meningitis from close to 500 cases annually before the immunisation to less than ten cases per year within five years after the vaccine was implemented. (82, 158)

During our study period vaccine against *Streptococcus pneumoniae* and *Neisseria meningitidis* had been implemented, with a comparative effect for each pathogen. In the U.S pneumococcal conjugate vaccine (PCV) reduce *S. Pneumoniae* bacteraemia with 95% in children 3–36 months of age from an incidence of 74.4/100,000 children before to 3.5/100,000 children 3–36 months of age after the implementation of PCV (Fig. 6a & 6b). (55) This induced a decline incidence for all bacteraemia from 97/100,000 children before to 21/100,000 after PCV. The drop in incidence resulted in reduced risk bacteraemia from up to 5% to less than 0.5% for a child 3–36 months of age visiting the emergency department because of fever in setting with high PCV coverage. (69) Universal coverage with the pneumococcal conjugate vaccine to children is estimated to avert up to 11.4 million days /year of antibiotics for pneumonia caused by *Streptococcus pneumoniae* in children under five years of age, a 47% reduction in days of antibiotics. (159)

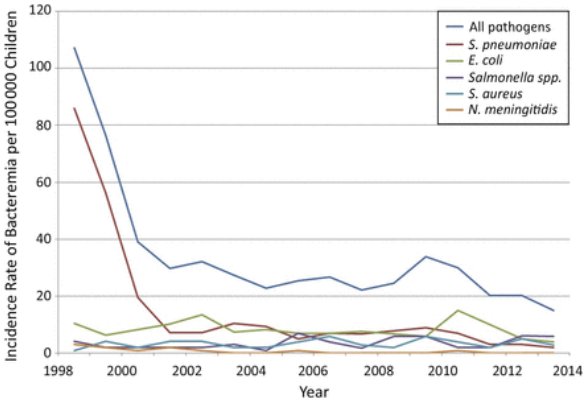

Figure 6a Rate of all bacteraemia by organism per 100 000 children per year between 1998 and 2014

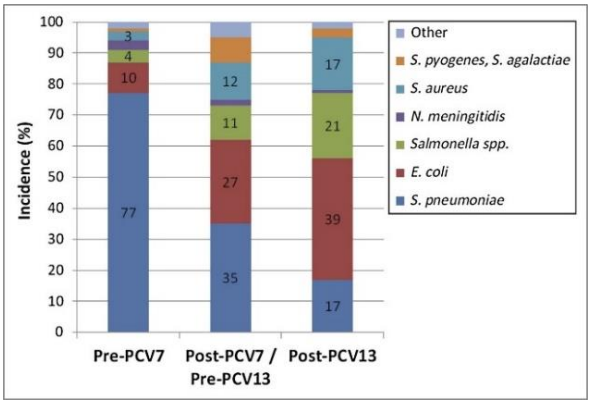

Figure 6b Relative incidence of bacteraemia by organism per study period (pre-PCV7, post-PCV7/pre-PCV13, and post-PCV13) Printed with permission

In Sweden incidence for invasive pneumococcal disease (IPD) (including positive culture from blood, from cerebrospinal fluid and other sterile fluids) declined from 28.4/100,000 children 0–2 years of age to 4.3/100,000 after vaccine introduction, and for bacteraemia from

6.3 to 2.0/100,000 children 0–2 years of age (160). For unknown reasons *N. meningitidis* is rare in Sweden but is still life-threatening for those contracted with invasive infection. The mortality rates are reported to be 5-10%. In countries with higher incidence, vaccines have been implemented. In the U.K the hospital admissions of *N meningitidis* infection decrease by 66% after the introduction of the meningococcal serogroup C vaccine, from 34.5 admission/ 100,000 children in 1999 to 12.4 /100,000 children in 2011. (61)

2.6.1.2 Prophylaxis to identified risk groups

Streptococcus agalactiae (GBS) emerged as the leading cause of new-born infection in the U.S. the 1970s. In the 1980s, clinical trials demonstrated that giving intrapartum intravenous ampicillin or penicillin to mothers at risk was highly effective at preventing invasive GBS disease in the first week of life (early-onset). Systematic review and meta-analysis found a risk of EOGBS of 1.1% (95% CI, .7%–1.6%) for new-borns born to women colonised with GBS in pregnancy without a policy of providing IAP for positive GBS screening. As IAP coverage increased the risk of EOGBS decreased, with a linear relationship.(76) The risk reduction ranged from 40–79% after implementation of IAP, with the most prevalent reduction in setting with high coverage of microbiological-screening-based IAP policy.(161) In Sweden, a risk-based IAP program was implemented in 2008.

Invasive fungal infections account for 10 % of all cases of late-onset invasive infection in very low birth weight (VLBW) infants. (162) Antifungal prophylaxis with intravenous fluconazole is proven to be effective for the prevention of invasive candida infection in VLBW and ELBW neonates.(163-165) Oral nystatin has been evaluated as effective as fluconazole in reducing invasive fungal infection in VLBW neonates. Still, these results have been interpreted as uncertain and larger, higher quality trials are recommended by Cochrane, to resolve this uncertainty. (166)

Invasive fungal infection is also a problem for paediatric patients with cancer and those undergoing haematopoietic stem cell transplantation. Evidence for antifungal prophylaxis in children is lacking but recommended for children undergoing haematopoietic stem cell transplantation, acute myeloid leukaemia/myelodysplastic syndrome and relapsed acute lymphoblastic leukaemia. (167, 168)

3 AIMS

3.1 GENERAL AIMS

The general aims of this thesis were to describe the aetiology, risk factors, and occurrence of bacteria resistant to antimicrobial therapy in children with bloodstream infections.

3.2 SPECIFIC AIMS

3.2.1 Paper I

To study the occurrence and aetiology of BSI in children aged 0–17 years, and to describe the influence of age and underlying comorbidity.

3.2.2 Paper II

To study the effect of introducing immunisation against *Streptococcus pneumoniae* on the occurrence of BSI, and the implementation of a risk-based strategy with intrapartal antibiotic prophylaxis targeting early-onset sepsis caused by *Streptococcus agalactiae*

3.2.3 Paper III

To evaluate the quantity and quality of antimicrobial prescriptions at our hospital with an aim to identify targets to improve the quality and reduce the quantity of antimicrobial use.

3.2.4 Paper IV

To study changes in aetiology and incidence of BSI over a 20-year period. Implementations of preventive measures and changes in patients mix for hospital admitted patients are hypothesised to result in change with implication for designing empiric antibiotic therapy regimes, and for the planning of targeted measures to improve therapy and preventions of BSI.

3.2.5 Paper V

To study the occurrence and trends of antimicrobial resistance and antibiotic prescription in children hospitalised with BSI during 20 years at Astrid Lindgren Children's Hospital.

4 MATERIAL AND METHODS

4.1 STUDY SUBJECTS AND METHODS

4.1.1 Paper I

4.1.1.1 Patients and study design

The study was a retrospective, single-centre descriptive analysis. We included all children aged 0–17 years, with positive blood cultures, admitted to Astrid Lindgren Children's Hospital between July 1, 1998, and June 30, 2008. The Hospital serves a primary paediatric population of 220,000 children in northern Stockholm and is the regional centre for cancer treatment, paediatric surgery, and intensive care serving 440,000 children. Patient identity in children with positive blood cultures was obtained from the laboratory information system (LIS). Information regarding comorbidity, focal sites of infection, paediatric intensive care unit admission, and mortality was retrieved from the hospital records.

4.1.1.2 Definitions and inclusion criteria

An episode of bloodstream infection was defined as the presence of an accepted pathogen isolated in blood culture, associated with clinical signs of infection. If the same pathogen was found in another sample taken 14 days after the previous sample, or if any other species were isolated on any other day, those isolates were considered to represent a separate episode. If the clinical pictures were not associated with infection or a targeted antimicrobial treatment was not prescribed, the recognised pathogen was regarded as contaminants or transient bacteraemia, not requiring treatment, and not included in the study. According to the CDC criterion, two or more positive blood cultures are required to include possible contaminants. In children, two blood cultures are rarely drawn before the start of antibiotic therapy. Possible contaminants like Coagulase-negative staphylococci (CoNS) viridans streptococci, *Bacillus* species, *Propionibacterium*, *Corynebacterium,* and *Micrococcus* species were therefore considered not-accepted pathogen and not included.

The children were categorised into three groups: (i) No underlying comorbidities, (ii) underlying comorbidities, including children with cancer, malformation, chronic diseases, and immune deficiency, and (iii) children at the neonatal unit. Children in this group were admitted shortly after birth to the neonatal care unit and had never been discharged from Hospital.

4.1.1.3 Microbiological methods

From each patient, 1–2 BacT/Alert P.F. paediatric FAN bottles (BioMérieux, Marcy l'Etoile, France) were drawn. Following transport to the laboratory, the bottles were monitored with the BacT/Alert 3D-blood culture system (BioMérieux). Any bottle with a positive signal was immediately removed from the system, and an aliquot was taken for gram staining and subculture, whereas susceptibility testing was performed with disc diffusion according to the recommendations from the Swedish Reference Group for Antibiotics.

4.1.2 Paper II

4.1.2.1 Patients and study design

The study was a retrospective review of all positive blood culture results during two five-year periods July 1, 2002–June 30, 2007, and July 1, 2008–June 30, 2013. Conjugated pneumococcal vaccination was introduced in the National Swedish Immunisation program in the Stockholm area in 2007, the same year when pneumococcal vaccine was introduced. The immunisation program started with a 7-valent vaccine but was replaced by a 13-valent vaccine in 2010. According to the recommendation from the National Board of Health and Welfare, a risk-based program to reduce early neonatal infections of *Streptococcus agalactiae* was implemented nationwide in 2008, according to recommendations from The National Board of Health and Welfare. As the new routines were gradually implemented, data from the year July 2007 to June 2008 were excluded. Blood culture results, patient identity and patient data was retrieved, as in paper one.

Definition, inclusions criteria and microbiological methods were similar to those in Paper I and not further described.

4.1.3 Paper III

4.1.3.1 Patients and study design

The study was a one-day, hospital-wide, point-prevalence survey conducted eight times between 2003 and 2017 at Astrid Lindgren Children's Hospital. For appropriate comparison between years, the surveys were performed within three weeks from the second half of November to the first week of December. The first five surveys performed between 2003 and 2010 were a part of a nationwide hospital point-prevalence survey initiated by the Swedish Strategic Program against Antibiotic Resistance (STRAMA) and included children and adults but did not include patients at the neonatal wards. The retrieved data were reported following a standardised protocol developed by STRAMA. The surveys in 2015 and 2016 were a local initiative using the same protocol as in the first five surveys. In the last survey in 2017, the data retrieved was reported in a similar, but new protocol developed by the Antibiotic Resistance and Prescribing in European Children (ARPEC) project. All inpatients admitted at 8:00 AM were included in the survey, and in children with ongoing antimicrobial therapy, the data were collected through chart review.

4.1.3.2 Patient data

According to the protocols, information about age, sex, underlying diseases, antimicrobial regimen, administered single-unit dose, the number of doses per 24 hours, route of administration, indications (community-acquired, hospital-acquired or prophylaxis), and diagnosis for treatment were collected. Underlying diseases were defined as a chronic condition with increased risk of infections such as cancer, malformation, and immune deficiency. In the surveys, 2003–2016 treatment was evaluated as appropriate or inappropriate. Incorrect dosing, choice of drug or other faults were evaluated as incorrect. In the 2017 surveys, compliance to local or national guidelines was evaluated as appropriate.

4.1.3.3 Classification of Antimicrobial Agents

Antimicrobial agents were classified according to the Anatomical Therapeutic Chemical (ATC) Classification System of the World Health Organization. The following antimicrobials agents were included in the study; antibacterial for systemic use (J01), antimycotics and antifungals for systemic use (J02 and D01BA02), drugs for the treatment of tuberculosis (J04A), antivirals for systemic use (J05), antibiotics used as intestinal anti-infectives (A07AA) and antiprotozoals used as antibacterial agents (P01AB).

4.1.4 Paper IV

4.1.4.1 Patients and study design

The study was a continuation of the review of BSI initiated in paper I. In this study, we retrospectively analysed all blood cultures obtained from patients between July 1998 and June 2018 at Astrid Lindgren Children's Hospital, Karolinska University Hospital. Blood cultures were obtained from children with clinical signs of infections, and with a suspicion of a serious infection. Results from the blood cultures were retrieved yearly since 1999. In this study, definitions, inclusion criteria, and microbiological methods were similar to those in Paper I. During the study period, the number of children aged <18 increased from 387,000 to 510,000 in Stockholm County, which is the population base for oncology and surgery patients, and from 198,000 to 255,000 in Northern Stockholm, which is the catchment area of the hospital for most other paediatric conditions. Additionally, the number of live births increased from 10,800 to 15,500 per year in Northern Stockholm.

4.1.5 Paper V

4.1.5.1 Patients and study design

The study was a retrospective analysis of the occurrence of isolates with acquired AMR in children with BSI admitted at Astrid Lindgren Children's hospital between July 1998 and June 2018. Results from blood cultures and patient information were retrieved as in paper I, II and IV.

4.1.5.2 Antibiotic susceptibility testing

Susceptibility testing was performed with disk diffusion, and the results were reported as "resistant" (R) or "susceptible, increased exposure" (I). For gram-positive bacteria, we reported *S. aureus*, resistant to methicillin and lincosamides; *S. pneumoniae* resistant to macrolides, lincosamides, and penicillin non-susceptibility; *S. agalactiae* resistant to macrolides; *Enterococcus faecium* resistant to vancomycin, and *E. faecalis* resistant to ampicillin. For gram-negatives bacteria, we reported *E. coli, Klebsiella*, and other *Enterobacteriaceae,* resistant to third generation cephalosporins, trimethoprim-sulfamethoxazole, aminoglycosides, and carbapenems. We also reported *Acinetobacter spp*, resistant to carbapenems, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole; and *P. aeruginosa*, resistant to ceftazidime, carbapenems, aminoglycosides, piperacillin-tazobactam, and fluoroquinolones. Susceptibility for antifungals was not evaluated, and *Candida spp*. were, therefore, not included in the denominator when calculating the crude prevalence of AMR.

4.1.5.3 Acquisition of reference data

To compare our collected data with existing data on antimicrobial susceptibility, we retrieved reference data from the European Antimicrobial Resistance Surveillance Network (EARS-Net), the Antibiotic Resistance and Prescribing in European Children (ARPEC) project, and the Swedish Surveillance of Antibiotic Resistance (SVEBAR). EARS-Net are based on routine clinical antimicrobial susceptibility data on invasive isolates from local and clinical laboratories reported to ECDC by appointed representatives from the E.U. member states. We retrieved data from the annual report on surveillance of antimicrobial resistance in Europe, 2018. SVEBAR is a platform that collects daily reports from the local and national network of microbiology laboratories. Susceptibility data on invasive isolates from selected pathogens are collected, and accessible from the Swedish Agency for Public Health's website. We retrieved data from 2017.

4.2 STATISTICS

Paper I. To compare the proportion of *Staphylococcus aureus* and *Streptococcus pneumoniae* in children with no underlying comorbidity, a comparison of categorical variables was performed using Fisher exact test and chi-square test, using the GraphPad software (GraphPad Software, Inc, CA, USA). The annual BSI incidence rate in children was calculated by dividing the number of children with BSI (numerator) by the total number children of the same age strata (denominator). For infants, the number with BSI incidences was divided by the total number of live-born children for that year.

Paper II. Incidence rates were calculated using the same formula as in paper I. To assess significant differences between the proportion in the two five-year periods, we used chi-square test for exact Clopper-Pearson binomial 95% confidence intervals (CI), or exact Poisson confidence intervals, to obtain a confidence interval for the outcome.

Paper III. Trends in CAI, HAI and prophylaxis proportions, the total number of antimicrobial prescriptions, the proportion of patients receiving antimicrobial treatment, and the proportion of patients with an underlying disease were analysed with non-parametric Mann-Kendall test, using the function *cor.test* in R version 3.5.0. (R Core Team. A language and environment for statistical Computing; 2018. Vienna, Austria: R Foundation for Statistical Computing; 2018. Available at: http://www.R-project.org).

Paper IV. Trends of incidence and proportions were tested using the Kendall tau rank correlation. The correlation was assessed as weak, moderate, or strong, depending on the tau (τ) value. τ varies from 1 to -1, and a value of 0 (zero) is interpreted as no monotone trend, negative values as a negative trend and positive values as a positive trend. The statistical significance level was set at $p < .05$. The annual BSI incidence in infants was calculated by dividing the number of children with BSI (numerator) with the total number of live-born children in Stockholm for that year (denominator). For older children, the total number of children with BSI was divided by the total number for the same age strata for that year. Information about the populations was retrieved from Statistic Sweden Database.

Paper V. Difference in the occurrence of AMR percentages between HAI vs CAI and in children before vs after one year of life were compared using a chi-square test. A *p*-value of less than 0.05 indicated statistically significant differences. Trends for the occurrence of acquired AMR and the consumption of antimicrobials in hospitals over the study period for different paediatric risk groups were tested with the Kendall tau rank correlations test.

4.3 ETHICAL CONSIDERATIONS

Approval for the studies reported in all papers was obtained from the Regional Ethical Review Board at Karolinska Institutet, Stockholm

For Paper I; Dnr 2009/1063-31/2

For Paper II; Dnr 2013/2056-32

For Paper III; Dnr 2018/2069-32

For Paper IV; Dnr 2013/2056-32, 2018/2069-32

For Paper V; Dnr 2019-05049, 2009/1063-31/2 and 2013/2056-32

The studies are retrospective register studies and spans over a long period. It was not considered reasonable, or feasible, to obtain approval from each individual who participated in the study. No identifiable data can be linked to any study subject.

5 RESULTS

Our main findings were:

- Age and risk factors correlate with BSI incidence.
- Age and risk factors correlate with the aetiology of BSI.
- Immunisation against *Streptococcus pneumoniae* is effective.
- Intrapartal antibiotic prophylaxis reduces the early onset of GBS BSI.
- A high proportion of hospitalised patients receive antimicrobial therapy.
- Rates of antimicrobial resistance are low, but there is an alarming increase in some of the most prevalent pathogens.
- Aetiology of BSI changes over time and needs continuous and long-term surveillance.

5.1 AGE AND CONCOMITANT RISK FACTORS INFLUENCE THE INCIDENCE OF BSI

In paper I, II and IV, 66,698 blood cultures retrieved from children with clinical signs of infections between July 01, 1998, and June 30, 2018, were analysed. As described in the materials and methods section, we decided to exclude coagulase-negative staphylococci (CoNS) as a possible contaminant. The rationale behind this exclusion was that two blood cultures were rarely drawn before starting antibiotic treatment in children. The lack of two blood cultures makes it impossible to include contaminants, according to the CDC's definition for possible contaminants. The number of retrieved and analysed blood cultures are presented in Figure 7. The majority of cultures were negative (83%; 55,590/66,986). Of the 11,396 positive cultures, 9317 were considered as possible contaminants, and thus excluded. In the end, 2079 or 3.1% of all retrieved blood cultures were positive and further analysed

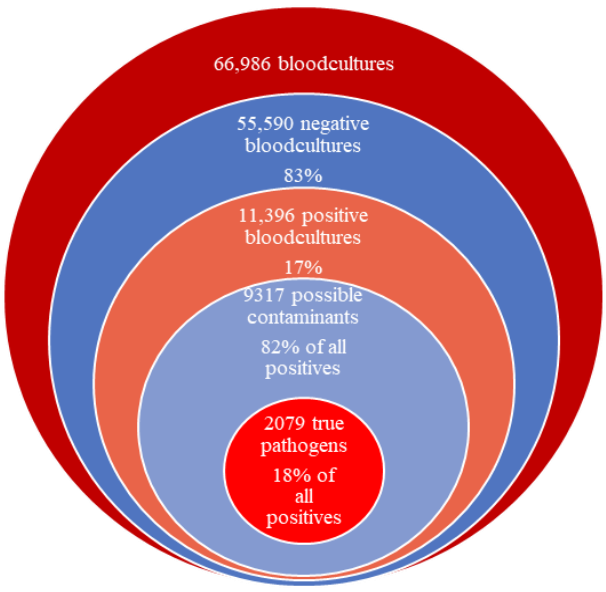

Figure 7 The number of retrieved and analysed blood cultures

During the preparation of **paper I**, we identified four different groups of children with different distribution of pathogens and different risk for BSI.

- Newborn children warded at the neonatal ward
- Oncology patients
- Children with different underlying co-morbidity
- Previously healthy children with no underlying co-morbidity

Each category compromise approximately 25% of the total BSI episodes, Figure 8. Although we hypothesized that the proportion of previously healthy children would gradually decrease, they continue to constitute 25% of the individuals. The number children in each group vary, from the small group of approximately hundred diagnosed oncology per year to the whole paediatric population in our catchment area who also constitutes the population at risk for children with no underlying co-morbidity.

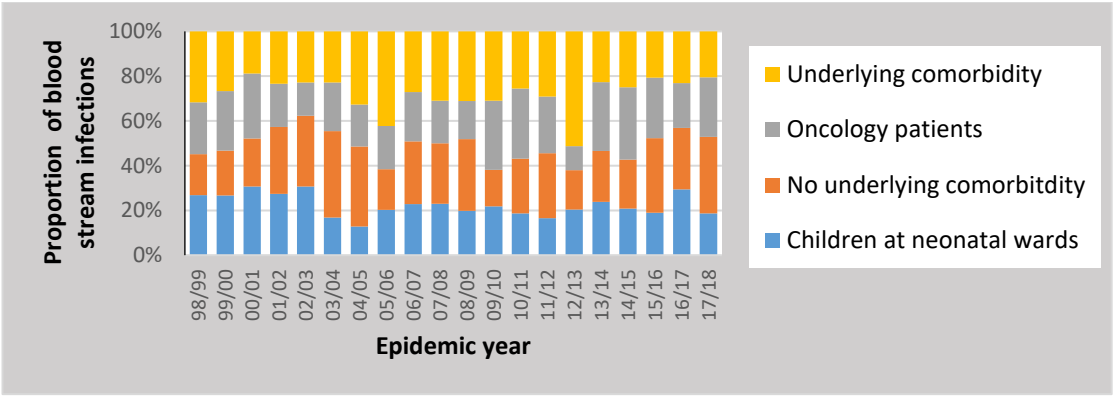

Figure 8 Proportion of BSI for each risk groups

In **paper I**, **II** and **IV**, we estimated BSI incidence in different age groups, but with some difference in age stratification. We also separated children, 0–2 months old, warded at the neonatal units, from the same age group not warded at neonatal units. The estimated incidences are presented in Figure 9.

The crude BSI incidence was 25.5/100,000 in children aged 0–17 years. Children 0–2 months old accounted for 34.4% (715/2079) of all children. A majority of children with BSI (64.1%; 458/715) aged 0–2 months, were admitted to neonatal wards, equalising an estimated incidence of 1.8/1,000 live births. Among children with BSI at the neonatal wards, 74.7% were born before gestational week 33. For 0–2 months old children warded outside neonatal wards, there was a 58% lower incidence, 0.7/1,000 live births children, which included both children with various underlying co-morbidity and children with no underlying co-morbidity. For a 0–2 months old child with no underlying co-morbidity, the estimated incidence for BSI was 0.4/1,000 live births. After the neonatal period, BSI incidence was 47.5/100,000 for children aged between 3 months and 2 years, and 15/100,000 for children and adolescents aged between 3 and 17 years. BSI risk was highest in children at the neonatal wards, followed by children with cancer. The latter constituted 23.2% of the entire cohort.

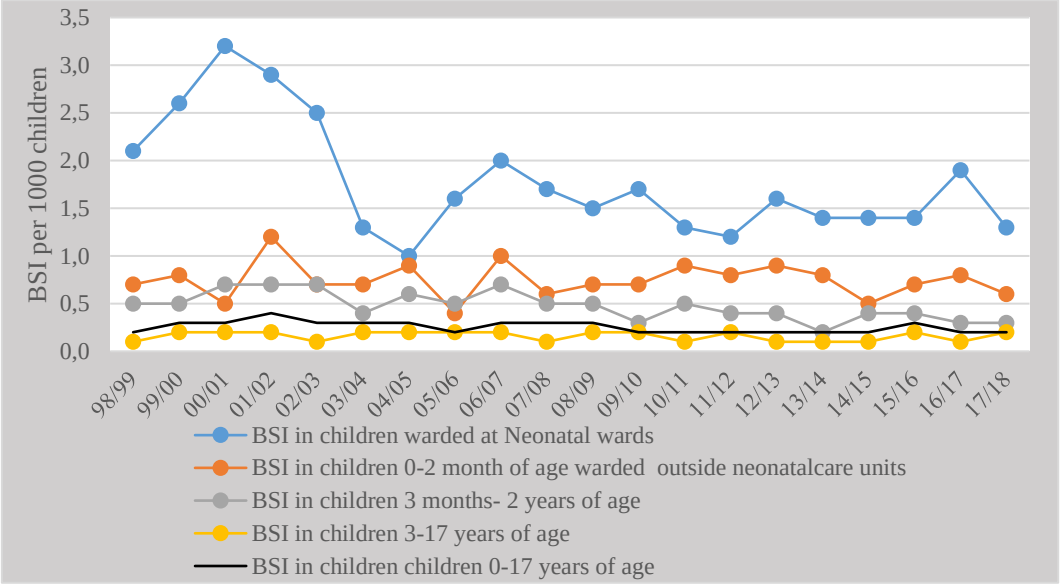

Figure 9 The estimated incidence of BSI for different age groups.

During the entire observation period, BSI incidence decreased, which was most evident in children aged between 3 months to 2 years (τ= -0.59, P =0.0006), and in neonates with early-onset BSI (τ= -0.44, P =0.0069). Comparing the first 10 years to the last 10 years of the study, the mean decline in BSI incidence in children warded at the neonatal wards was 29%, from 2.09/1,000 to 1.48/1,000 live births. For children 0–2 months old, warded outside neonatal wards, BSI incidence did not change. For children 3 months to 2 years old, BSI incidence declined 30%, from 0.53/1,000 to 0.37/1,000 children from the first to last ten years of the study period. This decrease explained the overall decrease for BSI in children aged between 0 and 17 years.

In conclusion, the risk for BSI inversely correlated with age, with a highest risk for the youngest individuals. Patients with cancer had the highest risk for BSI, but data of the total number of oncology patients was not collected, and therefore the incidence was not calculated.

5.2 THE AETIOLOGY OF BSI IS INFLUENCED BY AGE AND CONCOMITANT RISK FACTORS

The aetiology of BSI varies considerably, both with age and underlying risk factors, as shown in paper **I**, **II** and **IV**. Therefore, data representing previously healthy children will be separated from children with known risk factors for BSI. Regardless of age and underlying risk factors, *Staphylococcus aureus* was the most common pathogen. All positive cultures are presented in table 6.

Table 6 Distribution of all analysed pathogens

	Total		No underlying co-morbidities	Oncology patients	Patients with underlying co-morbidities*	Patients at neonatal wards
Pathogen	n,	(%)	n, (%)	n, (%)	n, (%)	n, (%)
S. aureus	657	(31,6)	184 (33,5)	127 (26,3)	209 (35,9)	137 (29,5)
S. pneumoniae	178	(8,6)	113 (20,5)	25 (5,2)	38 (6,5)	2 (0,4)
Enterococcus spp.	194	(9,3)	14 (2,5)	66 (13,7)	65 (11,2)	49 (10,5)
S. pyogenes	58	(2,8)	36 (6,5)	6 (1,2)	15 (2,6)	1 (0,2)
S. agalactiae	114	(5,5)	38 (6,9)	4 (0,8)	9 (1,5)	63 (13,5)
L. monocytes	4	(0,2)	0,0	2 (0,4)	1 (0,2)	1 (0,2)
Other gram-positive bacteria	37	(1,8)	10 (1,8)	12 (2,5)	12 (2,1)	3 (0,6)
Gram positives, total	1242	(59,7)	395 (71,8)	242 (50,2)	349 (60,0)	256 (55,1)
E. coli	217	(10,4)	56 (10,2)	53 (11,0)	51 (8,8)	57 (12,3)
Klebsiella spp.	123	(5,9)	4 (0,7)	47 (9,8)	41 (7,0)	31 (6,7)
Other Enterobacteriaceae	121	(5,8)	3 (0,5)	34 (7,1)	46 (7,9)	38 (8,2)
P. aeruginosa	86	(4,1)	1 (0,2)	42 (8,7)	28 (4,8)	15 (3,2)
N. meningitides	20	(1,0)	18 (3,3)	(0,0)	2 (0,3)	(0,0)
Other gram-negative bacteria	120	(5,8)	31 (5,6)	39 (8,1)	33 (5,7)	16 (3,4)
Salmonella	48	(2,3)	41 (7,5)	5 (1,0)	3 (0,5)	(0,0)
Gram negatives, total	735	(35,4)	154 (28,0)	220 (45,6)	204 (35,1)	157 (33,8)
Candida spp.	102	(4,9)	1 (0,2)	20 (4,1)	29 (5,0)	52 (11,2)
Total	2079		550	482	582	465

5.2.1 Aetiology in previously healthy children

For previously healthy children, the six most prevalent pathogens that accounted for 85% of all BSI episodes, with marked age-dependent variability, are shown in Figure 10. Over 80% of episodes were associated with signs of focal infection; meningitis for *Streptococcus agalactiae*, pneumonia and meningitis for *Streptococcus pneumoniae*, urinary tract infections for *Escherichia coli*, skin- and soft tissue infections or bone- and joint infection for *Staphylococcus aureus*, and respiratory tract infections for *Streptococcus pyogenes*.

The remaining 15% of BSI incidences comprised of a variety of bacteria; 20 episodes of *Neisseria meningitidis*, 19 episodes of *Haemophilus influenzae* pathogens (of whom one type B with fatal outcome and the rest non-typeable); eight episodes of *Fusobacterium necrophorum* with Lemierre's syndrome, and few episodes of other gram-negative bacteria, and *Enterobacteriaceae*, *Enterococcus faecalis* and other gram-positive bacteria. In conclusion, previously healthy children had a limited spectrum of well-known pathogens and for an unknown reason a low occurrence of *N. meningitidis*.

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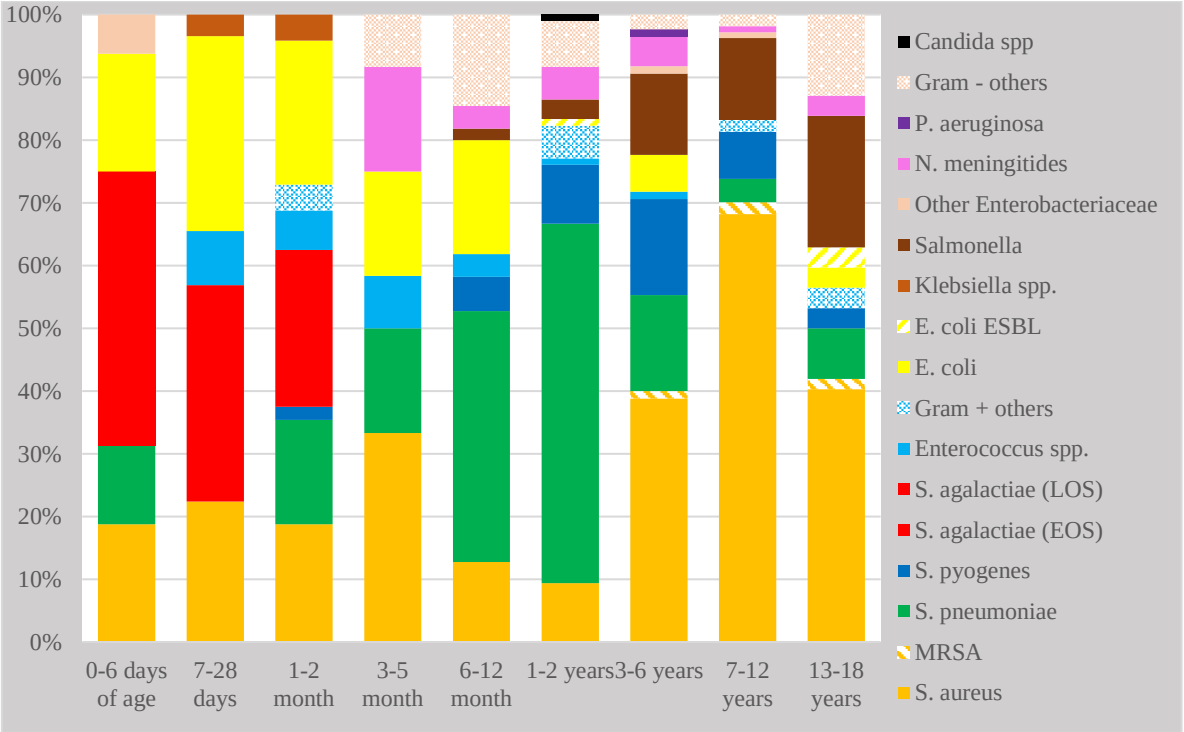

Figure 10. Proportion of pathogens at different age in previously healthy children

5.2.2 Aetiology in children with underlying co-morbidity and oncology patients

In children with underlying co-morbidities, BSI was more common, with a fever of unknown origin. There was no apparent age-dependent variation. *Staphylococcus aureus* was the dominant pathogen, present in 30% of all episodes, in all age groups. *S. aureus* infection in this category appeared as fever without focus and was associated with intravascular catheters. After *S. aureus*, the most prevalent isolates were *Enterococcus faecalis* and *faecium*, *E. coli*, *Klebsiella* spp, other *Enterobacteriaceae* and other gram-negative bacteria. Isolates of *Acinetobacter baumannii*, *Stenotrophomonas*, *Serratia marcescens*, and *Candida* spp. were only found in children with severe underlying comorbidity. The occurrence of *Acinetobacter* declined during the latter part of the study, possibly indicating an improved hygiene standard. Figure 11 shows the distribution of isolates. Classical paediatric pathogens like *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae* also occurred, but to a much lesser extent than in previously healthy children.

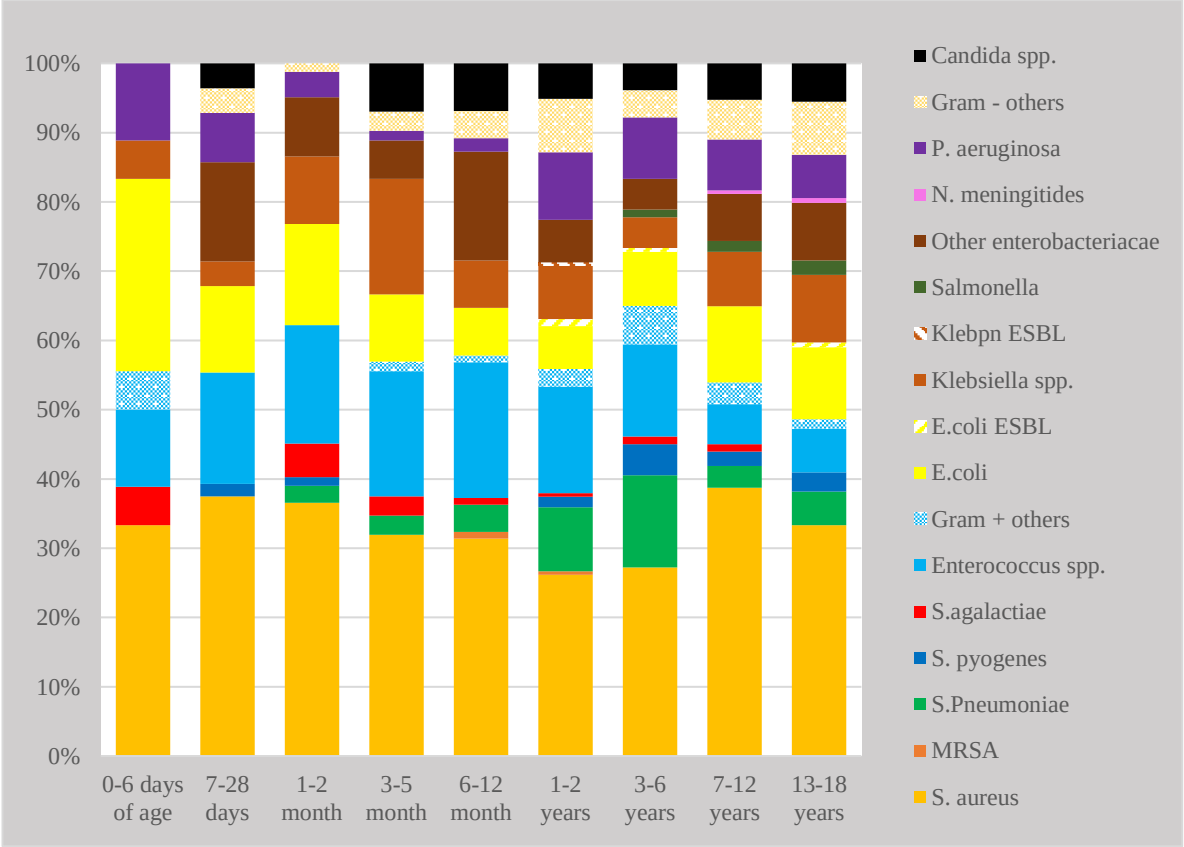

Figure 11. Proportion of pathogens in children with underlying diseases, including oncology patients.

5.2.3 Aetiology in children warded at the neonatal wards

During early-onset BSI, 50% of all incidences showed a positive bacterial culture within 48 hours of admission. *Streptococcus agalactiae* and *E. coli* were the most commonly isolated pathogens and constituted 72.8% of episodes, with *S. agalactiae* causing 45.6%, and *E. coli* causing 27.2% incidences respectively. All early-onset BSI due to *Streptococcus agalactiae* were culture-positive within 72 hours after births in children warded at the neonatal wards. Thereafter, *Staphylococcus aureus* was the most prevalent isolated pathogen, found in approximately 30% of cases. Also, here, *Staphylococcus aureus* was related to the presence of an intravascular catheter. *Enterococcus faecalis* was common, regardless of time since birth. *E. coli*, *Klebsiella*, other *Enterobacterales*, *Pseudomonas aeruginosa* and *Candida* became more frequently found in patients aged 1 week and beyond. Figure 12 shows the distribution of pathogens isolated in children warded at the neonatal wards.

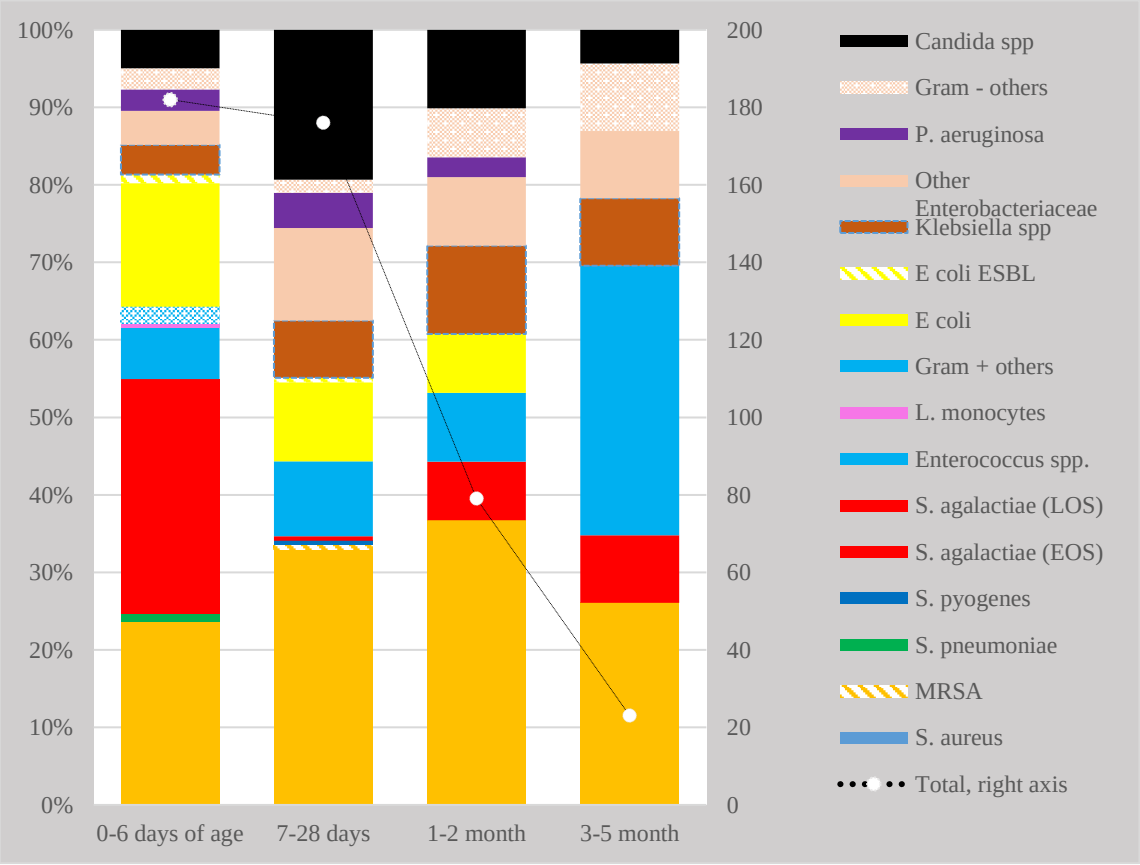

Figure 12. The proportion of each pathogen at different age for children warded at the neonatal ward in bars. The line joins the dots representing the total number of BSI for that age group.

5.2.4 Community-acquired or hospital-acquired infections

The aetiology for BSI differed considerably between children with the community- or hospital-acquired infections, which is related, to an extent, to the underlying co-morbidity and risk groups. Children without underlying co-morbidity had a CAI in 97.5% of all BSI episodes. *S. aureus* was the most frequently isolated pathogen for CAIs, and HAIs. In the case of CAI, a focal infection with osteomyelitis and septic arthritis was usually found in combination with clinical signs.

In children with underlying co-morbidity and in children at the neonatal units, HAI constituted 61.2% and 84.5% respectively. However, the distinction between CAI and HAI in children with underlying conditions is unclear and challenging, since neither of them predicts which pathogens to expect. Factor like patients' characteristics and underlying comorbidities predict the causative pathogen with higher accuracy than the timing of a positive culture. In children with no underlying diseases and neonates, there was a more marked difference between HAI and CAI. In Figure 13, the proportion of pathogens in BSI for CAI and HAI for different risk groups are presented

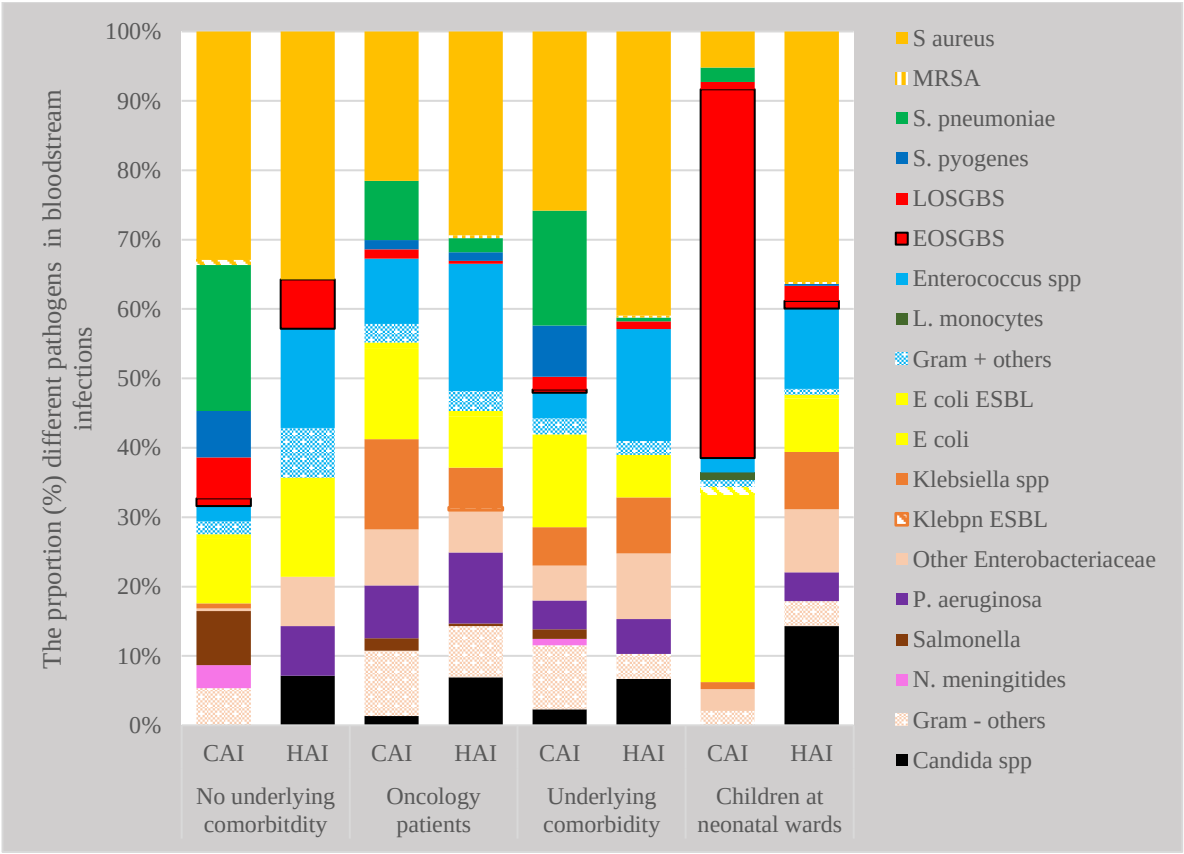

Figure 13 The proportions of pathogens in CAI and HAI BSI for different risk-groups.

5.3 PREVENTIVE STRATEGIES TO REDUCE BSI ARE EFFECTIVE

5.3.1 Immunisation against *Streptococcus pneumoniae*

In **paper II**, we evaluated BSI in relation to universal pneumococcal immunisation in children, and it was further analysed in **paper IV**. Before the introduction of the pneumococcal conjugate vaccine (PCV) in 2007, *Streptococcus pneumoniae* accounted for 30% of all isolated BSI pathogens in previously healthy children. Overall, BSI with *Streptococcus pneumoniae* declined by 68%, as shown in Figure 14. In children aged between 3 and 36 months (the most common age span for contracting *S. pneumoniae* infection); the incidence declined by 71% from 21.7 to 6.3/100,000 children in the ten-year study period after the introduction of PCV. The reduction of invasive *S. pneumoniae* infection was also reflected by fewer children presenting with *S pneumoniae* attributable meningitis, with 45 cases during the ten years before PCV introduction, and 10 cases in ten years after introduction.

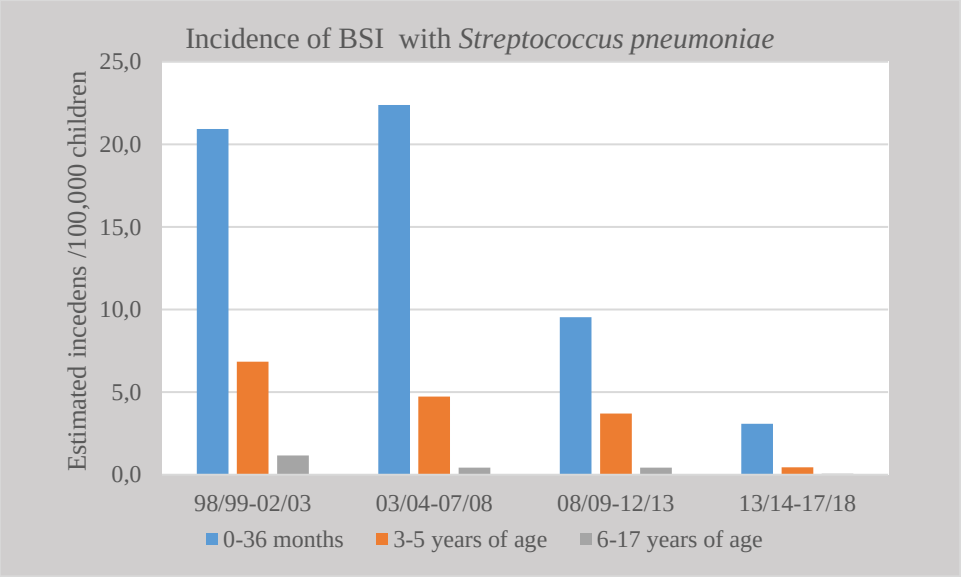

Figure 14 The estimated incidence for BSI with Streptococcus pneumoniae. Immunisation was introduced in 2007

5.3.2 Intrapartal antibiotic prophylaxis against Streptococcus agalactiae Early-Onset Sepsis

To prevent early-onset sepsis (EOS) infection by *Streptococcus agalactiae* (Group B streptococcus or GBS), a risk-based Intrapartal Antibiotic prophylaxis Programme (IAP) was implemented in Stockholm in 2008. In short, in this programme antibiotic prophylaxis is prescribed to women in labour, because of an identified risk of *S. agalactiae* colonisation, since maternal high colonisation rate of *S. agalactiae* increases the risk of early-onset sepsis with *S. agalactiae* (EOS GBS) in the new-born.

In **paper II**, *S. agalactiae* was identified as the dominant pathogen in severe neonatal infections during the first day of life. Our estimated incidence of EOS GBS before IAP implementation was 0.31/1,000 live births, which dropped to 0.06/1,000 during the first five years after AIP implementation, with only 3 cases between 2009 and 2012. In **paper IV**, it was shown that after 2012, the incidence of EOS GBS increased to 0.26/1,000 live births.

When the medical records were scrutinised for children with EOSGBS, we observed that 63% (14/22) of the pregnancy fulfilled criteria for IAP, but only 14.3% (2/14) received IAP.

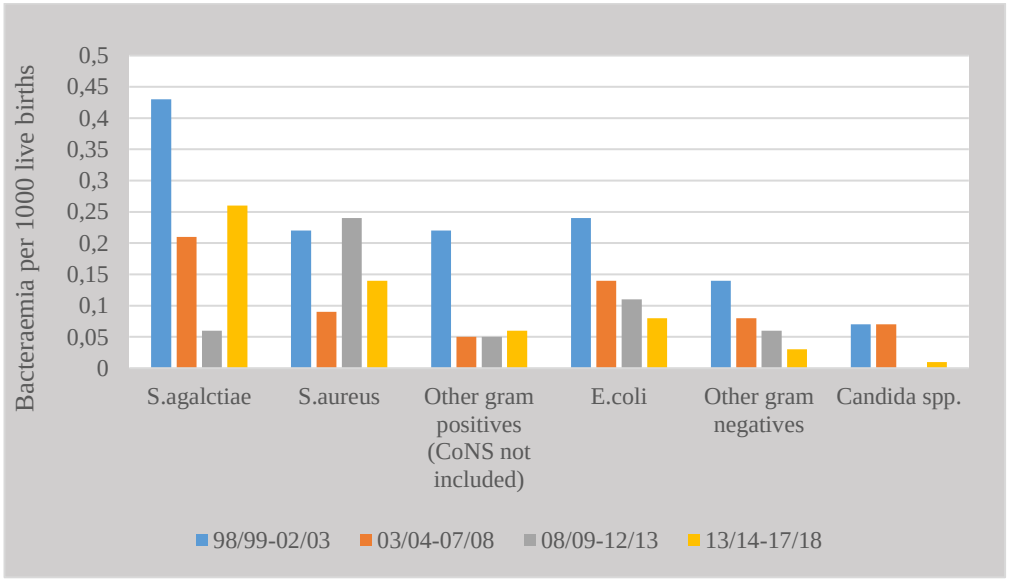

Figure 15 The estimated incidence of early-onset infection. Intrapartal Antibiotic prophylaxis programme was implemented in 2008

5.3.3 Candida prophylaxis

Besides the changes in early-onset BSI, due to *S. agalactiae*, in **paper II** we observed a decrease in early-onset BSI with *E. coli*, other gram-negative bacteria, and gram-positive bacteria others than *S. aureus*. In late-onset BSI (LOS), there was no trend for a changed overall incidence. However, BSI with Candida spp. BSI (τ= - 0.58, *P*= 0.0005) declined significantly after the introduction of candida prophylaxis in neonatal units in 2010. Since the introduction, only 3 episodes of candidemia were detected in the neonatal units. As opposed to the observed decrease for most pathogens during the neonatal period, *Staphylococcus aureus* was more frequently isolated towards the end of the study period, as reported in **paper IV**. In Figure 16, the estimated incidence of late-onset BSI are shown.

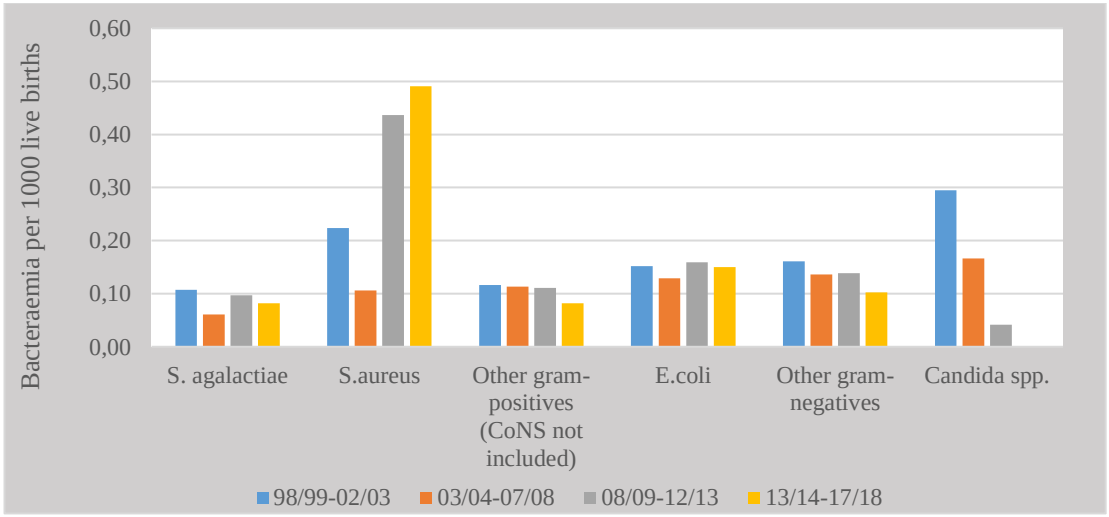

Figure 16. The estimated incidence of late-onset infection.

5.4 ANTIMICROBIAL THERAPY FOR HOSPITALISED PATIENTS

In **paper III**, we evaluated all in-hospital patients who were prescribed antibiotics in eight one-day point-prevalence surveys between 2003 to 2017. Overall, 35.5% (336/946) of patients received antimicrobial therapy. The top three indications for antimicrobial prescription, including sepsis (involving fever with neutropenia and fever of unknown origin), respiratory infections and, intra-abdominal infections attribute to 23.6%, 21.1% and 16.1% of BSI incidence, respectively. During the study period, there was a small increase in the number of patients who received antibiotics, but the proportion of patient receiving antimicrobials was not significant. The distribution of antimicrobials prescribed for treatment of community- and hospital-acquired infections, according to the Anatomical Therapeutic Chemical Classification (ATC)-System are presented in Figure 17 as the proportion of prescribed antimicrobials for each survey.

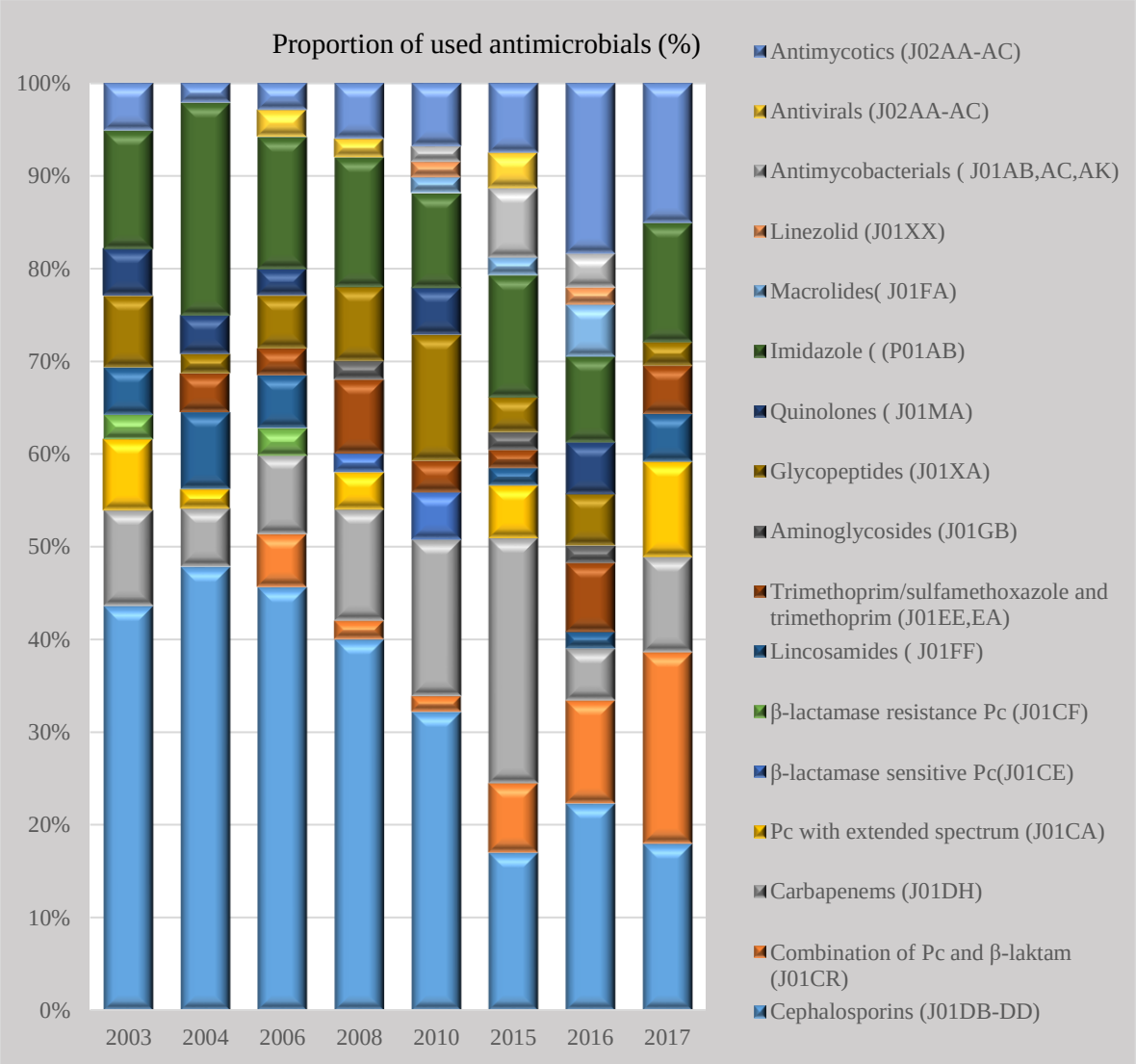

Figure 17 Proportion of used antimicrobials (%) according to the ATC system

5.4.1 Trends of antimicrobial therapy

The results concluded that there was a change in the prescription of antimicrobial therapy during 2003–2017. Prescription of third-generation cephalosporines declined concomitantly with community-acquired infections and an increase in prophylactic therapy to specific risk groups of patients. During the observation period, the total paediatric population in the hospital catchment area increased by 24.1%, but the number of hospital beds decreased by 35.9%, which contributed to a shift in patient mix. We observed an increase in patients with underlying comorbidity from 41.2% to 65.3% during the study period (p =0.031). Among those patients with underlying co-morbidities, 50% were oncologic patients.

During the study period, medical prophylaxis against fungal and other opportunistic infections in oncology patients and premature, very-low birthweight children, increased. The prescription for community-acquired infections decreased significantly, which is partly related to a concurrent decrease in BSI incidence due to *Streptococcus pneumoniae*.

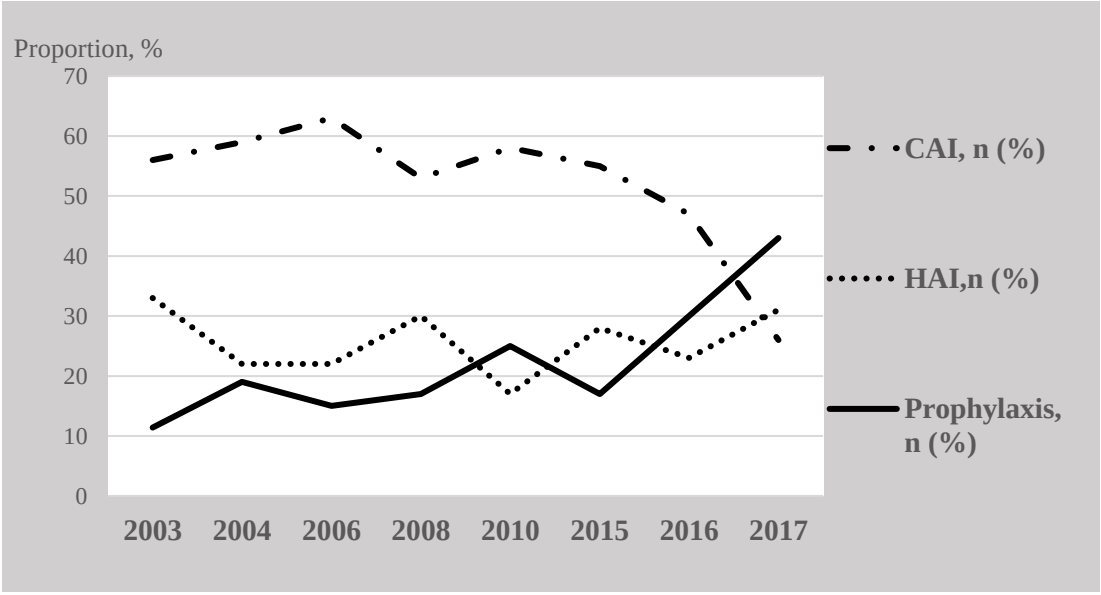

Figure18. Prevalence of antimicrobials prescribed per CAI, HAI, and prophylaxis

In **paper V,** we also look for a trend in antimicrobial therapy. We included data on delivered doses of the most common antimicrobials from 2010 to 2018. There we could not confirm the decrease in the use of third generation cephalosporines; the trend was increasing (τ = 0.5, p .007) rather than declining. As possible explanations for the different findings could be that point-prevalence surveys were conducted at non-representative days and that neonatal settings were excluded in the point-prevalence surveys. During the first five point-prevalence surveys, the neonatal antimicrobial prescription was excluded from the compiled presentation.

During the period between 2010 and 2018, the total antibiotic use did not change. The use of piperacillin with enzyme inhibitor increased (τ = 0.7, p .0048), partly because of a recommendation of Piperacillin-Tazobactam in cases of neutropenic fever in favour for Meropenem. The prescription of Benzylpenicillin, Aminoglycosides, and Vancomycin decreased

5.4.2 Antimicrobial prophylaxis

In **paper III**, one finding was the increased use of antimicrobial prophylaxis, mainly to oncology patients and surgical prophylaxis. The increase was attributed to medical prophylaxis to oncologic patients against fungal and other opportunistic infections. Oncology patients receive an overall 24% of all prescribed antimicrobials. Still, during the study period, the proportion increase, and in 2017, 48% of all prescribed antibiotics were directed to oncology patients (neonatal patients were omitted), with 42% of the prescriptions for medical prophylaxis. In Figure 19, the distribution of antimicrobial prophylaxis is presented.

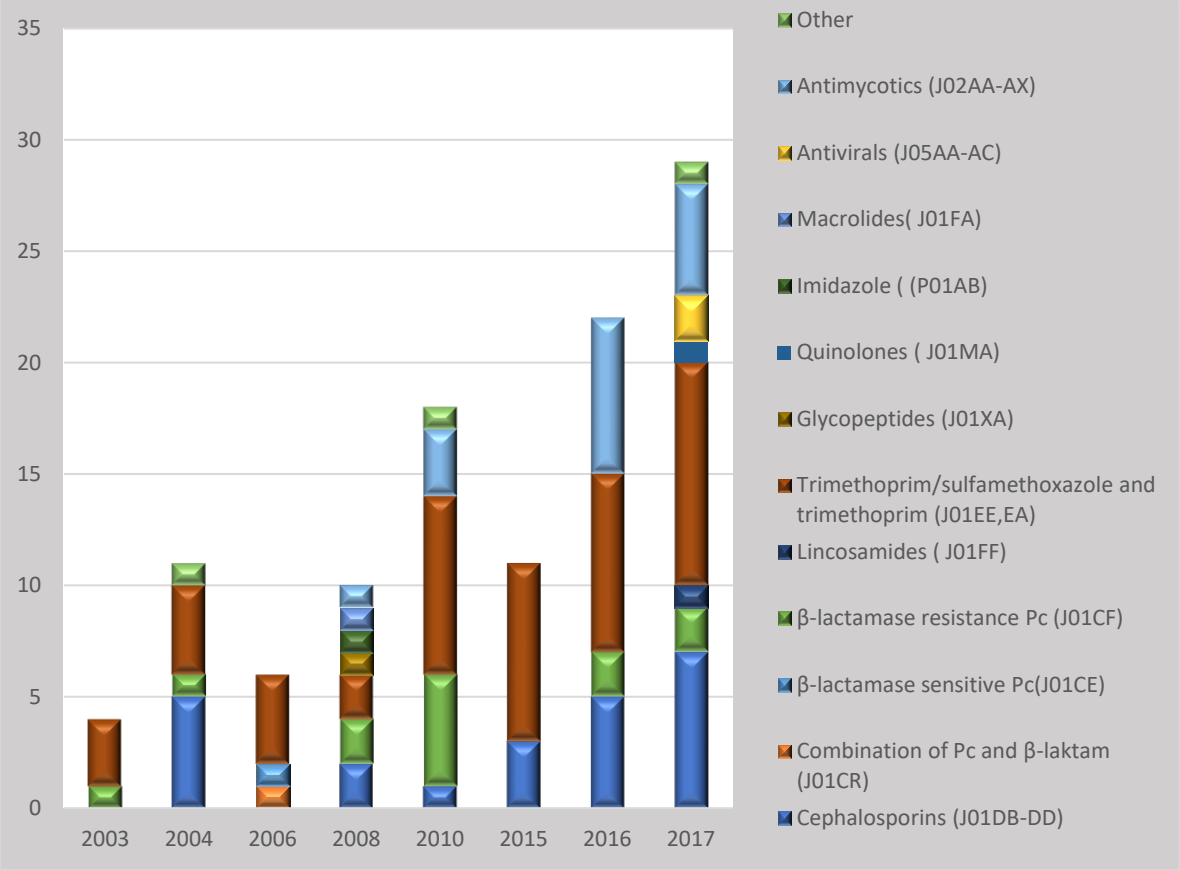

Figure 19. Antimicrobials prescribed for prophylactic treatment

5.5 RATES OF ANTIMICROBIAL RESISTANCE

5.5.1 Prevalence of antimicrobial resistance

In **paper V** we evaluated the prevalence of AMR in children n with BSI. AMR was present in 9.2% of all BSI isolates and was most prevalent in oncology patients, the patient group that also received most antimicrobial therapy. Our rates of AMR from the last five years are presented in Table 4, where we also present other paediatric data from Europe, and mixed adult and paediatric data from Sweden and Europe. Our prevalence was low compared to other European paediatric data, but almost in harmony with Swedish mixed adult and paediatric data.

	Paediatric data		*Mixed adult and paediatric data*		
Pathogen and antibiotic Class	ALB 2013–2018	ARPEC 2011–2012	EARS-net, European populations based, mean, 2018	EARS-net SWE 2018	SWE-BAR 2017
Staphylococcus aureus					
Methicillin-resistance	3.3	16.4	16.4	1.9	2.0
Lincosamides	8.4				4.3
Enterococcus faecalis					
High-level gentamicin	0.0	30.5	27.1	12.8	
Enterococcus faecium					
Vancomycin	0.0	8.3	17.3	1.4	
Streptococcus. pneumoniae					
PC-non-susceptibility	0.0	13.4	10.8	5.5	
Macrolides	7.1	33.1	15.3	4.5	
Escherichia coli					
3rd gen. Cephalosporins.	15.5	10.7	15.1	8.3	9.5
Aminoglycosides	5.2	13.5	11.1	7.7	7.0
Fluoroquinolones	15.5	8.5	25.3	18.1	20.5
Carbapenems	0.0	0.0	0.1	<0.1	0.1
Klebsiella pneumoniae					
3rd gen. Cephalosporins	11.1	29.9	31.7	5.5	11.5
Aminoglycosides	0.0	26.2	22.7	3	6.8
Fluoroquinolones	7.4	7.5	31.6	10.1	13.9
Carbapenems	0.0	1.9	7.5	0.2	0.3
Pseudomonas. aeruginosa					
Pip-tazobactams	8.7	36.0	17.6	7.8	
Ceftazidime	4.4	25.8	14.8	6.1	
Aminoglycosides	0.0	27.3	19.3	1.0	
Fluoroquinolones	4.4	23.4	23.1	7.1	
Carbapenems	13.1	32.8	20.5	4.4	

Table 7. Comparison of the prevalence of AMR found in our material and European paediatric and adult data and Swedish date with mixed adult and paediatric isolates.

5.5.2 Trends

In **paper V**, we describe a trend for increased antimicrobial resistance (AMR). The increase was significant for both gram-positive and gram-negative isolates. In gram-positive isolates, an increase of *Staphylococcus aureus* resistant to lincosamides and *Streptococcus pneumoniae* resistant to macrolide, tetracycline, and trimethoprim-sulfamethoxazole contributed to the increasing BSI trend. During the last period of our study, 13.4% of *Staphylococcus aureus* was found resistant to lincosamides. CAI in previously healthy

children comprises a large proportion of AMR in gram-positive bacteria. In gram-negative bacteria, 82.7% of isolates were AMR in oncology patients during the last five-years.

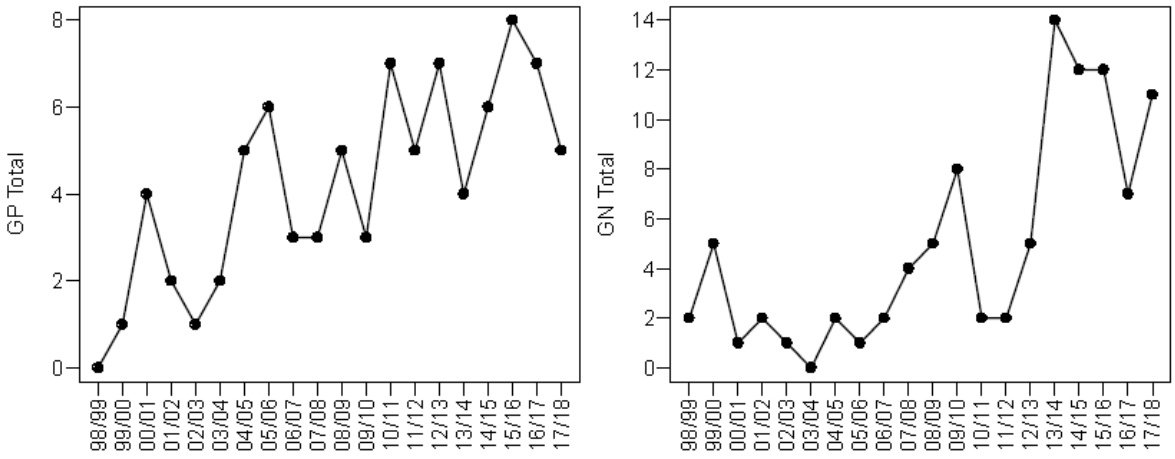

Figure 20 Number of Gram-positive (GP) and gram-negative (GN) with isolates express AMR

5.5.3 ESBL

The prevalence of extended-spectrum β-lactamase (ESBL) producing Enterobacterales increased during the study period and constituted 14.1% of the isolates in the last five-years of observation, 15.5% of *E. coli* and 11.1% of *Klebsiella pneumoniae* were ESBL-positive. A majority (64.7%) of ESBL isolates exhibited resistance to ≥ three classes of antibiotics and were, by definition, multidrug-resistant. More than half (52.9%) of ESBL producing Enterobacterales were found in children with no previous antibiotic therapy. Hospital-acquired infection accounted for 58% of cases, whereas 50% were oncologic patients. A high number of patients (82.4%) with ESBL had a history of travel, hospital-admission or a household member travelling or originated from a non-Nordic country.

ESBL	ESBL-E. coli	ESBL-Kleb.	MDR	HAI	Abx., six months	Non-Nordic history	Mortality
14.1%	15.5%	11.1%	64.7%	52.9%	47.1%	82.4%	5.8%

We found higher rates of ESBL than those reported by EARS-net with Swedish data, but our rates are similar to the EARS-net European mean rates of *E. coli* ESBL (15.1%), but lower for *Klebsiella* (31.7%). The mortality rate for children contracted with ESBL producing Enterobacterales was 5.8% (1/17), compare to 5.9% mortality in children with BSI with susceptible isolates.

5.5.4 MRSA

MRSA occurrence was 3.2% of all *S. aureus* BSI over the entire observation period. MRSA also increased and constituted 5.1% of BSI incidences during the last five-years. MRSA infection was more common in children without underlying comorbidity, and 75% were community-acquired infections. As for ESBL, a high proportion (75%) of affected individuals had a history of recent stay, hospital admission or a household member travelling to or originated from a non-Nordic country

6 DISCUSSION

The overall aim of the thesis was to describe the aetiology, risk factors, and occurrence of bacteria resistant to antimicrobial therapy in children with bloodstream infections (BSI). The following scientific question were asked.

- What is the current prevalence and distribution of BSI in children in our area?
- Has the incidence of BSI changed during the study period?
- Is there an aetiological difference in BSI between previously healthy children and children with underlying diseases?
- Can we identify targets for improving antimicrobial prescriptions to hospitalised children?
- How common is AMR among children with BSI?

6.1 STRENGTHS

Data presented in my thesis is based on the general paediatric population in Stockholm where patient information on BSI was collected continuously for over 20 years. Since the hospital serves as the primary hospital for approximately 12% of the Swedish paediatric population, 25 % of the paediatric population with surgical and oncologic conditions, we believe our data could be generalized for the total Swedish paediatric population. Our hospital is also a tertiary hospital with intensive care units and neonatal intensive care units that gave us the possibility to evaluate BSI for a general paediatric population as well a population of children with underlying conditions. All longitudinal data ware retrieved by only a few persons which we believe make the data-collection similar and comparable over time. This is the first characterization of BSI in a paediatric population in Sweden.

6.2 LIMITATION

The retrospective study design made it difficult to collect data on patient characteristics in a structured way. We depended on the quality of the medical records for our data collection on patient risk factors and antibiotic prescription. Data on aetiology and AMR were retrieved from the Clinical Microbiology database, which made the data on bacteria more reliable. To further analyse risk factors, it would have been appropriate to use a case-control study for comparison. Our hospital is a tertiary referral centre that skewed our patient mix to a higher occurrence of children with underlying co-morbidity, which could affect the appropriacy of generalization.

6.3 THE DISTRIBUTION OF BSI IN CHILDREN IN OUR AREA

During the preparation of **paper I**, four different groups of children were identified with significant differences in the distribution of pathogens with implications for empiric antibiotic therapy regimens as well as for planning targeted measurements to improve therapy and to prevent BSI. For this thesis, the following groups were used when analyzing data on epidemiology and aetiology of BSI, where each category compromised approximately 25% of total BSI episodes

- Newborn children warded at the neonatal ward
- Oncology patients
- Children with different underlying co-morbidities
- Previously healthy children with no underlying co-morbidities

Although we hypothesized that the proportion of previously healthy children with BSI would gradually decrease, this was not the case. However, the number of children in each group varied, with few children diagnosed with cancer each year (compared to the number of newborns), and the paediatric population in our catchment area without underlying co-morbidity

6.3.1 Definition of CAI or HAI in our cohort

In children with no underlying comorbidity, an overwhelming majority (97.5%) of BSI episodes were categorized as CAI. These episodes were characterized by a few causative pathogens and were often accompanied with focal signs of infection. On the other hand, BSI in children warded at neonatal wards were mainly categorised as HAI (84.5%). In children with underlying comorbidities and oncology patients, it was more difficult to classify the episodes but there were slightly more HAI (61%).

The distinction we made between CAI and HAI in children with underlying conditions may not be entirely appropriate. The classification system does not recognize the healthcare-associated infections that emerged in patients with complex conditions over the study period. These children and their episodes of BSI are managed as cases ranging between HAI and CAI. Our results, similar to other groups', reveal that only a few pathogenic infections can be categorized as CAI or HAI, with infections from *Streptococcus pneumoniae, Streptococcus pyogenes, Salmonella* spp., and *Neisseria meningitidis* predominantly appearing as CAI, and those from Enterococcus spp. as HAI. (96, 169) The timing of positive blood culture results, relative to hospital admissions, is insufficient to differentiate the aetiology of BSI. Typically, HAI pathogens are frequently isolated from samples taken within 48 hours after hospitalization. (96, 169) Patient's clinical characteristics, recent healthcare exposure, and recent invasive procedures could predict the possible pathogens with higher accuracy. This knowledge is important while taking an informed decision about empiric therapy.

The healthcare-associated infections (HCAI) category has been introduced to consider the patient's characteristics appropriately. The HCAI definition includes BSI in association with, a) occurrence of an indwelling medical device, b) surgery within 30 days, c) invasive instrumentation or incisions perform within 48 hours of the onset of symptoms, and, d) neutropenia induced by cytotoxic therapy (170)

6.4 OVERALL INCIDENCE OF BSI

We concluded that the incidence of BSI varies considerably with age. Prematurely born children have, by far, the highest incidence rate; tenfold higher than in children born after the neonatal age.

Overall, the incidence decreased slightly for the whole age span of 0–17 years. However, for two groups, the decline was more pronounced. In children 3 months–2 years of age, the group with the highest risk for pneumococcal diseases, the incidence of BSI decreased after the introduction of the conjugated pneumococcal vaccine (PCV) in 2007 has declined to below

0.5/1,000 children. A similar decrease is reported from both the U.S and Europe, and the rates for bacteraemia is <0.5% in feverish children 3–36 months of age presenting to the emergency department in areas with high PCV coverage. (69)

For neonates, the incidence of GBS EOS decreased after the implementation of a risk-based intrapartal antibiotic prophylaxis in 2008. In the U.S., IAP has been used since 1996, and it has reduced GBS EOS by 80%. Screening based IAP is in the U.S now recommended in favour of a risk-based screening (161) Moreover, BSI due to candida almost disappeared after the introduction of candida prophylaxis to very-low birth weight premature children. Taken together this led to

- 29% decrease in BSI in children 0–2 months of age between the first and last ten years of the study
- 30% decrease in BSI in children 3 months–2 years of age.

This finding contrasts with international studies on paediatric sepsis based on diagnosis codes, that have reported an increase in prevalence. (34, 35) (37, 38) These studies report an increase in sepsis and also describe a temporal correlation of a decline in mortality. (34, 35, 38) Although somewhat confusing, sepsis occurs because of several reasons other than BSI, which may impact both prevalence and mortality. The clinical definition of sepsis includes serious bacterial infections but does not require a positive blood culture. Sepsis also includes some severe viral infections and inflammatory conditions that could be life-threatening without accessibility to healthcare facilities but with near null in-hospital-mortality. Thus, when comparing studies that utilized different definitions for sepsis, we observed up to a tenfold difference in both the prevalence and mortality due to sepsis. A more uniform definition of paediatric sepsis would facilitate comparison between studies conducted in different parts of the world.

6.4.1 High and low-risk neonates

In **paper I** and **IV**, we concluded that new-born children warded at neonatal units have the highest incidence of BSI, despite the decreasing trend over the study period. Our finding of an estimated incidence of 1.46/1,000 live births is similar to a previously published Swedish report (171) that estimated an incidence rate of 1.2/1,000 new-born (when CoNS was excluded). For neonates admitted for BSI outside the neonatal units, the incidence was 0.41/1,000 live births, similar to the incidence rate of 0.37/1,000 in children 3 months to 2 years of age. This finding is consistent with other reports where community-acquired late-onset BSI is reported to be considerably lower for neonates outside the neonatal units than for the high-risk group of premature neonates warded at neonatal wards. (92) There is also evidence for a declining risk for invasive infections during the first month of life, with an 89% lower risk for invasive infections at week four compared to week one.(172) This may support a strategy of watchful waiting with serial clinical and laboratory assessments in the full-term infant, rather than aggressive early antimicrobial therapy for suspected sepsis. (156) Contrasting to adopting such a strategy there are other studies recommending empiric antibiotic therapy in all febrile children <21 days of age with fever duration less than 6 hours. regardless of their general appearance or blood test results. For children >21 days of age, with

good appearance and normal blood test result, it was possible to rule out invasive bacterial infection with high sensitivity. (173)

6.5 AETIOLOGY PATTERNS CHANGE OVER TIME

During the study period, we observed some important changes in the aetiology of BSI. On the one hand, changes correlate to the decreased incidence of BSI described above and relates to different targeted measures, also described above. On the other hand, the changes correlated to a change in patient composition.

6.5.1 A decline in the occurrence of *Streptococcus pneumoniae*

BSI due to *Streptococcus pneumoniae* declined by 71% among children 3–36 months of age, which was previously a high-risk age-group for severe *S. pneumoniae* infections during childhood. The decline was a result of the introduction of the pneumococcal conjugate vaccination (PCV) in the National Swedish Immunisation program. The same decline has been described extensively from other countries that implemented the immunisation programme. (56) (174, 175) The lower burden of *S. pneumoniae* infections also causes fewer fatalities. This was reflected by fewer children presenting with meningitis attributed to *S. pneumoniae*. (176)

In addition to the individual benefit of immunisation, the vaccines also have an impact on antimicrobial use and antibiotic resistance. A universal cover with a pneumococcal conjugate vaccine is estimated to contribute to a large (47%) reduction of days on antibiotics per year for pneumonia caused by *S. pneumoniae* in children < 5 years. (159) Consequently, a reduced burden of invasive pneumococcal disease (IPD) and reduced antimicrobial use could result in a decline in penicillin non-susceptible strains of *S. pneumoniae*, and perhaps also less resistance to erythromycin. *(175)* However, as reported from Sweden, a rise of AMR in IPD disease with non-vaccine serotypes is observed. (177) The current PCV immunisation leads to an expansion of non-vaccine serotypes, and cases of IPD after PCV introduction, caused by non-vaccines ST, are rising in Sweden. (160) However, the incidence of IPD is still much lower than before PCV, but increase in non PCV serotypes may increase and potentially compromise the benefits of the vaccine. (178) Thus *Streptococcus pneumoniae* remains a significant pathogen and should still be considered in paediatric BSI.

6.5.2 Early-onset sepsis due to *Streptococcus agalactiae* decline

Risk-based intrapartal antibiotic prophylaxis (IAP), where antibiotics directed to women in labour with a known risk for high-grade vaginal colonisation of *S. agalactiae,* reduced the risk for GBS EOS from 0.31/1,000 live births to 0.06/1,000 live births in the five first year after its implementation in 2008 (**paper II**). In a meta-analysis concerning the use of any intrapartum antibiotic prophylaxis, the incidence of GBS EOS was 0.23 /1,000 live births (95% CI 0.13–0.59). (179) However, when we extended the study period in **paper IV**, the incidence returned to 0.26/1,000 live births. The reason for this throwback is not apparent, but we speculate that it is due to decline in adherence to the IAP guidelines in the last period of our study. Even optimal adherence to either IAP strategy is estimated to reduce the incidence of 0.2/1,000 live births, at maximum but still, with no effect on late-onset GBS. (77, 180, 181) IAP is given either as a result of positive maternal antenatal screening or due

to risk of high maternal colonisation, where each strategy has advantages and disadvantages, e.g. false-negative screening cultures, fast and unexpected labour where a timely correct IAP is not possible, and increased antibiotic therapy. (161) Furthermore, the most adapted IAP strategy is screening based IAP. With screening based IAP up to 35% of all women in labour will receive antibiotics which will affects the infants' gut microbiome leading to a higher occurrence of intestinal bacteria with AMR. (182) LOS has not increased after the introduction of IAP, but the pattern has changed, and IAP is a possible risk factor for LOS with gram-negative bacteria expressing AMR. (181, 183, 184) Therefore, finding other prevention strategies is urgently required to decrease morbidity and mortality from invasive GBS disease, and vaccines are in development which could be delivered to pregnant women. (185)

6.5.3 Candida prophylaxis to very-low birth weight

Another example of targeted antimicrobial prophylaxis is fluconazole prophylaxis to very-low birth weight premature children. After the introduction of candida prophylaxis in 2010, candidemia decreased significantly in our cohort. Only three episodes of candidemia were detected in the neonatal wards after the introduction of prophylaxis, in contrast to 50 episodes, including four fatalities, before its introduction. The risk for invasive fungal infections is reported to be 2–4% of very-low birth weight infants and may affect 4–16% of extremely low birth weight infants with substantial morbidity and mortality. (186)

6.5.4 Increase in BSI with *Staphylococcus aureus*

With the decrease of the above-described pathogens, the proportion of the other isolated pathogens increased over time. Incidences of *S. aureus* isolation increased during the study period. It was the most frequently isolated bacteria in all age groups as well as in all risk groups, but with different clinical characteristics. BSI with *S. aureus* (SAB) increased in community-acquired infections in children with no underlying co-morbidity, and in children having signs of focal infection with arthritis, osteomyelitis, skin-or soft tissues infections or pulmonary involvement. Most SAB in children with HAI was without focal presentation, and was highly associated with the presence of an intravascular access, which has also been described among neonates. (187) Whether the high frequency of S. aureus reflects an actual systemic infection in all cases could be debatable. For instance, Denniston et al. suggested that up to 20% of all blood cultures positive for S. aureus were contaminants. (188) However, due to the retrospective design of my studies, it was impossible to distinguish between systemic infection and contamination in most cases.

6.6 ANTIMICROBIAL RESISTANCE INCREASE

During the study period, AMR increased, but the antimicrobial use did not increase. In **paper III**, we found a decrease in the prescription for 3rd generations cephalosporins. We did not find such a decrease in **paper V**. In **paper III**, neonates were omitted, and in **paper V**, only the most prescribed parenteral antibiotics were evaluated. This may hamper the comparison between the results. However, the increased AMR did not result in an increase in antimicrobial use at our hospital. We also know that antimicrobial out-patients prescriptions to children have decreased by 77% over the last three decades in Sweden. (110)

Somewhat surprisingly, we observed an increase in AMR among patients with no underlying comorbidity, with no previous antibiotic therapy, and with community-acquired infections, as well as in oncology patients, which is the paediatric group with the highest antimicrobial consumption (**paper III** and **V**). We also found a large proportion of children with a travel history to non-Nordic countries among children with isolates expressing AMR.

Increase in AMR in different groups of patients also represents the differences in the mechanism of AMR, in terms of both transmission and prevention.

Resistant species found in children with community-acquired infections reflects the occurrence of AMR in the community, which relates to the total consumption of antimicrobials in society (189) The import of antimicrobial-resistant bacteria through travellers returning from countries with higher rates of AMR is reported from several studies. (140, 141, 190). In oncology patients who are exposed to many episodes with antimicrobials, there is most likely a selection pressure on their microbiota, with increased risk of developing resistant bacteria subpopulations. (191-193). Nosocomial infections may increase the risk of AMR. Luckily, during the study period, there was only one outbreak of ESBL-producing *K. pneumoniae* in 2007. (194) The lack of spread of AMR in the NICU and PICU is in contrast to reports from other intensive care units (146, 195) could perhaps reflect a good hygiene standard.

Based on our studies, we could not make any conclusion of the reasons for the increased AMR in our hospital. Nevertheless, we do know that AMR is a natural evolutionary response to antimicrobial exposure where antimicrobial molecules produced naturally by microorganism has existed for millions of years. (196) In an ecosystem (e.g., the human microbiota) free from external antimicrobial selection pressure, antimicrobial-resistant and non-resistant species co-exist in a stable balance. (197) Antibiotic therapy changes this balance, and antimicrobial drugs exert selection pressure on the microorganism in favour of species that are resistant to the antimicrobials used. Increased use of antimicrobials will, therefore, naturally increase the occurrence of AMR, and lowering their use will reduce AMR (198). In Sweden, administering growth-promoting antibiotics and prophylactic antibiotics to healthy animals is banned since 1986. Sales of antibiotics for animals are stable and at a low level, with mostly narrow-spectrum antibiotics, and contribute to only 12 % of the total antimicrobial consumption in Sweden. The occurrence of AMR in isolates from animal samples of Swedish origin is low. (110) This low use and occurrence of AMR contrast with other some European countries where antibiotics are used in healthy food-producing animals. Countries with high antibiotic use in healthy life stocks also have a high occurrence of AMR. (113) Our results indicate that the increase of AMR is related to travel history to non-nordic countries with implications on strategies to reduce the increased AMR. To avoid inappropriate antimicrobial use in hospital is essential, but efforts to inform policymakers about the importance of political decisions to reduce the global threat of antimcrobial resistance is probably as important.

6.7 IMPLEMENTATION OF PREVENTION STRATEGIES

6.7.1 Antibiotic stewardship programme

In **paper III**, we found around 40% of all admitted patients receiving antimicrobial therapy whereof 70% of all received parenteral antibiotics were third-generation cephalosporins, piperacillin-tazobactam and meropenem. Still, it was mostly evaluated as appropriate prescription and in accordance with guidelines. However, we also concluded that guidelines were missing in almost half of the prescription for both CAI, HAI and surgical prophylaxis. From the result, it was also not possible to evaluate if initiating or continuation of the antibiotic prescription was appropriate or not. Similar surveys of antimicrobial prescription in European paediatric hospital reveal a similarly high rate of prescription where authors advocated antimicrobial stewardship program and continuous surveillance of antimicrobial use. (199, 200)

In the US, 84% of the hospitals reported to have a stewardship program, and the CDC reports a 5% decrease of antibiotic prescription in the outpatient settings and a 15% decline in paediatric prescriptions. (139) Although there is evidence in support the introduction of paediatric antimicrobial stewardship programmes in term of reduced antibiotic usage, improved prescriptions quality, and cost-savings, there is, at present, no consensus for the best strategy. Different interventions are required in different settings. (201) ASP targeting early consultation of an infectious specialist in *S. aureus* BSI had an effect, with an increased number of performed, prolonged intravenous antibiotic therapy as an indicator, improved management but did not affect outcomes. (131) The absence of effect on outcome may also reflect possible lower-risk catheter-related complications in children compared to adults (74) Reduction of antibiotic consumption, and limited use of broad-spectrum antibiotics has been reported in ASP program applied in neonatal settings (194). Finally, a significant impact on antimicrobial use and healthcare cost in pediatric stewardship programmes is reported in studies from the US. Surveillance data regarding antimicrobial use and antibiotic resistance is considered essential for planning and assessment of local stewardship programmes. (202)

6.7.2 Hygiene measurements

Our studies revealed high occurrence of *S. aureus;* 70% of SAB were HAI in children with underlying disease, cancer and children warded at the neonatal unit. Previous, implementation of national infections-control measurements showed a significantly decreased of catheter-associated bacteraemia where the decrease was most evident for CoNS but observed for all pathogens. (67) Similar significant reduction of CoNS is shown in neonatal settings after implementation of the infection control measurements, scrub the hub. (203). The use of a central line bundle protocol is well established, but adherence to the recommended time-bed measures could possible improve with education. (204) In addition to the type of intervention, the structure of the implementation is also of great importance. Designated, trained team leaders with skills to disseminate information to multi professionals teams are described to be important factors in achieving results. (205)

7 CONCLUSION

In summary, this thesis adds knowledge about the aetiology and epidemiology of BSI in children from a Swedish perspective. The findings are of high importance for designing empiric antibiotic therapy regimes and for planning targeted measures to improve therapy and preventions of BSI.'

We concluded that epidemiology and aetiology for BSI were age-related and varied with underlying co-morbidity over the study period. Nowadays, BSI in children without underlying co-morbidity is a rare condition, caused by a limited number of pathogens, but often associated with signs of a focal infection. Not surprisingly, children with cancer and premature neonates were the groups with the highest risk for BSI. On the contrary to healthy children, aetiology of BSI in children with cancer and underlying co-morbidity was diverse, closely related to risk factors and partly age-related. Despite the fact that the proportion of children with BSI and underlying co-morbidity increased during the study period, there was a decreasing trend of paediatric BSI and mortality attributed to BSI.

Over the study period, several strategies to reduce BSI was successfully implemented. The introduction of a vaccine against *Streptococcus pneumoniae* had most impact and decreased the incidence of BSI and the occurrence of meningitis in children. But also the implementation of a risk-based IAP program reduced the risk for GBS EOS. However, it was also evident that a risk-based IAP needs to be monitored carefully to ensure that it reaches all potential cases. Introduction of antifungal prophylaxis to selected neonates was also effective to prevent invasive candida infection.

The occurrence of resistant bacteria, in particular *E.coli* ESBL, increased during the last years of the study period but could not be attributed to an increase in hospital antimicrobial consumption. The most prescribed drugs were third-generation cephalosporins and piperacillin-tazobactam. Therefore, we conclude that changes in antimicrobial therapy prescribed for BSI is not believed to contribute to the increased AMR.

8 FUTURE PERSPECTIVE

8.1 CONTINUOUS MONITORING OF BSA AND AMR

- My studies show the importance of longitudinal monitoring of changes in aetiology. Continuous, structural, prospectively collection of aetiology and patients characteristics will continue to be of interest and importance.
- The attempts to cover a large area have reduced the possibility of analyzing interesting findings in more detail. The results are inspiring and raise several questions for the future.

8.2 PROPER USE OF ANTIBIOTICS

- Our results provide a baseline for antibiotic prescribing in our hospital and could serve as a ground to start an antibiotic stewardship program. I also believe that repeated point prevalence surveys need to be part of the paediatric antibiotic stewardship strategy to identify prescribing trends over time, to evaluate the efficacy of ASPs and to tackle the issue of suboptimal antibiotic use. International standardised PPS with a further linkage between antibiotic prescribing and AMR will be critical to characterise appropriate use of antibiotics in hospitalised children and to propose guidance on the management of paediatric infections taking into account resistance profiles.

- I hope that Sweden will establish a national network of paediatric hospitals to evaluate the antibiotic consumption for hospitalised children on a national level. The overall aim for such a network should be to identify areas for optimising antibiotic prescribing for hospitalized children.

8.3 NEW INSIGHT- NEW THERAPEUTIC APPROACHES AND POSSIBILITIES

- There is an enormous increase in knowledge on the immunological response to infections over the last two decades, both regarding individual differences but also regarding different response to different pathogens. The genetic contribution to the severity of infection may be substantial. This insight will probably lead to the possibility to identify individuals with increased risk for sepsis. Genome-wide studies are allowing research on the impact of genetics on the susceptibility to, and severity of infections. We need to include more of our patients in studies addressing these issues.

8.4 INTERACTION BETWEEN CLINICS AND ACADEMY

After over 20 years of clinical experience and ten years of efforts to combine clinical work with research, I am confident that it is possible to improve this collaboration further. Collaborative projects between clinicians and the academic society with mutual benefits should be the way forward for paediatric infectious disease research.

9 SUMMARY OF THE THESIS IN SWEDISH

Infektioner drabbar alla barn, är i allmänhet orsakade av virus och läker ofta ut inom loppet av dagar. Upp till 10% av alla infektioner är bakteriella och kan behöva antibiotika för att läka ut och inte utvecklas till en allvarlig infektion. Enligt beräkningar dör 3.2 miljoner barn varje år pga infektioner varav 44 % inträffar i nyföddhetsperioden. Över hälften bedöms vara möjliga att förebygga med bl.a. en fungerande sjukvård, vaccinationsprogram, tillgång till antibiotika och andra läkemedel. Majoriteten av all infektionsrelaterad dödlighet hos barn sker i länder med sämre tillgång till detta. Även i länder med god tillgång till sjukvård orsakar infektioner stort behov av sjukhusvård och dödsfall. Infektioner med växt av bakterier i blod, blodinfektioner, är en av de mer allvarliga infektionerna med en betydande risk för död.

Avgörande för framgångsrik behandling av blodinfektioner är tidig upptäckt och att snabbt inleda behandling. Ett stor hot mot behandling av infektioner är den globala ökningen av antibiotikaresistens. Effektiv antibiotikabehandlingen kräver kunskap om vilka bakterier som med störst sannolikhet orsakar blodinfektion och i vilken utsträckning dessa bakterier är resistenta mot förstalinjens antibiotikabehandling. Det finns avgörande skillnader mellan olika länders förekomst av bakterier och resistensmönster Det föreligger bristande kunskap om bakterier och antibiotikaresistens vid blodinfektioner hos barn i Sverige.

Vi har i detta avhandlingsarbete velat öka kunskapen om blodinfektioner hos barn genom att studera vilka typer av bakterier som drabbar vilka barn, underliggande risker för att drabbas och förekomsten av resistenta bakterier.

Avhandlingen beskriver förekomsten av blodinfektioner under en 20-årsperiod. Vi har utgått från resultatet av 60,000 blododlingar erhållna från barn 0-17 år som har vårdats på Astrid Lindgrens Barnsjukhus, Karolinska Universitetssjukhuset i Stockholm 1998-2018. Vi har även studerat hur antibiotikaförskrivningen har sett ut under perioden. Resultaten från forskningen har presenterats i fem delarbeten varav fyra har publicerats som artiklar i vetenskapliga tidskrifters och det femte delarbetet föreligger i manus.

I delarbete **I** analyserade vi resultatet av 1,097 blododlingar tagna på barn 0-17 år med misstanke om infektion under perioden 1998-2008. I genomsnitt insjuknande ett av 2500 barn med blodinfektion orsakad av någon typ av bakterie. Risken var dock avsevärt högre för nyfödda barn där risken var ett av 434 nyfödda barn medan endast ett av 5000 barn i åldersgruppen 6-17 år drabbades. Barn med cancer var den patientgrupp som hade störst risk för blodinfektion. Risken att dö vid blodinfektion var 14.4% bland nyfödda, 5.4% hos barn med underliggande sjukdomar och 1.7% hos tidigare friska barn. Stafylokocker var den vanligaste bakterien. Hos tidigare friska barn utgjorde några få bakterier närmast alla positiva blododlingar. Barn med underliggande sjukdomar riskerade att bli infekterade av många fler olika sorters bakterier vilket har betydelse för vilken antibiotika som skall väljas.

I delarbete **II** utvärderade vi effekten av två olika infektionsförebyggande åtgärder. 2007 introducerade vaccination för att skydda mot pneumokocker och 2008 infördes ett

åtgärdsprogram med att ge antibiotika till födande kvinnor med ökad risk för att överföra Grupp B streptokocker (GBS) till sina barn. Vi jämförde förekomsten av pneumokocker och GBS under två femårsperioder, före och efter att åtgärderna införts. Båda åtgärder var effektiva. För pneumokocker minskade risken med 62% bland de barn som haft störst risk för pneumokockinfektion, 3 månader- 3 års ålder. Bland nyfödda barn <7dgr minskade risken att få en allvarlig GBS infektion med 60%. För både pneumokocker och GBS minskade även hjärnhinneinflammationer med 70%.

I delarbete **III** genomförde vi åtta endagarsmätningar över antibiotikaförskrivningen för alla patienter som vårdades på sjukhuset den specifika dagen. Trots få mätpunkter anses den typen av undersökning ge en representativ bild. Vi fann en förändring av antibiotikaförskrivningen över tid. Förebyggande behandling till utvalda patientgrupper ökade. Andelen av de sjukhusvårdade som fick antibiotika mot samhällsförvärvade infektioner minskade. Av alla som vårdades fick i genomsnitt 36% antibiotika vilket ändrades? tydligt under perioden. Sepsis, oklar feber hos barn med cancerbehandling, bukinfektioner och lunginflammation var de vanligaste anledningarna till behandling.

Delarbete **IV** var en uppföljning av delarbete **I** och **II** för att undersöka om förändringarna bestod och upptäcka eventuellt nya förändringar. Sammanlagt analyserades 2,079 positiva blododlingar under perioden 1998-2018. Risken att insjukna med blodinfektion minskade. Effekten av de infektionsförebyggande åtgärderna beskrivna i arbete **II** kvarstod och var huvudorsaken till minskningen tillsammans med svampförebyggande behandling till mycket förtidigt födda barn med mycket låg födelsevikt. Förändringarna bidrog till en minskad dödlighet bland nyfödda barn. Samtidigt kunde vi notera en ökning av tidiga GBS infektioner slutet av studietiden men orsakerna har ännu inte studerats.

I delarbete **V** granskade vi förekomsten av resistenta bakterier bland alla barn som haft blodinfektioner under hela perioden. 9.2% av de 2,079 blodinfektionerna hade någon typ av resistens. Förekomsten av resistenta bakterier ökade. Bland barn utan underliggande sjukdomar ökade antalet stafylokocker resistenta mot clindamycin och antalet pneumokocker resistenta mot antibiotika-macrolider, tetracyklin och trimetoprim-sulfa. För barn med underliggande sjukdomar noterades 15% av E. coli producera ESBL under de sista 5 åren, då MRSA noterades bland 3 % av alla Stafylokock infektioner. Utlandsvistelse bedömdes som en riskfaktor för både ESBL och MRSA. Antibiotikaförbrukningen ökade inte under perioden och bedöms ej kunna förklara ökningen av resistens.

Sammanfattningsvis konstaterar vi att blodinfektioner bland barn utan underliggande sjukdomar är ovanligt och att vaccination mot de vanligaste och farligaste bakterierna är ett effektivt sätt att minska sjukdom. Förtidigt nyfödda barn med underliggande sjukdomar och barn som behandlas mot cancer har en betydligt större risk för blodinfektioner. De drabbas också delvis av andra bakterier där risken för resistenta bakterier ökar oroande. Åtgärder för att minska risken för infektioner hos dessa grupper behöver sannolikt rikta sig specifikt till respektive riskgrupp. En viktig förebyggande åtgärd är att försöka minska infektioner kopplade till intravenösa infarter som är en vanlig orsak till infektion för sjukhusvårdade barn.

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