ISBN 978-82-326-2440-9 (printed ver.)
ISBN 978-82-326-2441-6 (electronic ver.)
ISSN 1503-8181

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Incidence, mortality, antimicrobial resistance, antibiotic treatment, and impact of statin prophylaxis

Doctoral thesis

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Thesis for the Degree of
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Printed by NTNU Grafisk senter

Blodbaneinfeksjon ved Sykehuset Levanger 2002-2013

Ved infeksjonar på ulike stader i kroppen (til dømes i lunger, urinvegar gallevegar osb) kan bakteriar, og stundom gjærsopp, koma over i blodbanen, formeira seg der og spreiast til alle delar av kroppen. Slik oppstår ein blodbaneinfeksjon. Mikroorganismar kan også koma direkte inn i blodbanen, til dømes gjennom kanylar eller kateter som går inn i blodårer. Diagnosen blodbaneinfeksjon vert stilt ved at vi påviser bakteriar eller gjærsopp i blodprøve (blodkultur).

I perioden 2002-2013 vart det registrert 1995 episodar av blodbaneinfeksjon ved Sykehuset Levanger. Den totale insidensen av blodbaneinfeksjon (episodar pr. 100.000 person-år) auka frå 205 i 2002-2007 til 223 i 2008-2013. Insidensen av blodbaneinfeksjon auka mest hos pasientar som hadde helseteneste-assosiert blodbaneinfeksjon, d.v.s. dei som hadde mykje kontakt med helsetenesta, til dømes pasientar som fekk cellegiftbehandling, dialyse eller som budde på sjukeheim. Insidensen av blodbaneinfeksjon var høgast hos eldre menn.

Dei tre vanlegaste mikrobane som gav opphav til blodbaneinfeksjon var *Escherichia coli* (34,4%), *Streptococcus pneumoniae* (11,3%) og *Staphylococcus aureus* (10,9%). Mindre enn 5% av mikrobane vi fann i blodkulturar frå pasientar med blodbaneinfeksjon, var resistente mot den antibiotikakombinasjonen som vert tilrådd av norske helsestyresmakter til behandling av sepsis av ukjent årsak (penicillin pluss gentamicin, med tillegg av metronidazol dersom anaerob infeksjon er mistenkt). Førekomsten av resistente mikrobar var lågare enn det som vert rapportert frå dei fleste andre land. Men i dei siste åra av studieperioden fann vi ein liten auke av *E. coli* som produserte breispektra beta-laktamase (ESBL).

Antibiotikaresistens er eit aukande problem rundt om i verda, men også i vårt land. Skal vi greie å redusere førekomsten av antibiotikaresistens, må vi bruke mindre antibiotika. Å finne andre tiltak som kan førebyggje blodbaneinfeksjon og død som fylgje av blodbaneinfeksjon vil derfor bli viktig framover.

Vi undersøkte om det å overleve blodbaneinfeksjon kunne ha samanheng med om pasientane brukte statin, ein type medisin som reduserer kolesterol i blodet, men som også har betennelsesdempande effekt. Det viste seg at blant pasientane som hadde blodbaneinfeksjon med Gram-negativ bakterie, var prosentdel som døydde 50% lågare blant dei som brukte eit statin samanlikna med dei som ikkje brukte statin. Blant dei som hadde blodbaneinfeksjon

med Gram-positiv bakterie, var det derimot ingen skilnad mellom dei som brukte statin og dei som ikkje brukte statin.

Navn på kandidat: Arne Mehl

Disputas: Fredag 16. juni, 2017, HUNT Forskningssenter, Levanger

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Finansieringskilder:

- **Enhet for anvendt klinisk forsking, NTNU**
- **Samarbeidsorganet mellom Helse Midt-Norge og NTNU**
- **St Olavs Hospital**
- **Norsk overvåkingssystem for antibiotikaresistens hos mikrober (NORM), Tromsø**
- **Fondet for forskning og utvikling, Helse Nord-Trøndelag HF**

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1. ACKNOWLEDGEMENTS

This study was carried out at the Department of Medicine, Levanger Hospital, Nord-Trøndelag Hospital Trust during the years 2002-2013.

First of all, I want to thank those who inspired me to go ahead with this bloodstream infection (BSI) project. The forerunner of the present work was a study performed by Stein Hallan and Thor Naustdal, who investigated BSI at Innherred Hospital through the years 1988-90, assessing the occurrence of microbes and their resistance patterns and which antimicrobial agents that were used. The results were published in 1994. That same year, a second source of inspiration came from Bergen, where Jon Birger Haug, Stig Harthug, and co-workers published a study on BSI from Haukeland University Hospital. I met Jon Birger and Stig several times through the years, and Stig later on became my first co-supervisor, who has given me valuable input and advice in planning and designing the project. On-site, at Levanger Hospital, Tom-Harald Edna was my collaborator and adviser, and he became my main supervisor. He encouraged me to plan for a PhD project, and he has supported me and inspired me all the time throughout. I am very grateful to Stig and Tom-Harald for their support.

We designed a registration form, and through the years, each positive blood culture result sent from the microbiology laboratory to the hospital wards was accompanied by that registration form, which in turn was filled in by the doctors treating the patients at the wards. I owe the staff at the microbiology huge thanks for consecutively registering episodes of positive blood cultures, freezing down the microbial isolates, and sending the registration forms to the physicians at the wards. Particularly, I want to thank Anne Norunn Vada and Angela Kümmel for their participation and support. I also want to thank all the doctors who through the years have filled in the registration forms.

Bjørn Olav Åsvold was a subordinate doctor at the Department of Medicine, Levanger Hospital, and I was his supervisor in internal medicine. He has later on evolved into a skilled epidemiologic scientist, and he agreed to be my second co-adviser. His wise and relevant guidance has been vital for the progress of the project. Further, Bjørn Olav brought me into collaboration with Erik Solligård and Jan Kristian Damås, whose professional expertise and engagement in developing the Mid-Norway Sepsis Study resulted in a real boost to the BSI project at Levanger Hospital. I am very thankful to Bjørn Olav, Erik and Jan Kristian for their support. I also want to thank Stian Lydersen, the eminent statistician, for his support, and I am very grateful to Julie Paulsen and Ingvild Haugan, who were my subordinate doctors and who joined the project with great enthusiasm.

I am also thankful to Nord-Trøndelag Hospital Trust, Department of Medicine, Levanger Hospital, for supporting my work, and in particular to my former boss, Øystein Sende, who really understood that this type of work might be of value to the hospital.

Further, I would like to thank our research nurses for diligent and accurate work in the data collection process. Many have contributed more or less, but three of them have performed the majority the registration work, so I want to thank them in particular, namely Liv Jorun Vinje, Liv Anita Heggvik, and Tone Benedicte Nylund.

Last but not least, I would like to thank my wife Sigrid for her support, encouragement, and patience during this long period of time-consuming academic immersion.

Financial support was received from the Unit for Applied Clinical Research, Norwegian University of Science and Technology; the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU); St Olav's University Hospital; the Norwegian Surveillance Programme for Antimicrobial Resistance; and by Nord-Trøndelag Hospital Trust's Fund for Research and Improvement.

Levanger, February 28, 2016

Arne Mehl

2. LIST OF PAPERS

I. Arne Mehl, Bjørn Olav Åsvold, Stian Lydersen, Julie Paulsen, Erik Solligård, Jan Kristian Damås, Stig Harthug, and Tom-Harald Edna. **Burden of bloodstream infection in an area of Mid-Norway 2002-2013: a prospective, population-based observational study.** BMC Infectious Diseases 2017, ***17***(1): 205.

II. Arne Mehl, Bjørn Olav Åsvold, Angela Kümmel, Stian Lydersen, Julie Paulsen, Ingvild Haugan, Erik Solligård, Jan Kristian Damås, Stig Harthug, and Tom-Harald Edna. **Trends in antimicrobial resistance and empiric antibiotic therapy of bloodstream infections at a general hospital in Mid-Norway 2002-2013: a prospective observational study.** BMC Infectious Diseases 2017, ***17***(1): 116.

III. Arne Mehl, Stig Harthug, Stian Lydersen, Julie Paulsen, Bjørn Olav Åsvold, Erik Solligård, Jan Kristian Damås, Tom-Harald Edna. **Prior statin use and 90-day mortality in Gram-negative and Gram-positive bloodstream infection: a prospective observational study.** Eur J Clin Microbiol Infect Dis 2015, ***34***: 609–617.

3. ABBREVIATIONS

AAT	Appropriate antimicrobial therapy
ADR	Acquired drug resistance
AEAT	Appropriate empiric antibiotic therapy
AIS	Acute ischemic stroke
AMI	Acute myocardial infarction
APTT	Activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
AT	Antimicrobial therapy
BSI	Bloodstream infection
CA	Community acquired
CCI	Charlson comorbidity index
CI	Confidence interval
CO	Community onset
DAT	Definitive antibiotic therapy
DDD	Defined Daily Doses
DIC	Disseminated intravascular coagulation
EARS-Net	European Antimicrobial Resistance Surveillance Network
EAT	Empiric antibiotic therapy
ED	Emergency department
ESBL	Extended spectrum beta-lactamase
ESBL-E	Extended spectrum beta-lactamase producing *Enterobacteriaceae*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GGPP	Geranylgeranylpyrophosphate

HA	Hospital acquired
HCA	Health care-associated
HCAI	Health care-associated infection
HCAP	Health care-associated pneumonia
ICU	Intensive care unit
IDR	Inherent drug resistance
IEAT	Inappropriate empiric antibiotic therapy
IL	Interleukin
INR	International normalized ratio
IRR	Incidence rate ratio
LPS	Lipopolysaccharide (endotoxin, cell wall component in Gram-negative bacteria)
LTA	Lipoteichoic acid (cell wall component in Gram-positive bacteria)
MALDI-TOF	Matrix-Absorption-Laser-Desorption-Ionisation-Time of Flight
MDR	Multi-drug resistant
MRSA	Methicillin-resistant *Staphylococcus aureus*
NF-κB	Nuclear factor kappa B
NADPH	Nicotinamide adenine dinucleotide phosphate
NORM	Norwegian Surveillance Programme for Antimicrobial Resistance ()
NWGA	Norwegian Working Group on Antibiotics
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor 1
PAMPs	Pathogen associated molecular patterns
PCR	Polymerase chain reaction
PG	Penicillin plus gentamicin

PGM	Penicillin, gentamicin, and metronidazole
PGN	Peptidoglycan, cell wall component in bacteria (a thick layer in Gram-positive bacteria; a thin layer in Gram-negative bacteria)
PIP/TAZ	Piperacillin-tazobactam
PNSP	Penicillin-non-sensitive *Streptococcus pneumoniae*
RCT	Randomized controlled trial
PRRs	Pattern recognition receptors
SOFA	Sepsis (or sequential) organ failure assessment
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
VT	Venous thromboembolism

4. DEFINITIONS

4.1 Epidemiological and statistical terms

Incidence

The incidence rate is defined as the number of new cases of disease arising in a specified population over a given time period divided by the total person-time at risk during the period.

Incidence rate ratio

The incidence rate ratio (IRR) is defined as the ratio between two incidence rates.

Mediator

A mediator is an intermediate stage between exposure and outcome

Odds

Odds is the number of events to non-events, e.g., the number of diseased to the number of non-diseased, or the number of dead to the number of survivors.

Odds = x/n-x, where x is the number of events and n is the total number of study participants.

Odds ratio

Odds ratio (OR) is the ratio between two odds, e.g., the ratio between the odds of disease (or death) in exposed (or treated) and unexposed (or untreated) individuals. An OR of 1 means that there is no difference in risk of disease between exposed and unexposed individuals, an OR >1 means that the exposed individuals have a higher risk of death, and an OR <1 means that the exposed individuals have a lower risk of death.

Mortality

Mortality rate

The mortality rate is the number of deaths per 100,000 person-years (person-years is the number of persons in a population per calendar year)

In **Paper I**, the mortality rate was calculated as the total number of deaths within 30 days of a diagnosis of BSI per 100,000 person-years.

Case fatality rate

Case fatality rate is the number of deaths divided by the total number of patients with a disease (the proportion of patients who die).

30-day case fatality

The proportion of patients who died within 30 days of the time of BSI diagnosis (the time of the first positive blood culture). In **Paper I**, the case fatality rate was defined as the total number of deaths within 30 days of diagnosed BSI episodes divided by the total number of BSI episodes.

90-day case fatality

The proportion of patients who had died within 90 days of the time of BSI diagnosis (the time of the first positive blood culture). In **Paper III**, the 90-day case fatality rate is named 90-day mortality, because some journals, e.g., European Journal of Infectious Diseases and Clinical Microbiology, use the term 90-day mortality instead of 90-day case fatality rate.

4.2 Infection-related definitions

Infection

Infection is defined as a pathological process caused by the invasion of normally sterile tissue or fluid or body cavity by pathogenic or potentially pathogenic micro-organisms [1].

Bloodstream infection

Bloodstream infection is a condition where bacteria or fungi are isolated from blood cultures from a patient with clinical signs and symptoms of systemic infection, and where contamination has been ruled out [2]. Bloodstream infection includes the terms bacteremia and fungemia (see below).

Primary bloodstream infection

If a bloodstream infection arises because microbes are introduced directly into the bloodstream, e.g., through an intravascular catheter, the condition is named a primary bloodstream infection.

Secondary bloodstream infection

A secondary bloodstream infection occurs if microbes enter the bloodstream from infectious foci somewhere in the body, such as the lungs, the urinary tract, the biliary tract, the abdomen or the small pelvis, or from abscesses at any site.

Bacteremia

Bacteremia is a condition where viable bacteria are present in the bloodstream, as evidenced by blood cultures [3].

Fungemia

Fungemia is a state where fungi (most often yeasts) are present in the bloodstream, detected by blood cultures.

Infection severity

In this study, we have used the following scale for expressing the severity of infection (the terms are defined below):

(1) Bacteremia without sepsis

(2) Sepsis

(3) Severe sepsis

(4) Septic shock

Sepsis

Until 1990, sepsis was defined as the presence of pathogenic organisms or their toxins in the blood or tissues [4]. During the 1980s, however, an increasing knowledge in sepsis pathophysiology gave the understanding of sepsis as a state driven by a dysregulated host response [5]. Therefore, in 1991, a consensus conference [3] proposed a new definition of sepsis:

The sepsis-1 definition 1991

The conference introduced the term Systemic Inflammatory Response Syndrome (SIRS) and defined sepsis as a clinical syndrome with both infection (detected or suspected) and SIRS.

SIRS

If two or more of the following criteria are fulfilled, a patient has SIRS:

(1) Temperature >38.0^{o}C or lower than 36.0^{o}C

(2) Heart rate >90 per minute

(3) Hyperventilation evidenced by respiratory rate >20 per minute or pCO2 <4.3 kPa

(4) Leucocyte count >12x10^{-9} per Liter or <4x10^{-9} per Liter or >10% immature neutrophils

The Sepsis-2 definition 2001

Because of increasing discontentment [6] with the sepsis-1 definition, an extended consensus conference was held in 2001 [1]. The 2001-definition of sepsis was detected or suspected infection plus "some" criteria from the following categories of variables:

General variables

- Temperature > 38.3^{o}C or <36^{o}C
- Heart rate >90 beats/min
- Tachypnoe >20 breaths /min or $PaCO_2$ <4.3 kPa
- Altered mental status
- Significant new onset edema or positive fluid balance (>20 ml/kg over 24 h)
- Hyperglycemia in the abscence of diabetes. Plasma glucose > 7.7 mmol/L

Inflammatory variables

- Leukocytosis (>12 x 10^9/L) or leucopenia (< 4x10^9/L) or >10% immature neutrophils
- Plasma C reactive protein (CRP) >2 SD above the normal value
- Plasma procalcitonin >2 SD above the normal value

Hemodynamic variables

- Arterial hypotension (systolic blood pressure <90 mmHg, mean arterial pressure <70 mm Hg or a 40 mmHg decrease in systolic blood pressure)

Organ dysfunction variables

- Arterial hypoxemia. PaO_2/FiO_2 < 40. Partial oxygen pressure in arterial blood (kPa) divided by the fraction of oxygen in inspired air (0.2 in atmospheric air).
- Use of mechanical ventilation for acute respiratory failure
- Acute oliguria (urine output <0.5 mL/kg/hour or <45 mL/hour for at least 2 hours)

- Creatinine increase > 45 μmol/L
- Coagulation abnormalities (international normalized ratio (INR) >1.5 or activated partial thromboplastin time (APTT) >60 sec)
- Thrombocytopenia (platelet count <100 x 10^9/L)
- Ileus (absent bowel sounds)
- Hyperbilirubinemia (bilirubin > 70 μmol/L)

Tissue perfusion variables

- Decreased capillary refill (≥3 seconds)
- Mottling
- Hyperlactatemia (> 3 mmol/L)

The Sepsis-3 definition 2016 [7]

During the last 15 years, clinical studies and new insights into sepsis pathophysiology had made the Sepsis-2 definition outdated. The 2016-definition describes sepsis as a syndrome of physiologic, pathologic, and biochemical abnormalities induced by infection. Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction can be represented by an increase in the SOFA score of 2 points or more, which is associated with an in-hospital mortality greater than 10%.

A clinically useful set of criteria for diagnosing sepsis will necessarily be somewhat arbitrary. There is no gold standard against which the diagnostic criteria can be calibrated. Establishing working definitions for a syndrome is inherently an imperfect process and one that requires periodic updating on the basis of new insights into pathophysiology or the availability of diagnostic tests [7].

Severe sepsis

The term severe sepsis was defined in the Sepsis-1 and Sepsis-2 definitions and represented a state of suspected infection plus some degree of organ dysfunction [1]. In the Sepsis-3 definition, the term severe sepsis is no longer in use [7].

Septic shock

According to the Sepsis-1 and Sepsis-2 definitions [1, 3], septic shock was defined as a state of acute circulatory failure characterized by persistent arterial hypotension unexplained by other causes. Hypotension was defined by a systolic arterial pressure below 90 mmHg, mean arterial pressure lower than 60, or a reduction in systolic blood pressure of more than 40 mmHg from baseline, despite adequate volume resuscitation, in the absence of other cause of hypotension. According to the Sepsis-3 definition [7], patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and serum lactate level greater than 2 mmol/L in the absence of hypovolemia. This combination is associated with hospital mortality rates greater than 40%.

SOFA (Sepsis Organ Failure Assessment) **score**

Table 1.
Sepsis Organ Failure Assessment score [8]

Organ system	0	1	2	3	4
Respiratory: PaO_2/FiO_2, mmHg (kPa)	> 400 (>53.3)	≤400 (≤53.3)	≤300 (≤40)	≤200[a] (≤26.7) [a]	≤100 [a] (≤13.3) [a]
Renal: creatinine µmol/L	<110	110-170	171-299	300-440 or urine output <500 mL /day	>440 or urine output <200 mL /day
Hepatic: bilirubin µmol/L	<20	20-32	33-101	102-204	>204
Cardiovascular	MAP >70 mm Hg	MAP<70 mm Hg	Dopamine <5[b]	Dopamine >5 or norepinephrine ≤0.1 [b]	Dopamine >15 or norepinephrine >0.1 [b]
Hematologic: Platelets x 10^9/L	>150	<150	<100	<50	<20
Neurologic: Glasgow Coma Scale	15	13-14	10-12	6-9	<6

[a] With ventilatory support (invasive or non-invasive)
[b] Adrenergic agents administered for at least 1 h (doses are given in µg/kg/min)

In our BSI cohort, the SOFA score was not collected systematically in all patients, not even in all ICU patients. What we have done is to use **SOFA score >2 as a marker of severe organ failure**. For patients in the wards, it is unlikely that an organ failure corresponding to SOFA score grade 3 or 4 would not be detected. In **Paper III**, we have used SOFA score >2 as a variable expressing severe failure in any organ.

Quick SOFA (qSOFA)

In out-of-hospital, emergency department, or general hospital wards, adult patients with suspected infection can be rapidly identified as being more likely to have poor outcomes

typical of sepsis if they have at least 2 of the following clinical criteria that together constitute a new bedside clinical score termed quickSOFA (qSOFA) [7]:

-respiratory rate 22 per minute or greater,

-altered mentation, or

-systolic blood pressure of 100 mmHg or less.

The combination of suspected infection plus 2 qSOFA criteria is not sufficient for the diagnosis of sepsis, but shall lead to an immediate evaluation of the patient with regard to organ failure and sepsis [9].

In this dissertation, we have used the Sepsis-2 definitions of sepsis, severe sepsis, and septic shock, as these were the applicable definitions during the study period. Infection severity is discussed in **Paper III**. We defined sepsis as documented bacteremia or fungemia *plus* at least 2 variables mentioned under the Sepsis-2 definition. This was in accordance with the definition of sepsis used by Osborn et al. [10]. Figure 1 illustrates the difference between the 2016 sepsis definition and the sepsis definition from 1991.

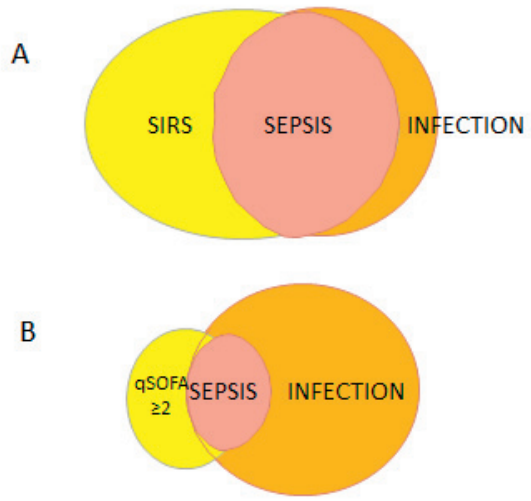

Figure 1.
Illustration of the 1991 and 2016 sepsis definitions. A. In the 1991 sepsis definition, a majority of infections belonged to the category of sepsis. **B**. According to the 2016 definition, a majority of infections in which ≥2 qSOFA criteria are fulfilled, fall into the category of sepsis. Adapted from Vincent et al., 2016 [9]

5. SUMMARY

Bloodstream infection (BSI) is a condition where microbes are isolated from blood cultures from patients with clinical signs and symptoms of systemic infection. BSI contributes substantially to morbidity and mortality worldwide.

Studies from several countries indicate that the incidence and mortality of bloodstream infection (BSI) as well as the antimicrobial resistance of BSI microbes have been increasing over time. We studied the occurrence of microbes and the burden of disease and death related to BSI (**Paper I**) in a defined geographical area of Mid-Norway (the catchment area of Levanger Hospital) during 2002-2013. In **Paper II**, we assessed trends in antimicrobial resistance and empiric antibiotic therapy at Levanger Hospital, which is a medium-sized general hospital in Mid-Norway.

In several studies on patients with bloodstream infection (BSI), prior use of statins has been associated with improved survival. As Gram-positive and Gram-negative bacteria alert the innate immune system in different ways, we studied whether the relationship between prior statin use and 90-day case fatality differed between Gram-positive and Gram-negative BSI (**Paper III**).

The BSI episodes were prospectively recorded at the microbiology laboratory, Levanger Hospital. Population data of the ten municipalities that constituted the catchment area of Levanger Hospital, were obtained from Statistics Norway. Demographic and clinical data, including use of statins and other medications at admission, comorbidities, functional status, and treatment, were collected from and from the patients' hospital records. Data on vital status were provided from the National Population Register. Death related to BSI was defined as death within 30 days of BSI detection (**Paper I**). In **Paper III**, death within 90 days was the study outcome.

Between 2002 and 2013, 1995 episodes of BSI in 1719 patients aged 16 to 99 years were included in the database. In **Paper I**, age and sex standardized incidence and mortality rates and case fatality rates were calculated. The overall incidence of BSI was 215 per 100,000 person-years. The incidence increased exponentially with age, particularly in males. The incidence increased from 205 to 223 per 100,000 person-years from 2002-07 to 2008-13. *Escherichia coli* was the most frequently isolated microbe, followed by *Streptococcus pneumoniae* and *Staphylococcus aureus*. The rate of *S. pneumoniae* BSI decreased over time in males (on average by 9.2% annually), but not in females. The total rate of BSI microbes with acquired resistance increased slightly over time, but did not exceed 2 episodes per 100,000 person-years. The mortality of BSI was 32 per 100,000 person-years. It was higher in males than in females (36 vs. 28 per 100,000 person-years) and significantly higher in old age, particularly in males. The total BSI mortality was similar in the first and second halves of the study period, but the mortality of *S. pneumoniae* BSI decreased in males (15.0% annually). The crude case fatality decreased from the first to the second half of the study period (17.2% to 13.1%; p=0.014). The rate of blood culture sampling increased more than twofold during the study period.

In **Paper II,** we analyzed the antimicrobial non-susceptibility of BSI microbes according to microbe group, place of acquisition, site of infection, and time period. There were 934 community-acquired (CA), 787 health care-associated (HCA) and 274 hospital-acquired (HA) BSIs. The urinary tract was the most common site of infection. Allover, 3.5% of the BSI microbes were non-susceptible to the antimicrobial regimen recommended by the National Professional Guidelines for Use of Antibiotics in Hospitals, consisting of penicillin, gentamicin, and metronidazole (PGM). In contrast, 17.8% of the BSI microbes were non-susceptible to cefotaxime and 27.8% were non-susceptible to ceftazidime.

Antimicrobial non-susceptibility differed by place of acquisition. For the PGM regimen, the proportions of non-susceptibility were 1.4% in CA-, 4.8% in HCA-, and 6.9% in HA-BSI ($p<0.001$), and increasing proportions of non-susceptibility over time were observed in HA-BSI (2.2% in 2002-2005, 6.2% in 2006-2009, and 11.7% in 2010-2013 [p=0.026]), mainly caused by inherently resistant microbes. We also observed increasing numbers of bacteria with acquired resistance, particularly *E. coli* producing ESBL or possessing gentamicin resistance, and these occurred predominantly in CA- and HCA-BSI.

The proportion of BSI episodes in which appropriate antibiotic therapy was given within 6 hours of the first positive blood culture, increased from 2002-2005 to 2010-2013 (62% to 67% in CA-BSI, 64% to 67% in HCA-BSI, and 44% to 59% in HA-BSI). For appropriate antibiotic therapy given within 24 hours, the corresponding proportions were 84% to 88% in CA, 77% to 84% in HCA, and 72% to 83% in HA-BSI. The use of cephalosporins decreased over time, whether the use of piperacillin-tazobactam and the combination penicillin-aminoglycoside increased.

In order to study the relationship of prior statin use with 90-day case fatality (**Paper III)**, we conducted a prospective observational cohort study of 1,408 adults with first time BSI admitted to Levanger Hospital between January 1, 2002, and December 31, 2011. The relationship of statin use with 90-day case fatality differed between Gram-negative and Gram-positive BSI (p-value for interaction 0.01). Among patients with Gram-negative BSI, statin users had significantly lower 90-day total case fatality (odds ratio [OR] 0.42, 95% confidence interval [CI] 0.23–0.75, p = 0.003). After adjustment for possible confounders (sex, age, functional status before the infection, underlying diseases, place of acquisition, focus of infection), the association remained essentially unchanged (adjusted OR 0.38, 95% CI 0.20–0.72, p = 0.003). A similar analysis of patients with Gram-positive BSI showed no association of statin use with mortality (adjusted OR 1.22, 95 % CI 0.69–2.17, p = 0.49).

Conclusions: The incidence rate of BSI episodes increased through the study period, but the mortality rate was mainly unchanged, and the case fatality rate decreased. A more than twofold increase in the rate of BSI sampling possibly contributed to the detection of milder and ultimately less fatal episodes, but earlier detection and improved treatment may have had impact.

Very low, yet slightly increasing rates of microbes with acquired resistance were observed. Generally, antimicrobial resistance was a far smaller problem in our BSI cohort than is reported from countries outside Scandinavia. In our cohort, appropriate empiric antibiotic therapy could be achieved to a larger extent by replacing second- and third-generation cephalosporins with penicillin-aminoglycoside or piperacillin-tazobactam.

In our BSI cohort, prior statin use was associated with a lower 90-day case fatality in Gram-negative BSI, but not in Gram-positive BSI.

6. INTRODUCTION

6.1 Bloodstream infection and sepsis

6.1.1 Opening perspective on bloodstream infection and sepsis

Invasive bacterial and fungal infections are commonly occurring, and if not treated effectively, they are life-threatening diseases. Before the antibiotic era, infectious disease constituted a leading cause of death, also in industrialized countries such as the US [11]. From 1920 to 1960, the mortality caused by infectious diseases decreased by 70%, but since then the curve has flattened [11]. In spite of great treatment efforts during the last decades, infections are still among the most common causes of death worldwide. In the last decades, a rising occurrence of microbes resistant to antimicrobial agents is of particular concern [12]. Patients infected with resistant microbes often receive appropriate antimicrobial therapy either too late or not at all. Consequently, more deaths are caused by infections that could formerly be treated successfully.

Bloodstream infection (BSI), a condition where bacteria or fungi invade the bloodstream, is still among the ten most common causes of deaths in developed countries [13]. Incidence rates between 80 and 257 per 100,000 person-years have been reported, with higher rates in the more recent years [14, 15], and the case fatality rate constitutes 10-40% [16-18].

The severity of BSI is not only caused by pathogenic microbes but is just as much a result of an uncontrolled host response that damages the host's own organs and tissues, a condition termed sepsis. If sepsis is defined according to the 2001-definition (see section 4.2), a large majority of BSI patients will have sepsis, i.e., infection with at least 2 criteria from a long list of various variables. About 25% of BSI patients develop severe sepsis [19], and 10%

to 16% deteriorate to septic shock [16, 20]. On the other hand, BSI has been found in 10% to 17% of patients with sepsis (suspected infection plus SIRS) [21, 22], in 25% to 31% of patients with severe sepsis [22, 23], and in nearly 70% of patients with septic shock [22]. In developed countries, the incidence of severe sepsis (corresponds approximately to *sepsis* according to the 2016-definition) has been estimated to 50 – 100 per 100,000 person-years [24]. The incidence of sepsis according to the 2001-definition is 3-4 times higher, as about 25% of sepsis patients develop organ dysfunction. The case fatality rates (the percentage of patients who die) are 10%-20% in sepsis, 20%-50% in severe sepsis, and 40%-80% in septic shock [24]. During the last decades, the incidence of sepsis has been increasing in all areas of the world where epidemiologic studies have been conducted [24]. Increasing number of older people with a high burden of comorbidity and more advanced health care, including invasive procedures and immunosuppressive therapy, are factors that contribute to increased incidence of BSI and sepsis. The increased incidence gives rise to increased mortality, despite a tendency of decreasing case fatality the last decades.

In the months following BSI or sepsis, survivors have significantly worse prognosis than population controls (secondary infections, persistent organ dysfunctions, vascular events, deaths [25-27].

6.1.2 Pathophysiology of sepsis

Pathogenic microorganisms have virulence factors that enable them to invade host tissues and cells. Such factors are the capsule of bacteria, which inhibits phagocytosis, and bacterial toxins and enzymes, that destroy cell membranes [28]. Invading micro-organisms are detected by immune cells (CD4 T-lymphocytes, monocytes/macrophages, dendritic cells, neutrophils), but also by other host cells, such as endothelial cells. Pathogen associated molecular patterns (PAMPs) on the surface of microbes are detected by pattern-recognition receptors (PRRs) on the outer cell membrane of host cells [29]. Among the different PAMPs are

lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria and lipoteichoic acid (LTA) and peptidoglycan (PGN) from the cell wall of Gram-positive bacteria (Figure 2).

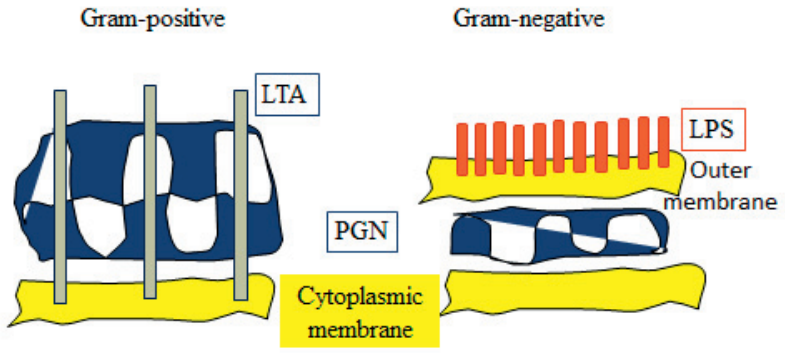

Figure 2.

Cell wall structure in Gram-positive and Gram-negative bacteria. In Gram-positive bacteria, the cell wall consists of a thick layer of peptidoglycan (PGN) anchored by elements of lipoteichoic acid (LTA). The Gram-negative cell wall has a thin layer of PGN and an outer membrane carrying lipopolysaccharide (LPS, also called endotoxin)

The recognition of PAMPs by PRRs activates intracellular pathways, inducing transcription of pro-inflammatory cytokine genes, giving rise to synthesis and secretion of inflammatory mediators such as interleukin (IL)1, IL-6 and tumor necrosis factor alpha (TNFα) [30] (Figure 3).

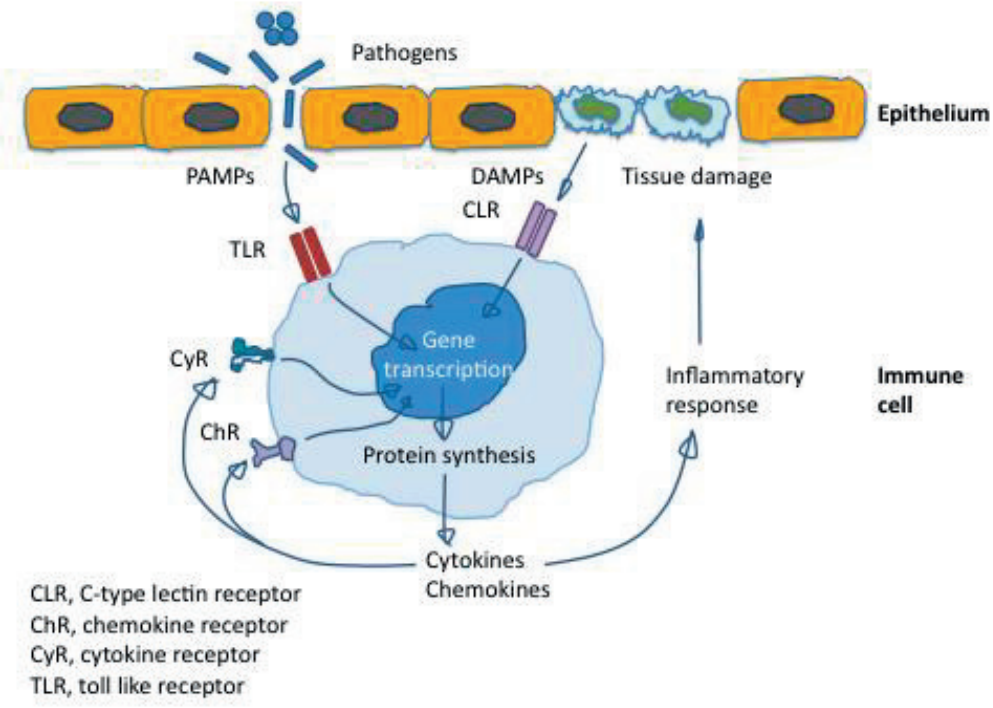

Figure 3.

The initial host response in sepsis**.** Microbes with their pathogen associated moleular patterns (PAMPs) penetrate a mucosal barrier and is recognized by immune cells with toll-like receptors (TLRs). This activates an intracellular signaling pathway that leads to transcription of inflammatory mediator genes and synthesis of cytokines and chemokines. These signaling molecules stimulate immune cells via cytokine- and chemokine receptors, causing further inflammatory mediator production, resulting in cell damage and release of damage associated molecular patterns (DAMPs), which further increase the inflammatory reaction. (Adapted from [31])

Many different PRRs are detected, among them the toll like receptors (TLRs), of which at least thirteen different types are known [32]. Of special interest for this thesis (**Paper III**) are TLR2, which detects LTA and PGN from Gram-positive bacteria and TLR4, which identify the presence of LPS from Gram-negative bacteria [30, 33] (Figure 4).

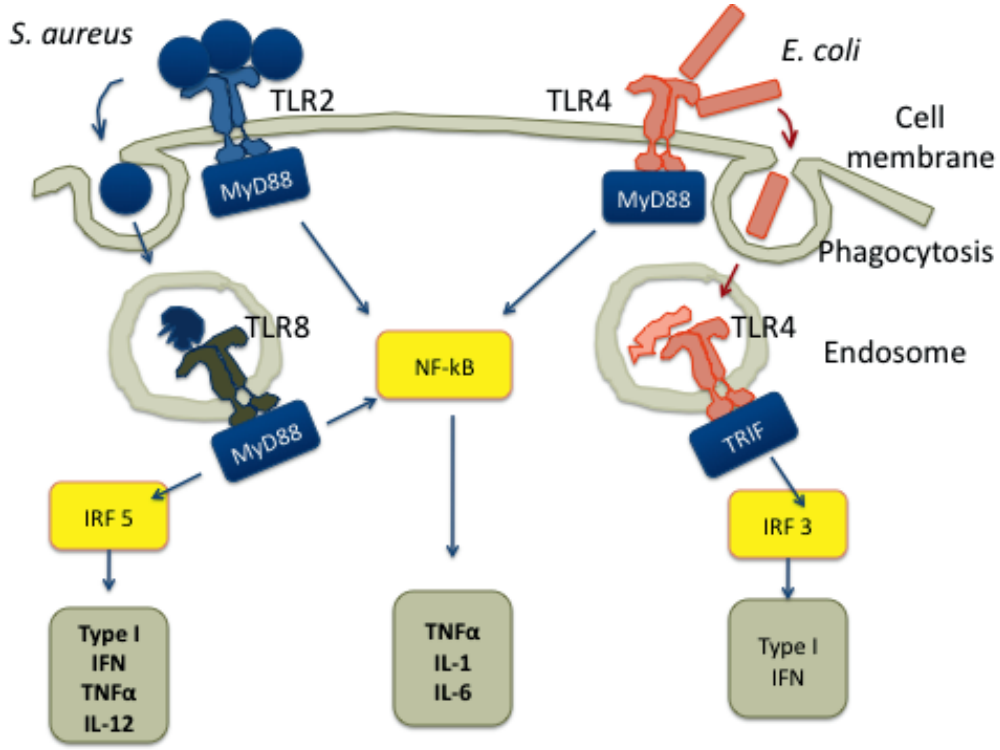

Figure 4.

Toll like receptor signaling pathways for Gram-positive and Gram-negative bacteria**.** Peptidoglycan (PGN) from Gram-positive bacteria (e.g., *S. aureus*) stimulates immune cells via TLR2, whereas lipopolysaccharide (LPS) from Gram-negative bacteria (e.g., *E. coli*) stimulates via TLR4. The initial stimulation via TLR 2 and 4 on the outer cell membrane switches on an intracellular pathway via the adapter protein MyD88, that activates the transcription factor NF-kB, inducing transcription of pro-inflammatory cytokine genes (TNFα, IL-1, IL-6). After phagocytosis, degrading bacteria inside endosomes continue to stimulate TLRs, but the signaling pathways in this late phase are different for Gram-positive and Gram-negative bacteria, resulting in transcription of different cytokine genes. Single-stranded RNA from Gram-positive bacteria stimulates TLR8, which is expressed within the endosome, switching on two signaling pathways, the one via NF-kB, inducing inflammatory cytokine genes, the other via IRF5, inducing immuno-modulatory (type I interferon (IFN), IL-12), but also pro-inflammatory cytokine genes (TNFα) [34]. When stimulated by LPS from

Gram-negative bacteria, TLR4 expressed inside endosomes switches on a pathway that activates IRF3, the latter inducing transcription of type I IFN genes. Interferons exert a multiplicity of immunomodulatory effects [35]. For simplicity, the protein kinases in the signaling pathways are not shown. Bacteria and receptors are not drawn to the same scale. IRF3, interferon regulatory factor 3; IRF5, interferon regulatory factor 5; LPS, lipopolysaccharide; MYD88, myeloid differentiation response gene 88; NF-kB, nuclear factor kappa B; PGN, peptidoglycan; TRIF, TIR-domain-containing adapter inducing interferon β. MyD88 and TRIF are adapter proteins. IRF3, IRF5, and NF-kB are transcription factors. IFN, TNFα, IL-1, IL-6, and IL-12 are cytokines.

The pro-inflammatory cytokines give rise to pathophysiological responses such as fever, mobilization of neutrophils, vasodilatation, hypotension, endothelial leakage, edema, and also release of tissue factor from endothelial- and immune cells, resulting in coagulation activation, which results in fibrin production. In a hyper-inflammatory state, the formation of plasmin, the enzyme that dissolves fibrin, is inhibited because of increased plasminogen activator inhibitor 1 (PAI-1). Therefore, fibrinolysis is hampered, and clotting takes place in small vessels around the body (disseminated intravascular coagulation (DIC) [29].

Cell damage caused by microbial toxins or enzymes or by hyper-inflammation releases intracellular substances such as DNA and RNA, which are recognized by the immune cells' PRRs as "alarmins", i.e., substances that activate the PRR-pathways in similar ways as the PAMPs, resulting in an even stronger inflammatory response. These "alarmins" are called damage associated molecular patterns (DAMPs) [29].

Contemporary and subsequent to the pro-inflammatory response, a compensatory, anti-inflammatory and immunomodulatory response is going on, driven by anti-inflammatory mediator substances such as IL10, IL13, and type I interferons, resulting in inhibition of TLR signaling pathways and immune cell apoptosis. A state of immunosuppression comes about, increasing the risk of secondary infections. Patients who survive the acute inflammatory stage

of sepsis, but who have longer stays in the ICU, often die from hospital-acquired infections [29].

The development of acute organ failure in sepsis is not primarily caused by cell damage but is a consequence of hypoxia. Oxygen uptake in the lungs is often impaired due to inflammation in alveoli, interstitial space, and lung vessel endothelium, the latter leading to vascular leakage and pulmonary edema. Inflammation and edema can give rise to acute respiratory distress syndrome (ARDS). The transport of oxygen in the blood is inhibited by hypotension, edema, or micro-vascular thrombosis, and the utilization of oxygen in the cells is disrupted by mitochondrial dysfunction [29].

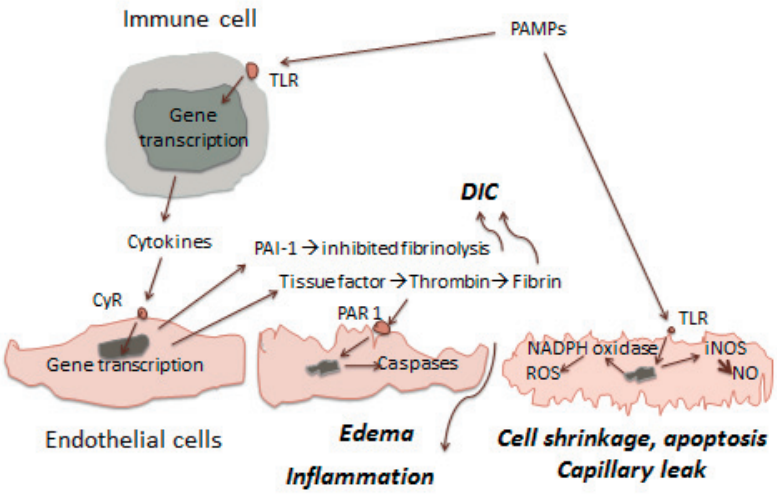

Figure 5.
The pathogenesis of endothelial inflammation, disseminated intravascular coagulation, and shock in sepsis**.** PAMPs are recognized by toll like receptors (TLRs) on immune cells, and intracellular signaling pathways are activated, resulting in gene transcription and synthesis of inflammatory cytokines. Cytokines stimulate endothelial cells. Genes of tissue factor (TF) and plasminogen activator inhibitor 1 (PAI-1) are induced, resulting in activated coagulation and inhibited fibrinolysis, which give rise to disseminated intravascular coagulation (DIC). Increased amounts of thrombin activate protease activating receptors (PAR 1), inducing genes

of enzymes (caspases) that provoke cell shrinkage and apoptosis (adapted from [29]). PAMPs also trigger TLRs on endothelial cells, inducing genes of enzymes (e.g., inducible nitric oxide synthase [iNOS] and NADPH oxidase), producing superfluous nitric oxide and reactive oxygen species (ROS), which give rise to vasodilatation and endothelial cell damage. The consequences are capillary leak, interstitial edema, perivascular inflammation, and eventually shock [36, 37].

CyR, cytokine receptor; NADPH, nicotinamide adenine dinucleotide phosphate; PAMPs, pathogen associated molecular patterns

6.1.3 Portal of entry and focus of BSI

In most cases, BSI originates from an infected organ or tissue, e.g. the lungs, the urinary tract, the biliary or gastrointestinal tract, from which microbes invade the bloodstream. BSI can also originate from an external portal of entry, e.g., a traumatic or surgical wound, instrumentations, or the insertion site of a cannula or intravascular catheter. A third mechanism is translocation of microbes from the intestinal flora, which may occur if local defense mechanisms fail, such as in bowel hypoxia or if the bowel mucosa is damaged by toxins or inflammation processes [38]. Hematogenous spread of microbes can result in secondary infection foci such as endocarditis, osteomyelitis, meningitis, and abscesses (in brain, psoas muscle, etc.) [16, 39].

6.1.4 Place of acquisition

From the 1980s, BSI has been classified as community onset (CO) if the infection was present at admission or appeared within 48 hours of the hospital stay. BSIs that occurred >48 hours after admission were categorized as hospital acquired (HA) [39]. BSIs secondary to surgical site infections were classified as HA infections if the surgery was performed in the last 30 days before admission [40]. Because patients who have frequent hospital visits, e.g. for

hemodialysis or chemotherapy, more often acquire infections with resistant microbes, a further division of CO-BSIs into two groups, health care-associated (HCA) BSI and community acquired (CA) BSI, was proposed in 2002 [41, 42]. To what amount the group HCA infection (HCAI) is associated with risk of infections with multidrug-resistant (MDR) microbes differ between studies, partly depending on geographical differences in the proportions of MDR infections [43]. Treatment guidelines have been proposed for HCA pneumonia (HCAP) [44], but treatment of HCAP based on the guidelines has resulted in overuse of broad-spectrum antibiotics (BSA), resulting in drug side effects, *Clostridium difficile* infection, and increased drug resistance, particularly in countries where the prevalence of MDR microbes is relatively low [45, 46]. Further, there has been some heterogeneity between researchers regarding which factors should be included in the definition of HCAI (Table 1; [47]).

Interestingly, prior antibiotic treatment has not been a separate criterion for HCAI in any of the studies reviewed by Cardoso et al. [47]. Prior invasive procedures are not a criterion in the most commonly used definition of HCA proposed by Friedman et al. [42], but was included in one of the first studies on the topic, performed by Siegman-Igra et al. [41]. According to Cardoso et al., recent invasive procedures, hospitalization in the last year or previous antibiotic treatment should be considered for inclusion in the definition [47]. Another relevant factor, which may define increased risk of infections with MDR microbes, is family members with MDR microbes [48]. Which patients with potential BSI acquired outside hospital should have broad-spectrum antibiotic treatment depends on local occurrence of MDR microbes and the severity of infection at the time of diagnosis.

Table 2.
Inclusion criteria for health care-associated (HCA) bloodstream infection in some studies and reviews (a large number of studies is reviewed by Cardoso [47])

Author (Year) [Ref]	Time (days) from the previous hospitalization	Nursing home residence included	Prior antibiotic treatment included	Prior surgery or instrumentation (days)
Friedman (2002) [42]	90	Yes	No	No
Siegman-Igra (2002) [41]	30	Yes	No	Yes (shortly before)
Schorr (2006) [49]	30	No	No	No
Søgaard (2011) [50]	30	No	No	No
Son (2010) [51]	90	Yes	No	No
Lenz (2012) [52]	90	Yes	No	No
Cardoso (2013) [53]	90	Yes	No	No

6.2 Causative microorganisms and antibiotic resistance

In most studies, *Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae* are the three most commonly occurring microbial agents in BSI, contributing to more than 50% of the total number of microbes [2, 50, 54-56]. *E. coli* is usually the most common BSI microbe in community acquired (CA) as well as health care-associated (HCA) and hospital acquired (HA) BSI [50]. *S. aureus* is more frequent in HCA and HA than in CA-BSI, whereas *S. pneumoniae* occurs mainly in CA–BSI. Opposed to *E. coli* and *S. aureus*, *S. pneumoniae* has been decreasing in many countries the last decade, concomitantly to the introduction of pneumococcal vaccine [2, 57].

Around the world, important differences in antibiotic resistance have been found with regard to place of acquisition [41, 43]. Hospitalized patients have a higher risk of acquiring BSI with inherently resistant microbes, such as *Pseudomonas aeruginosa* or *Candida* sp. Selection of resistant microbes due to antibiotic use is a challenge, particularly in hospitals.

Resistant pathogenic bacteria are found less frequently in Norway and other Nordic countries, compared to the rest of Europe and other world regions [58, 59]. In recent years, however, increasing numbers of infections with methicillin-resistant *Staphylococcus aureus*

(MRSA), expanded-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E), and vancomycin resistant enterococci have been detected [59].

6.3 Treatment and outcome

6.3.1 Main principles of treatment

The main principles of sepsis treatment include the following : 1. Early detection and diagnosis of sepsis; 2. ABC assessment (secure airways, respiration, circulation); 3. Ensure blood samples (biochemical analyses and blood cultures) and other microbiology samples; 4. Start antibiotic treatment within 1 hour of the time of suspected diagnosis of sepsis. The doctor in charge examines the patients and orders relevant imaging to confirm a potential source of infection.

At Levanger Hospital, patients are triaged in the Emergency Department (ED) based on SIRS and signs of organ failure. Use of qSOFA will be implemented in 2017. If sepsis (suspected infection plus any sign of organ failure), the patient is immediately examined by the doctor in charge. When indicated, sepsis treatment is initiated including nasal oxygen, intravenous fluid, blood sampling for biochemical analyses and blood cultures, and initiation of broad-spectrum antibiotic treatment within 1 hour of the time of suspected diagnosis of sepsis. In Norway, the antibiotic regimen of first choice in sepsis of unknown etiology is penicillin plus gentamicin, with addition of metronidazole if an anaerobic infection is suspected [60]. Clinical examination and imaging are performed to detect a potential source of infection. Patients in need of intensive care are transferred to the intensive care unit (ICU). Patients transferred to the wards are promptly re-triaged according to an observation scheme. If clinical signs indicating organ failure are detected, the doctor in charge is called upon to promptly re-evaluate the patient, including to re-examine for source of infection, start or adapt antibiotic treatment if indicated, to consider transfer to the ICU, or determine frequency of

further re-triaging [61]. Patients in the ICU are continuously evaluated for organ dysfunctions and are receiving supportive treatment as needed, such as ventilator treatment if respiratory failure, adequate amounts of intravenous fluid and vasopressor if hypotension or shock, or hemofiltration or dialysis in case of renal failure.

6.3.2 Appropriate antibiotic therapy

Early diagnosis and early appropriate treatment of BSI is crucial. In severe sepsis, the case fatality increases for each hour the antibiotic treatment is delayed [62, 63]. Therefore, empirical antibiotic treatment (EAT) has to be initiated before the results of blood cultures are available. However, as infections with resistant microbes is an escalating problem worldwide [12, 64, 65], it is increasingly challenging to maintain appropriate antibiotic regimens for initial empiric therapy. Updated knowledge about the distribution of microbes in serious infections and their resistance against antimicrobial agents is needed to ensure appropriate empiric antimicrobial treatment (AEAT) regimens.

Many studies have shown an association between inappropriate initial antibiotic therapy and death related to bloodstream infection [67]. Antibiotic therapy given before the blood culture result is available is called *empiric* antibiotic therapy [39, 66], whereas treatment started when microbe identity and resistance pattern is available is termed *definitive* antimicrobial therapy [66]. The proportion of BSI patients who received AEAT has been found to differ from <50% to >90% between studies, depending on geographical region, setting (community-onset, nosocomial, ICU), and time period [67]. In a recent meta-analysis of 13 BSI studies and 3 studies of pneumonia, the proportion of AEAT differed from 18% to 78% between studies [68].

The proportion of AEAT depends on whether only antibiotic coverage of the detected microbe is a criterion or if also dose and route of administration of antibiotics are included in the definition. In Denmark, Hanon et. al found that 75% of BSI patients received AEAT

(antibiotics covered the microbes) in 1996-1998 [69]. Søgaard et al. found 55.1% with AEAT in 1992-1996 and 58.3% in 2002-2006 (antibiotics covering microbes, correct dose and route of administration) [50]. As the epidemic of microbial resistance is growing worldwide, the proportion of patients receiving appropriate EAT is declining [70].

6.3.3 The importance of rapid appropriate empiric antibiotic treatment

Appropriate initial antibiotic therapy significantly reduces the case fatality in sepsis. In a meta-analysis of prospective observational studies of microbiologically confirmed bacterial sepsis, performed by Paul et al. [67], the pooled adjusted odds of death within 30 days was 1.6 times higher in patients receiving inappropriate empiric antibiotic (IEAT) therapy compared to those given appropriate empiric antibiotic therapy (AEAT) (OR 1.60, 95% CI 1.37-1.86). Appropriate treatment was in that study defined as empiric antibiotic treatment that matched the *in vitro* susceptibility of the detected microorganism. In a recent meta-analysis of verified Gram-negative infections, including 13 BSI studies and 3 studies of pneumonia (2493 patients all together), Raman et al. found that IEAT significantly increased the odds of death (pooled adjusted OR 3.30, 95% CI 2.42-4.49) [68].

A debated question, however, is how important rapid initiation of antibiotic treatment is in patients with sepsis. A retrospective cohort study by Kumar et al. showed that in patients with septic shock, the case fatality was significantly higher if the antibiotic treatment was delayed >1 hour after the initiation of hypotension (adjusted OR 7.33, 95% CI 5.44-9.97)[71]. In a large international observational study (Ferrer et al. [63]), including 28,150 patients with severe sepsis or septic shock in 165 ICUs in Europe, US, and South America, patients not receiving AEAT within 3 hours of triage in the emergency department (ED), had significantly higher odds of death (OR 1.23, 95% CI 1.14-1.32). A meta-analysis by Sterling et al. [72], including 11 observational studies, among them, Kumar's and Ferrer's studies and

several smaller studies, however, did not verify the significant benefit of AEAT initiated within 3 hours of triage in the ED in severe sepsis (OR 1.46, 95% CI 0.89-2.40) or within 1 hour of recognized septic shock (OR 1.16, 95% CI 0.92-1.46). The ORs were, however, in disfavor of IEAT. A timely editorial criticism [73] of the above meta-analysis did note that microbiological data had not been taken into account in the included studies, so that it could not be assessed whether the given antibiotic treatment had covered the bacteria causing the infections. In addition, the studies might have included many septic patients who did not have bacterial infections, and in such cases, antibiotics would not have given any benefit.

6.3.4 The Surviving Sepsis Campaign

In 2001, a randomized controlled trial (RCT) conducted by Rivers et al. compared usual patient care to a strict protocol-based care program (early goal-directed therapy; EGDT), in patients with severe sepsis or septic shock. The EGDT included a bundle of resuscitation goals that should be achieved within 6 hours. The case fatality was 46% among the control patients and 30% in the EGDT group [74]. Rivers' study initiated a broad international effort named the Surviving Sepsis Campaign (SSC), with the objective to reduce the case fatality of severe sepsis by 25% within 5 years [75]. International prospective observational multicenter studies have shown improved outcomes in hospitals with better compliance with the EGDT-protocols [76, 77]. However, recent RCTs have not verified the superior effect of strict protocol-based treatment compared to usual care [78-80]. The general quality of care has improved during the last 15 years, based on early recognition, early administration of antibiotics, adequate volume resuscitation, and circulation support [81]. Protocol-based care has not proved to add any beneficial effect. At our hospital, the 30-day case fatality rate in patients with bloodstream infection was reduced by >30% in 2012-2013 compared to 2008-

10, contemporary to a sepsis education and awareness campaign, including a defined clinical pathway for patients with SIRS and improved observation of patients by ward nurses [61].

6.3.5 Prognosis of bloodstream infection

Bloodstream infection (BSI) is a common and potentially lethal disease with a case fatality rate of 10-40% [16-18, 50, 82, 83]. More than 10% of the BSI patients develop septic shock, which has a case fatality of >50% [16, 20]. Patients who survive BSI are prone to secondary infections, and a substantial proportion of survivors have long-lasting or permanent organ dysfunctions such as mental or neurological disturbances or impairment of kidneys, lungs, or the cardiovascular system. Further, they have increased risk of vascular events, such as myocardial infarction, cerebral infarction, venous thromboses and pulmonary emboli. Thus, the long-term consequences of BSI are in many cases a reduced quality of life and death within one year [26, 27].

6.4 Clinical research and surveillance

6.4.1 Randomized controlled trials or observational studies?

Evidence based medicine should principally be based on results from randomized controlled trials (RCTs). In case of acute bacterial infection, however, it is often not possible to perform RCTs. If new treatment regimens are compared with current standard treatment, RCTs should be performed. Randomization to one group or another (treatment A or treatment B) ensures confounders be randomly distributed between the groups. The larger studies, the better the confounders will be balanced across the groups, and the less confounding[1] will occur. But as antibiotic treatment frequently has to be started before the diagnosis of is verified, e.g., in

[1] See Section 10.1.5

sepsis, far from all patients who receive antibiotic treatment for sepsis, have bacterial infections, which benefit from antibiotic treatment. One might perform subgroup analyses including patients who subsequently have been diagnosed with bacterial infection. In subgroups, however, it is less likely that confounders are balanced across the treatment groups, and despite randomization, confounding still may be a concern [84].

An additional problem is that RCTs on antibiotic treatment in many cases would be unethical. Because the effect of early appropriate antibiotic therapy is proven beyond all doubt, RCTs assessing antibiotic treatment versus placebo or early versus late initiation of antibiotic treatment would certainly not be approved. Therefore, guidelines on treatment of bacterial infections largely have to be based on observational studies, in which confounding may distort the results.

Even if RCTs are superior to observational studies with regard to the validity of the results, observational studies using large databases have several advantages over RCTs. Observational studies can evaluate the quality of usual clinical practice (real-world clinical settings), ethical concerns are limited as no intervention is made at the individual level, all patients can be included, and the number of interventions or comparisons is unlimited. Last, but not least, rare outcomes can be assessed in observational studies but not in RCTs, because the costs of so large-scaled RCTs would be too high [85].

6.4.2 End-points in BSI studies

When should we use 30-day case fatality and when is 90-day case fatality most reasonable in BSI studies? Within 30 days of the first positive blood culture, a large proportion of the deaths will be related to the current BSI episode. In epidemiologic studies, it is most reasonable to use death within 30 days, as the scope is to assess the burden of death related to BSI [50, 83].

In BSI treatment studies, on the other hand, the consensus is to use 90-day case fatality as the primary endpoint [86, 87]. Even if many deaths in the period from 31 to 90 days are not directly related to the current infection, pathophysiological processes during the BSI episode have made the patients prone to cardiovascular events and secondary infections [26, 27]. Thus, the purpose of the treatment is not only to help the patient to survive the first 30 days, but also to retain sufficient resilience to withstand the threats that lurk in the convalescence period. A follow up study in North Denmark found that working age adults who had been hospitalized for community acquired bacteremia, had 70% increased risk of death within 1 year compared to culture negative controls, and a fivefold increased risk of disability pension compared to population controls [88]. A multinational study followed up 2130 severe sepsis survivors (end point 28 days) who had participated in two RCTs and who had been independent prior to the severe sepsis episode. One third had died within 6 months, and another third had not returned to independent life. Therefore, long term mortality and quality of life should be assessed in studies on BSI or sepsis [25].

6.4.3 Why study bloodstream infection?

Sepsis is a condition with a multitude of causes, making it difficult to define criteria for study inclusion, whether it concerns surveillance of incidence and mortality or evaluation of treatment strategies. Sepsis patients with positive blood cultures, however, constitute a subgroup which can be more easily defined because the culprit organisms and their resistance patterns are known. Therefore, studies on the burden of BSI, the occurrence of BSI microbes and their resistance patterns, and the effect of treatment programs can be assessed, and these studies are “a window into the burden of sepsis” and an important means of examining the management of sepsis [89, 90]. Last, but not least, the patterns of resistance in BSI microbes form the basis for guidelines for empiric treatment of sepsis [91].

6.4.4 BSI registries and BSI surveillance

From the 1970s, computerized data collecting and analysis have resulted in important new insights in epidemiology, microbiology, risk factors and outcomes of BSI. Substantial contributions came from the UK [92, 93] and the US [17, 39, 94], later on also from Israel [20, 95, 96], Denmark [97], and Norway [16]. In some of these studies, data on BSI were collected over limited periods of time. Others established continuous registration of BSI episodes, e.g., at Beilinson Medical Center, Petah-Tiqva, Israel, from 1988 [95]. These registries were hospital-based, which means that epidemiological data, e.g., rates of BSI, were based on hospital data, such as number of admissions or hospital bed-days. The first population-based BSI registry, giving the possibility of calculating incidence rates (e.g., BSI episodes per 100,000 inhabitants per year) was, to our knowledge, established at Aalborg Hospital, Denmark. They have prospectively collected data on all cases of BSI in the North Denmark Region from 1992 (and retrospectively from 1981 to 1991 [98]. Another important example of a population-based registry is the electronic BSI surveillance database in Calgary, Canada. Since 2000, all residents of the Calgary Zone with a BSI have been registered [56]. In Finland, a nationwide registry with laboratory-based reporting of all BSI cases has been operating since 1995, giving opportunity to survey national BSI rates [83]. Recently, an international collaboration network has been established, including regions within Australia, Canada, Denmark, and Sweden in order to compare incidence and mortality rates, risk factors, outcomes, and resistance rates between different geographical areas and populations [99, 100].

At Levanger Hospital, Mid-Norway, a retrospective study of BSIs including the years 1988-90 was conducted [101]. Prospective registration of BSIs was initiated in 1994. Until the end of 2013, about 3200 BSI episodes had been included. So far, two microbe specific

cohorts (*S. aureus* and *S. pneumoniae* BSI) in patients >15 years have been analyzed and published [102, 103].

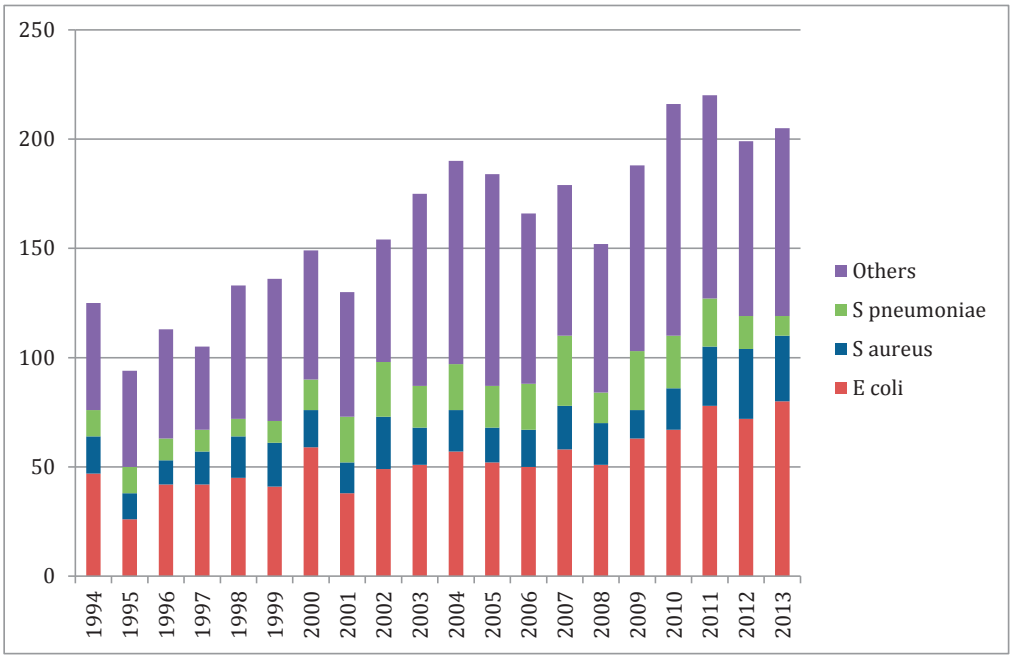

Figure 6.
Episodes of BSI per year at Levanger Hospital 1994-2013. Episodes in patients of all ages included.

6.4.5 Burden of BSI

Bloodstream infection (BSI) contributes substantially to morbidity and mortality worldwide [13]. The annual incidence has been reported between 80 and 257 per 100,000 person-years [14, 15, 50, 55, 83, 104-106]. In Europe, the annual numbers of BSI episodes and deaths associated with BSI have been estimated to 1.2 million and 157,000, respectively. The corresponding numbers of hospital-acquired BSIs were found to be 240,000 episodes and 29,000 deaths [13]. Most studies report increasing incidence rates [50, 83, 105], but a decreasing rate has also been described [107].

The comparison of incidence and mortality rates of BSI between studies is complicated by differences in the age group included, by whether the rates were age and sex-standardized, by differences in the classification of place of acquisition [41, 42, 50, 108], and by differences in the definition of incident episodes. In a previous study from Norway, a new episode with the same microbe was recorded if 30 days or more had lasted since the previous episode [16]. The same time span between episodes is used in the North Denmark Bacteremia Research Database [98]. In the nationwide BSI surveillance in Finland, a new episode with the same microbe is recorded if 3 months have lasted since the previous episode [105]. In the BSI database of the Calgary Zone, however, a new incident case is defined if a one-year period has lasted since the previous BSI episode with the same microbe [56, 100, 109]. Some studies have included only the first episode during the study period [50, 55, 107].

Table 3.

Incidence and mortality of BSI (cases per 100,000 person-years) in some studies. Concerning literature search, see Section 8.1

Geographical area, study period (Author, calendar year [Ref.])	Incidence	Mortality rate	Case fatality rate (CFR)
Charleston County, South Carolina, 1974-1976 (Filice, 1986 [14])	80 (average)		30%
North Jutland, Denmark (1981-1994) (Madsen, 1999 [104])	↑76 to 153	↑17 to 40	30-day CFR 23.6% (remained constant)
North Denmark Region, 1992-2006 (Søgaard, 2011 [50])	↑114 to 166*		30-day CFR ↓22.7 to 20.6
Funen County, Denmark2000-2008 (Nielsen, 2014 [107])	↓254 to 199* (patients ≥15 years included)		
Orebro county, Sweden, 1980-86 (Sjøberg, 1988 [110])	215 (average)		
Finland, 1995-2002 (Skogberg, 2008 [105])	↑104 to 145		
Finland, 2004-2007 (Skogberg, 2012 [83])	↑147 to 168	20.8 (average)	30-day CFR ↑12.6% to 13.2%
Olmsted County, Minnesota 2003-2005 (Uslan, 2007 [55])	189*		In hospital CFR 13.5%
England, 2008 (Wilson, 2011 [106])	189		
Calgary Health Region, Canada, (Laupland, 2013 [109]) 2000-2004 2005-2008 Average Age and sex-standardized to the population of South-Africa 2011	 119.5* 125.8* 122* 85#		In hospital CFR 13%

*age and sex-standardized to regional standard population

#demonstrates the importance of age- and sex standardization if incidence rates are compared between different geographical areas

↑,increased; ↓, decreased

The burden of BSI includes mortality as well as incidence, but few studies have reported both [13, 83, 104]. Monitoring the burden of BSI is important for reasons of resource allocation and for evaluating prevention and treatment strategies [2]. As the proportion of elderly people, more prone to infections, is increasing [111, 112], knowledge about their burden of severe infections is of particular importance. Growing antimicrobial resistance worldwide, associated with increased mortality [12, 64] makes surveillance of BSI microbes

and antimicrobial resistance essential. As different prevention and treatment strategies are needed dependent on place of acquisition (vaccination programs, antibiotic regimens, and infection control measures), it is necessary to survey CA, HCA, and HA-BSI separately [41, 42].

Only hospitalized patients are exposed to hospital stay. Therefore, the incidence and mortality of HA-BSI are presented as events per 100,000 hospital bed-days [50]). The incidence and mortality of community-onset (CO) BSI, on the other hand, should be related to the population (events per 100,000 person-years) [109].

Incidence and mortality rates depend on age and sex. BSI rates are reported to be particularly high in older males [55, 109]. Therefore, in order to compare rates between time periods or between geographical areas, age and sex standardization is mandatory. For comparisons over time in the same area, a national or regional standard population could be used. Comparisons of rates between geographical regions require use of a common international standard population [100, 109].

6.5 Diagnosis of BSI

6.5.1 Blood culture sampling

Blood culture is one of the most important types of specimens analyzed by microbiology laboratories [113]. When a microbe is detected from a blood culture and its antimicrobial resistance pattern is disclosed, antibiotic treatment can be targeted against that microbe.

For more than two decades, automated continuous-monitoring blood culture systems have been in common use [113-115]. Each blood culture bottle contains a sensor that detects CO_2 produced by growing bacteria [114]. Broth media containing charcoal or resins improves

the recovery of organisms in the presence of antimicrobials, and the use of specialized media enhances the recovery of anaerobes, mycobacteria, and fungi [113].

As most adult patients with BSI have very low circulating concentrations of microorganisms, the validity of blood cultures depends mainly on the volume of blood that is drawn [113]. In bloodstream infection, a sample of 20 ml blood detects the microbes in about 70% of cases, two samples of 20 ml detects about 90%, and three samples of 20 ml detects 95% of cases [116, 117]. Usually, one blood culture set consists of two bottles, one aerobic and one anaerobic, each taking 10 ml blood, both obtained from one venipuncture. The common recommendation if blood culture is indicated is to draw two blood culture sets from two different venipuncture sites [113]. Blood cultures should be drawn before onset of antibiotic treatment. Two or more sets can be drawn over a short period of time [113]. If endovascular infection, e.g., endocarditis, is suspected, and the patient is not septic, so that antibiotic treatment can be postponed, several blood culture sets should be drawn over a longer time period [113].

Proper skin antisepsis prior to sampling of blood cultures is mandatory. Recommended skin disinfectants with similar effect are tincture of iodine and chlorhexidine gluconate [113].

6.5.2 From time consuming to rapid identification and susceptibility testing of microbes

Even if automated blood culture systems have been in common use during the last 20 years, the time from blood culture sampling to the responsible clinicians receive the test result is often 24-48 hours, because both identification and antimicrobial susceptibility testing requires that the microbes are cultured in the microbiology laboratory. During this time period, empiric antibiotic treatment (EAT) has to be given. Many efforts are made to shorten the time of EAT by rapid methods for detection of microbial species and resistance testing [118, 119]. Methods that can rapidly identify growing microbes from positive blood culture bottles, such as

MALDI-TOF (Matrix-Absorption-Laser-Desorption-Ionisation-Time of Flight), a rapid technique that identifies bacterial ribosomal proteins [120], are now being adopted in the microbiology laboratories. Rapid molecular bacteriology techniques that can identify pathogens and their resistance genes directly in blood, such as polymerase chain reaction (PCR) [121], are being developed and eventually introduced in the microbiology laboratories. If we can rapidly direct antibiotic therapy towards a known target, we can avoid the use of broad-spectrum antibiotics if not necessary, and we can de-escalate broad spectrum treatment if yet initiated. In the context of increasing antimicrobial resistance worldwide, rapid diagnostics in the microbiology laboratories is one of the most important means of reducing the use of broad spectrum antibiotics, resulting in less resistance, less adverse effects, and less costs. See Section 12.2.3 for a more thorough discussion of this matter.

6.5.3 Indications for obtaining blood cultures

There are no consensual guidelines for when blood cultures should be sampled. Indications may differ with time and between geographical areas. The proportion of positive blood cultures varied between 3% and 13% in three Danish studies, but this does not necessarily reflect different indications for drawing blood cultures, as the definitions that had been used were not similar (no. of BSI episodes/no. of blood culture sets drawn (Nielsen, 2014[107]); no. of positive blood culture sets/no. of blood culture sets drawn (Roth, 2010 [122]); no. of first time BSIs/no. of patients with blood cultures drawn (Søgaard, 2011) [50]). The American Society for Microbiology, using the same definition as Roth et al., has recommended that the rate of true positive blood cultures should be between 5% and 15% [123].

Two positive SIRS criteria have been proposed as an indication for blood culture sampling [124]. Shapiro et al. suggested a decision rule that took into account major criteria (suspect endocarditis, indwelling vascular catheter, and temperature >39.4^{o}C) and minor criteria (temperature >38.3^{o}C, age >65 years, chills, vomiting, hypotension (systolic blood

pressure <90 mmHg), leucocytes >18 x 10^9/L, bands >5%, platelets <150 x 10^9/L, and creatinine >2.0 mg/dl [177 μmol/L]). If either one major criterion or at least 2 minor criteria were fulfilled, it was an indication to obtain blood cultures [125]. Sometimes, blood cultures may be indicated even if the patient has no fever, as a systemic infection may develop without a febrile response, e.g., in immunocompromised patients, infants, and older individuals [124].

6.5.4 Blood culture contamination

According to the literature [122, 126], 2.5% to 6% of blood cultures are contaminated. That means that 30-50% of positive blood cultures are false positives. False positive blood cultures result in increased laboratory work, increased length of patient stay in hospital, and over-use of broad-spectrum antibiotics, which in turn give rise to increased antimicrobial resistance, resulting in increased morbidity and even mortality. A target is that the rate of contaminated blood cultures should not exceed 3% [123, 127]. The main way of contamination is that personnel performing phlebotomy introduce exogenous bacteria into the blood. It is justified to invest considerable resources in reducing blood culture contamination. Effective measures have been shown to be education of personnel, monitoring programs, and feedback. Implementation of specialized phlebotomy teams has reduced contamination rates [126], but also education of ward nurses has shown to be effective [122]. To know why thorough skin disinfection before phlebotomy is necessary and to practice this knowledge is mandatory.

6.5.5 Interpretation of positive blood cultures

To judge whether a positive blood culture represents a true bloodstream infection or a contamination, several factors have to be considered [16, 39]: number of positive blood cultures out of blood cultures obtained, the patient's history, clinical and laboratory findings, and isolates obtained from other body sites. If a known pathogen microbe is isolated from blood culture, this microbe is usually considered to be the cause of the actual infection. If

common skin contaminants, e.g., coagulase negative staphylococci, *Corynebacterium* sp., *Bacillus* sp., *Propionibacterium acnes*, are detected, they should be found from at least two separate blood culture sets in order to be regarded as culprit microbes [16, 50].

6.5.6 Communication of blood culture results

As blood culture results give guidance for antibiotic treatment, and as early appropriate antibiotic treatment is associated with survival [62, 63], it is important that results of positive blood cultures are communicated as fast as possible to the doctors responsible for the treatment of the patients. A Gram-stain from a blood culture bottle that has indicated bacterial growth may give important guidance for treatment if Gram-negative or Gram-positive bacteria, shaped as rods or cocci, the latter located in clusters or chains, are found [128]. Even more information is available if the microbe's identity is established, but a sufficient treatment guidance can be provided only when the test for antimicrobial resistance is completed. At each of these three stages, the laboratory personnel should report to the physician in charge.

6.6 Prior statin use and BSI related case fatality

6.6.1 The search for adjunctive sepsis therapy: a series of shattered hopes

In spite of antibiotic and supportive therapy, bloodstream infection (BSI) is still a major cause of mortality and morbidity [13, 129, 130]. Measures to improve outcomes from BSI are necessary. In case of bacterial sepsis, the host's reaction against the microbe results in organ dysfunction [7]. Therefore, adjunctive as well as antibiotic therapy is mandatory. Many attempts have been performed to manage the exaggerated host reaction with drugs that were tailored to hamper specific steps in various inflammatory cascades, such as anti-LPS [131,

132], anti-thrombin III [133], activated protein C [134-136], and the MD2-TLR4 antagonist eritoran [137], but none of them have proved useful and safe in clinical practice [138, 139].

6.6.2 Statins as adjuvant therapy in sepsis: once again a hope that burst

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors), which were adopted thirty years ago as cholesterol-lowering drugs and have had a great impact on cardiovascular disease morbidity and mortality [140-142], have proved to have anti-inflammatory and immuno-modulatory properties [143-145] as well. Even *in vitro* antibacterial effects of statins have been detected [146-148]. Therefore, many attempts have been made to use statins as adjunctive drugs in septic infections. Whereas observational studies have shown promising results [149, 150], randomized controlled trials (RCTs) have not verified the beneficial effect of statins [151-153]. The authors of a recent review and meta-analysis conclude that further RCTs on sepsis and statin treatment would offer little value [153].

6.6.3 Effect of *prior* statin use – so far not refuted

Another approach has been to study the impact of statin *prophylaxis* on sepsis severity and outcome. Also in this context, many observational studies have shown reduced fatality rates in statin users [149, 154, 155]. RCTs designed to assess the impact of prophylactic statin use on infectious disease outcomes have, to our knowledge, not been performed so far. In a multicenter RCT conducted in Australia and New Zealand, however, prior use of atorvastatin was associated with improved 28-day survival in patients who continued to take their statin during the hospital stay [156].

6.6.4 Pleiotropic effects of statins: proposed mode of action

A main mechanism of the pleiotropic (anti-inflammatory and immuno-modulatory) effects of statins seems to be inhibition of the prenylation of small GTPases. Inhibition of mevalonate synthesis is a well-known effect of statins. Mevalonate is a precursor of cholesterol, but it also is a step in the pathway of geranylgeranylpyrophosphate (GGPP) synthesis, a phosphate containing molecule that prenylates small GTPases (guanosine triphosphate hydrolases). The prenylated GTPases (a geranylgeranylgroup is coupled to the protein) translocate to the cell membrane where they act as "on and off" switches for signal transmission from receptors on the cell surface to intracellular signaling pathways that induce transcription of genes coding for signaling molecules, (e.g., inflammatory cytokines and chemokines), and enzymes [144, 157] (Figure 8). Protein prenylation is dampened by statins, but not abolished. A reduction in the prenylated protein concentration reduces the response magnitude of the affected signaling pathway [144]. The small GTPases are involved in signaling via different receptors relevant for sepsis, such as TLRs, cytokine receptors, chemokine receptors, protease activating receptor (PAR) 1 etc. [36, 157-161]. Statins have been shown to influence TLR4 expression and signaling via inhibition of protein prenylation [158].

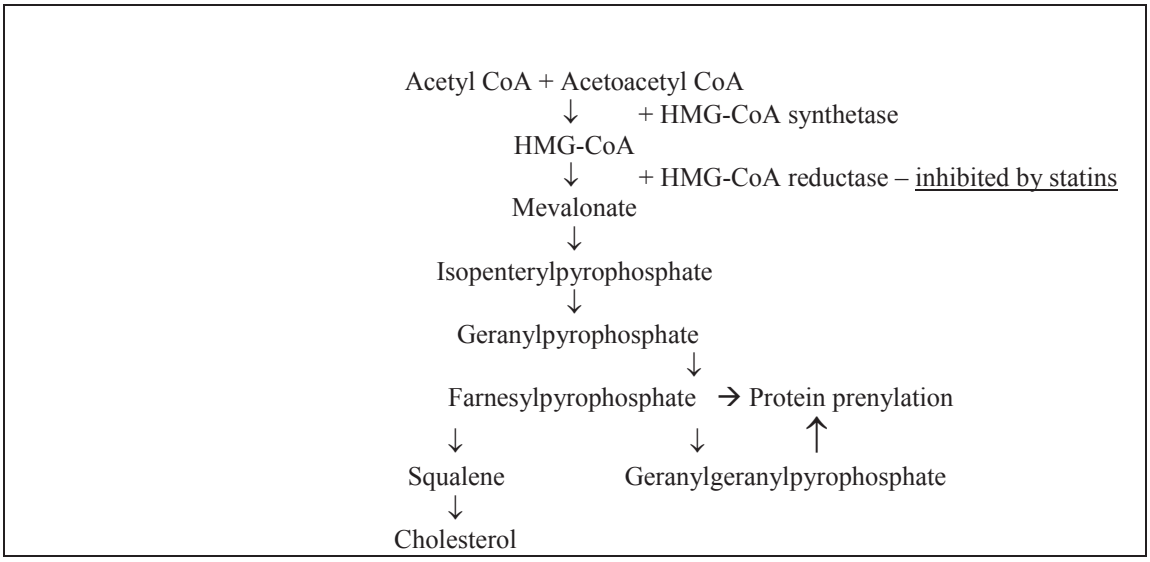

Figure 7.
The mevalonate biosynthesis pathway inhibited by statins**.** Adapted from [144].

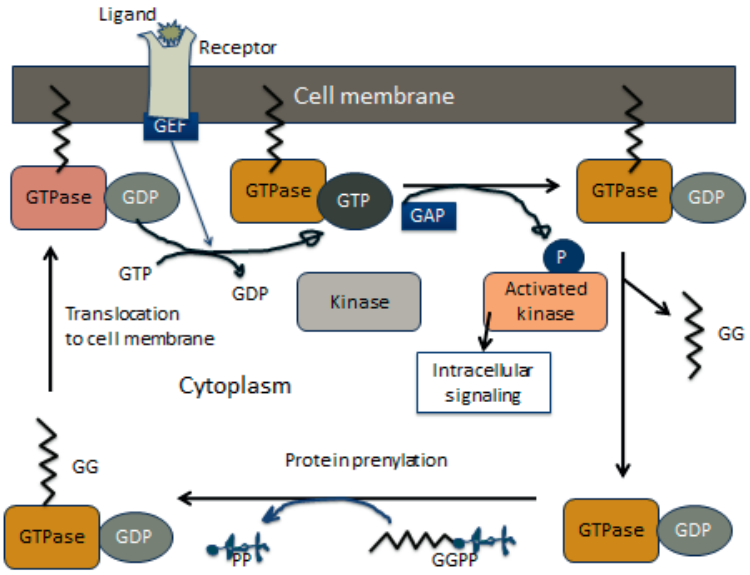

Figure 8.

The ”on and off” function of small GTPases**.** Small GTPases function as “molecular switches” or relay molecules, which cycles between an active, GTP-bound state, and an inactive GDP-bound state. A ligand stimulates a membrane receptor, which changes conformation and activates a guanosine nucleotide exchange factor (GEF), replacing GDP with GTP. Then a GTPase activating protein (GAP) hydrolyses GTP and activates a protein kinase, thereby initiating an intracellular signaling cascade (Adapted from [157]).

GAP, GTPase activating protein; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GG, geranylgeranyl; GGPP, geranylgeranylpyrophosphate; GTP, guanosine triphosphate; P, phosphate; PP, pyrophosphate

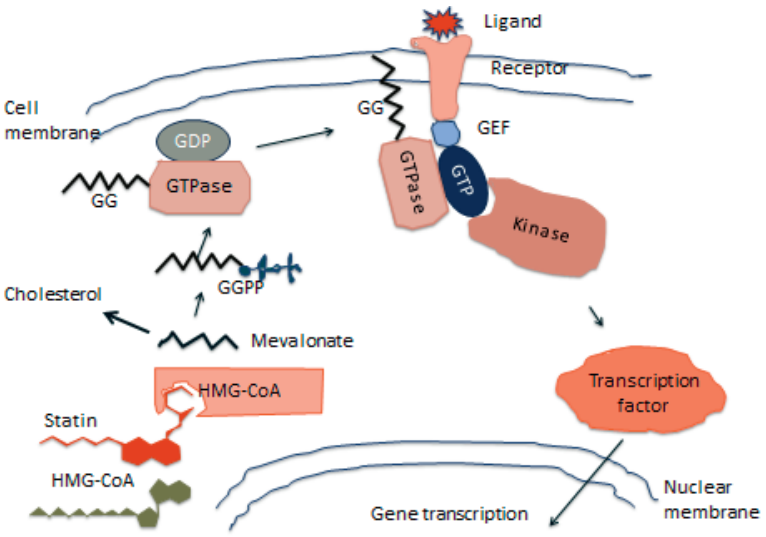

Figure 9.

The proposed intracellular effect of statins. Statins exert their effects in different cells, e.g., immune cells, endothelial cells, and smooth muscle cells. Due to structure similarity, they compete with HMG-CoA for the enzyme HMG-CoA reductase, thereby reducing the available amounts of mevalonate, cholesterol, and geranylgeranylpyrophosphate (GGPP). Thus, the prenylation of small GTPases is limited, resulting in lower amounts of membrane attached GTPases, which downregulate the signal transduction. ([162]).
GDP, guanosine diphosphate; GEF, guanosine nucleotide exchange factor; GTP, guanosine triphospate.

Table 4.
Pleiotropic effects of statins (adapted from [152, 163, 164])

Statins dampen intracellular signaling by limiting the availability of prenylated small GTPases. Receptor pathways indicated on Figure 5 (Section 6.1.2) are examples of action sites of statins. Gene transcription is inhibited, resulting in different effects:

Anti-inflammatory	Immunomodulatory	Antioxydant	Endothelial function	Antithrombotic
↓CRP ↓Chemokines ↓Adhesion molecules ↓Cytokines	↓MHC II expression ↓TLR4 expression ↓T-cell activation ↓proliferation (mono-cytes, macrophages)	↓NADPH oxidase ↑Haem oxygenase activation	↑eNOS expression ↓iNOS expression ↓Leucocyte adhesion	↓Platelet activity ↓TF ↓PAI-1 ↑TPA ↑TM expression and activity

CRP, C-reactive protein; iNOS, inducible nitrogen oxide synthase; MHC II, major histocompatibility complex II; NADPH, nicotinamide adenine dinucleotide phosphate; PAI-1, plasminogen activator inhibitor 1; TF, tissue factor; TLR4, toll-like receptor 4; TM, thrombomodulin; TPA, tissue plasminogen activator;

Mechanisms of endothelial protection by statins

The endothelial cells line the inside of blood- and lymphatic vessels. They are many more than the circulating immune cells and constitute a major source of cytokines and other signaling molecules [32]. It is well known that statins have endothelium protective effects [37, 157, 165]. In sepsis, bacteria and bacterial products stimulate TLRs on endothelial cells, activating pathways that induce enzymes such as inducible nitrogen oxide synthase (iNOS) and NADPH oxidase, which give rise to superfluous amounts of nitrogen oxide (NO) and free oxygen radicals (ROS, reactive oxygen species), substances that contribute substantially to endothelial dysfunction and development of shock. The increased iNOS expression can be induced by LPS from Gram-negative bacteria as well as lipoproteins from Gram-positive bacteria [165, 166]. Statins increase the activity of eNOS and dampen the activity of iNOS, thus counteracting endothelial cell damage [144]. Further, sepsis evokes a pro-coagulant state in the vascular endothelium. Thrombin, produced by coagulation, stimulates protease-activated receptor 1 (PAR 1) on endothelial cells, switching on pathways resulting in tight junction loosening, cell shrinkage, and apoptosis [167]. By dampening the prenylation of

small GTPases, thereby modulating a multitude of signaling pathways, including the PAR 1, statins may protect the endothelial integrity during sepsis.

6.6.5 Statins and outcome of BSI

Several observational studies have assessed the relationship between prior use of statins and the outcome of BSI. The majority of the studies suggest that statin use could have a beneficial effect in patients with BSI [168-172], whereas some studies have shown no difference in mortality between statin users and non-users [173-175]. One study showed that long-term statin use prior to BSI improved survival more than short-term use [176]. In a recent meta-analysis of six eligible cohort studies [168, 169, 171, 172, 175, 176] comprising 7553 BSI patients [177], the overall hospital mortality was 15.4% in patients on statin vs. 22.3% in patients not on statin (pooled OR 0.49, 95% CI 0.30-0.81).

6.6.6 Statins and outcome in Gram-negative and Gram-positive BSI

Many former studies assessing the relation between statin use and outcome of infection have not had verified BSI, nor have they discriminated between Gram-positive and Gram-negative etiology. As Gram-positive and Gram-negative bacteria alert the innate immune system in different ways [30, 178], drugs that have anti-inflammatory properties may not have the same effect in Gram-positive infection as they do in Gram-negative infection. Only a few authors have attempted to study this issue in patients [168, 171, 172, 174, 175, 179], and the results diverge. An important reason for the discrepancies is that the studies were observational and therefore more or less biased by confounding.

7. AIMS OF THE STUDY

The specific aims of the present study were as follows:

I To estimate the burden (incidence and mortality) of BSI, with emphasis on age- and sex differences and time trends, in an area of Mid-Norway (the catchment area of Levanger Hospital) during a 12-year period (**Paper I**).

II To assess the occurrence and distribution of BSI microbes and their non-susceptibility to some common antibiotic regimens for initial empiric antimicrobial treatment of sepsis of unknown etiology, with regard to microbe, place of acquisition (CA-, HCA- and HA-BSIs), and time trends (**Paper II**).

III To evaluate which antibiotic regimens were used for initial empiric treatment during the same time period and to what degree they were appropriate (**Paper II**).

IV To study whether the relationship between prior statin use and 90-day case fatality differed between Gram-positive and Gram-negative BSI (**Paper III)**

8. MATERIAL AND METHODS

8.1 Literature search strategies

Relevant literature for this thesis and the Papers I-III were identified through search in PubMed, MEDLINE, EMBASE, Google Scholar, and Cochrane Library through January 2017.

The search criteria used were

(I):”incidence or mortality or case fatality” and ”population-based” and ”bloodstream infection or bacteremia or bacteraemia” **(Paper I)**

(II): ”antimicrobial resistance or MRSA or ESBL” and ”bloodstream infection or bacteremia or bacteraemia” (**Paper II)**

(III): ”statins” and ”bloodstream infection or bacteremia or bacteraemia” and ”mortality or case fatality” **(Paper III)**

In addition, many references were found in the reference lists of selected articles. Titles were screened, and if of relevance, abstracts were read. Articles that dealt with the research questions under investigation were retrieved.

8.1 Study setting and cases included

Levanger Hospital is one of two hospitals in Nord-Trøndelag County in the Region of Mid-Norway. The hospital is an emergency hospital serving the population in a defined geographical area of 10 municipalities, with 68 491 inhabitants aged 16 years and above at the start of the study, and 75 858 at the end of the study.

The population is primarily Caucasian, living mainly in rural areas. Four small cities are located in the area. As in all Norway, the entire population in the area is provided with

hospital healthcare that that is free at the point of delivery. All patients with acute infectious diseases in the area are admitted to this hospital.

BSI episodes for the present thesis were prospectively recorded between 2002 and 2013 and included 1995 episodes in 1719 patients aged 16-99 years. In **Paper I-II,** both first-time and repeat BSI episodes were included. Regarding **Paper III,** a cohort comprising 1408 patients who had their first BSI episode between 2002 and 2011, was selected. BSI with Gram-negative or Gram-positive bacteremia accounted for 1356 episodes. The remaining cases consisted of 8 candidemias and 44 with mixed polymicrobial infection.

8.2 Microbiology laboratory diagnostics

The microbiology laboratory at Levanger Hospital is ISO 15189 accredited and participates in the national quality assurance schemes (ring tests). According to the ISO 15189 standard for medical laboratories, the laboratory has approved guidelines for the collection and handling of microbiological samples, including blood cultures. The laboratory handles all blood cultures from patients with community onset infections in the hospital's catchment area and nearly all blood cultures from patients with hospital acquired infections. The blood cultures were performed in an automated system, BACTEC 9240 Vacutainer Culture Bottles (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) [114], which in 2010 was replaced by BACTEC FX. Before venipuncture, skin disinfection with chlorhexidine gluconate and ethyl alcohol was performed. The volume of blood sampled in each bottle was 10 ml. A blood culture set consisted of one aerobic and one anaerobic BACTEC bottle obtained from a single draw. If antibiotic treatment was indicated immediately, a second draw comprising one aerobic bottle was taken simultaneously from another venipuncture site. In other cases, a second blood culture set (one aerobic and one anaerobic bottle) was taken after 2-3 hours. The volume of blood drawn was the same during the study period. No obvious changes in blood

culture techniques or indications for drawing blood cultures were done during the study period, but an increased focus on early detection of sepsis may have influenced the rate of blood culture sampling (**Paper I).**

Isolates were identified and resistance tested using standard methods [180]. Antimicrobial susceptibility testing was performed by the disc diffusion method (Neo-Sensitabs [Rosco Diagnostica, Taastrup, Denmark]). For measurement of MIC, E-test (AB Biodisk, Solna, Sweden) was used. The results of antibacterial susceptibility testing were interpreted according to the Norwegian Working Group on Antibiotics (NWGA). For the antibiotics included in this study, the Norwegian breakpoints correspond to EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints [59, 181]. In this study, microbes intermediately susceptible to antibiotics were classified as non-susceptible [54, 182, 183], as only susceptible microbes were regarded possible to manage by means of the respective antibiotic regimens. Microbes not tested in the laboratory because of known inherent non-susceptibility (e.g. enterococci are inherently resistant to cephalosporins) were classified as non-susceptible (Paper II, Additional file 1: Appendix 1 On inherent (natural) resistance in microbes).

8.3 Study design

Paper I

We conducted a prospective observational population-based study in an area of Mid-Norway (the catchment area of Levanger Hospital) to estimate the burden of BSI (incidence and mortality)

Paper II
In a prospective observational cohort study, we assessed the proportion of antimicrobial resistance (non-susceptibility) and the proportion of appropriate empiric antibiotic therapy over a 12-year period.

Paper III

In a prospective observational cohort study, including 1408 patients with first time episodes of BSI during 2002 to 2011, we studied whether the relationship between prior statin use and 90-day case fatality differed between Gram-positive and Gram-negative BSI.

8.4 Data sources

Population data

Population data of the ten municipalities (catchment area of Levanger Hospital) for every year between 2002 and 2013, with age and sex distribution, were obtained from Statistics Norway.

Data on the BSI cohort (Paper I-III)

Since 1994, all positive blood cultures at the hospital have been prospectively recorded for surveillance purposes, and clinical information has been recorded, in the following way: Whenever a positive blood culture was reported, a physician at the clinical ward filled out a registration form. A team of three research nurses, a subordinate doctor, and the main investigator reviewed the patients' records to verify the data and record additional variables.

The present thesis is based on data from BSI episodes prospectively recorded in patients aged 16 years or older admitted to Levanger Hospital between January 1, 2002 and December 31, 2013 (**Paper I-II**). Data from the years 2002 to 2011 were used in **Paper III**.

Data on vital status. The patient administrative system at the hospital receives updated information on vital status from the National Population Register, and thus, information on fatal outcome of BSI was complete even if the patients were discharged from hospital.

Data on statin use was prospectively included in the database from 2005, when we became aware of the early studies on statin use and sepsis. Information on the use of statins during 2002-2005 was recorded retrospectively from hospital records.

8.5 Definitions of study variables

BSI episode (Paper I-III)

An episode of BSI was defined by growth of one or more microbes from blood culture combined with clinical evidence of systemic infection [16]. A new BSI episode with the same microbe in the same patient was recorded if an interval of at least 30 days had passed without signs of infection since an earlier episode [16]. If more than one organism was isolated from one or more blood cultures within a 72-h period, the BSI episode was classified as polymicrobial [184]. One positive blood culture for organisms usually regarded as etiological agents was the requirement for inclusion. For coagulase-negative staphylococci or other possible skin contaminants, at least two identical isolates from separate venipunctures were required. Among alpha-hemolytic streptococci, *S. pneumoniae* and streptococci belonging to the *S. milleri* group were not considered as skin contaminants. Other alpha-hemolytic streptococci were included if they were found in two or more blood cultures from different venipuncture sites.

Place of acquisition (Paper I-III)

The BSI episodes were classified as hospital-acquired (HA), health care-associated (HCA) or community acquired (CA) [41, 42]. HA-BSI was diagnosed if the infection was detected >48

hours after admission [39]. Patients who during the 30 days prior to hospital admission had (1) been hospitalized two or more days or (2) had received intravenous therapy or wound care at home or (3) hemodialysis or chemotherapy at hospital visits or (4) were nursing home residents, were categorized as having HCA-BSI. CA-BSI was diagnosed if the infection was detected <48 hours after admission and none of the criteria for HCA-BSI were fulfilled.

Infection focus (Paper I-III)

A urinary focus was assigned when the same microbe was isolated from urine as well as from blood culture along with clinical signs/symptoms or risk factors for urinary infection, and no other source of infection was identified. A presumed pulmonary focus was diagnosed with clinical signs of lower respiratory infection accompanied by positive radiologic findings. Focus in the biliary tract was ascertained based on clinical, biochemical and radiological findings. Signs of infection along with focal growth of the same microbe as in blood culture were taken as a confirmation of infection in abdomen, skin, soft tissue or other sites. An unknown focus of infection was assigned when none of the criteria for ascertaining a focus were met.

Prior statin use (Paper III)

In this study, prior statin use, defined as taking a statin in the week before the time of positive blood culture [185], was the *exposure variable.* Two patients, whose statins were discontinued more than one week before the date of the positive blood culture, were categorized as non-statin users. We recorded statin use and the specific statin and dosage from the patients' hospital charts.

Outcome (Paper III)

The *outcome variable* was death from all causes within 90 days after the first positive blood culture [86]. Date of death was obtained from the patients' electronic records, which is updated by the Civil Registration System in Norway.

Variables assessed as confounders (Paper III)

The following variables were *a priori determined as possible confounders* because they might be associated with both statin use and mortality from BSI (Table 1): Age (<65 years, 65-79 years, ≥80 years); sex; comorbidities; Charlson comorbidity index [186], categorized as low (no underlying disease score), medium (score 1-2), or high (score >2) [171]; nursing home resident (yes/no); functional status (independent, partly independent, dependent); immunosuppressive therapy; alcohol abuse; smoking (no smoking, former smoker, present smoker); focus of infection (urinary tract, lungs, biliary tract, gastrointestinal tract, other, unknown); use of antibiotics before admission; place of acquisition (community, healthcare, hospital).

Variables considered mediators (Paper III)

Variables expressing the *severity of the current BSI* (systemic inflammatory response syndrome, organ failure, hypotension, hypoperfusion, sepsis, severe sepsis, and septic shock [1], severe organ failure (defined as SOFA score >2 in any organ [185, 187]), and admission to an Intensive Care Unit (ICU) were recorded, but not considered confounders. Instead, they may be *mediators* in the pathway between prior statin use and death, and, therefore, should not be adjusted for in the analyses [84, 171, 188].

Appropriate empiric antibiotic therapy (AEAT; Paper II-III)

AEAT was defined as correctly dosed intravenous antibiotic therapy with a regimen that was active in vitro against the microbe(s) isolated from blood culture(s). In **Paper II**, we assessed AEAT within 6 hours and within 24 hours of the time that the blood culture specimen was obtained. In **Paper III**, AEAT was defined as correctly dosed intravenous antibiotic therapy given within 24 hours of the time that the blood culture specimen was obtained, with a regimen that was active in vitro against the microbe(s) isolated from blood culture(s) [189, 190]. This variable was not adjusted for in the main analyses, as it was not considered a confounder. In section 10.1.5, the role of AEAT as a co-variable is further discussed.

8.6 Statistics

In **Paper I**, incidence and mortality rates were calculated for the population between 16 and 99 years. Population data during 2002–13 (Statistics Norway) were used as denominators to calculate age-specific and sex-specific rates of BSI episodes and of deaths within 30 days of BSI episodes. The incidence rate of HA-BSI was reported as the number of patients with HA-BSI in a time period divided by the number of hospital bed-days in that same time period. Observed incidence and mortality rates were standardized to the age and sex distribution of the population of Norway 2010, and the reported rates are age and sex standardized unless otherwise noted. Age and sex standardization was based on sex and 5-year age group specific rates and performed as described by Greenland and Rothman [191].

Proportions, such as case fatality rates in two time periods (**Paper I**) and proportion of statin users in different subgroups of patients (**Paper III: Table 1**) were compared using the unconditional z-pooled test, which is the unconditional version of the Pearson Chi-square test [192].

Trends in proportions, such as non-susceptibility to antibiotics and appropriate empiric antibiotic therapy across place of acquisition categories and time periods (**Paper II**) were analyzed using the exact Cochran-Armitage test.

Poisson regression was used to assess time trends in BSI incidence and mortality rates (average rate ratio per calendar year), adjusted for age (in 5-year intervals) and stratified by sex (**Paper I**).

Univariable analysis of mortality curves was done with Kaplan-Meier analysis.

The relationship between prior statin use and 90-day case fatality was analyzed using logistic regression. Univariable analyses of exposure and outcome were performed as well as multivariable analyses with proposed confounders included (**Paper III**).

Two-sided p-values <0.05 were considered significant and 95 % confidence intervals reported where relevant (**Paper I-III**).

Confidence intervals of incidence and mortality rates were calculated based on assumed Poisson distribution (**Paper I**). Confidence intervals for case fatality (**Paper I**) and for proportions of non-susceptibility (**Paper II**) were calculated using Wilson's approximation to the binominal distribution [193].

The analyses were performed using SPSS 18 (**Paper III**), StatXact 9 (**Paper I-III**), SPSS 22, and STATA 13 (**Paper I-II**).

8.7 Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics, Health Region IV, Norway. The Ethics Committee waived the need for informed consent, as this was an observational study, the treatment of the patients was standard, and no samples

were taken for the purposes of the research. The use of the data was approved by Nord-Trøndelag Hospital Trust (**Paper I-III**).

9. SUMMARY OF RESULTS

9.1 Paper I

We studied the burden of disease and death related to BSI in a defined geographical area of Mid-Norway where BSI episodes were prospectively recorded by the same microbiological department. Death from BSI was defined as death within 30 days of BSI detection. Between 2002 and 2013, 1995 episodes of BSI in 1719 patients aged 16 to 99 years were included. The overall incidence of BSI was 215 per 100,000 person-years. The incidence increased exponentially with age, particularly in males. The incidence increased from 205 to 223 per 100,000 person-years from 2002-07 to 2008-13. *Escherichia coli* was the most frequently isolated infective agent, followed by *Streptococcus pneumoniae* and *Staphylococcus aureus*. The rate of *S. pneumoniae* BSI decreased over time in males (on average by 9.2% annually), but not in females. The total incidence of BSI microbes with acquired resistance increased slightly over time, but did not exceed 2 episodes per 100,000 person-years. The mortality of BSI was 32 per 100,000 person-years, higher in males than in females (36 vs. 28 per 100,000 person-years) and was significantly higher in old age, particularly in males. The total BSI mortality was similar in the first and second halves of the study period, but the mortality of *S. pneumoniae* BSI decreased in males (15.0% annually). The crude case fatality decreased from the first to the second half of the study period (17.2% to 13.1%; p=0.014). The rate of blood culture sampling increased more than twofold during the study period, and the number of BSI episodes per 100 blood culture sets decreased from 8.7 to 5.6.

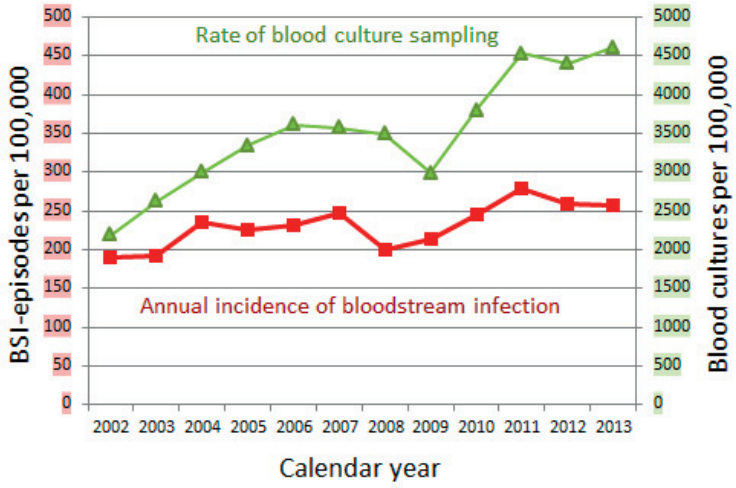

Figure 10.
Annual incidence of bloodstream infections (□) and rate of blood culture sampling (△) in an area of Mid-Norway 2002-2013.

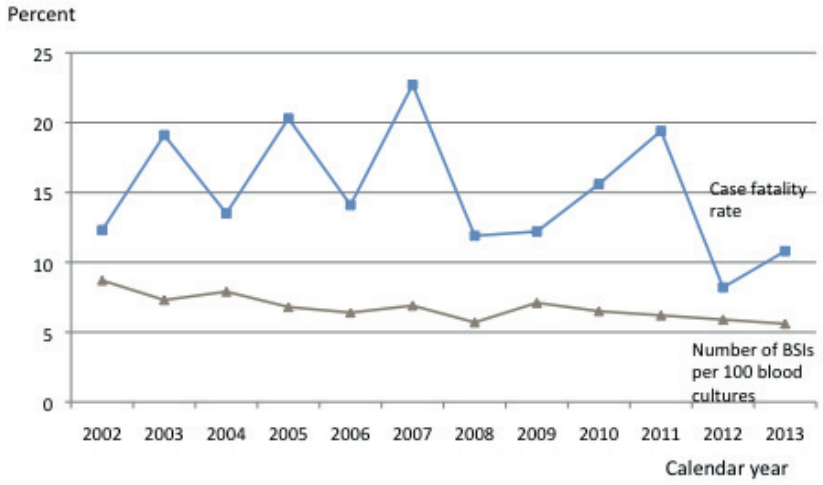

Figure 11.
The case fatality rate and the number of BSI episodes per 100 blood cultures drawn, year by year, during the study period

9.2 Paper II

We studied trends in antimicrobial resistance and empiric antibiotic therapy at a medium-sized general hospital in Mid-Norway. Between 2002 and 2013, 1995 prospectively recorded episodes of BSI in 1719 patients aged 16-99 years were included. We analyzed the antimicrobial non-susceptibility according to microbe, place of acquisition, site of infection, and time period. There were 934 community-acquired (CA), 787 health care-associated (HCA) and 274 hospital-acquired (HA) BSIs. The urinary tract was the most common site of infection. *Escherichia coli* was the most frequently isolated infective agent in all three places of acquisition. Second in frequency was *Streptococcus pneumoniae* in CA and *Staphylococcus aureus* in both HCA and HA. Allover, 3.5% of the BSI microbes were non-susceptible to the antimicrobial regimen recommended by the National Professional Guidelines for Use of Antibiotics in Hospitals, consisting of penicillin, gentamicin, and metronidazole (PGM). Antimicrobial non-susceptibility differed by place of acquisition. For the PGM regimen, the proportions of non-susceptibility were 1.4% in CA-, 4.8% in HCA-, and 6.9% in HA-BSI ($p<0.001$), and increasing proportions of non-susceptibility over time were observed in HA-BSI, 2.2% in 2002-2005, 6.2% in 2006-2009, and 11.7% in 2010-2013 ($p=0.026$), mainly caused by inherently resistant microbes. We also observed increasing numbers of bacteria with acquired resistance, particularly *E. coli* producing ESBL or possessing gentamicin resistance, and these occurred predominantly in CA- and HCA-BSI.

Initial empiric antibiotic therapy

The use of second- and third generation cephalosporins as initial empiric therapy decreased through the study periods. Cefuroxime was used as monotherapy or in combination in 19.6% and 5.3% of the BSI episodes in the first and in the third time period, respectively. The corresponding proportions for cefotaxime were 18.1% and 15.7% (Table 4). In contrast, the

use of ampicillin or penicillin plus gentamicin increased from 14.1% to 19.9%. PIP/TAZ was not used in the first period, but was the second most used empiric therapy in the third period (17.3%). The proportions of patients who received appropriate empiric antibiotic therapy (AEAT) within 6 h and within 24 h were larger in the third period than in the first and second periods (**Paper II**: Table 5).

9.3 Paper III

In order to assess the relationship between prior statin use and 90-day case fatality in Gram-negative and Gram-positive BSI, we conducted a prospective observational cohort study of 1408 adults with first time BSI admitted to Levanger Hospital between January 1, 2002, and December 31, 2011. Data on use of statin and other medication at admission, comorbidities, functional status, treatment, and outcome were obtained from the patients' hospital records. The relationship of statin use with 90-day case fatality differed between Gram-negative and Gram-positive BSI (p-value for interaction 0.01). Among patients with Gram-negative BSI, statin users had significantly lower 90-day total case fatality (10.1% vs. 21.4%, odds ratio [OR] 0.42, 95% CI 0.23-0.75, p=0.003). The association remained essentially unchanged after adjusting for the effect of sex, age, functional status before the infection, and underlying diseases that were considered confounders (adjusted OR 0.38, 95% CI 0.20-0.72, p=0.003). A similar analysis of patients with Gram-positive BSI showed no association of statin use with mortality (case fatality 28.6% vs. 29.7%, OR 1.08, 95% CI 0.67-1.75, adjusted OR 1.22, 95% CI 0.69-2.17, p=0.49). The present study suggests that prior statin use is associated with a lower 90-day total mortality in Gram-negative BSI, but not in Gram-positive BSI.

10. DISCUSSION

10.1 Strengths and limitations

The objective of an epidemiologic study is to obtain a valid and precise estimate of the frequency of a disease or of the effect of an exposure on the occurrence or the outcome of a disease in the source population of the study [194]. The estimates may be distorted by random error, that reduces the precision, or by systematic error, that interfere with the validity of the results [84].

10.1.1 Precision (lack of random error)

A precise estimate has little random error [194]. As epidemiological studies are influenced by chance, the random error will be large if the sample size is small. The larger sample size a study has, the less the random error will be. Tests of statistical significance evaluate whether an association between an exposure and an outcome is likely to be caused by chance. The p-value shows the probability that an association at least as strong as the observed association could be caused by chance alone. Confidence intervals are more informative than p-values, as they do not only show the precision, but also the size of the association [84].

In the present studies, the overall incidence and mortality rates are relatively precise with narrow confidence intervals, whether in subgroups of BSI, the confidence intervals are wider (**Paper I**). Correspondingly, the proportions of BSIs constituted by e.g., different microbes, resistant microbes, or patients receiving appropriate antibiotic treatment are more imprecise the smaller the numbers are that each of them contributes (**Paper II**). Regarding **Paper III**, our cohort has a sufficient number of patients in the different groups, which makes it possible to assess the relationship between statin use and 90-day case fatality. Also in Gram-negative BSI, the estimated negative association between prior statin use and 90-day case fatality was relatively precise with a low p-value and a narrow confidence interval. The

estimate of the lacking association between prior statin use and 90-day case fatality in Gram-positive BSI is somewhat less precise because of a smaller sample size.

10.1.2 Validity (lack of systematic error)

A valid estimate has little systematic error [194]. Three types of bias affect epidemiological studies: Selection bias, information bias, and confounding.

10.1.3 Selection bias

Selection bias is a systematic error that stems from the procedures used to select subjects and from factors that influence study participation [84]. In the present studies, the included patients had one or more positive blood culture(s), judged to be of clinical significance, and the possibility of contamination was ruled out. To determine what is contamination is not always quite simple. If for example a coagulase negative staphylococcus was detected in one blood culture and only one blood culture set was obtained from that patient, the case would not have been included in the study. The patient might yet have had a bloodstream infection, which might have been detected if more blood cultures had been drawn. The threshold for taking blood cultures influences the occurrence of bloodstream infection. Defined consensus criteria for when blood cultures are indicated do not exist [124]. In **Paper I**, trends in burden of BSI may have been influenced by a lowering of the threshold for ordering blood cultures, driven by a campaign for increased awareness of sepsis. In some patients, too few blood culture sets have been drawn, e.g, in cases of time pressure. Thus, the total volume of blood may have been too small in a proportion of the patients. As most adult patients with BSI have very low circulating concentrations of microorganisms [113], the blood volume sampled is the most important factor for the validity of blood cultures, as mentioned above (Section 6.5.1). Further, some cases of BSI may have been lost because antibiotic therapy that inhibited the growth of bacteria, had been started before blood culture sampling.

In population-based studies of incidence and mortality, all cases in the population must be included, and individuals not belonging to the population must be excluded. Limitations in **Paper I** are that some inhabitants in this area will likely have had BSI during stays at St Olav's Hospital, the closest university hospital, so that the true rate of HA-BSI in our area is slightly higher than is reported in this paper. We did not exclude the very small number of episodes that occurred in persons who were visitors to the area, as a similar number of BSIs is likely to have occurred among inhabitants of our area being on travel elsewhere.

Strengths of the studies (**Paper I-II**) include the prospective registration of BSIs within a well-defined source population and the handling of blood cultures at one microbiology laboratory.

10.1.4 Information bias

Systematic error can arise because the information collected about study subjects is erroneous. Such information is often referred to as *misclassification* if the error leads to a person being placed in an incorrect category [84]. Variables to consider with regard to misclassification are primarily exposure and outcome, but incorrect categorization of potential confounders may also give rise to error in estimates. In **Paper III,** data on outcome (90-day case fatality) are collected from the National Population Register, and the follow up was complete, so it is unlikely that the outcome variable has been misclassified. The information about exposure (statin use) was recorded from the patients' hospital records, which may in some cases have been incomplete. Noncompliance with statin medication may have occurred in some cases. Data entry errors may have occurred, but in a very small scale, as all cases were also checked by one of the responsible physicians.

A strength of the study presented in **Paper III** is that our bacteremia database contains confirmed diagnoses and variables collected from the patients' hospital data, which include medical records. Thus, we have more reliable clinical data than if information had been

collected only from discharge databases. The Charlson comorbidity index (CCI) [186] was used as a variable representing comorbidity. Not all diagnoses associated with adverse outcome are included in the CCI, so an underestimating of comorbidity, resulting in under-adjusting, may have occurred.

Regarding alcohol abuse and smoking, data are collected from the patients' hospital records. The patients have undoubtedly underreported their true use, so that an under-adjusting has taken place.

Differential bias, e.g. that exposure (statin use) may have been misclassified according to the value of the outcome variable, have certainly not occurred, either have any other variables been misclassified because of known vital status. The data have been collected and categorized independently of the outcome variable, which has been collected afterwards. Lastly, it is very unlikely that exposure data have been misclassified dependent on categories of possible confounders, as the data collectors were not aware of the study question.

10.1.5 Confounding

Confounding is the confusion of effects. That means that the effect of the exposure is mixed with the effect of another variable, leading to a bias [84]. Confounding arises from imbalances in risk factors for the outcome across the exposure categories. A confounder must have an effect, and it must be imbalanced between the exposure groups. Therefore, a confounder must have two associations:

1. A confounder is a variable that is associated with the exposure but not as an effect of the exposure
2. A confounder is a variable that is associated with the outcome, but not as an effect of the outcome

In RCTs, confounders are distributed by chance between the study groups. In large RCTs, therefore, confounders will be well balanced across the groups. As mentioned in section 6.4.1, many important research questions regarding BSI cannot be assessed by RCTs due to practical or ethical limitations. Therefore, confounding is a major challenge in studies on exposure effects in bacterial infection.

In **Paper III,** an observational study assessing the effect of prior statin use on outcome of BSI, adjustments for confounding variables are crucial. Confounding variables have to be selected *a priori*, based on pathophysiological, clinical, and epidemiological knowledge [84]. We adjusted for age, sex, comorbidity, functional status, nursing home residence, immunosuppressive therapy, alcohol abuse, smoking, focus of infection, use of antibiotics before admission, and place of acquisition. However, we have not collected data on some variables that are known to be associated with adverse outcome, such as marital status, income, education, occupation, and nutritional status [195, 196]. Severe confounding by socioeconomic or related differences may seem unlikely because statin prescriptions are reimbursed, and access to hospital treatment is the same for all citizens in Norway. In a study from Denmark, however, where the healthcare system is not quite different, they found clear indications of a socioeconomic gradient in statin use in men but not in women [195]. Further, we have not adjusted for vaccinations, e.g. pneumococcus vaccine or influenza vaccine, which some previous authors have regarded as "healthy user" markers [197]. Confounding by indication, (e.g., patients with a poor prognosis are less likely to receive a statin or patients with cardiovascular risk factors are more likely to be given a statin), may likely have occurred. Nonetheless, we are not aware of confounders that would be likely to cause a strong association of statin use with reduced mortality in Gram-negative, but not in Gram-positive, BSI. We cannot, however, completely exclude that unmeasured confounders may have

enhanced the effect estimate in Gram-negative BSI and attenuated the effect estimate in Gram-positive BSI.

In **Paper III**, we have not adjusted for factors expressing the severity of infection, such as severe sepsis or septic shock, as these variables must be considered as mediators (intermediate stages) between exposure and outcome. If mediators are included in the analysis, the association between the exposure and the outcome will be falsely lowered [198]. It must be emphasized that in a study assessing the relationship between statin use and outcome of BSI, statin use should be considered an explanatory variable and not a predictor. It is important to distinguish between studies assessing the effect of an exposure, i.e., an explanatory study, and studies investigating predictive impact of different variables, i.e., a predictive study. In predictive studies, stepwise regression analyses are performed to find the variables that are most strongly associated with the outcome. In explanatory studies, on the other hand, stepwise regression gives rise to misleading results, as the explanatory variable under study may be ousted by other variables that are more strongly associated with the outcome [84]. Thus, in predictive studies, intermediate stages can be adjusted for (e.g., septic shock is a strong predictor of death). In explanatory studies, on the other hand, intermediate stages should not be adjusted for [84, 198].

A somewhat intricate question is whether appropriate empiric antibiotic therapy (AEAT) is a confounder or not. AEAT is indeed strongly associated with outcome of BSI, but it is not associated with prior statin use in such a way that it influences whether a person has been prescribed statin medication. Prior statin use may be associated with the initial antibiotic therapy if statin use mitigates the inflammatory response so that symptoms are masked and appropriate initial antibiotic therapy, therefore, is delayed. In this case, appropriate initial antibiotic therapy is a mediator in the pathway between statin use and death, and not a confounder, and therefore it should not be adjusted for (Figure 12 A) [84, 198]. On the other

hand, one might postulate some unknown variable that influences whether people are prescribed statin medication and also influences whether they receive AEAT (e.g., some underlying condition that is not included in the Charlson comorbidity index – rapidly fatal disease [199] could be an example) (Figure 12 B). To reduce the influence of such an unknown confounder, we also performed an analysis including adjustment for AEAT, but the effect estimate remained of similar size.

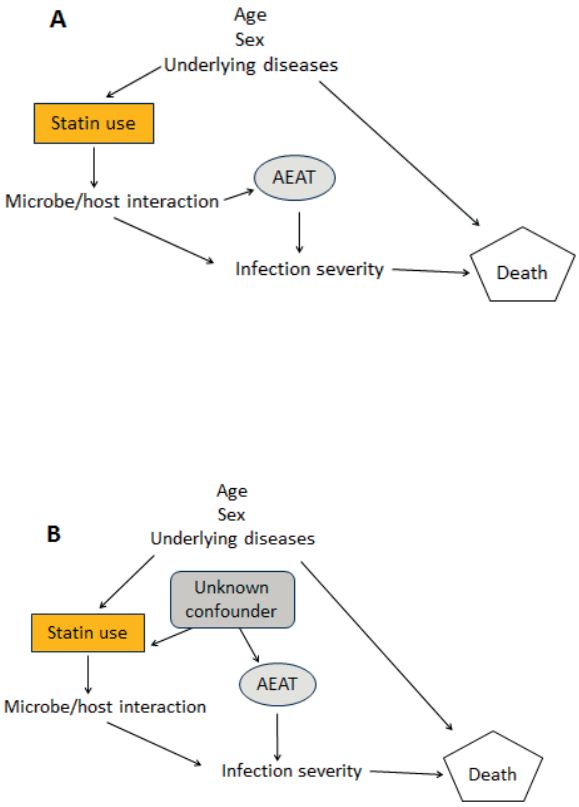

Figure 12.

Directed acyclic graph (DAG) analysis of the relationship between statin use and death. Statin use is the exposure, and death is the outcome. Age, sex, and underlying diseases are confounders. Microbe/host interaction and infection severity are presumed mediators. **A**. Appropriate empiric antibiotic therapy (AEAT) may be considered a mediator if statin attenuates the microbe/host interaction so that the clinical signs are altered, influencing the decision on AEAT initiation. **B**. An unknown confounder influences whether people are prescribed statin and also whether they receive AEAT

Intern and extern validity

Intern validity means lack of systematic error. Extern validity refers to generalizability, that is to what extent findings apply to other populations. Intern validity is a prerequisite for extern validity [194].

Standardization for the age and sex distribution in **Paper I** is representative for Norway 2010. An international standard population should rather have been used [100, 109], but so far, the majority of published studies have used regional or national standard populations [50, 55, 107].

Our use of one single institution as the study site limits the generalizability of our results, but regarding antibiotic resistance patterns as basis for treatment guidelines (**Paper II**), our results may be relevant for other general hospitals in Scandinavia.

As regards **Paper III**, our population is not very different from other countryside and small city populations in North-Western Europe. If the intern validity of the study is acceptable, the results should reflect exposure-outcome associations that exist in similar populations.

10.2 Discussion in relation to previous studies

Paper I

This paper provides information on the incidence and mortality of BSI in an area of Mid-Norway, both overall, by age and sex, by time period, and for specific subgroups of BSI. The burden of BSI, both in terms of incidence and mortality, increased strongly with age, particularly in males. The incidence increased during the 12-year study period, and the increase was strongest in females, for HCA-BSI, and for urinary tract and Gram-negative BSIs. Over the same period, the mortality remained stable and case fatality rate decreased, possibly because an increased rate of blood culture sampling may have led to improved detection of milder BSI episodes. A shift towards higher proportions of female sex, Gram-

negative etiology, and urinary tract site may also have influenced the case fatality rate to some degree. In addition, earlier detection of sepsis and improved treatment may have had impact. Very low, but slightly increasing rates of microbes with acquired resistance were observed.

To our knowledge, this is the first study estimating the overall burden of BSI in a Norwegian population. Incidence rates of invasive infections with single microbes have been reported [200-204], but no population-based study has focused on BSI as a whole. One previous study described the epidemiology of sepsis in Norway in 1999 [205], and two studies published more than 20 years ago [16, 206] described BSI incidence and mortality related to hospital admissions and bed-days, but did not include population statistics.

Compared to our results, incidence rates (166-189 per 100,000 person-years) [50, 83, 106] and mortality rates (22 per 100,000 person-years) [83] of BSI were somewhat lower in other studies that also included all BSI episodes. This difference is expected, as these studies also included children, which are generally at low risk of BSI.

An increase in BSI incidence over time have also been reported in Finland [83] and in Northern Denmark [50], although a study from Funen, Denmark, reported a decrease in BSI incidence. Similar to our findings, the increase in BSI incidence was accompanied by a decreasing case fatality rate in Denmark (22.7% to 20.6%) [50] but not in Finland (12.6% to 13.2%) [83]. At the same time, we observed a relatively stable mortality rate of BSI, as has also been reported in Finland [83]. We and others [105, 107] have observed increasing rates of blood culture sampling over time, and the number of BSI episodes per 100 blood culture sets decreased with time in our study as was found in Funen, Denmark [107], though not in Northern Denmark or in Finland [50, 105]. In 2007, we updated our local recommendations on sepsis diagnosis and treatment, based on the guidelines of the international Surviving Sepsis Campaign [10, 207], and we conducted an education and awareness campaign about

sepsis for physicians and nurses in 2011. Earlier detection and treatment may have improved survival of BSI towards the end of the study period [61].

The high incidence and mortality of BSI in the older ages in our study, particularly in men, corresponds to what has also been reported by others [55, 56, 83, 208]. An increasing rate of HCA-BSI with time, similar to our results, was also recently reported from Denmark [50].

In the present study as well as in a Finnish study [83], Gram-negative BSI was most frequent in females whereas Gram-positive BSI predominated in males. During the study period, Gram-negative BSIs increased whereas Gram-positive BSIs decreased. In Finland, however, both Gram-positive and Gram-negative BSIs increased during 2004-2007 [83]. In most other BSI studies as well as in the present study, [2, 50, 54-56] *E. coli*, *S. pneumoniae*, and *S. aureus* were the three most commonly occurring BSI microbes, accounting for more than one-half of all BSI episodes.

The incidence rate of *E. coli* was higher in the current study than in studies from Finland [83] and England [106]. A decreasing incidence [2, 57, 59, 209, 210] and mortality [57] of *S. pneumoniae* is reported in Norway and other countries after the introduction of pneumococcal conjugate vaccines. Noteworthy, the decreased rate of *S. pneumoniae* BSI in our study was observed in males but not in females. A possible explanation of this sex difference may be differences in smoking habits [211], as the peak prevalence of smoking in Norwegian males occurred 20 years earlier than in females [212]. The incidence of *S. aureus* BSI in our study was similar to a recent study from an area of South-East Norway [203]. The present study as well as others [83, 203] found a higher rate of *S. aureus* BSI in males than in females.

The total rate of BSI microbes with acquired resistance increased slightly over time, but did not exceed 2 episodes per 100,000 person-years. The incidence rate of MRSA in our

study was similar to what has been found in Denmark, Finland, and Sweden [100], and much lower than in Canada and Australia [100], and in the US and the UK [213]. Regarding ESBL-producing *Enterobacteriaceae,* substantially higher incidences have been found in studies from other parts of the world [214-216].

Paper II

In this prospective study of 1995 consecutive BSI episodes at a medium-sized Norwegian hospital between 2002 and 2013, antimicrobial resistance was a far smaller problem than reported in most studies [54, 58, 217]. Except for third generation cephalosporins, antimicrobial resistance to regimens recommended for sepsis of unknown etiology was low. In less than 4 percent of BSI episodes, microbes were non-susceptible to PGM, consistent with previous findings at our hospital [101] and in other Norwegian studies [218, 219]. However, the proportion of antibiotic non-susceptibility was higher in HA- and HCA- than in CA-BSI. For PGM, an increase in non-susceptibility through the study period was observed in HA-BSI, mainly caused by inherently resistant microbes. A slightly increasing number of bacteria with acquired resistance was also detected, particularly *E. coli* producing ESBL or possessing gentamicin resistance. In our cohort, appropriate empiric antibiotic therapy could be achieved to a larger extent by replacing cephalosporins with PG or PIP/TAZ.

The low proportions of non-susceptibility in our BSI microbes are likely explained by a relatively strict use of antibiotics in Norway [59, 220, 221]. All antibiotics used in humans are prescribed by physicians, and penicillins and aminoglycosides are the preferred drugs in severe bacterial infections. The increasing non-susceptibility in BSI microbes by place of acquisition and, for HA-BSI, by time period, was mainly due to a shift towards microbes with natural (inherent) resistance, particularly *Candida* spp., *Enterococcus faecium*, and *Staphylococcus epidermidis*. We attribute this shift to the increasing use of chemotherapy and

other immunosuppressive treatments (**Paper II**: Additional file 1, Table S9), which results in more prevalent infections and antibiotic treatments, giving rise to selection of resistant microbes. A higher proportion of non-susceptible microbes in HCA- than in CA-BSI is well known from other studies [41, 43], but this knowledge does not seem to have been sufficiently heeded by clinicians and guideline makers. The national Norwegian surveillance data on the distribution of different microbes isolated from blood cultures show no time trend towards increasing occurrence of natural resistant microbes from 2004 to 2014 [59, 222], but do not distinguish between CA, HCA, and HA infection.

Acquired resistance was uncommon in our BSI cohort, but an increasing proportion of *E. coli* was non-susceptible to gentamicin, and they occurred in CA- as well as in HCA-BSIs. Nationwide, a worrying increase in resistance to gentamicin has emerged from 2003 to 2014 (0.6% to 7.7%) [59]. The EARS-Net has reported 5.2% and 9.9% aminoglycoside resistance in *E. coli* in 2002 and 2013, respectively [58, 217], but the proportion of aminoglycoside resistance was much higher in the south-eastern region (32.1% in Bulgaria in 2013). The proportion of *E. coli* producing ESBL has increased from 2008 to 2014 (1.5% to 5.8%) according to the national data [59], and according to EARS-Net data, the proportion of ESBL-producing *E. coli* in Europe increased from 2.0% in 2002 [217] to 12% in 2013 (39.6% in Bulgaria) [58].

One single isolate of MRSA and two pneumococci intermediately susceptible to penicillin were found in our cohort. Nationwide, the proportion of MRSA in blood cultures has been low in the corresponding time period (0.3% and 0.8% in 2002 and 2014, respectively). The low occurrence of MRSA in the Nordic countries and in the Netherlands clearly distinguishes from the other European countries, where MRSA accounted for >25% of the *S. aureus* BSIs in 2007 [223] but had decreased to 18% in 2013 [58]. The nationwide proportion of invasive pneumococci non-susceptible to penicillin was 0.9% in 2002 and 5.5% in 2014 [59, 222]. In

Europe, the proportion of penicillin-non-susceptible isolates in 2013 ranged from 1.1% (the Netherlands) to 40.0% (Cyprus) [58].

Empirical antibiotic treatment regimens have to be continuously evaluated in accordance with national and local microbe resistance patterns. The initial empiric treatment for sepsis of unknown origin recommended by the National Professional Guidelines for Use of Antibiotics in Hospitals in Norway consists of penicillin and gentamicin, plus metronidazole (PGM) if an anaerobic infection is suspected [60]. PGM was not effective in vitro against 3.5% of the microbes isolated from blood cultures in the present study. In patients with HA-BSI, however, the proportion of microbes not susceptible to PGM was 11.7% in the third time period.

Enterococci are inherently resistant to cephalosporins and staphylococci are not susceptible to ceftazidime. In our BSI cohort, staphylococci and enterococci contributed to 30% of HA-BSI episodes. Noteworthy, the percentages of microbes non-susceptible to cefotaxime and ceftazidime in HA-BSI in the third period were as high as 37.9 and 55.3% respectively. Therefore, none of these appear suitable for use as mono-therapy in sepsis of unknown microbial origin. The emergence of ESBL in Gram-negative bacteria has made it even more risky to choose a third generation cephalosporin as monotherapy for severe infections with unknown etiology.

Even though the National Professional Guidelines [11] recommend PG (or PGM) as the regimen of first choice in sepsis of unknown etiology, the PG (or PGM) combination was given in no more than 20% of the episodes in our BSI cohort, yet the proportion was increasing with time. There are mainly two reasons for prescribing antibiotic treatment that does not include an aminoglycoside: (1) Aminoglycosides are potentially nephrotoxic and ototoxic. Therefore, there is a tendency to avoid them even in cases where they should not be contraindicated. (2) Use of an aminoglycoside requires measurement of aminoglycoside

serum concentrations, which is resource consuming, and knowledge and experience is needed for assessment of the results. In a busy day, it is much simpler to administer a beta-lactam-antibiotic, where dosing is simple and the risk of toxicity is negligible.

In Norway, we lack national data for antibacterial treatment given in cases of bloodstream infection or sepsis. Regarding antibiotic use in Norwegian hospitals, the national surveillance data [8] show that the proportion of aminoglycoside use was less than 5% in 2015 (3.3 out of 73 DDD/100 bed-days), indicating that avoiding aminoglycosides in favor of beta-lactam antibiotics is a common mode of acting countrywide.

The drawback of avoiding aminoglycosides is increased risk of antimicrobial resistance, as particularly cephalosporins are far more resistance driving than aminoglycosides. Therefore, a further shift in favor of aminoglycosides is desirable. As the use of aminoglycosides in our hospital and nationwide is still relatively low, overuse of aminoglycosides is unlikely to explain the observed increase in non-susceptibility to gentamicin in *E. coli.*

During the three time periods, we observed that the use of second- and third-generation cephalosporins decreased, whereas ampicillin or penicillin plus gentamicin were more frequently given. PIP/TAZ was introduced at our hospital in 2006, and the use of it has been increasing, particularly in the third period. Nationwide, the use of aminoglycosides and particularly piperacillin/tazobactam has increased during the last ten years, whereas the use of second-generation cephalosporins has decreased. The use of third-generation cephalosporins and fluoroquinolones peaked in 2011- 2012 and have since then declined [59]. These changes are in accordance with the national [60] and local antibiotic policy, in order to achieve regimens that are less resistance driving and also cover the BSI microbes to a larger extent.

In **Paper II**, we present susceptibility data by microbe, by place of acquisition, and by site of infection. Most authors have presented susceptibility data only by microbe, which is

generally unknown at the time the physician has to decide on the initial antimicrobial therapy. We included microbes with known inherent resistance in the presentation in order to give guidance for empirical treatment before the microbial etiology is known.

Regarding the proportion of patients receiving AEAT, reasonable comparisons between studies are impeded by different definitions. Some studies use *in vitro* effect against the culprit microbe as the only criterion [69, 97], others also include dose and route of administration [50], and still others state that treatment must be timely [66]. The most often time limits used are 8 hours [224] or 24 hours [70, 189, 190, 225]. In the present study, we have assessed AEAT within 6 hours and 24 hours.

The proportion of AEAT within 24 hours (>80%) was higher in our BSI cohort than in other studies, and the proportion was increasing with time. In a US cohort of BSI patients at a community hospital from 2003-2006, the proportion of AEAT was 67% [70]. In two former US studies, the first performed at a university hospital 1975-1977 and the second at a community hospital 1995, the proportions of AEAT were 78% and 80%, respectively. An increasing proportion of AEAT with time was also seen in Denmark, but the detected proportions were much lower than in our study (55.1% in 1992-1996, 58.3% in 2002-2006 [50], presumably because the Danish study recorded the very first antibiotic prescribed after the first positive blood culture, while we have collected data on antibiotic treatment after 6 hours and 24 hours.

Paper III

In this cohort study of patients with BSI, prior statin use was associated with reduced 90-day case fatality in Gram-negative, but not in Gram-positive, BSI. To our best knowledge, no previous research has reported this finding. The effect measures were not changed by

adjustment for variables that were considered confounding factors. In the total cohort of BSI, the unadjusted odds ratio for mortality among statin users compared with non-users did not differ much from what has been found by other investigators [171, 173, 175].

Impact of statins – a healthy user effect?

A population based prospective cohort study on statin use and community acquired pneumonia [197] raised concerns that previous studies indicating benefits of statins in patients with sepsis had been measuring and reporting a healthy user effect. In that study, statin users were less likely to die or to be admitted to an intensive care unit. After adjusting for confounding factors that reflect patient frailty or healthy user behavior, no reduction in either mortality or need for admission to an intensive care unit in statin users was found. In the present study, a significantly lower proportion of statin users were functionally dependent patients or nursing home residents compared with non-users. The proportion of former smokers was significantly higher among statin users. However, these associations of statin use were essentially similar in Gram-negative and Gram-positive BSI. Therefore, it seems unlikely that the observed difference in possible preventive effect of statins between Gram-negative BSI and Gram-positive BSI in our study could be explained by a healthy user effect.

In order to diminish the healthy user effect, recent studies [179, 226-228] of the association between statin use and outcomes of infections have excluded prevalent statin users (those who have adhered to statin therapy for a longer time period, e.g., >30 days [179]). Patients who adhere to preventive therapies, are more likely to behave in accordance with a healthy lifestyle [229]. Prevalent statin use has been found to be associated with lower occurrence of events that can obviously not be prevented by statins, such as motor vehicle and work place accidents [230]. It has been shown that where prevalent statin users are included, stronger effect estimates are found in observational studies than in RCTs, even in studies of

cardiovascular events [228, 231]. The greater the proportion of prevalent users in observational studies has been, the larger discrepancies between estimates from observational studies and RCTs have been found [231]. Excluding prevalent users reduces the proportion of individuals with high degree of health seeking behavior. Thereby, the influence of healthy user bias is diminished. A recent study on prior statin use and 30-day case fatality after *S. aureus* BSI excluded prevalent users and included as incident users those who had initiated statin treatment in the 30 days prior to the first positive blood culture [179].

The phases of sepsis

The course of sepsis can be described as three phases (Table 6) with different patterns of threats to the patients [232]. During the first 5 days, acute inflammation provokes organ failure, shock, and early death. Later on, secondary infections contribute substantially to organ failure and death [232]. From the third week and for half a year, vascular events, due to endothelial damage caused by inflammation, cause death and disability [26, 27].

Table 5.
The early acute, late acute, and late manifestations and consequences of sepsis

	Early acute phase	Late acute phase	Late phase
Time period	0-5 days	6-15 days	16-180 days
Pathophysiology	Acute inflammation	Inflammation and immunosuppression	Damaged vascular endothelium
Clinical events	Organ failure DIC MOF Septic shock Early deaths	Organ failure Secondary infections Late early deaths	Sustained organ impairment Secondary infections Vascular events (MI, AIS, VT) Late deaths

AIS, acute ischemic stroke; DIC, disseminated intravascular coagulation; MI, myocardial infarction; MOF, multi-organ failure; VT, venous thromboembolism

Possible late phase effects of statins

The beneficial effect of statins in patients with sepsis or bloodstream infection may not become evidently apparent in the acute phase of sepsis. In a Danish study on statin use and BSI case fatality [171], the survival curves followed the same track until about 30 days. After that, the curve of the statin users flattened, whereas the curve of the non-users continued to rise until the end of the follow-up time. In our study, the same phenomenon is apparent (Figure 13), but only for statin users with Gram-negative BSI. The flattening starts already after 4-5 days, but is more marked from about day 30.

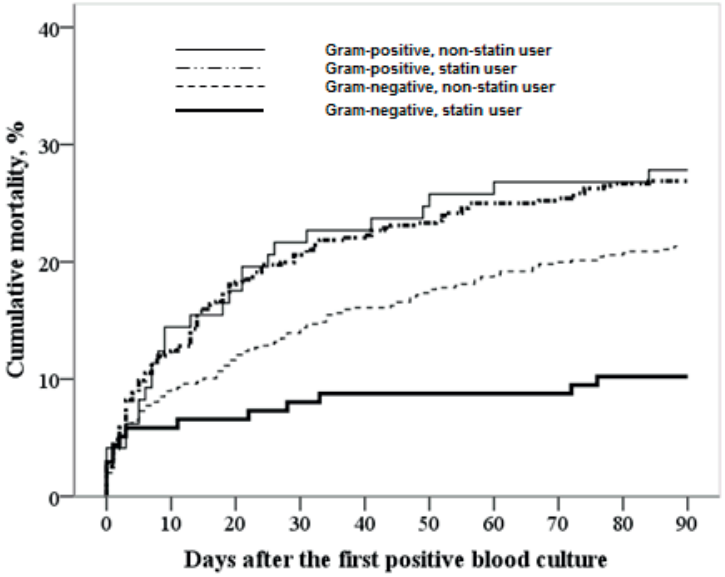

Figure 13.
Mortality curves for patients with Gram-negative and Gram-positive BSI stratified by statin use. Levanger Hospital, Mid-Norway 2002-2013 (**Paper III**)

It has been shown [26, 27] that patients who have survived a BSI episode, have a higher risk of death during the next 30 to 180 days. The causes of death were vascular events (myocardial infarction, acute ischemic stroke, venous thromboembolism) and secondary infections. Whether the immune-modulatory effects of statins may have impact on the risk of

acquiring secondary infections in the later phases of sepsis is an exciting question. Interestingly, a Danish study found that fewer new episodes of BSI occurred in statin users that in non-users (6.3% vs. 10.3%), indicating a possible role of statins in preventing secondary infections [171].

As described in section 6.6.4 (see also section 6.1.2, Figure 5), endothelial cells are major producers of inflammatory cytokines, and endothelial inflammation is a hub in the pathogenesis sepsis, provoking life-threatening conditions, such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), other organ failures, and shock. In the weeks and months after a sepsis episode, endothelial damage caused by inflammation may give rise to vascular sequela, such as acute myocardial infarction and other cardiovascular events, acute ischemic strokes and venous thromboembolism [26, 27]. Statins have well-known endothelium protecting effects, and vascular events following BSI or sepsis may be prevented because statins have dampened the endothelial inflammation during the infection [171].

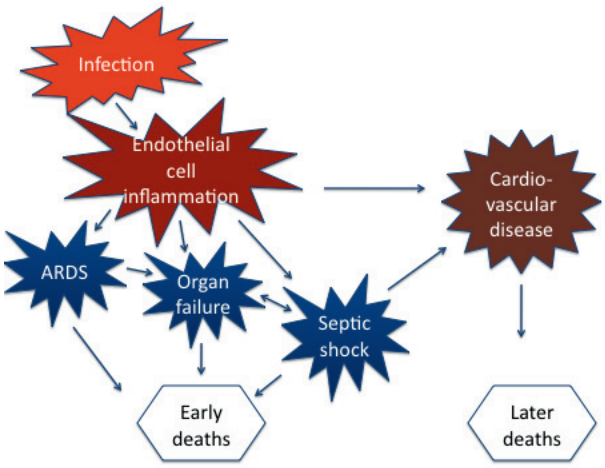

Figure 14.
The central role of endothelial cell inflammation in sepsis pathophysiology

Different effects of statins in Gram-positive and Gram-negative infection?

LPS-induced endothelial damage is a well known cause of shock and of diseases related to atherosclerosis [233, 234]. If vascular complications (hypotension, shock, and later events, such as acute myocardial infarction (AMI), acute ischemic stroke (AIS), and venous thromboembolism (VT) were more commonly occurring in Gram-negative than in Gram-positive infection, that could explain why statin use might have greater impact in Gram-negative infection. However, endothelial inflammation is induced in Gram-positive as well as in Gram-negative infection. The proportion of patients who develop septic shock is similar in both Gram-positive and Gram-negative BSI [20]. Regarding late phase vascular events, the risk of AMI, AIS or VT after BSI compared to population controls has been found to be at least as large in *S. aureus*- or other Gram-positive infections as in *E. coli*- or other Gram-negative infections [26, 27].

Most previous investigators have dealt with infection, sepsis, or BSI as if these categories represent homogenous groups with regard to pathogenesis or assumed preventive effect of statins. A few articles reported some data on the relations between statin use and death in Gram-positive and Gram-negative BSI separately. Liappis et al. [168] reported trends of reduced mortality rates in statin users with Gram-negative infection (1/19 vs. 48/223, p=0.13) or *Staphylococcus aureus* infection (0/15 vs. 22/130, p=0.13). Yang et al. [174] found a trend of lower mortality in statin users with Gram-negative bacteremia (10.3% vs. 16.4%, p=0.25), but not in Gram-positive bacteremia (33.3% vs. 27.8%, p=0.50). Thomsen et al. [171] did not find such a trend in the enterobacterial group of Gram-negative bacteria (adjusted 30-day mortality rate ratio 1.07, 95% CI 0.61-1.86, p=0.82), nor did Leung find a negative association between statin use and death in Gram-negative BSI (adjusted hazard ratio 1.10, 95% CI 0.87-1.39) [175]. However, the latter had adjusted for variables expressing the severity of the current infection, i.e. factors that should be considered as mediators rather than

confounders, and such adjustment may attenuate the true associations [84]. López-Cortes et al. found a negative association between statin use and death within 14 days from *S. aureus* BSI (adjusted OR 0.08, 95% CI 0.01-0.66, p=0.02) [172]. Recently, Caffrey et al. demonstrated that incident statin users (those who had initiated statin therapy within the 30 days prior to blood culture sampling) with *S. aureus* BSI who continued to receive statin at least 3 days after the positive blood culture, had significantly lower risk of death within 30 days than non-users (HRR 0.46, 95% CI 0.25-0.84). In patients who did not continue statin therapy after the time of positive blood culture and in patients who initiated statin therapy at the time of positive blood culture, no association between statin use and outcome was seen. Conclusively, other clinical studies do not support the finding that the effect of statins is limited to Gram-negative infection.

Table 6.

Observational studies on the association between statin use and outcome of bloodstream infection in which single microbes or microbe groups are assessed. Literature search strategy is discussed in section 8.1

Author, year (country) Study period	Study design	Microbe/ microbe group	Follow up time, days	Statin use	Statin users	Non-users	Adjusted effect estimate
					No. of deaths/ patients (%)	No. of deaths/ patients (%)	
Liappis, 2001 (USA) 1995-2000 [168]	RC	Gram-negative rods	In hospital	At the time of PBC and during HS	1/19 (5)	48/223 (22) (p=0.13)	
		Staph. aureus			0/15	22/130 (17) (p=0.13)	
Thomsen, 2006 (Denmark) 1997-2002 [171]	PC	Enterobacteria	30	≥1 pre-scription in the year before PBC	/78	/2193	MRR 1.07 (0.61-1.86)
		S. aureus			/28	/724	MRR 0.74 (0.30-1.81)
		Enterobacteria	31-180				MRR 0.59 (0.26-1.33)
		S. aureus					MRR 0.24 (0.03-1.74)
Yang, 2007 (Taiwan) 2002-2002 [174]	RC	Gram-negative bacteria	30	≥30 days before and during HS	6/58 (10.3)	40/244 (16.4) (p=0.25)	
		Gram-positive bacteria			14/42 (33.3)	30/108 (27.8) (p=0.50)	
Leung, 2012 (China) 2008-2009 [175]	RC	Gram-negative bacteria	90	Before or at the time of PBC	/307	/820	HRR 1.10 (0.87-1.39)$
		Gram-positive bacteria			/285	/727	HRR 0.91 (0.72-1.15)$
Lopez-Cortez, 2013 (Spain) 2008-2011 [172]		*S. aureus* BSI (SAB)	14	≥30 days and until PBC	2/33	32/127	OR 0.08 (0.01-0.66)
Mehl, 2014 (Norway) 2002-2011 [Paper III]	PC	Gram-negative bacteria	90	In the week before PBC	14/138 (10.1)	138/646 (21.4)	OR 0.38 (0.20-0.72)
		Gram-positive bacteria			28/98 (28.6)	128/474 (27.0)	OR 1.22 (0.69-2.17)
Caffrey, 2017 (USA) 2002-13 [179]	RC	*S. aureus* BSI	30	*PC§	19/141	33/141	HRR **0.46 (0.25-0.84)**
				*PNC#	63/331	70/331	HRR 0.92 (0.64-1.32)
				De novo	27/177	27/177	HRR 1.04 (0.60-1.82)

*prevalent users excluded (those who had used statin therapy for >30 days prior to positive blood culture)

§Pretreated with continuation

#Pretreated without continuation

$Adjusted also for mediators (severity of infection variables)

CFR, case fatality rate; HRR, hazard rate ratio; HS, hospital stay; MRR, mortality rate ratio; OR, odds ratio; PBC, positive blood culture; PC, prospective cohort; RC, retrospective cohort

Support from biological knowledge or *in vitro* or animal studies?

As we can see from Figure 4, Section 6.1.2, Gram-positive and Gram-negative bacteria alert different TLRs, activating partially different signaling pathways that induce production of different patterns of inflammatory and immunomodulatory cytokines [34, 35]. We cannot, however, from a model of signaling pathways, infer anything about which pathway might be most influenced by the dampening effect of statins.

In vitro and animal studies have demonstrated that statins attenuate LPS-induced inflammatory responses [165]. Similarly, statins have been found to diminish inflammatory responses induced by *Staphylococcus aureus* lipoteichoic acid [235]. However, there are few studies that have explored the mechanisms for differential effects of statins in Gram-positive and Gram-negative BSI as observed in the present study. Interestingly, there are several studies showing that statins decrease LPS signaling through down-regulation of TLR4 expression and signaling [158]. Although some studies suggest that statins may also suppress TLR2-signaling in Gram-positive infection [159], statins may not inhibit the lipoprotein induced pathway via TLR2 and the LPS-induced pathway via TLR4 to the same extent. One study found that statins inhibited TLR4-mediated signaling via the TRIF/IRF3 pathway but not via the MyD88/NF-kB pathway [236]. IRF3-deficient mice have been found to be more resistant to LPS-induced septic shock, indicating an essential role of IRF3 in LPS-induced shock [233]. Another interesting study has shown that during Gram-negative sepsis, LPS may induce endothelial damage and shock independent of TLR4 signaling by activating caspase-11, an enzyme inducing apoptosis [237]. To conclude, basal *in vitro* and animal studies may give some, but not particularly strong support to the hypothesis of different statin effect in Gram-positive and Gram-negative infection.

Whether a negative association between statin use and mortality in Gram-negative BSI really exists as a biological interaction should be further investigated, preferably by RCTs.

The RCTs that have been conducted [152, 153], were designed to study the effect of statin treatment in ongoing infection, not the prophylactic effect of statin use prior to and during infections. RCTs have so far not assessed whether different etiologic agents may influence the relation between prior statin use and the outcome of infections.

Other questions that should be further investigated are which of the different statins should be used and in which doses, and whether the effect differs between specific etiological agents [148], between different sites of infection [226], or between groups of patients dependent on host factors [227].

The impact of statin medication prior to and during sepsis

Some evidence has come up indicating that statin use may have impact on outcome of BSI or sepsis in patients who are treated with a statin before the start of infection and who continue to take the statin during the course of the infection [156, 179]. Caffrey et al., found a beneficial effect in *S. aureus* BSI in patients who were pretreated with statin and continued to take statin for at least 3 days after the time of sepsis diagnosis [179]. One RCT has shown improved survival in atorvastatin-treated ICU patients with severe sepsis if they had been prior statin users and continued statin medication, but no effect was found in patients who received *de novo* statin therapy [156].

It seems reasonable, therefore, that a prerequisite for beneficial effect of statins in BSI or sepsis is that the host's immune- and endothelial cells are pre-treated with a statin.

11. CONCLUSIONS

- Overall, both the incidence and the mortality rates of BSI increased significantly by age, particularly in males. As the proportion of older people increases, geriatric BSIs will be an escalating challenge.
- The incidence rate of BSI episodes increased through the study period, but the mortality rate was mainly unchanged, and the case fatality rate decreased. A more than twofold increase in the rate of BSI sampling possibly contributed to the detection of milder and ultimately less fatal episodes, but earlier detection and improved treatment may have had impact.
- In pneumococcal BSI, the incidence as well as the mortality decreased in males but not in females. Pneumococcal vaccine most likely has contributed, and the difference between sexes is possibly due to different changes in smoking habits.
- We observed very low but slightly increasing rates of microbes with acquired resistance.
- Antimicrobial resistance was a far smaller problem in our cohort than is reported from countries outside Scandinavia.
- The antibiotic regimen recommended by Norwegian Health Authorities [60], consisting of penicillin and gentamicin, and with metronidazole added when an anaerobic infection is suspected, is so far effective in vitro against a great majority of microbes isolated from BSI patients in this region.
- Appropriate empiric antibiotic therapy was achieved to a larger extent by replacing second- and third-generation cephalosporins with piperacillin/tazobactam or penicillin/ampicillin plus gentamicin.

- We must be aware of an increasing occurrence of inherently resistant microbes, particularly in HA infection.
- We have indications of increasing numbers of bacteria with acquired resistance, particularly *E. coli* producing ESBL and/or possessing gentamicin resistance, and these occurred predominantly in CA and HCA infections.
- In our BSI cohort, the relationship between statin use and 90-day total mortality was different in Gram-positive and in Gram-negative BSI. In Gram-negative BSI, statin use was associated with lower 90-day case fatality, which was not the case in Gram-positive BSI. Whether this finding reflects a biological mechanism or is due to some kind of unmeasured confounding, remains to be elucidated.

12. PERSPECTIVES

12.1 Further exploitation of the BSI database

12.1.1 Improvement of patient care

In order to improve the quality of care for patients with sepsis, it is mandatory to continuously update the recommendations for early detection and treatment of sepsis and to monitor both the compliance with the recommendations and the outcomes for sepsis patients.

At Levanger Hospital, sepsis has been an area of quality improvement initiatives since 2007, when we revised our guidelines for sepsis diagnosis and treatment in accordance with the Surviving Sepsis Campaign [10]. In 2011, we implemented an education and awareness program for nurses and physicians (See Section 6.3.4). We evaluated the compliance with guidelines and the 30-day case fatality in patients with detected BSI, as patients who have positive blood cultures constitute a group that is reasonably easy to define (See Section 6.4.3). The case fatality rate (CFR) was significantly lower in the period 2012-2013 than in 2008-2010, perhaps partly due to an increased rate of blood culture sampling (See Figures 10-11, Section 9.1), but earlier detection and treatment due to improved observation by ward nurses may have contributed to the low CFR during 2012 to 2013 [61]. For this work, Levanger Hospital won the 2016 National Improvement Price Competition arranged by the Norwegian Directorate of Health (Gustad, LT. Stop Sepsis Nurse. Tromsø, Sept 21, 2016).

12.1.2 Delayed appropriate antibiotic therapy and death

Delayed appropriate antibiotic therapy in BSI is associated with increased case fatality (see Section 6.3.3). Assessment of the relationship between delayed appropriate antibiotic therapy

and case fatality in the present BSI cohort has been performed and a paper is in progress, but it could not be completed before the deadline of this thesis.

12.1.3 Continuous surveillance of microbes, resistance, and outcome

The time period in which the data of this study have been recorded may in the future be regarded as a golden time with low resistance and low occurrence of deaths related to inactive antimicrobial treatment. The rising rates of antimicrobial resistance and excess deaths related to inappropriate antimicrobial treatment reported from elsewhere in the world [64], the extensive international travel which also we participate, and the rising number of migrants who arrive the country, will most likely bring us out of the state of being an "otherwise country". To monitor the occurrence of resistance and resistance-related deaths will be mandatory in the years to come. It is important to use a population-based surveillance of the burden of resistant microbes, measured as incidence, not only as the proportion of resistant microbes within a species. A resistant microbe may cause infections that do not replace but comes in addition to the cases caused by sensitive microbes, as have somewhere been the case for MRSA-BSI [100].

Microbiological diagnostics usually includes culturing techniques, which are time-consuming. Therefore, continuous surveillance data on causative microbes and local resistance patterns is mandatory for determining local guidelines for empiric antibiotic treatment [238].

Until the end of 2013, we have collected clinical data and analyzed BSI episodes in patients >15 years. Episodes in children (0-15 years) have been included in the database, but the collection of clinical data is so far not completed. In the future, we intend to survey BSI in all age groups at or Hospital Trust.

To exploit the potential of the hospital's BSI database, we should improve our registration system to achieve more timely reports of occurrence, outcomes and antimicrobial

resistance of BSIs, which would give access to real-time analyzed data necessary for updated treatment guidelines and also timely information of importance to health care administrators [239].

12.1.4 The Mid-Norway Sepsis Study

Our study population belongs to Nord-Trøndelag County. In this geographical area, the HUNT studies (I, II, and III) [240] have been performed. About 60% of the patients in the present BSI cohort participated in the HUNT II study. Linkage of clinical data from the BSI Registry and the HUNT database have been performed, making it possible to study associations of influenceable life style factors [241] and other risk factors with occurrence and outcome of BSI.

In order to further investigate the impact of statins on outcomes of BSI, we intend to perform a study in which we link the BSI database to the HUNT database, the Norwegian Prescription database (established January 1, 2004), and the national Cause of Death Registry. We might then assess the incidence, the case fatality, the long-term prognosis (secondary infections, cardiovascular and cerebrovascular events, death within 6 or 12 months) of BSI in statin users and non-users, and subgroup analyses (Gram-positive, Gram-negative, specific bacteria such as *S. aureus*, *E. coli* etc.) could be performed. Another question that should be investigated is whether statins may prevent secondary infections, possibly by attenuating the anti-inflammatory response in sepsis.

The Mid-Norway Sepsis Study Group has also linked the BSI Registry to the HUNT database and to the HUNT biobank, making it possible to study associations of genetic markers with occurrence and outcomes of BSI [Julie Paulsen. Article in progress].

A further upcoming project is to study interactions between microbes' virulence genes and hosts' genetic markers. As the isolated BSI microbes are stored in glycerol broth at -80^{o}C,

genome sequencing can be performed, and genetic data from microbes and patients can be linked. That project has been in progress since 2016 [Jan Egil Afset, Erik Solligaard et al.].

12.2 Main challenges in the field

12.2.1 National patient care improvement

In 2016, the Norwegian Directorate of Health has initiated a Patient Security Program. Early detection and treatment of sepsis is one of the designated priority areas in this program. A national expert group has proposed four measures for improved care of patients with sepsis: 1. Early detection and diagnosis of sepsis; 2. ABC assessment (secure airways, respiration, circulation); 3. Ensure blood samples (biochemical analyses and blood cultures) and other microbiology samples; 4. Start antibiotic treatment within 1 hour of the time of suspected diagnosis of sepsis. These measures are currently evaluated at three different hospitals in Norway. In 2017, a nationwide learning network will be established, based on the experience from the ongoing evaluation (www.helsedirektoratet.no).

During 2016, the Norwegian Board of Health (www.helsetilsynet.no) conducted a nationwide inspection of emergency departments in Norwegian hospitals regarding compliance with guidelines for early detection and treatment of sepsis. All hospitals were found to have some deviation from the guidelines.

12.2.2 Strategy against Antibiotic Resistance

Two main measures are needed to restrict the epidemic of antibiotic resistance. Firstly, improved infection control is mandatory to prevent spread of resistant microbes in the population, particularly among patients and health care workers. Secondly, antimicrobial resistance is strongly associated with the usage of antimicrobial agents. Therefore, a major

task is to reduce antibiotic consumption in human and veterinary medicine and in food production.

The World Health Organization, the European Union, and national governments have initiated efforts to reduce antimicrobial resistance. The Norwegian government launched a national strategy (2015-2020) in June 2015 including a target of 30% reduction in antibiotic consumption by 2020 compared to 2012 [242]. For Norwegian hospitals, the goal is to reduce the combined use of five selected antibiotic groups (quinolones, carbapenems, 3rd gen. cephlosporins, 2nd gen. cephalosporins, and piperacillin/tazobactam) by 30%.

In Norway, the first version of the National Professional Guidelines for Use of Antibiotics in Hospitals was published by the Directorate of Health in 2001. In 2013, a new, updated and extended online version of the guidelines was published [60]. A National Competence Service for Antibiotic Use in Hospitals was established in 2011, located to Haukeland University Hospital, Bergen. An online version of National Professional Guidelines for Use of Antibiotics in Primary Health Care came in 2012 (www.helsedirektoratet.no/retningslinjer). From 2016, antibiotic stewardship programs are being implemented in all Norwegian Hospitals.

The total use of antibiotics can be reduced by raising the threshold for antibiotic treatment of banal infections, such as probable viral airway infections. Further measures are rapid tests that exclude or substantiate bacterial infection (e.g., procalcitonin [243], rapid methods for microbe identification and resistance testing, which shorten the time of empiric treatment, de-escalation (narrowing the antimicrobial coverage) of the treatment regimen as fast as the result of the resistance test is available, and shorter duration of treatment.

12.2.3 Towards rapid identification and susceptibility testing of microbes

To shorten the time of empiric treatment is perhaps the most effective way of reducing overuse of broad-spectrum antibiotics. Traditionally, microbiology laboratories use culturing techniques for species identification and resistance testing. This implies that 48-72 hours last until the results are available to the clinicians. The use of rapid methods might reduce mortality, hospital LOS, antibiotic use, and costs [119]. MALDI-TOF (Matrix-Absorption-Laser-Desorption-Ionisation-Time of Flight) is a rapid technique which identifies bacterial ribosomal proteins [120]. By this method, the result of microbe identification is available within 30 min of the time when the blood culture bottle has "flagged" positive [244]. Another technique is FilmArray (bioMerieux), a multiplex-PCR technique [245], which can identify a large number of Gram-positive and Gram-negative bacteria, five *Candida* spp., and several resistance genes (*mecA*, *vanA*, *vanB*, *KPC*)[2] within 1 hour in blood culture bottles that have "flagged" positive. Vitek is a method of rapid resistance testing (results within 6-8 hours) [246]. PCR-based tests for detection of resistance genes may allow a further use of penicillin plus gentamicin as empiric antibiotic treatment in Norway. Examples of a gene possible to detect in the routine microbiology laboratory is the *mec A* gene in methicillin resistant *Staphylococcus aureus*. Other genes that would be very useful to detect are resistance genes in *E. coli* and *Klebsiella* spp. encoding resistance to aminoglycosides and cephalosporins [247]. The microbiology laboratory at Haukeland University Hospital identifies *E. coli* and *Klebsiella* by MALDI-TOF and detects the resistance genes AAC-3, CTX-m, SHV5/12, and CMY2, and they have the resistance result available 1 hour after the bacterial growth in blood culture is detected [244]. Even New Dehli Metallo-betalactamase (NDM), a carbapenemase first discovered in India, can be detected by real-time PCR [248].

[2] *mecA* gene causes methicillin resistance in *S. aureus*; *vanA* and *vanB* are resistance genes in enterococci; *KPC*, *Klebsiella pneumoniae* carbapenemase

12.2.4 The challenging balance between saving lives now and saving lives in the future

During the era of antibiotic treatment, millions of lives have been saved in patients with bacterial infections. The epidemic rise of resistant microbes forces physicians to use broad-spectrum antibiotics in order to save their patients' lives. An ethical dilemma is that the goal of saving more lives now may result in more infection-related deaths in the future. Optimal empiric antimicrobial regimens tailored to save as many lives as possible in the present time will include resistance driving broad spectrum drugs that will necessarily increase the burden of antimicrobial resistance, and treatment failure and death will be the consequence for millions of patients in the future [249, 250]. Individual physicians may be pressed to give empiric treatment that covers a wider spectrum of microbes than the national guidelines' recommend. Deaths related to inappropriate empiric therapy will undoubtedly become an increasingly common event. Who is responsible when a patient dies and the treatment has been inappropriate? The individual physician? The institution? The guideline makers? Leonhard Leibovici, one of the world's foremost BSI researchers, states that authorized guidelines should govern clinical practice. He recommends that patients with severe infections should be preferred for broad-spectrum antibiotic treatment, and that patients with short expected remaining lifetime (severe untreatable disease, advanced frailty due to age/disease burden) should not receive such treatment [250].

How can we balance the concerns for our current patients against the risks of future patients? What we need is updated statistics on microbes, resistance, and outcomes, giving background knowledge for timely treatment guidelines. Further improvement of early infection diagnostics as well as and strengthening of infectious disease and microbiology specialist service is mandatory. A high quality infection control system in the primary health care and in hospitals is the fundament of all modern health care, and compliance with infection control procedures must be evaluated and improved in order to prevent spread of

resistant microbes. Vaccination programs and strategies promoting a healthy lifestyle in individuals and in the population may attenuate occurrence and outcomes of infections. Whether drugs, such as statins, might have prophylactic effect, at least in subgroups of patients, should be further investigated (Figure 15).

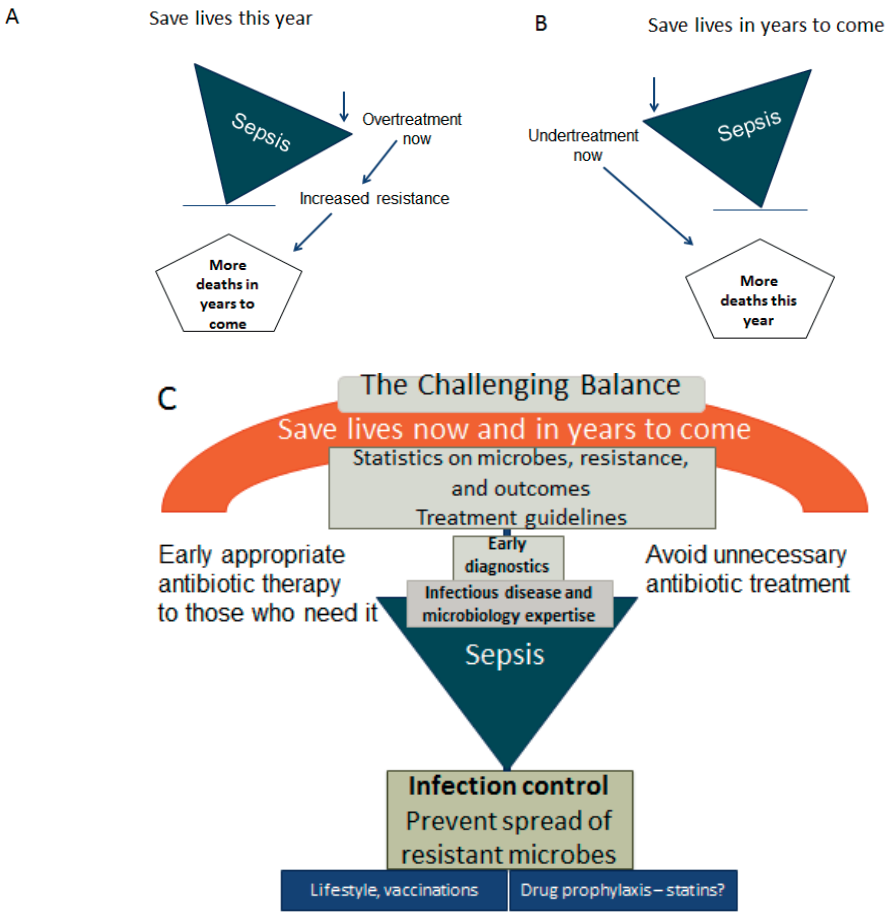

Figure 15.
The ethical dilemma of infection treatment. **A**. Concerns for current patients and focus on early covering antibiotic treatment may lead to overtreatment now and give rise to more deaths in years to come. **B**. An exclusive focus on achieving goals for limited antibiotic use may cause under-treatment and excess deaths in the present time. **C**. To balance the needs of current patients against the risks of future patients, we need increased and updated knowledge and technology, strengthened professional expertise in research, education and clinical practice, and strong improvement efforts on many levels

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14. ERRATA

Thesis:

The term "mortality" has been used instead of the term "case fatality" the following places in the manuscript:

Page 65, section 6.6.5, line 4 and line 7

Page 98, line 2 from the top; 2nd paragraph, line 7

Page 100, in the legend of Figure 13 and on the Y-axis of the figure

Page 102, 2nd paragraph, lines 5, 7, and 10

Page 105, 2nd paragraph, first line

Page 108, lower paragraph, first line

Thesis page 61, Figure 9: The enzyme HMG-CoA reductase has been labeled HMG-CoA

Thesis, References:

Reference 198 is wrong. The correct reference in this place is reference 84.

Paper II:

In Fig. 3, there is a substantial error. The Y-axis has been given the label Percent. That is wrong. The correct label of the Y-axis should be Number of episodes. (The percentages of episodes with microbe(s) non-susceptible to penicillin-gentamicin-metronidazole were 1.9%, 3.0%, and 5.2% in 2002-2005, 2006-2009, and 2010-2013, respectively)

15. PAPERS I-III

Paper I

Mehl et al. BMC Infectious Diseases (2017) 17:205
DOI 10.1186/s12879-017-2291-2

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Burden of bloodstream infection in an area of Mid-Norway 2002-2013: a prospective population-based observational study

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Abstract

Background: Studies from several countries indicate that the incidence and mortality of bloodstream infection (BSI) have been increasing over time.

Methods: We studied the burden of disease and death related to BSI in a defined geographical area of Mid-Norway, where BSI episodes were prospectively recorded by the same microbiological department during 12 consecutive years. Death from BSI was defined as death within 30 days of BSI detection. Age and sex standardized incidence and mortality rates and case fatality rates were calculated.

Results: Between 2002 and 2013, 1995 episodes of BSI in 1719 patients aged 16 to 99 years were included. The overall incidence of BSI was 215 per 100,000 person-years. The incidence increased exponentially with age, particularly in males. The incidence increased from 205 to 223 per 100,000 person-years from 2002–07 to 2008–13. *Escherichia coli* was the most frequently isolated infective agent, followed by *Streptococcus pneumoniae* and *Staphylococcus aureus*. The rate of *S. pneumoniae* BSI decreased over time in males (on average by 9.2% annually), but not in females. The total rate of BSI microbes with acquired resistance increased slightly over time, but did not exceed 2 episodes per 100,000 person-years. The mortality of BSI was 32 per 100,000 person-years, higher in males than in females (36 vs. 28 per 100,000 person-years) and was significantly higher in old age, particularly in males. The total BSI mortality was similar in the first and second halves of the study period, but the mortality of *S. pneumoniae* BSI decreased in males (15.0% annually). The crude case fatality decreased from the first to the second half of the study period (17.2% to 13.1%; $p = 0.014$). The rate of blood culture sampling increased more than twofold during the study period.

Conclusions: The mortality of BSI remained stable during 2002–2013. At the same time, BSI incidence increased and case fatality rate decreased, perhaps because an increased rate of blood culture sampling may have led to improved detection of milder BSI episodes. Very low, yet slightly increasing rates of microbes with acquired resistance were observed.

Keywords: Bloodstream infection, Bacteremia, Bacteraemia, Sepsis, Population-based, Incidence, Mortality, Case fatality

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Background

Bloodstream infection (BSI) contributes substantially to morbidity and mortality worldwide [1]. The annual incidence has been reported between 80 and 257 per 100,000 person-years [2–9]. In Europe, the annual number of BSI episodes and deaths associated with BSI has been estimated at 1.2 million and 157,000, respectively. The corresponding numbers of hospital-acquired BSIs were found to be 240,000 episodes and 29,000 deaths [1]. Most studies report increasing incidence rates [5, 6, 8], but a decreasing rate has also been described [10].

The burden of BSI includes mortality as well as incidence, but few studies have reported both [1, 3, 6]. Monitoring the burden of BSI is important for reasons of resource allocation and for evaluating prevention and treatment strategies [11]. As the proportion of elderly people, more prone to infections, is increasing [12, 13], knowledge about their burden of severe infections is of particular importance. Growing antimicrobial resistance worldwide, associated with increased mortality [14, 15], make surveillance of BSI microbes and antimicrobial resistance essential. As different prevention and treatment

Table 1 Incidence of bloodstream infection (BSI) stratified by sex in an area of Mid-Norway 2002-2013. Number of episodes and observed and standardized incidence rates, allover and in various subgroups, are shown

Group of BSI	Total			Females			Males		
	n	Observed BSI rate[a] (95% C.I.)	Age and sex standar- dized rate[b]	*n*	Observed BSI rate[a] (95% CI)	Age standar-dized rate [c]	*n*	Observed BSI rate[a] (95% CI)	Age standar-dizedrate [d]
All BSIs	1995	232 (222–242)	215	961	221 (208–236)	209	1034	242 (228–258)	222
Age[e]									
16–64 years	578	84 (78–92)	82	284	84 (75–94)	83	294	84 (75–95)	82
65–79 years	692	564 (523–607)	555	306	477 (425–534)	473	386	658 (594–727)	646
≥80 years	725	1379 (1280–1483)	1373	371	1122 (1011–1242)	1126	354	1813 (1629–2012)	1826
Place of acquisition									
CA-BSI	934	109 (102–116)	102	502	116 (106–126)	110	432	101 (92–111)	94
HCA-BSI	787	91 (85–98)	85	356	82 (74–91)	78	431	101 (92–111)	91
HA-BSI	274	32 (28–36)	30	103	24 (19–29)	23	171	40 (34–47)	37
Infection focus									
Urinary tract	752	87 (81–94)	81	429	99 (90–109)	94	323	76 (68–84)	68
Lungs	331	38 (34–43)	36	142	33 (28–39)	31	189	44 (38–51)	41
Biliary tract	220	26 (22–29)	24	93	21 (17–26)	21	127	30 (25–35)	27
Gastrointestinal tract	101	12 (10–14)	11	44	10 (7–14)	9.8	57	13 (10–17)	13
Skin or soft tissue	143	17 (14–20)	16	61	14 (11–18)	13	82	19 (15–24)	18
Other	247	29 (25–33)	27	104	24 (20–29)	23	143	34 (28–39)	31
Unknown	201	23 (20–27)	22	88	20 (16–25)	19	113	26 (22–32)	24
Microbe group									
Gram-negative BSI	1133	132 (124–140)	123	616	142 (131–154)	135	517	121 (111–132)	110
Gram-positive BSI	777	90 (84 to 97)	85	310	71 (64–80)	68	467	109 (100–120)	101
Polymicrobial or fungal BSI	85	10 (8–12)	9	35	8 (6–11)	8	50	12 (8–15)	11
Microbes (the four most common)									
Escherichia coli	686	80 (74–86)	74	421	97 (88–107)	93	265	62 (55–70)	56
Streptococcuspneumoniae	226	26 (23–30)	25	109	25 (21–30)	24	117	27 (23–33)	25
Staphylococcusaureus	218	25 (22–29)	24	75	17 (14–22)	16	143	34 (28–39)	31
Klebsiella spp.	134	16 (13–18)	14	65	15 (12–19)	14	69	16 (13–20)	15

[a]BSI episodes per 100,000 person-years (totally 860,630 person-years in individuals ≥16 years, 426, 517 in males, 434,113 in females)
[b]males and females standardized to the age distribution of the male and female population of Norway 2010, respectively
[c]standardized to the age distribution of females in Norway 2010
[d]standardized to the age distribution of males in Norway 2010
[e]Person-years in the three age groups were: 16–64 years: males 348,342; females 336,950; 65–79 years: males 58,645; females 64,100; ≥80 years: males 19,530; females 33,063

strategies are needed (vaccination programs, antibiotic regimens, and infection control measures), it is necessary to separately survey community acquired, health care-associated, and hospital acquired BSIs [16, 17].

We conducted a prospective study within an area of Mid-Norway to assess the BSI incidence and mortality, with emphasis on age and sex differences and time trends.

Methods

As part of the Mid-Norway Sepsis Study we prospectively recorded episodes of BSI in patients aged 16 years or older admitted to Levanger Hospital between January 1, 2002 and December 31, 2013. This BSI cohort or parts of it have previously been used in studies on other aspects of BSI [18–20], and two studies, describing BSIs with *Staphylococcus aureus* 1995–2011 [21] and *Streptococcus pneumoniae* 1993–2011 [22], have included the respective bacterial species from the current cohort. Levanger Hospital is one of two hospitals in Nord-Trøndelag County. The hospital is an emergency hospital serving the population in a defined geographical area of 10 municipalities, with 68,491 inhabitants aged 16 years and above at the start of the study, and 75,858 at the end of the study. Population data of the ten municipalities around Levanger Hospital for every year between 2002 and 2013, with age and sex distribution, was obtained from Statistics Norway.

The microbiology laboratory at Levanger Hospital exclusively provided all microbiology services in Nord-Trøndelag County. Patients hospitalized with BSI were identified and prospectively registered at the microbiology laboratory. For each BSI, a registration form was completed by the responsible physician. The main investigator, two subordinate doctors and three research nurses reviewed the patients' records to verify the data and record additional variables. Blood cultures were performed in BACTEC 9240 Vacutainer Culture Bottles (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) [23], which in 2010 was replaced by BACTEC FX. The volume of blood drawn was the same during the study period. A blood culture set consisted of one aerobic and one anaerobic BACTEC bottle obtained from a single draw. If a second draw was taken simultaneously from another site, one aerobic bottle was used. Isolates were identified using standard methods [24]. Antimicrobial susceptibility testing was performed by the disc diffusion method (Neo-Sensitabs, Rosco Diagnostica, Taastrup, Denmark). The microbiology laboratory at Levanger Hospital is ISO 15189 accredited.

An episode of BSI was defined by growth of one or more microbes from blood culture combined with clinical evidence of systemic infection [25]. A new BSI episode with the same microbe in the same patient was recorded if an interval of at least 30 days had passed without signs of infection since an earlier episode [26]. If more than one organism was isolated from one or more blood cultures within a 72-h period, the BSI episode was classified as polymicrobial. One positive blood culture for organisms regarded as etiological agents was the requirement for inclusion. For coagulase-negative staphylococci, alpha-hemolytic streptococci, or other possible skin contaminants, at least two identical isolates from separate venipunctures were required.

The BSI episodes were classified as hospital-acquired (HA), health care-associated (HCA) or community acquired (CA) [16, 17]. HA-BSI was diagnosed if the infection was detected >48 h after admission [27]. Patients who during the 30 days prior to hospital admission had (1) been hospitalized two or more days or (2) had received intravenous therapy or wound care at home or (3) hemodialysis or chemotherapy at hospital visits or (4) were nursing home residents, were categorized as having HCA-BSI. CA-BSI was diagnosed if the infection was detected <48 h after admission and none of the criteria for HCA-BSI were fulfilled.

We defined death from BSI as death within 30 days of BSI detection. The patient administrative system at the hospital receives updated information on vital status from the national population register, and thus, information on

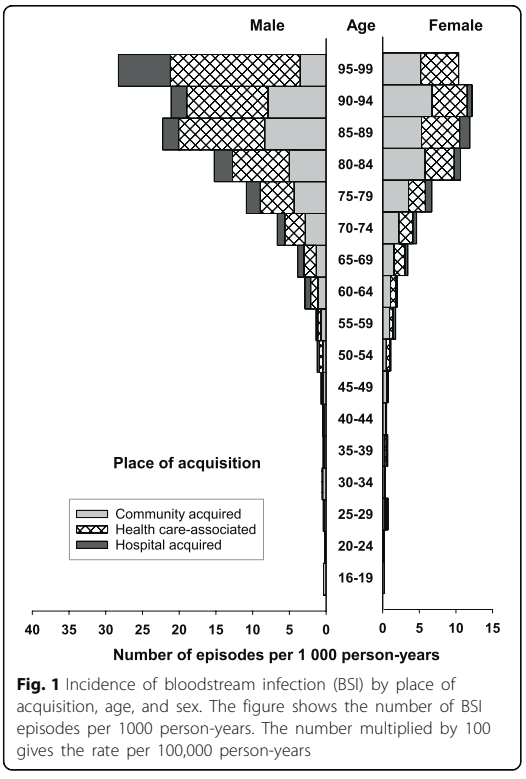

Fig. 1 Incidence of bloodstream infection (BSI) by place of acquisition, age, and sex. The figure shows the number of BSI episodes per 1000 person-years. The number multiplied by 100 gives the rate per 100,000 person-years

fatal outcome of BSI was complete even if the patient was discharged from hospital.

A urinary focus was assigned when the same microbe was isolated from urine and as well as from blood culture along with clinical signs/symptoms or risk factors for urinary infection, and no other source of infection was identified. A presumed pulmonary focus was diagnosed with clinical signs of lower respiratory infection accompanied by positive radiologic findings. Focus in the biliary tract was ascertained based on clinical, biochemical and radiological findings. Signs of infection along with focal growth of the same microbe as in blood culture were taken as a confirmation of infection in abdomen, skin, soft tissue or other sites. An unknown focus of infection was assigned when none of the criteria for ascertaining a focus were met.

Statistical analyses

BSI included both first-time and repeat episodes [6, 7]. The mortality rate was calculated as the total number of deaths within 30 days of a diagnosis of BSI per 100,000 person-years. Incidence and mortality rates were calculated for the population between 16 and 99 years. Population data during 2002–13 (Statistics Norway), were used as denominators to calculate age-specific and sex-specific rates of BSI episodes and BSI related deaths. The incidence rate of HA-BSI was also reported as the number of patients with HA-BSI in a time period divided by the number of hospital bed-days in that same time period. Observed incidence and mortality rates were standardized to the age and sex distribution of the population of Norway 2010. Confidence intervals (CIs) of rates were

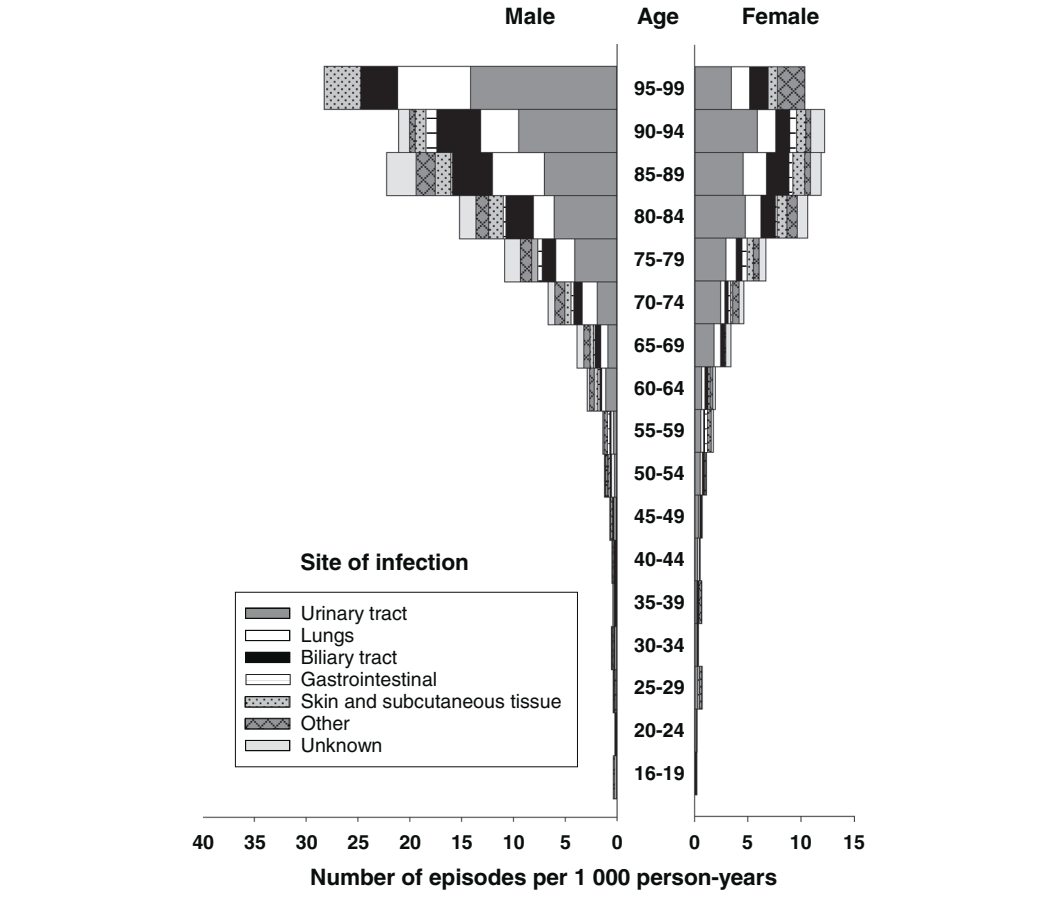

Fig. 2 Incidence of bloodstream infection (BSI) by site of infection, age, and sex. Number of BSI episodes per 1000 person-years is shown. The number multiplied by 100 gives the rate per 100,000 person-years

calculated based on assumed Poisson distribution. Poisson regression was used to assess time trends in BSI incidence and mortality rates (average rate ratio per calendar year), adjusted for age (in 5-year intervals) and stratified by sex.

The case fatality rate was defined as the total number of deaths within 30 days of diagnosed BSI episodes divided by the total number of BSI episodes. Confidence intervals were calculated using Wilson's approximation to the binominal distribution [28]. Case fatality rates for two time periods were compared using chi-square test. Two-sided p-values <0.05 were considered significant and 95% confidence intervals (CI) have been reported where relevant. The analyses were performed using SPSS 22, Stata 13, and StatXact 9.

Results

During the 12-year study period, a total of 1995 episodes of BSI occurred, 1034 in males and 961 in females, among 1719 individuals.

Incidence rates allover and by sex and age

The overall incidence rate of BSI (first-time and repeat episodes) was 215 per 100,000 person-years (Table 1), and the rate was higher in males than in females (222 vs. 209 per 100,000 person-years). Age-specific BSI rates were substantially higher in males than in females, particularly in older age groups (Figs. 1, 2 and 3; Additional file 1: Table S1). Whereas the incidence was similar for males and females 16–64 years (82 vs. 83 per 100,000 person- years), males had substantially higher incidence than females in the age group ≥80 years (1826 vs. 1126 per 100,000 person-years).

Place of acquisition

The rates of CA-, HCA-, and HA-BSI were 102, 85, and 30 per 100,000 person-years, respectively (Table 1). HCA- and HA-BSIs constituted larger parts of the BSIs in men than in women, and HCA- and HA-BSI predominated among men in the oldest age groups (Fig. 1). The total rate of HA-BSI was 38 per 100,000 hospital bed-days (Additional file 1: Table S2).

Site of infection

The urinary tract was the predominant site of infection (no. of episodes per 100,000 person-years: 81 overall, 94 in females and 68 in males) (Table 1), followed by the lungs and the biliary tract. Rates of BSI from the urinary

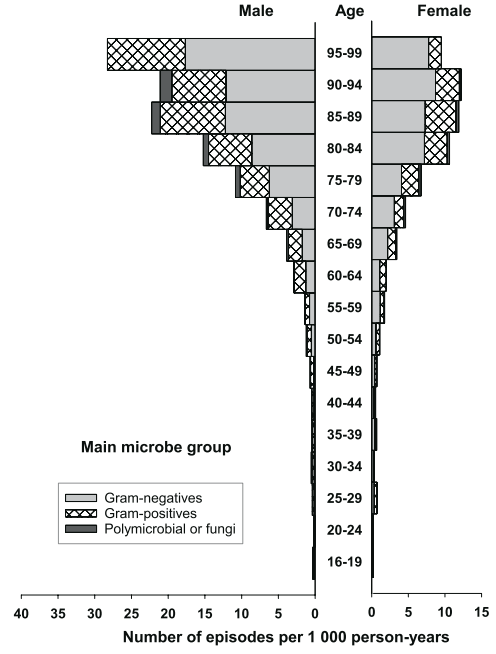

Fig. 3 Incidence of bloodstream infection (BSI) by main microbe group, age, and sex. The figure shows the number of BSI episodes per 1000 person-years. The number multiplied by 100 gives the rate per 100,000 person-years

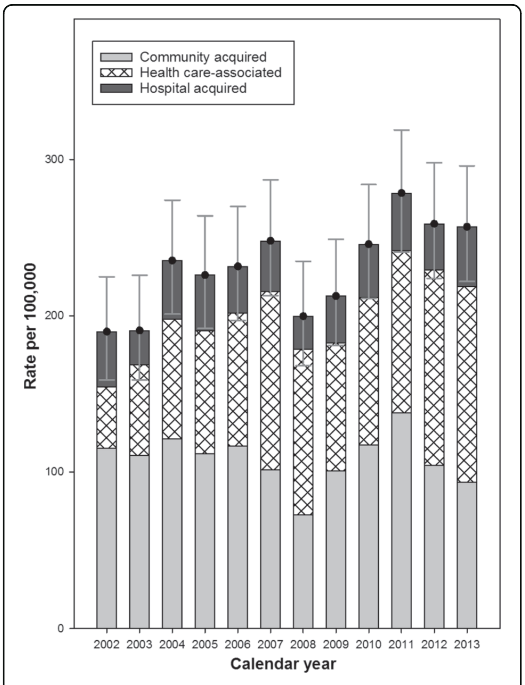

Fig. 4 Observed incidence of bloodstream infection (BSI) per calendar year by different places of acquisition. Number of BSI episodes per 100,000 person-years is shown

tract, lungs, and biliary tract were higher in old age, particularly in males (Fig. 2).

Microbes

Gram-negative BSI was more common in females than in males (135 vs. 110 per 100,000 person-years) (Table 1), whereas Gram-positive BSI most often occurred in males (101 vs. 68 per 100,000 person-years). *Escherichia coli* was the most commonly isolated BSI microbe from all three places of acquisition and predominated in females (93 vs. 56 per 100,000 person-years) (Table 1). The second most common microbe was *Streptococcus pneumoniae* (25 episodes per 100,000 person-years), which was evenly distributed between the sexes and mainly occurred in CA-BSI. *Staphylococcus aureus*, close to *S. pneumoniae* in total rate (24 per 100,000 person-years), was mostly represented in HCA-BSI and was significantly more frequent in males than in females (31 vs. 16 episodes per 100,000 person-years).

Time trends in incidence

The observed incidence rate of BSI increased from 190 per 100,000 person-years in 2002 to 257 in 2013 (with a peak at 278 in 2011) (Fig. 4; Additional file 1: Table S3). Standardized incidence rates for two time periods, 2002-2007 and 2008–2013, are shown in Table 2, and age-adjusted time trends in BSI rate by sex are shown in Table 3. Overall, the incidence rate increased from 205 to 223 per 100,000 person-years from 2002–07 to 2008–13. The incidence rate increased on average by 2.8% annually in females but not significantly in males. HCA-BSIs increased from 72 to 96 per 100,000 person-years from 2002–07 to 2008–13. The HA-BSI rate, calculated as episodes per 100,000 hospital bed-days, increased from 36 to 40 (Additional file 1: Table S4).

Table 2 Incidence of bloodstream infection (BSI) in two time periods in an area of Mid-Norway. Number of episodes and observed and standardized incidence rates are shown

Group of BSI	2002–2007			2008–2013		
	n	Observed incidence rate[a] (95% CI)	Age and sex standardized incidence rate[b]	*n*	Observed incidence rate[a] (95% CI)	Age and sex standardized incidence rate[b]
All BSIs	921	221(206–235)	205	1074	242 (228–257)	223
Place of acquisition						
CA-BSI	471	113 (103–123)	106	463	105 (95–115)	98
HCA-BSI	316	76 (68–84)	72	471	106 (97–116)	96
HA-BSI	134	32 (27–38)	30	140	32 (27–37)	29
Infection focus						
Urinary tract	316	76 (68–84)	70	436	98 (89–108)	90
Lungs	169	40 (35–47)	38	162	37 (31–43)	34
Biliary tract	106	25 (21–31)	23	114	26 (21–31)	23
Gastrointestinal tract	47	11 (8–15)	11	54	12 (9–16)	12
Skin or soft tissue	81	19 (15–24)	18	62	14 (11–18)	13
Other	108	25 (21–31)	24	139	31 (26–37)	30
Unknown	94	22 (18–28)	21	107	24 (20–29)	22
Microbe group						
Gram-negative BSI	499	119 (109–130)	111	634	143 (132–155)	131
Gram-positive BSI	386	92 (83–102)	87	391	88 (80–97)	81
Polymicrobial or fungal BSI	36	9 (6–12)	8	49	11 (8–15)	10
Microbes (the four most common)[c]						
Escherichia coli	303	73 (65–81)	68	383	86 (78–96)	79
Streptococcus pneumoniae	127	30 (25–36)	29	99	22 (18–27)	21
Staphylococcus aureus	100	24 (19–29	22	118	27 (22–32)	25
Klebsiella spp.	47	11(8–15)	10	88	20 (16–24)	18

[a]BSI episodes per 100,000 person-years (417,682 person-years in 2002–2007; 442,948 person-years in 2008–2013)
[b]age and sex standardized to the population of Norway 2010
[c]less common BSI microbes are listed in Additional file 1: Table S5

Table 3 Age-adjusted time trends in bloodstream infection (BSI) incidence stratified by sex. The table shows BSI rate ratios per calendar year by Poisson regression

	Females		Males	
	BSI rate ratio (95% CI)	*p*-value	BSI rate ratio (95% CI)	*p*-value
BSI, allover	1.028 (1.010–1.047)	0.003	1.011(0.993–1.029)	0.22
Place of acquisition				
Community acquired	0.998 (0.973–1.024)	0.88	0.976 (0.950–1.004)	0.09
Health care-associated	1.080 (1.047–1.114)	<0.001	1.053 (1.024–1.083)	<0.001
Hospital acquired	1.009 (0.954–1.067)	0.76	0.999 (0.956–1.043)	0.96
Infection focus				
Urinary tract	1.056 (1.027–1.086)	<0.001	1.041 (1.008–1.075)	0.013
Lungs	1.021 (0.974–1.071)	0.39	0.969 (0.930–1.010)	0.14
Biliary tract	1.017 (0.959–1.079)	0.57	0.993 (0.944–1.045)	0.80
Gastrointestinal tract	1.017 (0.933–1.108)	0.70	1.052 (0.975–1.136)	0.19
Skin or soft tissue	0.934 (0.868–1.005)	0.069	0.979 (0.920–1.042)	0.51
Other	0.989 (0.935–1.045)	0.69	1.040 (0.991–1.091)	0.11
Unknown	1.043 (0.981–1.109)	0.17	0.987 (0.935–1.041)	0.64
Microbe group				
Gram-negative BSI	1.043 (1.019–1.068)	<0.001	1.020 (0.995–1.046)	0.12
Gram-positive BSI	0.997 (0.966–1.030)	0.87	0.993 (0.968–1.020)	0.62
Polymicrobial or fungal BSI	1.031 (0.936–1.135)	0.54	1.095 (1.007–1.190)	0.034
Microbes (the four most common)				
Escherichia coli	1.044 (1.015–1.073)	0.003	1.002 (0.967–1.037)	0.92
Streptococcus pneumoniae	1.007 (0.954–1.063)	0.80	0.908 (0.860–0.958)	<0.001
Staphylococcus aureus	0.988 (0.926–1.055)	0.73	1.032 (0.984–1.082)	0.20
Klebsiella spp.	1.136 (1.053–1.224)	0.001	1.094 (1.020–1.175)	0.012

BSIs from the urinary tract increased from 70 to 90 per 100,000 person-years. Gram-negative BSI increased from 111 to 131 per 100,000 person-years, most evident for *E. coli* (68 to 79 per 100,000 person-years) and *Klebsiella* spp. (10 to 18 per 100,000 person-years). Polymicrobial or fungal BSI increased from 8 to 10 per 100,000 person-years, whereas *Streptococcus pneumoniae* BSI decreased from 29 to 21 per 100,000 person years. The rate of *Pseudomonas aeruginosa* BSI was fairly stable (6.7 to 5.8 per 100,000 person-years), while the rate of candida BSI increased slightly over time (1.4 to 2.3 in the first and second period, respectively) (Additional file 1: Table S5).

BSI microbes with acquired drug resistance

The rate of BSI microbes with acquired drug resistance (ADR) was very low but it increased slightly over time in our population (Additional file 1: Table S5). In the first and second time period, the total rates of ADR microbes were 0.6 and 2.0 per 100,000 person-years. Methicillin-resistant *S. aureus* (MRSA) contributed 0 and 0.2 per 100,000 person-years, penicillin-non-susceptible pneumococci (PNSP) 0 and 0.4 per 100,000 person-years, and *Enterobacteriaceae* producing extended spectrum beta-lactamase (ESBL-E) 0.6 and 1.4 per 100,000 person-years in the first and second time period, respectively.

BSI mortality

Death within 30 days occurred in 299 of the BSI episodes, 172 in males and 127 in females. The overall mortality rate of BSI was 32 per 100,000 person-years (Table 4). The mortality rate was higher in males than in females (36 vs. 28 per 100,000 person-years) and increased more with age in males than in females (Fig. 5; Additional file 1: Table S6). The mortality rate was 35 and 29 per 100,000 person-years in 2002-07 and 2008-13, respectively (Table 5), but no significant age-adjusted annual change was observed (Table 6). Among subgroups of BSI, the mortality rate of HCA-BSI increased in females (7.4% annually), and the mortality rate of BSI from the urinary tract increased in males (11.4% annually). The mortality rate of BSI from pulmonary infection decreased from 10.4 to 5.7 per 100,000 person-years (annually by 9.0% in males). For *Streptococcus pneumoniae* BSI, the mortality rate decreased from 4.8 to 1.9 per 100,000 person-years, with an average annual decrease of 15.0% in males.

Table 4 Mortality of bloodstream infection (BSI) stratified by sex in an area of Mid-Norway 2002–2013. Number of deaths and observed and standardized mortality rates, allover and in various subgroups, are shown

Group of BSI	Total			Females			Males		
	n	Observed mortality rate[a] (95% C.I.)	Age and sex standar-dized mortality rate[b]	*n*	Observed mortality rate[a] (95% CI)	Age standar-dized rate[c]	*n*	Observed mortality rate[a] (95% CI)	Age standar-dized rate[d]
All BSIs	299	35 (31–39)	32	127	29 (24–35)	28	172	40 (35–47)	36
Place of acquisition									
CA-BSI	85	10 (8–12)	9	42	10 (7–13)	9	43	10 (7–14)	9
HCA-BSI	163	19 (16–22)	18	65	15 (12–19)	14	98	23 (19–28)	22
HA-BSI	51	6 (4–8)	6	20	5 (3–7)	4	31	7 (5–10)	7
Infection focus									
Urinary tract	63	7 (6–9)	7	31	7 (5–10)	7	32	8 (5–11)	7
Lungs	75	9 (7–11)	8	25	6 (4–9)	6	50	12 (9–15)	11
Biliary tract	15	2 (1–3)	1	4	1 (0–2)	0.4	11	3 (1–5)	2
Gastrointestinal tract	16	2 (1–3)	2	8	2 (0–4)	2	8	2 (0–3)	2
Skin or soft tissue	34	4 (3–6)	4	12	3 (1–5)	3	22	5 (3–8)	5
Other	28	3 (2–5)	3	14	3 (2–5)	3	14	3 (2–5)	3
Unknown	68	8 (6–10)	7	33	8 (5–11)	7	35	8 (6–11)	8
Microbe group									
Gram-negative BSI	128	15 (12–18)	14	64	15 (11–19)	14	64	15 (12–19)	14
Gram-positive BSI	144	17 (14–20)	15	48	11 (8–15)	11	96	23 (18–27)	20
Polymicrobial or fungal BSI	27	3 (2–5)	3	15	3 (2–6)	3	12	3 (1–5)	3
Microbes (the four most common)									
Escherichia coli	59	7 (5–9)	6	28	6 (4–9)	6	31	7 (5–10)	6
Streptococcus pneumoniae	31	4 (2–5)	3	10	2 (1–4)	2	21	5 (3–8)	4
Staphylococcus aureus	60	7 (5–9)	6	25	6 (4–9)	5	35	8 (6–11)	7
Klebsiella spp.	20	2 (1–4)	2	12	3 (1–5)	2	8	2 (0.7–4)	2

[a]Deaths within 30 days of BSI episodes per 100,000 person-years (totally 860,630 person-years in individuals ≥16 years, 426,517 in males, 434,113 in females)
[b]males and females standardized to the age distribution of the male and female population of Norway 2010, respectively
[c]standardized to the age distribution of females in Norway 2010
[d]standardized to the age distribution of males in Norway 2010

Time trends in case fatality

Allover, the case fatality rate decreased from 17.2% to 13.1% ($p = 0.014$) between 2002–07 and 2008–13, with similar decreases across the three places of acquisition. Among the specific infection foci, the case fatality rate of BSI from pulmonary infection decreased from 28% to 17% ($p = 0.026$) (Additional file 1: Table S8).

Time trends in blood culture sampling rate

The rate of blood culture sampling increased more than twofold from 2002 to 2013 (2189 to 4605 blood culture sets per 100,000 person-years), and the rate of BSI episodes per 100 blood culture sets decreased from 8.7 in 2002 to 5.6 in 2013 (Additional file 1: Table S3). In the first (2002–07) and second (2008–13) halves of the study period, the average rates of blood culture sampling were 3062 and 3977 sets per 100,000 person-years, and the average rates of BSI episodes per 100 blood culture sets were 7.2 and 6.1, respectively (Additional file 1: Table S9).

Discussion

The present study provides information on the incidence and mortality of BSI in an area of Mid-Norway, overall, by age and sex, by time period, and for specific subgroups of BSI. The burden of BSI, in terms of both incidence and mortality, increased strongly with age, particularly in males. The incidence increased during the 12-year study period, and the increase was strongest in females, for HCA-BSI, and for urinary tract and Gram-negative BSIs. Over the same period, the mortality remained stable and case fatality rate decreased, possibly because an increased rate of blood culture sampling may have led to improved

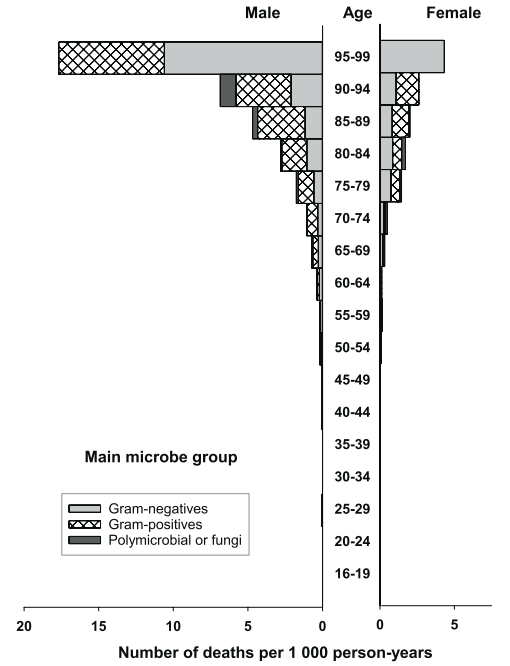

Fig. 5 Mortality of bloodstream infection (BSI) by main microbe group, age, and sex. The figure shows number of deaths within 30 days of BSI episodes per 1000 person-years. The number multiplied by 100 gives the rate per 100,000 person-years

detection of milder BSI episodes. A shift towards higher proportions of female sex, Gram-negative etiology, and urinary tract site may also have influenced the case fatality rate to some degree. In addition, earlier detection of sepsis and improved treatment may have had impact. Very low, yet slightly increasing rates of microbes with acquired resistance were observed.

To our knowledge, this is the first study estimating the overall burden of BSI in a Norwegian population. Incidence rates of invasive infections with single microbes have been reported [29–33], but no population-based study has focused on BSI as a whole. One previous study described the epidemiology of sepsis in Norway in 1999 [34], and two studies published more than 20 years ago [26, 35] described BSI incidence and mortality related to hospital admissions and bed-days, but did not include population statistics. Strengths of the present study include the prospective registration of BSIs within a well-defined source population. All patients with a BSI acquired outside hospital (CA- and HCA-BSI) in our geographical area were admitted to Levanger Hospital, and the blood cultures were handled at one microbiology laboratory. Some inhabitants in this area will likely have had BSI during stays at St Olav's Hospital, the closest university hospital, so that the true rate of HA-BSI in our area is slightly higher than is reported in this article. We did not exclude the very small number of episodes that occurred in persons who were visitors to the area, as a similar number of BSIs is likely to have occurred among inhabitants of our area being on travel elsewhere. Standardization for the age and sex distribution is representative for Norway 2010. Use of international population standards could have eased comparison between studies [36, 37], but most studies in this field have used regional or national population standards [4, 8, 10].

The comparison of incidence and mortality rates of BSI between studies is challenging because of differences in the age groups included and in age- and sex distribution of populations [37], differences in the classification of place of acquisition [8, 16, 17, 38], and differences in the definition of incident episodes: first-time vs. total number of BSIs, and different time periods since last BSI required to define a new episode (after 30 days [26], 3 months [6], first episode in another calendar year [36, 37], first episode during the study period [4, 8, 10]). BSI can affect the same individual several times, and the episodes are most often independent of each other. To inform about the total burden of BSI, we chose to estimate the total rate of BSI rather than the first-time episodes only [11]. Compared with our results, incidence rates (166–189 per 100,000 person-years) [6–8] and mortality rates (22 per 100,000 person-years) [6] of BSI are somewhat lower in other studies that also included all BSI episodes. This difference is expected, as these studies also included children, who are generally at low risk of BSI.

An increase in BSI incidence over time have also been reported in Finland (from 147 to 168 per 100,000 person-years from 2004 to 2007) [6] and in Northern Denmark (from 120 to 201 per 100,000 person-years from 1992 to 2006) [8], although a study from Funen, Denmark, [10] of first-time BSI among people ≥16 years of age reported a decrease in BSI incidence from 254 to 199 per 100,000 person-years through 2000 to 2008. Similar to our findings, the increase in BSI incidence was accompanied by a decreasing case fatality rate in Denmark (22.7% to 20.6%) [8], but not in Finland (12.6% to 13.2%) [6]. At the same time, we observed a relatively stable mortality rate of BSI, as has also been reported in Finland [6]. The combination of increased incidence, reduced case fatality, and stable mortality may be explained by improvements in the detection of milder, less fatal BSI episodes. In support of that explanation, we and others (5, 10) have observed increasing rates of blood culture sampling over time, and the number of BSI episodes per 100 blood culture sets decreased with time in our study as was found in Funen, Denmark [10], though not in Northern Denmark or in Finland [5, 8].

Table 5 Mortality of bloodstream infection (BSI) in two time periods in an area of Mid-Norway. Number of deaths and observed and standardized mortality rates are shown

Group of BSI	2002–2007			2008–2013		
	n	Observed mortality rate[a] (95% CI)	Age and sex standardized mortality rate[b]	*n*	Observed mortality rate[a] (95% CI)	Age and sex standardized mortality rate[b]
All BSIs	158	37 (32–44)	35	141	32 (17–53)	29
Place of acquisition						
CA-BSI	50	12 (9–16)	11.0	35	7.9 (5.5–11.0)	7.1
HCA-BSI	77	18 (15–23)	17.0	86	19 (16–24)	17.3
HA-BSI	31	7 (5–11)	7.0	20	4.5 (2.8–7.0)	4.1
Infection focus						
Urinary tract	28	7 (4–10)	6.2	35	7.9 (5.5–11.0)	7.0
Lungs	47	11 (8–15)	10.4	28	6.3 (4.2–9.1)	5.7
Biliary tract	7	1.7 (0.7–3.5)	1.6	8	1.8 (0.78–3.6)	1.5
Gastrointestinal tract	11	2.6 (1.2–4.7)	2.4	5	1.1 (0.37–2.6)	1.0
Skin or soft tissue	16	3.8 (2.2–6.2)	3.5	18	4.1 (2.4–6.4)	3.7
Other	14	3.4 (1.8–5.6)	3.0	14	3.2 (1.7–5.3)	2.9
Unknown	35	8 (6–12)	7.8	33	7.5 (5.1–10.4)	6.8
Microbe group						
Gram-negative BSI	64	15 (12–20)	14.4	64	14 (11–18)	13.0
Gram-positive BSI	81	19 (15–24)	17.9	63	14 (11–18)	12.7
Polymicrobial or fungal BSI	13	3.1 (1.7–5.3)	2.8	14	3.2 (1.7–5.3)	2.8
Microbes (the four most common)[c]						
Escherichia coli	31	7 (5–11)	7.1	28	6.3 (4.2–9.1)	5.7
Streptococcus pneumoniae	22	5 (3–8)	4.8	9	2.1 (0.9–3.8)	1.9
Staphylococcus aureus	30	7 (5–10)	6.7	30	7 (5–10)	6.1
Klebsiella spp.	8	1.7 (0.7–3.5)	1.6	12	2.7 (1.4–4.7)	2.4

[a]Death within 30 days of BSI episodes per 100,000 person-years (417,682 person-years in 2002–2007; 442,948 person-years in 2008–2013)
[b]age and sex standardized to the population of Norway 2010
[c]mortality in BSI episodes with less common microbes is shown in Additional file 1: Table S7

Alternatively, the true mortality rate of BSI may have decreased, but the higher detection rate of BSIs may have led to more deaths being attributed to BSI, thus masking a true decline in mortality. In 2007, we updated our local recommendations on sepsis diagnosis and treatment, based on the guidelines of the international Surviving Sepsis Campaign [39], and we performed regular teaching sessions about sepsis for physicians and nurses and implemented standardized observation of patients with suspected sepsis at the wards. Earlier detection and treatment may have improved survival of BSI towards the end of the study period. An in-depth discussion of case fatality rate in the present BSI cohort is given elsewhere [18–22].

The high incidence and mortality of BSI in the older ages in our study, particularly in men, corresponds to what has also been reported by others [4, 6, 40, 41]. While the absolute number of BSIs decreased beyond 85 years, the population beyond this age is progressively smaller and the incidence continued to increase. As the proportion of older people will rise in our part of the world in the decades to come [12, 13], the challenges associated with BSI will escalate.

Compared to our results, HA-BSI accounted for a higher proportion of BSIs (15–58%) in most recent publications, whereas CA-BSI accounted for a lower proportion (18–44%) [1, 42]. An increasing rate of HCA-BSI with time, similar to our results, was also recently reported from Denmark [8], whereas another Danish study found no change with time in HCA-BSI, though reported decreasing incidence rates for both CA-BSI and HA-BSI [10]. In the present study as well as in a Finnish study [6], Gram-negative BSI was most frequent in females whereas Gram-positive BSI predominated in males. During the study period, Gram-negative BSIs increased whereas Gram-positive BSIs decreased. Similar trends were found in Australia [43]. In Finland, however, both Gram-positive and Gram-negative BSIs increased

Table 6 Age-adjusted time trends in bloodstream infection (BSI) mortality stratified by sex. BSI rate ratios per calendar year by Poisson regression are shown

	Females		Males	
	Mortality rate ratio (95% CI)	*p*-value	Mortality rate ratio (95% CI)	*p*-value
BSI, all	1.014 (0 .965–1.067)	0.58	0.974 (0.933–1.017)	0.23
Place of acquisition				
Community acquired	0.917 (0.838–1.002)	0.056	0.979 (0.8980–1.068)	0.64
Health care-associated	1.074 (0.999–1.154)	0.052	0.982 (0.928–1.039)	0.53
Hospital acquired	0.998 (0.882–1.129)	0.97	0.915 (0.825–1.015)	0.095
Infection focus				
Urinary tract	0.989 (0.893–1.095)	0.83	1.114 (1.001–1.239)	0.047
Lungs	1.018 (0.908–1.140)	0.76	0.910 (0.838–0.988)	0.024
Biliary tract	0.982 (0.740–1.303)	0.90	1.030 (0.867–1.226)	0.73
Gastrointestinal tract	0.978 (0.800–1.195)	0.83	0.891 (0.724–1.097)	0.28
Skin or soft tissue	1.007 (0.855–1.186)	0.93	0.994 (0.880–1.122)	0.92
Other	0.913 (0.782–1.065)	0.25	1.024 (0.879–1.193)	0.76
Unknown	1.086 (0.981–1.202)	0.11	0.922 (0.836–1.017)	0.10
Microbe group				
Gram-negative BSI	1.023 (0.953–1.098)	0.53	1.011 (0.941–1.085)	0.77
Gram-positive BSI	0.999 (0.921–1.084)	0.99	0.937 (0.884–0.993)	0.027
Polymicrobial or fungal BSI	0.952 (0.829–1.093)	0.49	1.072 (0.911–1.260)	0.40
Microbes (the four most common)				
Escherichia coli	1.014 (0.911–1.129)	0.79	0.957 (0.964–1.060)	0.40
Streptococcus pneumoniae	0.971 (0.812–1.161)	0.75	0.850 (0.745–0.971)	0.017
Staphylococcus aureus	0.985 (0.880–1.103)	0.80	0.972 (0.882–1.070)	0.56
Klebsiella spp.	1.169 (0.960–1.425)	0.12	1.107 (0.916–1.338)	0.29

during 2004–2007 [6]. In most other BSI studies as well as in the present study, [4, 8, 11, 38, 44] *E. coli*, *S. pneumoniae*, and *S. aureus* were the three most commonly occurring BSI microbes, accounting for more than one-half of all BSI episodes. The incidence rate of *E. coli* was higher in our study than in other studies (43 per 100,000 person-years reported both from Finland [6] and England [7]), which, however, also included children. One Danish study [10] found a decreasing incidence rate of *E. coli* (70 to 57 per 100,000 person-years). However, another Danish study [8] reported an increasing rate of *E. coli* and reported increased rates of BSI from the urinary tract, similar to the pattern observed by us.

A decreasing occurrence [11, 45–48] and mortality [47] of *S. pneumoniae* is reported in Norway and other countries after the introduction of pneumococcal conjugate vaccines. In Norway, the vaccine was implemented in the immunization program for children in 2006. However, a reduced occurrence of invasive pneumococcal infection is seen also among adults and even among elderly people, due to a herd effect [11, 47]. Furthermore, pneumococcal vaccine is recommended for people >65 years and for those who have undergone an invasive pneumococcal infection. Noteworthy, the decreased rate of *S. pneumoniae* BSI in our study was observed in males but not in females. A possible explanation of this sex difference may be differences in smoking habits, which is a risk factor for invasive pneumococcal disease [49]. The peak prevalence of smoking in Norwegian males occurred 20 years earlier than in females, whose peak prevalence cohort is now in the age group 65–79 years [50].

The incidence of *S. aureus* BSI in our study was similar to a recent study from an area of South-East Norway (27.6 per 100,000 person-years) [32]. The present study as well as others [6, 32] found a higher rate of *S. aureus* BSI in males than in females. The large proportion of HCA infections among the *S. aureus* BSIs was also described in a previous report from our catchment area [21]. *Klebsiella* spp. showed a higher and more increasing (10 to 18 per 100,000 person-years) incidence rate in our study than was found in a Canadian study (7 per 100,000 during 2000–2007) [51]. A nationwide study of invasive *Pseudomonas aeruginosa* infection in Norway 1999–2002 found an incidence rate of 3.2 per 100,000 person-years [31], which was lower than was found in the present study. The incidence rate of

candida BSI in our study was slightly lower than what was found in a nationwide study from Norway 2004–2012 (3.9 per 100,000 person-years) [30].

The incidence rate of MRSA in our study was similar to what was found in Copenhagen, Finland, and Western Sweden 2005–08 (<1 per 100,000 person-years) [36], and much lower than what was found in Canada and Australia 2005–08 (7.4 and 4.9 per 100,000 person-years) [36] and in the US and the UK 2006–08 (22 and 3.5 per 100,000 person-years) [52]. Even though ESBL-E has been a rapidly increasing challenge the last 15 years [53, 54], population-based incidence rates for ESBL-E are, so far, not commonly reported. Two Canadian studies found substantially higher and more increasing rates of community onset infections with ESBL-producing microbes (5.5 per 100,000 person-years in 2000–2002 [55], 10.6 per 100,000 person-years some years later [53]) than in the present study.

Conclusions

Overall, both the incidence and the mortality rates of BSI increased significantly by age, particularly in males. As the proportion of older people increases, geriatric BSIs will be an escalating challenge. The rate of BSI episodes increased through the study period, but the mortality rate was mainly unchanged, and the case fatality rate decreased. A more than twofold increase in the rate of BSI sampling may have contributed to the detection of milder and ultimately less fatal episodes and a shift towards higher proportions of female sex, Gram-negative etiology, and urinary tract site, but earlier detection and improved treatment may have had impact. In pneumococcal BSI, the incidence as well as the mortality decreased in males but not in females. Pneumococcal vaccine probably has contributed, and the difference between sexes is possibly due to sex-specific changes in smoking habits. We observed very low yet slightly increasing rates of microbes with acquired resistance.

Additional file

Additional file 1: Table S1. Incidence of bloodstream infection in 5-year age groups stratified by sex. **Table S2.** Incidence and mortality of hospital acquired bloodstream infection. **Table S3.** Incidence of bloodstream infection and blood culture sampling by calendar year. **Table S4.** Incidence and mortality of hospital acquired bloodstream infection in 2002–2007 compared to 2008–2013. **Table S5.** Less common bloodstream infection microbes in two time periods in an area of Mid-Norway. **Table S6.** Mortality of bloodstream infection in 5-year age groups stratified by sex. **Table S7.** Mortality of bloodstream infection with less common microbes. **Table S8.** Case fatality rate of bloodstream infection in two time periods. **Table S9.** Rate of blood culture sampling in 2002–2007 compared to 2008–2013. (DOCX 60 kb)

Abbreviations

ADR: Acquired drug resistance; BSI: Bloodstream infection; CA: Community acquired; ESBL-E: Extended-spectrum beta-lactamase producing *Enterobacteriaceae*; HA: Hospital acquired; HCA: Healthcare-associated; MRSA: Methicillin-resistant *Staphylococcus aureus*; PNSP: Penicillin-non-susceptible pneumococci

Acknowledgments

We would like to thank our research nurses for diligent and accurate work in the data collection process. We would also like to thank the staff at the Microbiology Laboratory, Levanger Hospital, for consecutively including cases and sending registration forms to physicians treating the patients at the wards.

Funding

This work is supported by the Unit for Applied Clinical Research, Norwegian University of Science and Technology; the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU); St Olav's University Hospital; the Norwegian Surveillance Programme for Antimicrobial Resistance; and by Nord-Trøndelag Hospital Trust's Fund for Research and Improvement.

Availability of data and material

The data that support the findings of this study are available from Nord-Trøndelag Hospital Trust but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Nord-Trøndelag Hospital Trust.

Authors' contributions

AM conceived the study and participated in design, data collection, statistical analysis, interpretation of the data, and drafting of the manuscript. BOÅ participated in design, statistical analysis, data interpretation, and drafting of the manuscript. SL participated in design, statistical analysis, data interpretation, and drafting of the manuscript. JP participated in design, data collection and drafting of the manuscript. ES contributed to design, interpretation of the data, and drafting of the manuscript. JKD participated in study design, data interpretation and drafting of the manuscript. SH participated in design, data interpretation, and drafting of the manuscript. THE participated in design, data collection, statistical analysis, and drafting of the manuscript. All the authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the Regional Committee for Medical and Health Research Ethics, Health Region IV, Norway. The Ethics Committee waived the need for informed consent, as this was an observational study, the treatment of the patients was standard, and no samples were taken for the purposes of the research.

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Received: 7 September 2016 Accepted: 24 February 2017
Published online: 11 March 2017

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Additional file 1

Burden of bloodstream infection in an area of Mid-Norway 2002-2013.
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Table S1 Incidence of bloodstream infection (BSI) in 5-year age groups stratified by sex. Number of BSI episodes per 100,000 person-years is shown

	Males		Females	
		BSI rate		BSI rate
	n	n/100,000 person-years (95% CI)	n	n/100,000 person-years (95% CI)
Age group (years)				
16-19	11	35 (18-63)	7	23 (9-48)
20-24	7	20 (8-42)	8	24 (11-48)
25-29	11	38 (19-68)	20	72 (44-111)
30-34	17	53 (31-85)	12	38 (20-67)
35-39	15	40 (23-66)	25	69 (44-101)
40-44	19	48 (29-75)	20	53 (33-82)
50-54	45	120 (88-161)	40	112 (80-115)
55-59	50	136 (101-180)	63	176 (136-226)
60-64	93	288 (232-353)	62	196 (151-252)
65-69	95	387 (313-473)	86	342 (274-423)
70-74	124	664 (552-792)	94	463 (374-566)
75-79	167	1085 (926-1262)	125	670 (558-798)
80-84	173	1521 (1303-1766)	174	1063 (911-1233)
85-89	133	2225 (1863-2637)	130	1189 (993-1411)
90-94	40	2109 (1506-2871)	56	1220 (921-1584)
95-99	8	2827 (1220-5570)	12	1037 (536-1812)

Table S2 Incidence and mortality of hospital acquired bloodstream infection (HA-BSI). HA-BSI episodes and deaths within 30 days of episodes per 100,000 hospital bed-days by calendar year and overall are shown

Calendar year	Hospital bed-days	HA-BSI episodes	HA-BSI episodes per 100 000 hospital bed-days	Deaths within 30 days	Mortality per 100 000 hospital bed-days
2002	59,906	24	40.1 (25.7-59.6)	4	6.7 (1.8-17.1)
2003	62,114	15	24.1 (13.5-39.8)	4	6.4 (1.8-16.5)
2004	63,810	26	40.7 (26.6-59.7)	2	3.1 (0.38-11.3)
2005	61,837	25	40.4 (26.2-59.7)	7	11.3 (4.6-23.3)
2006	61,874	21	33.9 (21.1-51.9)	6	9.7 (3.6-21.1)
2007	60,577	23	38.0 (24.1-57.0)	8	13.2 (5.7-26.0)
2008	62,222	15	24.1 (13.5-39.8)	1	1.6 (0.41-9.0)
2009	56,655	22	38.8 (24.3-58.8)	4	7.1 (1.9-18.1)
2010	56,601	25	44.2 (28.6-65.2)	6	10.6 (3.9-23.1)
2011	58,642	27	46.0 (30.3-67.0)	4	6.8 (1.9-17.4)
2012	59,113	22	37.2 (23.3-56.4)	3	5.1 (1.1-14.8)
2013	58,731	29	49.4 (33.1-70.9)	2	3.4 (0.41-12.3)
Total	722,082	274	37.9 (33.6-42.7)	51	7.1 (5.3-9.3)

Table S3 Incidence of bloodstream infection (BSI) and blood culture sampling by calendar year. The observed incidence of BSI episodes, the rate of blood culture sampling, and the number of BSI episodes per 100 blood culture sets are shown.

Calendar year	Population*	BSI episodes	BSI episodes per 100,000 person-years	Blood culture sets	Blood culture sets per 100,000 person-years	BSI episodes per 100 blood culture sets
2002	68,491	130	190 (159-225)	1499	2189 (2079-2302)	8.7 (7.2-10.3)
2003	68,746	131	191 (159-226)	1803	2623 (2503-2747)	7.3 (6.1-8.6)
2004	69,236	163	235 (201-274)	2075	2997 (2869-3129)	7.9 (6.7-9.2)
2005	69,883	158	226 (192-264)	2340	3348 (3214-3487)	6.8 (5.7-7.9)
2006	70,363	163	232 (197-270)	2539	3608 (3469-3752)	6.4 (5.5-7.5)
2007	70,963	176	248 (213-287)	2535	3572 (3435-3714)	6.9 (6.0-8.0)
2008	71,612	143	200 (168-235)	2499	3490 (3354-3629)	5.7 (4.8-6.7)
2009	73,357	156	213 (181-249)	2195	2992 (2868-3120)	7.1 (6.0-8.3)
2010	73,225	180	246 (211-284)	2782	3799 (3659-3943)	6.5 (5.6-7.5)
2011	73,976	206	278 (242-319)	3348	4526 (4374-4682)	6.2 (5.3-7.1)
2012	74,920	194	259 (224-298)	3299	4403 (4254-4556)	5.9 (5.1-6.8)
2013	75,858	195	257 (222-296)	3493	4605 (4453-4760159)	5.6 (4.8-6.4)

*≥16 years

Table S4 Incidence and mortality of hospital acquired bloodstream infection (HA-BSI) in 2002-2007 compared to 2008-2013 (the denominator is hospital bed-days)

	2002-2007	2008-2013
HA-BSI episodes	134	140
Hospital bed-days	370,118	351,964
HA-BSI episodes per 100,000 hospital bed-days	36.2 (30.3-42.9)	39.8 (33.5-46.9)
Deaths within 30 days in HA-BSI episodes	31	20
HA-BSI mortality per 100,000 hospital bed-days	8.4 (5.7-11.9)	5.7 (3.5-8.8)

Table S5 Less common bloodstream infection (BSI) microbes in two time periods in an area of Mid-Norway. Numbers of episodes and observed and standardized incidence rates of microbes not listed in Table 2 are shown. Microbes with inherent or acquired resistance are included

	2002-2007			2008-2013		
Other BSI microbes#	n	Observed incidence rate* (95% CI)	Age and sex standardized incidence rate§	n	Observed incidence rate* (95% CI)	Age and sex standardized incidence rate§
Other *Enterobacteriaceae*	57	13.7 (10.3-17.7)	13	64	14.4 (11.1-18.4)	14
Beta-hemolytic streptococci						
Group A	21	5.0 (3.1-7.7)	4.7	15	3.4 (1.8-5.6)	3.1
Group B	18	4.3 (2.6-6.8)	4.0	18	4.1 (2.4-6.4)	3.8
Group C or G	16	3.8 (2.2-6.2)	3.5	16	3.6 (2.1-5.9)	3.1
Enterococcus spp.	38	9.1 (6.4-12.5)	8.7	51	11.5 (8.6-15.1)	10.6
E. faecalis	31	7.4 (5.0-10.5)	7.1	45	10.2 (7.4-13.6)	9.3
E. faecium	3	0.7 (0.15-3.1)	0.6	5	1.1 (0.4-2.6)	1.1
Anaerobic bacteria	25	6.0 (3.9-8.8)	5.3	31	7.0 (4.8-9.9)	6.5
Coagulase-negative staphylococci	28	6.7 (4.5-9.7)	6.3	26	5.9 (3.8-8.6)	5.3
Haemophilus influenzae	11	2.6 (1.3-4.7)	2.4	6	1.4 (0.5-3.0)	1.3
Neisseria meningitidis	6	1.4 (0.5-3.1)	1.5	3	0.7 (1-2.0)	0.7
Microbes with inherent resistance						
Pseudomonas aeruginosa	33	7.9 (5.4-11.1)	6.7	29	6.5 (4.4-9.4)	5.8
Acinetobacter spp.	2	0.5 (0.06-1.7)	0.5	5	1.1 (0.4-2.6)	1.0
Stenotrophomonas maltophilia	0	0	0	2	0.5 (0.1-1.6)	0.4
Elisabethkingia meningo-septica	0	0	0	1	0.2 (0.06-1.2)	0.2
Microbes with acquired resistance						
MRSA	0	0	0	1	0.2 (0.06-1.2)	0.2
ESBL-E	3	0.7 (0.15-2.1)	0.6	7	1.6 (0.6-3.3)	1.4

PNSP	0	0	0	2	0.5 (0.1-1.6)	0.4
Fungi						
Candida spp.	6	1.4 (0.5-3.1)	1.4	11	2.5 (1.2-4.4)	2.3

#microbes not noted in Table 2
*BSI episodes per 100,000 person-years (417,682 person-years in 2002-2007; 442,948 person-years in 2008-2013)
§age and sex standardized to the population of Norway 2010
ESBL-E, extended spectrum beta-lactamase producing *Enterobacteriaceae;* MRSA, methicillin-resistant *Staphylococcus aureus*; PNSP, penicillin non-susceptible pneumococci

Table S6 Mortality of bloodstream infection (BSI) in 5-year age groups stratified by sex. The table shows the number of deaths within 30 days of BSI episodes per 100,000 person-years

	Males		**Females**	
Age group	n	BSI mortality rate n/100,000 person-years (95% CI)	n	BSI mortality rate n/100,000 person-years (95% CI)
16-19	0	0	1	3.3 (0.08-18.6)
20-24	0	0	0	0
25-29	1	3.4 (0.09-19.2)	0	0
30-34	0	0	0	0
35-39	0	0	0	0
40-44	1	2.5 (0.06-14.2)	0	0
45-49	1	2.6 (0.07-14.6)	1	2.7 (0.07-14.8)
50-54	7	18.7 (7.5-38.6)	3	8.4 (1.7-24.4)
55-59	7	19.0 (7.6-39.1)	6	16.8 (6.2-36.5)
60-64	12	37.1 (19.2-64.9)	4	12.7 (3.5-32.4)
65-69	18	73.3 (43.4-115.8)	8	31.8 (13.7-62.7)
70-74	20	107.1 (65.4-165.4)	10	49.2 (23.6-90.5)
75-79	27	175.3 (115.6-255.2)	27	144.7 (95.4-210.6)
80-84	32	281.4 (192.5-397.2)	28	171.0 (113.6-247.1)
85-89	28	468.4 (311.2-677.0)	22	201.1 (126.1-304.5)
90-94	13	685.3 (365.9-1171.9)	12	261.4 (135.1-456.6)
95-99	5	1766.8 (573.7-412.3)	5	432.2 (140.3-1008.5)

Table S7 Mortality of bloodstream infection (BSI) with less common microbes. Number of deaths and observed and standardized mortality rates in 2002-2007 and 2008-2013 are shown

	2002-2007			2008-2013		
Other BSI microbes#	n	Observed mortality rate* (95% CI)	Age and sex standardized mortality rate§	n	Observed mortality rate* (95% CI)	Age and sex standardized mortality rate§
Other *Enterobacteriaceae*	10	2.4 (1.2-4.4)	2.1	7	1.6 (0.6-3.3)	1.4
Beta-hemolytic streptococci	9	2.2 (1.0-4.1)	2.0	8	1.8 (0.8-3.6)	1.6
Enterococcus spp.	5	1.2 (0.4-2.8)	1.2	5	1.1 (0.4-2.6)	1.0
Anaerobic bacteria	5	1.2 (0.4-2.8)	1.1	3	0.7 (0.1-2.0)	0.6
Pseudomonas spp.	6	1.4 (0.5-3.1)	1.3	5	1.1 (0.4-2.6)	1.0
Coagulase-negative staphylococci	4	1.0 (0.3-2.5)	0.8	4	0.9 (0.25-2.3)	0.8
Haemophilus influenzae	3	0.7 (0.1-2.1)	0.7	1	0.2 (0.006-1.3)	0.2
Neisseria meningitidis	2	0.5 (0.06-1.7)	0.5	0	0	0
Fungi						
Candida spp.	3	0.7 (0.1-2.1)	0.7	6	1.4 (0.5-3.0)	1.2

#mortality in BSI episodes with the four most common microbes is shown in Table 4
*Death within 30 days of BSI episodes per 100,000 person-years (417,682 person-years in 2002-2007; 442,948 person-years in 2008-2013)
§age and sex standardized to the population of Norway 2010

Table S8 Case fatality rate (CFR) of bloodstream infection (BSI) in two time periods. Number of deaths and observed CFR, overall and in subgroups, are shown

	Total		2002-2007		2008-2013		p-value
	No.	CFR (95% CI)	No.	CFR (95% CI)	No.	CFR (95% CI)	
All BSI episodes	299	15.0 (13.5-16.6)	158	17.2 (14.9-19.7)	141	13.1 (11.2-15.3)	0.014
Place of acquisition							
CA-BSI	85	9.1 (7.4-11.1)	50	10.6 (8.1-13.7)	35	7.6 (5.5-10.3)	0.11
HCA-BSI	163	20.7 (18.0-23.7)	77	24.4 (20.0-29.4)	86	18.3 (15.0-22.0)	0.040
HA-BSI	51	18.6 (14.4-23.6)	31	23.1 (16.8-31.0)	20	14.3 (9.4-21.0)	0.064
Infection focus							
Urinary tract	63	8.3 (6.6-10.6)	28	8.9 (6.2-12.5)	35	8.0 (5.8-11.0)	0.69
Lungs	75	22.7 (18.5-27.5)	47	27.8 (21.6-35.0)	28	17.3 (12.2-23.8)	0.026
Biliary tract	15	6.8 (4.2-10.9)	7	6.6 (3.2-13.0)	8	7.0 (3.6-13.2)	>0.99
Gastrointestinal tract	16	15.8 (10.0-24.2)	11	23.4 (13.6-37.2)	5	9.3 (4.0-19.9)	0.061
Skin or soft tissue	34	23.8 (17.5-31.4)	16	19.8 (12.5-29.7)	18	29.0 (19.2-41.3)	0.24
Other	28	11.3 (8.0-15.9)	14	13.0 (7.9-20.6)	14	10.1 (6.1-16.2)	0.55

Unknown	68	33.8 (27.6-40.6)	35	37.2 (28.1-47.3)	33	30.8 (22.9-40.1)	0.327
Microbe group							
Gram-negative BSI	128	11.3 (9.6-13.3)	64	12.8 (10.2-16.0)	64	10.1 (8.0-12.7)	0.16
Gram-positive BSI	144	18.5 (16.0-21.4)	81	21.0 (17.2-25.3)	63	16.1 (12.8-20.1)	0.096
Polymicrobial or fungal BSI	27	31.8 (22.8-42.3)	13	36.1 (22.4-52.4)	14	28.6 (17.8-42.4)	0.49
Microbes							
Escherichia coli	59	8.6 (6.7-10.9)	31	10.2 (7.3-14.2)	28	7.3 (5.1-10.4)	0.22
Streptococcus pneumoniae	31	13.7 (9.8-18.8)	22	17.3 (11.7-24.8)	9	9.1 (4.9-16.4)	0.082
Staphylococcus aureus	60	27.5 (22.0-33.8)	30	30.0 (21.9-39.6)	30	25.4 (18.4-34.0)	0.53
Klebsiella spp.	20	14.8 (9.8-21.8)	8	17.0 (8.9-30.1)	12	13.6 (8.0-22.3)	0.62
Other *Entero-bacteriaceae*	16	13.2 (8.3-20.4)	9	15.8 (8.5-27.4	7	10.9 (5.4-20.9)	0.59
Beta-hemolytic streptococci	17	16.3 (10.5-24.6)	9	16.4 (8.9-28.3)	8	16.3 (8.5-29.0)	>0.99
Enterococcus spp.	10	11.2 (6.2-19.5)	5	13.2 (5.8-27.3)	5	9.8 (4.3-21.0)	0.74
Anaerobic bacteria	8	14.3 (7.4-25.7)	5	20.0 (8.9-39.1)	3	9.7 (3.3-24.9)	0.45
Pseudomonas spp.	11	19.0 (10.9-30.9)	6	20.0 (9.5-37.3)	5	17.9 (7.9-35.6)	>0.99
Coagulase-negative staphylococci	8	14.8 (7.7-26.6)	4	14.3 (5.7-31.5)	4	15.4 (6.1-33.5)	>0.99

Table S9 Rate of blood culture sampling in 2002-2007 compared to 2008-2013

	2002-2007	2008-2013
Person-years	417,682	442,948
Blood culture sets	12,791	17,616
Blood culture sets per 100,000 person-years	3062	3977
BSI episodes	921	1074
BSI episodes per 100 blood culture sets	7.2 (6.7-7.7)	6.1 (5.7-6.5)

Paper II

Mehl et al. BMC Infectious Diseases (2017) 17:116
DOI 10.1186/s12879-017-2210-6

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Trends in antimicrobial resistance and empiric antibiotic therapy of bloodstream infections at a general hospital in Mid-Norway: a prospective observational study

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Abstract

Background: The occurrence of bloodstream infection (BSI) and antimicrobial resistance have been increasing in many countries. We studied trends in antimicrobial resistance and empiric antibiotic therapy at a medium-sized general hospital in Mid-Norway.

Methods: Between 2002 and 2013, 1995 prospectively recorded episodes of BSI in 1719 patients aged 16–99 years were included. We analyzed the antimicrobial non-susceptibility according to place of acquisition, site of infection, microbe group, and time period.

Results: There were 934 community-acquired (CA), 787 health care-associated (HCA) and 274 hospital-acquired (HA) BSIs. The urinary tract was the most common site of infection. *Escherichia coli* was the most frequently isolated infective agent in all three places of acquisition. Second in frequency was *Streptococcus pneumoniae* in CA and *Staphylococcus aureus* in both HCA and HA. Of the BSI microbes, 3.5% were non-susceptible to the antimicrobial regimen recommended by the National Professional Guidelines for Use of Antibiotics in Hospitals, consisting of penicillin, gentamicin, and metronidazole (PGM). In contrast, 17.8% of the BSI microbes were non-susceptible to cefotaxime and 27.8% were non-susceptible to ceftazidime.
Antimicrobial non-susceptibility differed by place of acquisition. For the PGM regimen, the proportions of non-susceptibility were 1.4% in CA, 4.8% in HCA, and 6.9% in HA-BSI ($p < 0.001$), and increasing proportions of non-susceptibility over time were observed in HA-BSI, 2.2% in 2002–2005, 6.2% in 2006–2009, and 11.7% in 2010–2013 ($p = 0.026$), mainly caused by inherently resistant microbes. We also observed increasing numbers of bacteria with acquired resistance, particularly *E. coli* producing ESBL or possessing gentamicin resistance, and these occurred predominantly in CA- and HCA-BSI.

Conclusions: Generally, antimicrobial resistance was a far smaller problem in our BSI cohort than is reported from countries outside Scandinavia. In our cohort, appropriate empiric antibiotic therapy could be achieved to a larger extent by replacing second- and third-generation cephalosporins with penicillin-gentamicin or piperacillin-tazobactam.

Keywords: Antibiotic therapy, Antimicrobial resistance, Bacteremia, Bacteraemia, Bloodstream infection, Empiric antibiotic treatment, Non-susceptibility, Sepsis

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Background

Bloodstream infection (BSI) contributes substantially to morbidity and mortality worldwide [1]. In Europe, the annual number of BSI episodes and deaths associated with BSI has been estimated to 1.2 million and 157,000, respectively [1]. Early diagnosis and early appropriate treatment is crucial. In severe sepsis, the case fatality increases for each hour the antibiotic treatment is delayed [2, 3]. Therefore, empirical antibiotic treatment has to be initiated before the results of blood cultures are available. However, as infections with resistant microbes is an escalating problem worldwide [4–6], it is increasingly challenging to maintain appropriate antibiotic regimens for initial empiric therapy. Resistant pathogenic bacteria are found less frequently in Norway and other Nordic countries, compared to the rest of Europe and other world regions [7, 8]. This probably reflects a relatively restrictive use of antimicrobial agents. In Norway, a regimen containing penicillin and gentamicin (PG), plus metronidazole (PGM) if an anaerobic infection is suspected, has been recommended for more than thirty years in sepsis with unknown focus and etiology [9–11]. In recent years, however, increasing numbers of infections with methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E), and vancomycin resistant enterococci have been detected [8]. Selection of inherently resistant microbes due to antibiotic use is also a challenge. Updated knowledge about the distribution of microbes in serious infections and their resistance against antimicrobial agents is needed to ensure appropriate empiric antimicrobial treatment regimens. It is also important to identify subgroups in which tailored regimens are required. Important differences in antibiotic resistance have been found with regard to place of acquisition [12, 13], and therefore, resistance statistics should specify results for community acquired (CA), health care-associated (HCA), and hospital acquired (HA) infections.

We conducted a prospective study to assess the occurrence and distribution of BSI microbes and their non-susceptibility to some common antibiotic regimens for initial empiric antimicrobial treatment of sepsis of unknown etiology. Particularly, we assessed microbes and antimicrobial resistance by place of acquisition (CA, HCA and HA-BSIs) and with regard to time trends over a 12-year period. We also studied the antibiotic regimens that were used for initial empiric treatment during the same time period and the degree to which they were appropriate.

Methods

Levanger Hospital serves a population of about 90,000 as an emergency facility in a defined geographical area of Mid-Norway. Since 1994, all positive blood cultures at the hospital have been prospectively recorded for surveillance purposes, and clinical information has been recorded, in the following way: whenever a positive blood culture was reported, a physician at the clinical ward filled out a registration form. A team of three research nurses, two subordinate doctors, and the main investigator reviewed all patients' records to verify the data and record additional variables. The present study includes BSIs that occurred between January 1, 2002 and December 31, 2013 in patients who were ≥16 years of age, and it is part of the Mid-Norway Sepsis Study.

The microbiology laboratory at Levanger Hospital is ISO 15189 accredited and participates in the national quality assurance schemes (ring tests). Blood cultures were performed in BACTEC 9240 Vacutainer Culture Bottles (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) [14], which in 2010 was replaced by BACTEC FX. No obvious changes in blood culture techniques or indications for drawing blood cultures have been done during the study period, but an increased focus on early detection of sepsis may have influenced the rate of blood culture sampling. Over the study period, the number of blood culture sets per 1000 hospital bed-days increased from 25.0 in 2002 to 59.5 in 2013.

Isolates were identified using standard methods [15]. Antimicrobial susceptibility testing was performed by the disc diffusion method (Neo-Sensitabs, Rosco Diagnostica, Taastrup, Denmark). For measurement of MIC, E-test (AB Biodisk, Solna, Sweden) was used. The results of antibacterial susceptibility testing were interpreted according to the Norwegian Working Group on Antibiotics (NWGA). For the antibiotics included in this study, the Norwegian breakpoints correspond to EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints [8, 16]. In this study, microbes intermediately susceptible to antibiotics were classified as non-susceptible [17–19], as only susceptible microbes can be regarded as being able to be managed by means of the respective antibiotic regimens. Microbes not tested in the laboratory because of known inherent non-susceptibility (e.g. enterococci are inherently resistant to cephalosporins) were classified as non-susceptible (see Additional file 1: Appendix 1 On inherent (natural) resistance in microbes).

An episode of BSI was defined by growth of one or more microbes from blood culture combined with clinical evidence of systemic infection. A new BSI episode with the same microbe in the same patient was recorded if an interval of at least 30 days had passed without signs of infection since an earlier episode [20]. If more than one organism was isolated from one or more blood cultures within a 72-h period, the BSI episode was classified as polymicrobial. One positive blood culture for organisms usually regarded as etiological agents was the requirement for inclusion. For coagulase-negative staphylococci or other possible skin contaminants, at least two identical

isolates from separate venipunctures were required. Among alpha-hemolytic streptococci, *S. pneumoniae* and streptococci belonging to the *S. milleri* group were not considered as skin contaminants. Other alpha-hemolytic streptococci were included if they were found in two or more blood cultures from different venipuncture sites.

The place of acquisition was classified as hospital-acquired (HA), health care-associated (HCA) or community acquired (CA) [12, 21]. HA-BSI was diagnosed if the infection was detected >48 h after admission [22]. Patients who during the 30 days prior to hospital admission had (1) been hospitalized two or more days or (2) had received intravenous therapy or wound care at home or (3) hemodialysis or chemotherapy at hospital visits or (4) were nursing home residents, were categorized as having HCA-BSI. CA-BSI was diagnosed if the infection was detected <48 h after admission and none of the criteria for HCA-BSI were fulfilled.

A urinary focus was assigned when there was growth of the same microbe (s) from urine as well as from blood culture along with clinical signs/symptoms or risk factor for urinary infection, and no other source of infection was identified. A presumed pulmonary focus was diagnosed with clinical signs of lower respiratory infection accompanied by positive radiological findings. Focus in the biliary tract was ascertained based on clinical, biochemical and radiological findings. Signs of infection along with focal growth of the same microbe as in blood culture were

Table 1 Bloodstream infection (BSI) episodes in three time periods

	Total	2002–2005	2006–2009	2010–2013
All BSIs	1995 (100,0)	582 (100.0)	638 (100.0)	775 (100.0)
Place of acquisition				
Community acquired	934 (46.8)	317 (54.5)	280 (43.9)	337 (43.5)
Health care-associated	787 (39.4)	175 (30.1)	277 (43.4)	335 (43.2)
Hospital acquired	274 (13.7)	90 (15.5)	81 (12.7)	103 (13.3)
Microbial agent(s)				
Escherichia coli	686 (34.4)	186 (32.0)	231 (36.2)	269 (34.7)
Streptococcus pneumoniae	225 (11.3)	80 (13.8)	80 (12.5)	65 (8.4)
Staphylococcus aureus	218 (10.9)	72 (12.4)	55 (8.6)	91 (11.7)
Klebsiella spp.	135 (6.8)	22 (3.8)	44 (6.9)	69 (8.9)
Beta-hemolytic streptococci	104 (5.2)	33 (5.7)	35 (5.5)	36 (4.6)
Enterococcus spp.	89 (4.5)	28 (4.8)	26 (4.1)	35 (4.5)
Other mixed bacterial infections	68 (3.4)	21 (3.6)	19 (3.0)	28 (3.6)
Pseudomonas spp.	58 (2.9)	20 (3.4)	21 (3.3)	17 (2.2)
Viridans group streptococci	57 (2.9)	15 (2.6)	19 (3.0)	23 (3.0)
Coagulase-negative staphylococci	54 (2.7)	23 (4.0)	11 (1.7)	20 (2.6)
Proteus spp.	48 (2.4)	17 (2.9)	15 (2.3)	16 (2.1)
Anaerobic Gram-negative bacteria	45 (2.3)	14 (2.4)	16 (2.5)	15 (1.9)
Mixed Gram-negative aerobic or anaerobic bacteria	42 (2.1)	13 (2.2)	11 (1.7)	18 (2.3)
Enterobacter spp.	37 (1.9)	8 (1.4)	14 (2.2)	15 (1.9)
Other *Enterobacteriaceae*	37 (1.9)	7 (1.2)	12 (1.9)	18 (2.3)
Other aerobic Gram-negative bacteria	19 (1.0)	5 (0.9)	4 (0.6)	10 (1.3)
Haemophilus influenzae	17 (0.9)	6 (1.0)	8 (1.3)	3 (0.4)
Candida spp.	14 (0.7)	1 (0.2)	4 (0.6)	9 (1.3)
Anaerobic Gram-positive bacteria	11 (0.6)	3 (0.5)	4 (0.6)	4 (0.5)
Mixed gram-positive aerobic or anaerobic bacteria	11 (0.6)	1 (0.2)	4 (0.6)	6 (0.8)
Neisseria meningitidis	9 (0.5)	4 (0.7)	2 (0.3)	3 (0.4)
Listeria monocytogenes	8 (0.4)	2 (0.3)	2 (0.3)	4 (0.5)
Mixed bacterial and fungal infections	3 (0.2)	1 (0.2)	1 (0.2)	1 (0.1)

The table shows number (percent) of BSIs overall, by place of acquisition, and by microbial agent(s)

taken as a confirmation of infection in abdomen, skin, soft tissue or other sites. An unknown focus of infection was assigned when none of the criteria for ascertaining a focus were met.

Appropriate empiric antibiotic therapy (AEAT) was defined as correctly dosed intravenous antibiotic therapy with a regimen that was active in vitro against the microbe(s) isolated from blood culture (s). We assessed AEAT within 6 h and within 24 h of the time that the blood culture specimen was obtained.

Statistical analyses

Proportions of non-susceptibility across place of acquisition categories and time periods were assessed by a two-sided chi-square test. Trends in proportions were analyzed using Cochran-Armitage test. Two-sided p-values <0.05 were considered significant. Confidence intervals were calculated using Wilson's approximation to the binominal distribution [23]. The analyses were performed using SPSS 22, STATA 13, and StatXact 9.

Results

During the 12-year study period, a total of 1995 episodes of BSI occurred among 1719 individuals. CA-BSI episodes amounted to 46.8% of the total, HCA- and HA-BSI contributed 39.4% and 13.7%, respectively (Table 1). *Escherichia coli* was the predominating microbe (34.4%), followed by *Streptococcus pneumoniae* (11.3%) and *Staphylococcus aureus* (10.9%). The distribution of microbes by place of acquisition is shown in Fig. 1 and Additional file 1: Table S1. The distribution of microbes by infection site is shown in Additional file 1: Table S2. Totally, the number of BSIs increased across the three 4-year periods 2002–2005, 2006–2009, and 2010–2013 (Table 1). Most microbes contributed essentially similar proportions of the BSIs in each of the three time periods. However, the proportions of BSI from *Klebsiella* spp. (3.8% vs. 8.9%) and *Candida* spp. (0.3% vs. 1.3%) increased from the first to the third period. Conversely, the proportion of BSI from *Streptococcus pneumoniae* decreased from 13.8 to 8.4%.

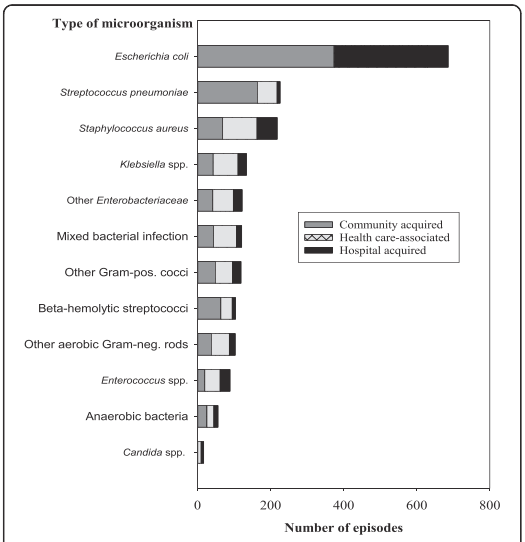

Fig. 1 Distribution of microbes from 1995 bloodstream infection episodes by place of acquisition. (Additional file 1: Table S1 shows microbial agents by place of acquisition in more detail)

In vitro susceptibility to antibiotics

Overall, 6.1% of the microbes were non-susceptible to a regimen consisting of penicillin and gentamicin (PG), and 3.5% were non-susceptible to a triple agent regimen including penicillin, gentamicin, and metronidazole (PGM) (Table 2). The proportions not susceptible to imipenem, piperacillin-tazobactam (PIP/TAZ), and cefotaxime, were 4.5%, 7.6%, and 17.8%, respectively. A broad-spectrum combination containing PIP/TAZ, gentamicin, and metronidazole had the lowest degree of non-susceptibility (2.6%), whereas ceftazidime had the highest (27.8%) (Table 2).

The non-susceptibility to PGM was higher in HA-BSI (6.9%) and HCA-BSI (4.8%) than in CA-BSI (1.4%) ($p < 0.001$). Similar differences across place of acquisition were seen for imipenem, PIP/TAZ, and cefotaxime (Fig. 2, Table 2, Additional file 1: Table S3). The proportions of microbes non-susceptible to PGM increased through the three time periods in HA-BSI (2.2%, 6.2%, and 11.7%; $p = 0.026$), but we observed no significant time trends in antibiotic susceptibility for other antibiotic regimens (Table 2). Proportions of non-susceptibility by microbe are shown in Table 3. Non-susceptibility by site of infection is shown in Additional file 1: Table S4.

Among seventy BSI episodes with microbes non-susceptible to PGM (Fig. 3; Additional file 1: Table S5), *Candida* spp. accounted for 17 episodes, *E. coli* 16, and *Enterococcus faecium* and *Staphylococcus epidermidis* for 9 episodes each. The great majority of episodes with *Candida* spp., *Enterococcus faecium* or *Staphylococcus epidermidis* occurred in HCA or HA-BSI, and the highest numbers were recorded in the third time period. Regarding *E. coli* not susceptible to PGM (which means not susceptible to gentamicin), seven episodes occurred in CA, eight in HCA, and one in HA-BSI. The number of *E. coli* isolates non-susceptible to gentamicin was one (0.5%) in 2002–2005, six (2.6%) in 2006–2009, and nine (3.3%) in 2010–2013 (Additional file 1: Table S6).

The proportions of *E. coli* producing extended-spectrum beta-lactamase (ESBL) were 0% in 2002–05,

Table 2 The proportion (%) of bloodstream infection (BSI) episodes with microbe(s) non-susceptible to some common antibiotic regimens

	Total	2002–2005[a]	2006–2009	2010–2013	p for trend
Penicillin-gentamicin					
Total	6.1	4.4	6.1	7.5	0.011
Community acquired	4.3	2.2	5.7	5.0	0.22
Health care-associated	6.7	7.4	5.8	7.2	0.16
Hospital acquired	10.2	4.4	8.6	16.5	0.023
p for trend	0.006	0.058	0.46	0.003	
Penicillin-gentamicin-metronidazole					
Total	3.5	1.9	3.0	5.2	<0.001
Community acquired	1.4	0.3	1.8	2.1	0.28
Health care-associated	4.8	4.6	3.2	6.3	0.045
Hospital acquired	6.9	2.2	6.2	11.7	0.026
p for trend	<0.001	0.007	0.15	0.001	
Piperacillin/tazobactam					
Total ($n = 1413$)	7.6		7.4	7.7	0.25
Community acquired ($n = 617$)	3.6		2.9	4.2	0.27
Health care-associated ($n = 612$)	8.8		9.4	8.4	0.31
Hospital acquired ($n = 184$)	8.8		9.4	17.0	0.31
p for trend	<0.001		<0.001	<0.001	
Piperacillin/tazobactam-gentamicin					
Total ($n = 1413$)	3.0		2.4	3.6	0.21
Community acquired ($n = 617$)	1.0		0.7	1.2	0.69
Health care-associated ($n = 612$)	3.3		3.2	3.3	0.98
Hospital acquired ($n = 184$)	9.2		4.9	12.6	0.12
p for trend	<0.001		0.037	<0.001	
Piperacillin/tazobactam-gentamicin-metronidazole					
Total ($n = 1413$)	2.6		1.7	3.4	0.065
Community acquired ($n = 617$)	0.5		0.0	0.9	0.26
Health care-associated ($n = 612$)	2.9		2.5	3.3	0.64
Hospital acquired ($n = 184$)	8.7		4.9	11.7	0.12
p for trend	<0.001		0.004	<0.001	
Imipenem					
Community acquired	1.1	1.6	0.7	0.9	0.59
Health care-associated	6.5	9.1	5.8	5.7	0.39
Hospital acquired	10.6	11.1	7.4	12.6	0.14
p for trend	<0.001	<0.001	0.001	<0.001	

Table 2 The proportion (%) of bloodstream infection (BSI) episodes with microbe(s) non-susceptible to some common antibiotic regimens *(Continued)*

Cefotaxime					
Total	17.8	17.5	18.7	17.3	0.68
Community acquired	10.8	11.0	11.4	10.1	0.69
Health care-associated	21.3	25.1	22.7	18.2	0.18
Hospital acquired	31.4	25.6	29.6	37.9	0.31
p for trend	<0.001	<0.001	<0.001	<0.001	
Ceftazidime					
Total	27.8	29.0	24.8	29.4	0.25
Community acquired	18.5	20.2	15.4	19.6	0.42
Health care-associated	31.3	36.0	28.2	31.3	0.37
Hospital acquired	49.6	46.7	45.7	55.3	0.46
p for trend	<0.001	<0.001	<0.001	<0.001	

[a]Piperacillin/tazobactam was not adopted in the first time period
The BSIs are stratified by place of acquisition and by time period (the total number in each cell is shown under the heading Place of acquisition in Table 1)

2.0% in 2006–09, and 1.7% in 2010–13 (Additional file 1: Table S7). Only one episode of methicillin-resistant *Staphylococcus aureus* (MRSA) was found, and only in two (0.9%) of 225 BSIs with *Streptococcus pneumoniae*, the microbe was non-susceptible to penicillin (Additional file 1: Table S3).

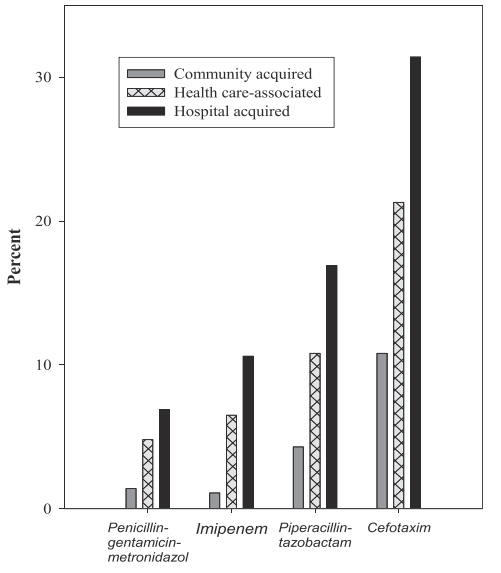

Fig. 2 Proportion of bloodstream infection microbes non-susceptible to four antibiotic regimens by place of acquisition

Initial empiric antibiotic therapy

The use of second- and third generation cephalosporins as initial empiric therapy decreased through the study periods. Cefuroxime was used as monotherapy or in combination in 19.6% and 5.3% of the BSI episodes in the first and in the third time period, respectively. The corresponding proportions for cefotaxime were 18.1% and 15.7% (Table 4). In contrast, the use of ampicillin or penicillin plus gentamicin increased from 14.1% to 19.9%. PIP/TAZ was not used in the first period, but was the second most used empiric therapy in the third period (17.3%). The proportions of patients who received appropriate empiric antibiotic therapy (AEAT) within 6 h and within 24 h were larger in the third period than in the first and second periods (Table 5). The proportions of patients who received AEAT within 6 h in the third period were 67.1% in CA, 67.2% in HCA, and 59.0% in HA-BSI. The corresponding proportions who received AEAT within 24 h were 88.4%, 84.4%, and 83.1%, respectively.

Discussion

In this prospective study of 1995 consecutive BSIs at a medium-sized Norwegian hospital between 2002 and 2013, antimicrobial resistance was a far smaller problem than reported in most studies [7, 17, 24, 25]. Except for third-generation cephalosporins, antimicrobial resistance to regimens recommended for sepsis of unknown etiology was low. In less than 4% of BSI episodes, microbes were non-susceptible to PGM, consistent with previous findings at our hospital [26] and in other Norwegian studies [10, 27]. However, the proportion of antibiotic non-susceptibility was higher in HA and HCA than in CA-BSI. For PGM, an increase in non-susceptibility

Table 3 Proportions (%) of different microbes or microbe groups non-susceptible to some commonly recommended antibiotics and antibiotic regimens

Microbe/microbe group	Antibiotics and antibiotic combinations (2002–2013)							P/T alone or in combinations (2006–2013)			
	PG	PGM	Imi-penem	Cefo-taxime	Cefta-zidime	Cipro-floxa-cin	Total N	P/T	P/T-G	P/T-G-M	Total N
Streptococcus pneumoniae§	0.9	0.9	0.0	0.4	0.4	NRT	226	0.7	0.7	0.7	146
Beta-hemolytic streptococci	0.0	0.0	0.0	0.0	100.0	NRT	104	0.0	0.0	0.0	71
Viridans streptococci	0.0	0.0	0.0	3.5	8.8	NRT	57	0.0	0.0	0.0	42
Staphylococcus aureus#	0.5	0.5	0.0	0.0	100.0 (IR)	NRT	218	0.0	0.0	0.0	146
Coagulase-negative staphylococci	13.0	13.0	38.9	38.9	100.0 (IR)	NRT	54	41.9	19.4	19.4	31
Enterococcus spp.	9.0	9.0	11.2	100.0 (IR)	100.0 (IR)	NRT	89	11.5	9.8	9.8	61
Listeria monocytogenes	0.0	0.0	0.0	100.0 (IR)	100.0 (IR)	NRT	8	0.0	0.0	0.0	6
Escherichia coli	1.9	1.9	0.1	1.9	1.9	3.3	686	6.0	0.8	0.8	500
Klebsiella spp. or *Proteus* spp.	0.0	0.0	5.5	2.2	2.2	4.7	182	7.0	0.0	0.0	143
Other enterobacteria	1.4	1.4	9.5	20.3	21.6	2.9	74	16.9	100.0	100.0	59
Pseudomonas spp.	1.7	1.7	6.9	100.0 (IR)	3.4	8.2	58	5.3	2.6	2.6	38
Other aerobic Gram-negatives	8.9	8.9	8.9	31.1	28.9	6.7	45	23.3	0.0	0.0	30
Anaerobic bacteria	66.1	3.6	0.0	96.4	96.4	NRT	56	5.1	7.7	0.0	39
Mixed bacterial infection	24.8	11.6	13.2	48.8	50.4	NRT	121	11.6	8.1	4.7	86
Candida spp. single or in mixed infection	100.0 (IR)	100.0 (IR)	100.0 (IR)	100.0 (IR)	100.0 (IR)	100.0 (IR)	17	100.0 (IR)	100.0 (IR)	100.0 (IR)	15
Total	6.1	3.5	4.5	17.8	27.8	NRT	1995	7.6	3.0	2.6	1413

§two isolates of penicillin-non-susceptible pneumococci were detected
#one single isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) and one single isolate of gentamicin-resistant *S. aureus* were detected
IR, inherently resistant (see Additional file 1: Appendix 1 On inherent (natural) resistance in microbes); NRT, not routinely tested; PG, penicillin-gentamicin; PGM, penicillin-gentamicin-metronidazole; P/T, piperacillin-tazobactam; P/T-G, piperacillin-tazobactam plus gentamicin; P/T-G-M, piperacillin-tazobactam plus gentamicin plus metronidazole
Piperacillin-tazobactam (P/T) was adopted in 2006

through the study period was observed in HA-BSI, mainly caused by inherently resistant microbes. A slightly increasing number of bacteria with acquired resistance was also detected, particularly *E. coli* producing ESBL or possessing gentamicin resistance. In our cohort, appropriate empiric antibiotic therapy could be achieved to a larger extent by replacing cephalosporins with PG or PIP/TAZ.

Strengths of this study include the prospective registration of BSIs within a well-defined area, and the handling of blood cultures at one microbiology laboratory. We present susceptibility data by microbe, by place of acquisition, and by site of infection. Most authors have presented susceptibility data only by microbe, which is generally unknown at the time the physician has to decide on the initial antimicrobial therapy. We included microbes with known inherent resistance in the presentation in order to give guidance for empirical treatment before the microbial etiology is known. Our use of one single institution as the study site limits the generalizability of our results, but regarding antibiotic resistance patterns as basis for treatment guidelines, our results may be relevant for other general hospitals in Scandinavia.

The low proportions of non-susceptibility in our BSI microbes are likely explained by a relatively strict use of antibiotics in Norway [8, 28, 29]. All antibiotics used in humans are prescribed by physicians, and penicillins and aminoglycosides are the preferred drugs in severe bacterial infections. The increasing non-susceptibility in BSI microbes by place of acquisition and, for HA-BSI, by time period, was mainly due to a shift towards microbes with natural (inherent) resistance, particularly *Candida* spp., *Enterococcus faecium*, and *Staphylococcus epidermidis*. We attribute this shift to the increasing use of chemotherapy and other immunosuppressive treatments (Additional file 1, Table S9), which results in more prevalent infections and antibiotic treatments, giving rise to selection of

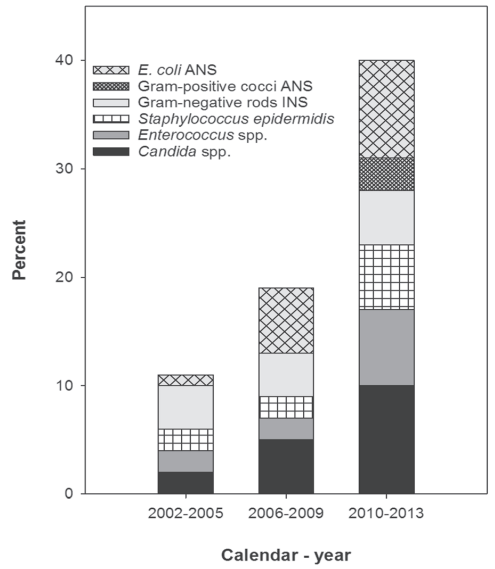

Fig. 3 Microbes non-susceptible to penicillin-gentamicin-metronidazole (PGM) through three time periods. (Additional file 1: Table S5 shows the PGM non-susceptible microbes in detail) ANS, acquired non-susceptibility; INS, inherent non-susceptibilty

resistant microbes. A higher proportion of non-susceptible microbes in HA and HCA than in CA-BSI is well known from other studies [12, 13], but this cautionary knowledge does not seem to have been sufficiently heeded by clinicians and guideline makers. The national Norwegian surveillance data on the distribution of different microbes isolated from blood cultures show no time trend towards increasing occurrence of natural resistant microbes from 2004 to 2014

Table 4 The most commonly used initial empiric antibiotic regimens (percent) through three time periods

Initial antibiotic regimen	2002–2005 ($n = 582$)	2006–2009 ($n = 638$)	2010–2013 ($n = 775$)
Cefotaxime	18.1	18.2	15.7
Penicillin/ampicillin-gentamicin	14.1	14.6	19.9
Penicillin	14.1	14.3	8.1
Piperacillin-tazobactam	0	8.6	17.3
Cefuroxime-metronidazole	9.8	8.1	4.1
Cefotaxime-metronidazole	3.6	5.6	5.5
Mecillinam	5.0	4.7	4.9
Cefuroxime	9.8	3.4	1.2
Penicillin-gentamicin-metronidazole	5.9	3.4	1.7

A complete table showing initial treatment in 1995 bloodstream infection episodes is found in Additional file 1: Table S8

Table 5 Appropriate empiric antibiotic therapy (AEAT) through three time periods by place of acquisition

	2002–2005 ($n = 582$)	2006–2009 ($n = 638$)	2010–2013 ($n = 775$)
AEAT within 6 h			
Community acquired BSI	61.7	63.4	67.1
Health care-associated BSI	64.0	53.6	67.2
Hospital acquired BSI	43.9	46.4	59.0
AEAT within 24 h			
Community acquired BSI	84.2	89.4	88.4
Health care-associated BSI	76.7	81.3	84.4
Hospital acquired BSI	72.2	73.9	83.1

Percent of patients with bloodstream infection (BSI) receiving AEAT within 6 h and within 24 h

[8, 30], but do not distinguish between CA, HCA, and HA infection. The European Antimicrobial Resistance Surveillance Network (EARS-Net) reported increasing occurrence of *Enterococcus faecium* from 2002 to 2008 [7], but *Candida* spp. and *S. epidermidis* are not included in the EARS-Net surveillance.

Acquired resistance was uncommon in our BSI cohort, but an increasing proportion of *E. coli* was non-susceptible to gentamicin, and they occurred in CA as well as in HCA-BSIs. Nationwide, a worrying increase in resistance to gentamicin has emerged from 2003 to 2014 (0.6% to 7.7%) [8]. The EARS-Net has reported 5.2% and 9.9% aminoglycoside resistance in *E. coli* in 2002 and 2013, respectively [7, 24], but the proportion of aminoglycoside resistance was much higher in the south-eastern region (32.1% in Bulgaria in 2013). The proportion of *E. coli* producing ESBL has increased from 2008 to 2014 (1.5% to 5.8%) according to the national data [8], and according to EARS-Net data, the proportion of ESBL-producing *E. coli* in Europe increased from 2.0% in 2002 [24] to 12.6% in 2013 (39.6% in Bulgaria) [7].

One single isolate of MRSA and two pneumococci intermediately susceptible to penicillin were found in our cohort. Nationwide, the proportion of MRSA in blood cultures has been low in the corresponding time period (0.3% and 0.8% in 2002 and 2014, respectively). The low occurrence of MRSA in the Nordic countries and in the Netherlands clearly distinguishes from the other European countries, where MRSA accounted for >25% of the *S. aureus* BSIs in 2007 [25] but had decreased to 18% in 2013 [7]. The nationwide proportion of invasive pneumococci non-susceptible to penicillin was 0.9% in 2002 and 5.5% in 2014 [8, 30]. In Europe, the proportion of penicillin-non-susceptible isolates in 2013 ranged from 1.1% (the Netherlands) to 40.0% (Cyprus) [7]. Comparisons of trends in acquired non-susceptibility in the current study and in surveillance

data from Norway and other European countries are shown in Table 6.

Empirical antibiotic treatment regimens have to be continuously evaluated in accordance with national and local microbe resistance patterns. The initial empiric treatment for sepsis of unknown origin recommended by the National Professional Guidelines for Use of Antibiotics in Hospitals in Norway consists of penicillin and gentamicin, plus metronidazole (PGM) if an anaerobic infection is suspected [11]. PGM was not effective in vitro against 3.5% of the microbes isolated from blood cultures in the present study. In patients with HA-BSI, however, the proportion of microbes not susceptible to PGM was 11.7% in the third time period. In defined subgroups we have to be aware of PGM resistant microbes and include vancomycin (e.g., suspected central venous catheter infection) or an antifungal drug (if suspected candida infection, e.g., long lasting broad-spectrum antibiotic therapy, long time stay in a ICU, particularly after gastrointestinal surgery), in our recommendations. Use of carbapenems in empiric therapy should be restricted to patients infected with bacteria resistant (known or suspected) to PIP/TAZ and in whom aminoglycoside therapy is contraindicated.

Enterococci are inherently resistant to cephalosporins and staphylococci are not susceptible to ceftazidime. In our BSI cohort, staphylococci and enterococci contributed to 30% of HA-BSI episodes. Noteworthy, the percentages of microbes non-susceptible to cefotaxime and ceftazidime in HA-BSI in the third period were as high as 37.9 and 55.3%, respectively. Therefore, none of these appears suitable for use as monotherapy in sepsis of unknown microbial origin. The emergence of ESBL in Gram-negative bacteria has made it even more risky to choose a third-generation cephalosporin as monotherapy for severe infections with unknown etiology.

Even though the National Professional Guidelines [11] recommend PG (or PGM) as the regimen of first choice in sepsis of unknown etiology, the PG (or PGM) combination was given in no more than 20% of the episodes in our BSI cohort, yet the proportion was increasing with time. There are mainly two reasons for prescribing antibiotic treatment that does not include an aminoglycoside: (1) Aminoglycosides are potentially nephrotoxic and ototoxic. Therefore, there is a tendency to avoid them even in cases where they should not be contraindicated. (2) Use of an aminoglycoside requires measurement of aminoglycoside serum concentrations, which is resource consuming, and knowledge and experience is needed for assessment of the results. In a busy day, it is much simpler to administer a beta-lactam-antibiotic, where dosing is simple and the risk of toxicity is negligible.

In Norway, we lack national data for antibacterial treatment given in cases of bloodstream infection or sepsis.

Table 6 Comparisons of trends in acquired non-susceptibility in *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*

Microbe	Type of non-susceptibility	Surveillance Area	Trends in proportions of non-susceptibility (Time period)	
E. coli	Gentamicin non-susceptibility	Levanger Hospital	0.5% (2002–05)	3.3% (2010–13)
		Iceland	2.9% (2010)	4.1% (2013)
		Norway	0.6% (2003)	7.7% (2014)
		EU/EEA	5.2% (2002)	9.9% (2013)
		Bulgaria	15.8% (2003)	32.1% (2013)
E. coli	Resistance to 3rd generation cephalosporins	Levanger Hospital	0 (2002–05)	1.7% (2010–13)
		Norway	1.5% (2008)	5.8% (2014)
		EU/EEA	2.0% (2002)	12.6% (2013)
		Bulgaria	24.8% (2010)	39.6% (2013)
S. aureus	MRSA	Levanger Hospital	0 (2002–05)	1.0% (2010–13)
		Norway	0.3% (2002)	0.8% (2014)
		EU/EEA	25.6% (2007)	18.0% (2013)
		Malta	52.0% (2007)	51.8% (2013)
		Romania	39.1% (2010)	64.5% (2013)
S. pneumoniae	PNSP	Levanger Hospital	0 (2002–05)	3.0% (2010–13)
		Netherlands	2.0% (2010)	1.1% (2013)
		Norway	0.9% (2002)	5.5% (2014)
		Cyprus	41.7% (2010)	40.0% (2013)

MRSA, meticillin-resistant *Staphylococcus aureus*; PNSP, penicillin non-susceptible pneumococci
Proportions of non-susceptibility from the current study (Levanger Hospital) are compared with data from Norway (the national surveillance system [8, 30], the European Union/the European Economic Area (EU/EEA), and with countries which have extraordinary low or high proportions of non-susceptibility [7, 24, 25]

Regarding antibiotic use in Norwegian hospitals, the national surveillance data [8] show that the proportion of aminoglycoside use was less than 5% in 2015 (3.3 out of 73 DDD/100 bed-days), indicating that avoiding aminoglycosides in favor of beta-lactam antibiotics is a common mode of acting countrywide.

The drawback of avoiding aminoglycosides is increased risk of antimicrobial resistance, as particularly cephalosporins are far more resistance driving than aminoglycosides. Therefore, a further shift in favor of aminoglycosides is desirable. As the use of aminoglycosides in our hospital and nationwide is still relatively low, overuse of aminoglycosides is unlikely to explain the observed increase in non-susceptibility to gentamicin in *E. coli*.

During the three time periods, we observed that the use of second- and third- generation cephalosporins decreased, whereas ampicillin or penicillin plus gentamicin were more frequently given. PIP/TAZ was introduced at our hospital in 2006, and the use of it has been increasing, particularly in the third period. Nationwide, the use of aminoglycosides and particularly piperacillin/tazobactam has increased during the last ten years, whereas the use of second-generation cephalosporins has decreased. The use of third-generation cephalosporins and fluoroquinolones peaked in 2011–2012 and have since then declined [8]. These changes are in accordance with the national [11] and local antibiotic policy, in order to achieve regimens that are less resistance driving and also cover the BSI microbes to a larger extent.

Conclusions

Antimicrobial resistance was a far smaller problem in our BSI cohort than is reported from countries outside Scandinavia. The antibiotic regimen recommended by Norwegian Health Authorities [11], consisting of penicillin and gentamicin, and with metronidazole added when an anaerobic infection is suspected, is so far effective in vitro against a great majority of microbes isolated from BSI patients in this region. In our cohort, appropriate empiric antibiotic therapy was achieved to a larger extent by replacing second- and third-generation cephalosporins with penicillins and gentamicin. We must, however, be aware of an increasing occurrence of inherently resistant microbes, particularly in HA infection. There are also indications of increasing numbers of bacteria with acquired resistance, particularly *E. coli* producing ESBL and/or possessing gentamicin resistance, and these occurred predominantly in CA and HCA infections.

Additional file

Additional file 1: Table S1. Number (percent) of bloodstream infection episodes stratified by microbe(s)/microbe group and by place of acquisition. **Table S2.** Number (percent) of bloodstream infection episodes stratified by microbe(s)/microbe group and by infection focus. **Table S3.** Proportions of bloodstream infection episodes with microbe(s) non-susceptible to four commonly used antibiotic regimens by place of acquisition. **Table S4.** Percent of bloodstream infection episodes with microbe(s) non-susceptible to commonly recommended sepsis regimens by site of infection. **Table S5.** Number of microbes not susceptible to penicillin-gentamicin-metronidazole by place of acquisition through three time periods. **Table S6.** Number of BSIs with *Escherichia coli* susceptible, intermediately susceptible or resistant to gentamicin through three time periods. **Table S7.** Number of BSIs with *Escherichia coli* non-susceptible to cefotaxime through three time periods. **Table S8.** Antimicrobial agents (single or in combinations) given as initial treatment in 1995 episodes of bloodstream infection. **Table S9.** Use of antibacterial agents and antineoplastic agents, measured in DDD/100 bed-days, at Levanger Hospital 2006 to 2013. **Appendix 1 On inherent (natural) resistance in microbes**. Rules for assessment of non-susceptibility in microbes not tested against antimicrobial agents in the laboratory. (DOCX 78 kb)

Abbreviations

AEAT: Appropriate empiric antibiotic therapy; BSI: Bloodstream infection; CA: Community acquired; EARS-Net: The European Antimicrobial Resistance Surveillance Network; ESBL: Extended-spectrum beta-lactamase; ESBL-E: Extended-spectrum beta-lactamase producing *Enterobacteriaceae*; EUCAST: European Committee on Antimicrobial Susceptibility Testing; HA: Hospital acquired; HCA: Health care-associated; NWGA: Norwegian Working Group on Antibiotics; PG: Penicillin plus gentamicin; PGM: Penicillin, gentamicin, and metronidazole; PIP/TAZ: Piperacillin-tazobactam

Acknowledgments

We would like to thank our research nurses for diligent and accurate work in the data collection process. We would also like to thank the staff at the Microbiology Laboratory, Levanger Hospital, for consecutively including cases and sending registration forms to physicians treating the patients at the wards.

Funding

This work is supported by the Unit for Applied Clinical Research, Norwegian University of Science and Technology; the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU); St Olav's University Hospital; the Norwegian Surveillance Programme for Antimicrobial Resistance; and by Nord-Trøndelag Hospital Trust's Fund for Research and Improvement.

Availability of data and materials

The data that support the findings of this study are available from Nord-Trøndelag Hospital Trust but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of Nord-Trøndelag Hospital Trust.

Authors' contributions

AM conceived the study and participated in design, data collection, statistical analysis, interpretation of the data, and drafting of the manuscript. BOÅ participated in design, statistical analysis, data interpretation, and drafting of the manuscript. AK participated in design, data interpretation, and drafting of the manuscript. SL participated in design, statistical analysis, data interpretation, and drafting of the manuscript. JP participated in design, data collection and drafting of the manuscript. ES contributed to design, interpretation of the data, and drafting of the manuscript. JKD participated in study design, data interpretation and drafting of the manuscript. SH participated in design, data interpretation, and drafting of the manuscript. THE participated in design, data collection, statistical analysis, and drafting of the manuscript. All the authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Ethics approval and consent to participate
The study was approved by the Regional Committee for Medical and Health Research Ethics, Health Region IV, Norway. The Ethics Committee waived the need for informed consent as this was an observational study, the treatment of the patients was standard, and no samples were taken for the purposes of the research. The use of the data for the present study was approved by Nord-Trøndelag Hospital Trust.

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Received: 18 September 2016 Accepted: 18 January 2017
Published online: 02 February 2017

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Additional file 1

Bloodstream infection at a general hospital in Mid-Norway 2002-2013: Trends in antimicrobial resistance and empiric antibiotic therapy. A prospective observational study

A. Mehl et al.

Tables

Table S1 Number (percent) of bloodstream infection episodes stratified by microbe(s)/microbe group and by place of acquisition

		Place of acquisition		
	Total n (%)	Community acquired n (%)	Health care-associated n (%)	Hospital acquired n (%)
	(n=1995)	(n=934)	(n=787)	(n=274)
Escherichia coli	686 (34.4)	374 (40.0)	255 (32.4)	57 (20.8)
Streptococcus pneumoniae	225 (11.3)	164 (17.6)	53 (6.7)	8 (2.9)
Staphylococcus aureus	218 (10.9)	69 (7.4)	94 (11.9)	55 (20.1)
Klebsiella spp.	135 (6.8)	43 (4.6)	69 (8.8)	23 (8.4)
Beta-hemolytic streptococci	104 (5.2)	64 (6.9)	31 (3.9)	9 (3.3)
Enterococcus spp.	89 (4.5)	20 (2.1)	42 (5.3)	27 (9.9)
Other mixed bacterial infections	68 (3.4)	24 (2.6)	37 (4.7)	7 (2.6)
Pseudomonas spp.	58 (2.9)	12 (1.3)	33(4.2)	13 (4.7)
Viridans group streptococci	57 (2.9)	29 (3.1)	22 (2.8)	6 (2.2)
Coagulase-negative staphylococci	54 (2.7)	19 (2.0)	19 (2.4)	16 (5.8)
Proteus spp.	48 (2.4)	15 (1.6)	25 (3.2)	8 (2.9)
Anaerobic Gram-negative bacteria	45 (2.3)	23 (2.5)	13 (1.7)	9 (3.3)
Mixed Gram-negative aerobic or anaerobic bacteria	42 (2.1)	18 (1.9)	20 (2.5)	4 (1.5)
Enterobacter spp.	37 (1.9)	10 (1.1)	20 (2.5)	7 (2.6)
Other *Enterobacteriaceae*	37 (1.9)	17 (1.8)	11 (1.4)	9 (3.3)
Other aerobic Gram-negative bacteria	19 (1.0)	9 (1.0)	9 (1.1)	1 (0.4)

Haemophilus influenzae	17 (0.9)	8 (0.9)	7 (0.9)	2 (0.7)
Candida spp.	14 (0.7)	1 (0.1)	7 (0.9)	6 (2.2)
Anaerobic Gram-positive bacteria	11 (0.6)	3 (0.3)	6 (0.8)	2 (0.7)
Mixed gram-positive aerobic or anaerobic bacteria	11 (0.6)	2 (0.2)	6 (0.8)	3 (1.1)
Neisseria meningitidis	9 (0.5)	9 (1.0)	0	0
Listeria monocytogenes	8 (0.4)	1 (0.1)	6 (0.8)	1 (0.4)
Mixed bacterial and fungal infections	3 (0.2)	0	2 (0.3)	1 (0.4)

Data are presented as number of BSI episodes (%)

Table S2 Number (percent) of bloodstream infection episodes stratified by microbe(s)/microbe group and by infection focus

Microbes	Infection focus							
	Total	Urinary tract	Lungs	Biliary tract	Gastro-intestinal tract	Skin or soft tissue	Other	Unknown
Escherichia coli	686 (34.4)	462 (61.4)	22 (6.6)	113 (51.4)	24 (23.8)	9 (6.3)	14 (5.7)	42 (20.9)
Klebsiella spp.	135 (6.8)	63 (8.4)	20 (6.0)	29 (13.2)	6 (5.9)	0	2 (0.8)	15 (7.5)
Proteus spp.	48 (2.4)	42 (5.6)	1 (0.3)	1 (0.5)	0	3 (2.1)	0	1 (0.5)
Enterobacter spp.	37 (1.9)	14 (1.9)	3 (0.9)	12 (5.5)	1 (1.0)	1 (0.7)	3 (1.2)	3 (1.5)
Other *Enterobacteriaceae*	37 (1.9)	11 (1.5)	1 (0.3)	4 (1.8)	10 (9.9)	4 (2.8)	1 (0.4)	6 (3.0)
Pseudomonas spp.	58 (2.9)	26 (3.5)	10 (3.0)	0	3 (3.0)	6 (4.2)	3 (1.2)	10 (5.0)
Other aerobic Gram-negative bacteria	19 (1.0)	5 (0.7)	2 (0.6)	2 (0.9)	5 (5.0)	2 (1.4)	0	3 (1.5)
Anaerobic Gram-negative bacteria	45 (2.3)	1 (0.1)	5 (1.5)	4 (1.8)	22 (21.8)	1 (0.7)	8 (3.2)	4 (2.0)
Mixed Gram-negative aerobic or anaerobic bacteria	42 (2.1)	15 (2.0)	2 (0.6)	9 (4.1)	4 (4.0)	1 (0.7)	3 (1.2)	8 (4.0)
Streptococcus pneumoniae	225 (11.3)	0 (0.0)	189 (57.1)	1 (0.5)	2 (2.0)	3 (2.1)	23 (9.3)	7 (3.5)
Staphylococcus aureus	218 (10.9)	18 (2.4)	29 (8.8)	1 (0.5)	0	49 (34.3)	87 (35.2)	34 (16.9)
Beta-hemolytic streptococci	104 (5.2)	7 (0.9)	10 (3.0)	0	2 (2.0)	46 (32.2)	23 (9.3)	16 (8.0)
Enterococcus spp.	89 (4.5)	43 (5.7)	4 (1.2)	8 (3.6)	3 (3.0)	6 (4.2)	14 (5.7)	11 (5.5)
Viridans group streptococci	57 (2.9)	6 (0.8)	4 (1.2)	10 (4.5)	5 (5.0)	2 (1.4)	23 (9.3)	7 (3.5)
Coagulase-negative staphylococci	54 (2.7)	10 (1.3)	5 (1.5)	1 (0.5)	1 (1.0)	3 (2.1)	17 (6.9)	17 (8.5)
Listeria monocytogenes	8 (0.4)	0 (0.0)	1 (0.3)	1 (0.5)	0	0	1 (0.4)	5 (2.5)

Anaerobic Gram-positive bacteria	11 (0.6)	0	0	2 (0.9)	4 (4.0)	1 (0.7)	3 (1.2)	1 (0.5)
Mixed gram-positive aerobic or anaerobic bacteria	11 (0.6)	3 (0.4)	1 (0.3)	3 (1.4)	0	1 (0.7)	3 (1.2)	0
Other mixed bacterial infections	68 (3.4)	21 (2.8)	5 (1.5)	16 (7.3)	9 (8.9)	5 (3.5)	6 (2.4)	6 (3.0)
Mixed bacterial and fungal infections	3 (0.2)	1 (0.1)	0	0	0	0	0	2 (1.0)
Candida spp.	14 (0.7)	4 (0.5)	6 (1.8)	1 (0.5)	0	0	1 (0.4)	2 (1.0)
Haemophilus influenzae	17 (0.9)	0 (0.0)	11 (3.3)	2 (0.9)	0	0	3 (1.2)	1 (0.5)
Neisseria meningitidis	9 (0.5)	0 (0.0)	0	0	0	0	9 (3.6)	0
Total	1995 (100.0)	752 (100.0)	331 (100.0)	220 (100.0)	101 (100.0)	143 (100.0)	247 (100.0)	201 (100.0

Table S3 Proportions of bloodstream infection episodes with microbe(s) non-susceptible to four commonly used antibiotic regimens by place of acquisition (data for Fig. 2)

	n/N	Percent	(95% CI)
Penicillin-gentamicin-metronidazole			
Community acquired	13/934	1.4	(0.8-2.4)
Health care-associated	38/787	4.8	(3.5-6.6)
Hospital acquired	19/274	6.9	(4.5-10.6)
p for trend	<0.001		
Imipenem			
Community acquired	10/934	1.1	(0.6-2.0)
Health care-associated	51/787	6.5	(5.0-8.4)
Hospital acquired	29/274	10.6	(7.5-14.8)
p for trend	<0.001		
Piperacillin-tazobactam*			
Community acquired	22/617	3.6	(2.4-5.3)
Health care-associated	54/612	8.8	(6.8-11.3)
Hospital acquired	31/184	16.8	(12.1-22.9)
p for trend		<0.001	
Cefotaxime			
Community acquired	101/934	10.8	(9.0-13.0)
Health care-associated	168/787	21.3	(18.6-24.3)
Hospital acquired	86/274	31.4	(26.2-37.1)
p for trend		<0.001	

*smaller numbers because piperacillin-tazobactam was not used in 2002-2005

Table S4 Percent of bloodstream infection episodes with microbe(s) non-susceptible to commonly recommended sepsis regimens by site of infection. Piperacillin-tazobactam (P/T) was adopted in 2006

Infection site	Antibiotics used 2002-2013						P/T used 2006-2013			
	PG	PGM	Imi-penem	Cefo-taxime	Ceftazidime	Total N	P/T	P/T-G	P/T-G-M	Total N
Urinary tract	4.1	3.9	4.8	15.7	16.0	752	9.1	2.9	2.7	560
Lungs	4.8	3.3	3.0	10.6	17.5	331	5.0	3.6	3.2	222
Biliary tract	5.5	3.6	5.9	17.3	18.2	220	9.2	3.3	2.6	152
Gastrointestinal tract	29.7	5.0	4.0	47.5	45.5	101	8.6	3.7	1.2	81
Skin or soft tissue	4.2	2.8	2.8	14.0	46.2	143	2.4	2.4	2.4	85
Other	6.5	2.4	4.9	19.0	56.3	247	6.0	3.0	2.4	168
Unknown	5.0	3.5	5.5	24.4	42.8	201	8.3	2.8	2.8	145
Total	6.1	3.5	4.5	17.8	27.8	1995	7.6	3.0	2.6	1413

PG, penicillin-gentamicin; PGM, penicillin-gentamicin-metronidazole; P/T-G, piperacillin-tazobactam plus gentamicin; P/T-G-M, piperacillin-tazobactam plus gentamicin plus metronidazole

Table S5 Number of microbes non-susceptible to penicillin-gentamicin-metronidazole by place of acquisition through three time periods. Isolates from mixed infections included

	Community acquired			Health care-associated			Hospital acquired			Total
	2002-2005	2006-2009	2010-2013	2002-2005	2006-2009	2010-2013	2002-2005	2006-2009	2010-2013	
Candida spp.*	0	0	1	2	4	3	0	1	6	17
Escherichia coli	1	2	4	0	3	5	0	1	0	16
Enterococcus spp.#	0	0	0	1	2	5	1	0	2	11
Staphylococcus epidermidis	0	0	1	2	0	3	0	2	2	10
Other *Enterobacteriaceae*§	0	1	0	2	0	1	1	0	0	5
Anaerobic bacteria¤	0	1	0	0	0	2	0	0	0	3
Gram-negative rod non-fermenters£	0	0	0	0	0	1	0	1	1	3
Fastidious Gram-negative rods$	0	1	0	1	0	0	0	0	0	2
Streptococcus pneumoniae	0	0	1	0	0	0	0	0	1	2
Staphylococcus aureus	0	0	0	0	0	1	0	0	0	1
Total	1	5	7	8	9	21	2	5	12	70

**Candida albicans* 12; *Candida glabrata* 3; *Candida parapsilosis* 2

#*Enterococcus faecium* 9; *Enterococcus faecalis* 1; *Enterococcus gallinarium* 1

§*Citrobacter sp*. 1; *Hafnia alvei* 1; *Klebsiella oxytoca* 1; *Proteus mirabilis* 1; *Salmonella typhimurium* 1

¤*Bacteroides* sp. 1; other anaerobic Gram-negative rod 1; anaerobic Gram-positive rod 1

£*Elisabethkingia meningoseptica* 1; *Pseudomonas aeruginosa* 1; *Stenotrophomonas maltophilia* 1

$ *Aggrigatibacter actimomycetemcomitans* 1; *Haemophilus influenzae* 1

Table S6 Number of bloodstream infection episodes with *Escherichia coli* susceptible, intermediately susceptible or resistant to gentamicin through three time periods. Isolates from mixed infections included

Microbe	Time period	Penicillin-gentamicin-metronidazole			Total
		Susceptible	Intermediately susceptible	Resistant	
Escherichia coli	2002-2005	186	1*	0	186+1
	2006-2009	227	1	3+2*	231+2
	2010-2013	260	2	7	269
	Total	673	4	12	686+3

* *E. coli* isolated from mixed infection. The numbers of E. coli isolates not susceptible to gentamicin in the three time periods were, therefore, one intermediate in 2002-2005, one intermediate and five resistant in 2006-2009, and two intermediate and seven resistant in 2010-2013. The proportions of *E. coli* isolates non-susceptible to gentamicin in the three time periods were 1/187 (0.5%), 6/233 (2.6%), and 9/269 (3.3%)

Table S7 Number of bloodstream infection episodes with *Escherichia coli* not susceptible to cefotaxime through three time periods. *E. coli* isolates from mixed infections included*

	ESBL#/Total *E. coli* (percent ESBL)	Total res to cefotaxime (percent resistant)
2002-2005	0/206 (0.0)	0
2006-2009	5/247 (2.0)	8/247 (3.2)
2010-2013	5/293 (1.7)	5/293 (1.7)
Total	10/746	13/746

*E. coli in monoculture 686; E. coli in mixed culture 60; total E. coli 746
#Documented ESBL-producing *E. coli*: 5 in 2006-2009 and 5 in 2010-2013.
Three isolates of E coli (two in monoculture and one in mixed infection), all occurring in 2006-2009, were not susceptible to cefotaxime although ESBL could not be detected

Table S8 Antimicrobial agents (single or in combinations) given as initial treatment in 1995 episodes of bloodstream infection

	2002-2005		2006-2009		2010-2013		Total
	N	%	N	%	N	%	N
All BSI episodes	582		638		775		1995
Cefotaxime	105	18.0	116	18.2	122	15.7	343
Penicillin-gentamicin	79	13.6	85	13.3	102	13.2	266
Penicillin	82	14.1	91	14.3	63	8.1	236
Piperacillin-tazobactam	0		55	8.6	134	17.3	189
Cefuroxime-metronidazole	57	9.8	52	8.2	32	4.1	141
Cefotaxime-metronidazole	21	3.6	36	5.6	43	5.5	100
Mecillinam	29	5.0	30	4.7	38	4.9	97
Cefuroxime	57	9.8	22	3.4	9	1.2	88
Penicillin-gentamicin-metronidazole	34	5.8	22	3.4	13	1.7	69
Ampicillin-gentamicin	3	0.5	8	1.3	52	6.7	63
Dicloxacillin	20	3.4	14	2.2	24	3.1	58
Ciprofloxacin	9	1.5	30	4.7	15	1.9	54
Other antimicrobial(s)	32	5.5	16	2.5	2	0.3	50
Antimicrobial therapy not given	14	2.4	6	0.9	9	1.2	27
Ciproflaxacin-metronidazole	2	0.3	7	1.1	17	2.2	26
Dicloxacillin-gentamicin	9	1.5	2	0.3	4	0.5	15
Ampicillin	1	0.2	5	0.8	9	1.2	15
Imipenem	3	0.5	6	0.9	5	0.6	14
Penicillin-klindamycin	0		1	0.2	11	1.4	12
Gentamicin	2	0.3	5	0.8	4		11
Penicillin-cefotaxime	0		2	0.3	6	0.8	8
Pivmecillinam po	0		1	0.2	7	0.9	8
Erytromycin	0		3	0.5	4	0.5	7
Metronidazole	0		1	0.2	6	0.8	7
Penicillin-kloramfenikol	5	0.9	1	0.2	0		6
Ceftazidime	2	0.3	2	0.3	1	0.1	5
Klindamycin	0		2	0.3	3	0.4	5
Meropenem	1	0.2	1	0.2	2	0.3	4
Trimetoprim-sulfa	4	0.7	0		0		4
Penicillin-dicloxacillin	1	0.2	1	0.2	2	0.3	4
Penicillin-ciprofloxacin	3	0.5	0		0		3
Penicillin-metronidazole	1	0.2	1	0.2	1		3
Erytromycin-gentamicin	0	0.0	2	0.3	1		3
Penicillin-tobramycin	1	0.2	1	0.2	1	0.1	3
Cefuroxime-gentamicin-metronidazole	0		1	0.2	2	0.3	3
Vancomycin	0		1	0.2	2	0.3	3
Pip/taz-metronidazole	0		1	0.2	2	0.3	3
Klindamycin-gentamicin	0		1	0.2	2	0.3	3
Pip/taz-gentamicin	0		1	0.2	1	0.1	2
Gentamicin-metronidazole	0		1	0.2	1	0.1	2
Ampicillin-cefotaxime	0		0		2	0.3	2
Ampicillin-gentamicin-metronidazole	0		0		2	0.3	2
Ciprofloxacin po-metronidazole po	0		0		2	0.3	2

Dicloxacillin-klindamycin	0		0		2	0.3	2
Ceftriaxone	0		0		2	0.3	2
Doxycyclin	2	0.3	0		0		2
Dicloxacillin-cefotaxime	0		0		2	0.3	2
Penicillin-cefotaxime-gentamicin	0		1	0.2	1	0.1	2
Clindamycin-gentamicin-metronidazole	0	%	1	0.2	0		1
Ampicillin-metronidazole	0		1	0.2	0		1
Ampicillin-mecillinam	1	0.2%	0		0		1
Ampicillin-ciprofloxacin- metronidazole	0		0		1	0.1	1
Amphotericin B	0		0		1	0.1	1
Pip/tazo-ciproflaxacin	0		1	0.2	0		1
Pip/tazo-tobramycin	0		1	0.2	0		1
Cefotaxime-fluconazole	0		0		1	0.1	1
Cefotaxime-metronidazole-fluconazole	0		0		1	0.1	1
Meropenem-vancomycin-fluconazole	0		0		1	0.1	1
Penicillin-ciprofloxacin-clindamycin	0		0		1	0.1	1
Penicillin-cefotaxime-clindamycin	0		0		1	0.1	1
Aztreonam	1	0.2	0		0		1
Ceftazidim-ciprofloxacin	1	0.2	0		0		1
Klindamycin-tobramycin	0		0		1	0.1	1
Penicillin-gentamicin-kloramfenikol	0		0		1	0.1	1
Caspofungin	0		0		1	0.1	1

Table S9 Use of antibacterial agents (ATC code J01) and antineoplastic agents (ATC code L01), measured in DDD/100 bed-days, at Levanger Hospital 2006 to 2013. Data from Levanger Hospital Pharmacy

Year	Antibacterial agents	Antineoplastic agents
2006	76.11	4.40
2007	81.91	4.49
2008	72.27	4.82
2009	71.12	5.32
2010	75.70	4.99
2011	91.53	5.67
2012	82.82	5.78
2013	80.90	6.98

DDD, Defined Daily Doses

Appendix 1: On inherent (natural) resistance in microbes

Rules for assessment of non-susceptibility in microbes not tested against antimicrobial agents in the laboratory

Many microbes have an inherent resistance against various antimicrobial agents. In the microbiology laboratory, therefore, such microbes are not tested against agents that we know will not work. A common example is that enterococci are inherently resistant to cephalosporins.

Acquired resistance – or resistance development, is the phenomenon that a microbe that has earlier been susceptible to an antimicrobial agent, becomes resistant. A well known example is that staphylococci were initially susceptible to penicillin, but soon they became resistant because they acquired the property to produce penicillinase, an enzyme that destroys the penicillin molecule. The field of acquired resistance will not be further discussed here.

In the study "Bloodstream infection at a general hospital in Mid-Norway 2002-2013: Trends in antimicrobial resistance and empiric antibiotic therapy", knowledge about natural resistance has been necessary for two purposes: (1) to assess whether a microbe not tested against an antimicrobial agent was susceptible to that agent or not. And (2) to determine whether an antibiotic regimen if given initially would have been sufficient to control an infection with a given microbe until the result of the susceptibility test was available. The decision rules are mainly based on "The Sanford Guide To Antimicrobial Therapy" [1], which has been available in yearly updated editions through the study period. The antimicrobial spectra of inherently resistant microbes, however, have not changed.

Decisions on susceptibility in microbes not tested in the laboratory

S Sensitive

CS Considered sufficient to manage the infection until the result of the susceptibility test is available

NS Not sufficient to manage the infection until the result of the susceptibility test is available

R Resistant (no effect or considered insufficient to manage the infection)

Microbe	Decision	Antimicrobials against which the microbe was not tested
Acinetobacter spp.	R	Cefotaxime
Anaerobic bacteria	R	Cefuroxime Cefotaxime Ceftriaxone Ceftazidime Aminoglycosides Mecillinam
Enterococci	CS if susceptible to ampicillin	Penicillin, high dose iv Piperacillin/tazobactam Imipenem Meropenem
	NS	Ciprofloxacin
	R	Mecillinam
Campylobacter jejuni	S	Aminoglycosides Carbapenems Fluoroquinolones
	R	Penicillins Cephalosporins
Candida spp.	R	Antibacterial agents
Listeria monocytogenes	CS	Piperacillin/tazobactam
	R	Cephalosporins
Pseudomonas spp.	R	Cefuroxime, Cefotaxime
Gram-positive bacteria	R	Mecillinam
Gram-negative bacteria	R	Macrolides Clindamycin Vancomycin
Staphylococci	CS if not methicillin-	Cefotaxime

	resistant	
	NS	Ceftazidime
Streptococci Group A, B, C, G	S	Ceftazidime
	CS	Cloxa-/dicloxacillin
	R	Aminoglycosides
Viridans streptococci	NS	Ceftazidime
	NS	Cloxa-/dicloxacillin
	R	Aminoglycosides

Comments on some antimicrobial agents

Mecillinam

Mecillinam is an antibiotic active against *Enterobacteriaceae*. It is rapidly excreted in high concentrations to the urine, and is a useful drug in lower urinary tract infections.

In Norway, mecillinam was formerly recommended as a drug useful in the treatment of pyelonephritis if sepsis was not suspected [2] . In the National Professional Guidelines 2013 [3], mecillinam and pivmecillinam are no longer recommended for treatment of pyelonephritis. If the microbe was sensitive to mecillinam in vitro, we have, in the present study, considered mecillinam given intravenously sufficient until the result of positive blood culture was available. For definitive therapy, (when the identity and resistance pattern of the microbe was known) mecillinam had to be changed to an antibiotic known to give higher blood- and tissue concentrations.

Cefuroxime

The wild strains/types of *E coli* and *Klebsiella* spp. are intermediately sensitive to cefuroxime. Intravenous dosing of cefuroxime 1.5 g x 3 was considered appropriate when *E. coli* or *Klebsiella* spp. were intermediately sensitive to cefuroxime.

Ciprofloxacin

A column for fluoroquinolone (ciprofloxacin) non-susceptibility is included in Table 3. Gram-negative aerobic bacteria have been tested routinely with ciprofloxacin, whereas Gram-positive bacteria have not. For microbes not routinely tested with ciprofloxacin, NRT (not routinely tested) is written in the respective table cells. As non-susceptibility to ciprofloxacin differs among Gram-positive microbes, we have not attempted to make decision rules about presumed non-susceptibility.

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Paper III

Eur J Clin Microbiol Infect Dis (2015) 34:609–617
DOI 10.1007/s10096-014-2269-6

ARTICLE

Prior statin use and 90-day mortality in Gram-negative and Gram-positive bloodstream infection: a prospective observational study

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Received: 18 July 2014 / Accepted: 20 October 2014 / Published online: 6 November 2014

Abstract In several studies on patients with bloodstream infection (BSI), prior use of statins has been associated with improved survival. Gram-positive and Gram-negative bacteria alert the innate immune system in different ways. We, therefore, studied whether the relation between prior statin use and 90-day total mortality differed between Gram-positive and Gram-negative BSI. We conducted a prospective observational cohort study of 1,408 adults with BSI admitted to Levanger Hospital between January 1, 2002, and December 31, 2011. Data on the use of statins and other medications at admission, comorbidities, functional status, treatment, and outcome were obtained from the patients' hospital records. The relation of

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statin use with 90-day mortality differed between Gram-negative and Gram-positive BSI (p-value for interaction 0.01). Among patients with Gram-negative BSI, statin users had significantly lower 90-day total mortality [odds ratio (OR) 0.42, 95 % confidence interval (CI) 0.23–0.75, p=0.003]. The association remained essentially unchanged after adjusting for the effect of sex, age, functional status before the infection, and underlying diseases that were considered confounders (adjusted OR 0.38, 95 % CI 0.20–0.72, p=0.003). A similar analysis of patients with Gram-positive BSI showed no association of statin use with mortality (adjusted OR 1.22, 95 % CI 0.69–2.17, p=0.49). The present study suggests that prior statin use is associated with a lower 90-day total mortality in Gram-negative BSI, but not in Gram-positive BSI.

Background

In spite of antibiotic and supportive therapy, bloodstream infection (BSI) is still a major cause of mortality and morbidity [1–3]. Measures to improve outcomes from BSI are necessary. Several observational studies have assessed the relation between prior use of statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors] and the outcome of BSI. The majority of the studies suggest that statin use could have a beneficial effect in patients with BSI [4–8], whereas some studies have shown no difference in mortality between statin users and non-users [9–11].

Many former studies assessing the relation between statin use and outcome of infection have not had verified BSI, nor have they discriminated between Gram-positive and Gram-negative etiology. As Gram-positive and Gram-negative bacteria alert the innate immune system in different ways [12, 13], drugs that have anti-inflammatory properties may not have the same effect in Gram-positive infection as they do in Gram-negative infection. Only a few authors have attempted to study this issue in patients [4, 7, 10, 11], and the results diverge. In this prospective observational cohort study on BSI, we chose to investigate the relation between prior statin use and 90-day mortality in Gram-positive and Gram-negative BSI separately.

Materials and methods

Levanger Hospital serves a population of 87,000 as an emergency facility in a defined geographical area. Since 1994, all positive blood cultures at the hospital have been prospectively recorded for surveillance purposes, and clinical information has been recorded, in the following way: whenever a positive blood culture was reported, a physician at the clinical ward filled out a registration form. A team of three research nurses, a subordinate doctor, and the main investigator reviewed the patients' records to verify the data and record additional variables. Data on statin use were prospectively included in the database from 2005, when we became aware of the early studies on statin use and sepsis. Information on the use of statins during the period 2002–2005 was recorded retrospectively from hospital records. The present study includes BSIs occurring between January 1, 2002 and December 31, 2011 in patients ≥16 years of age. BACTEC 9240 (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) was used for blood culture testing [14]. If a blood culture was positive for bacteria known to cause sepsis, only one positive vial was required for inclusion in the present study. For common skin contaminants (coagulase-negative staphylococci, alpha-hemolytic streptococci, corynebacteria, etc.), at least two positive vials from separate venipunctures were required. An episode of BSI was classified as polymicrobial if more than one organism was isolated from one or more blood cultures within a 72-h period.

For patients who had more than one episode with positive blood culture in the 10-year period, only the first episode was selected for this study. This cohort consisted of 1,408 patients. BSI with Gram-negative or Gram-positive bacteremia accounted for 1,356 episodes. The remaining cases consisted of eight candidemias and 44 with mixed polymicrobial infection.

The *exposure variable* was prior statin use, defined as taking a statin in the week before the time of positive blood culture [15]. Two patients, whose statins were discontinued more than one week before the date of the positive blood culture, were categorized as non-statin users. We recorded statin use and the specific statin and dosage from the patients' hospital charts. The *outcome variable* was death from all causes within 90 days after the first positive blood culture [16]. The date of death was obtained from the patients' electronic records, which is updated by the Civil Registration System in Norway. The following variables were *a priori determined as possible confounders* because they might be associated with both statin use and mortality from BSI (Table 1): age (<65 years, 65–79 years, ≥80 years); sex; comorbidities; Charlson comorbidity index [17], categorized as low (no underlying disease score), medium (score 1–2), or high (score >2) [7]; nursing home resident (yes/no); functional status (independent, partly independent, dependent, unknown); immunosuppressive therapy; alcohol abuse; smoking (no smoking, former smoker, present smoker); focus of infection (urinary tract, lungs, biliary tract, gastrointestinal tract, other, unknown); use of antibiotics before admission; and place of acquisition (community, healthcare, hospital). Whether the current BSI episode was nosocomial, healthcare-associated, or community-acquired was determined according to commonly used definitions [18, 19].

Variables expressing the *severity of the current BSI* (systemic inflammatory response syndrome, organ failure, hypotension, hypoperfusion, sepsis, severe sepsis, and septic shock [20]), severe organ failure (defined as SOFA score >2 in any

Table 1 Baseline characteristics of 1,356 adult patients with Gram-negative or Gram-positive bloodstream infection (BSI) by statin use at Levanger Hospital, Norway, 2002–2011

Variable	Gram-negative BSI (*n*=784)			Gram-positive BSI (*n*=572)		
	No statin use (*n*=646)	Statin use (*n*=138)	*p*-Value	No statin use (*n*=474)	Statin use (*n*=98)	*p*-Value
Age			<0.001			0.021
<65 years	185 (28.6)	25 (18.1)		159 (33.5)	26 (26.5)	
65–79 years	189 (29.3)	71 (51.4)		153 (32.3)	45 (45.9)	
≥80 years	272 (42.1)	42 (30.4)		162 (34.2)	27 (27.6)	
Female sex	367 (56.8)	67 (48.6)	0.060	196 (41.4)	35 (35.7)	0.29
Chronic renal insufficiency	52 (8.0)	16 (11.6)	0.17	36 (7.6)	19 (19.4)	<0.001
Malignancy						
Solid tumor	148 (22.9)	32 (23.2)	0.94	99 (20.9)	21 (21.4)	0.93
Hematological cancer	35 (5.4)	3 (2.2)	0.11	38 (8.0)	3 (3.1)	0.085
Diabetes mellitus	87 (13.5)	41 (29.7)	<0.001	70 (14.8)	35 (35.7)	<0.001
Hypertension	186 (28.8)	77 (55.8)	<0.001	127 (26.8)	40 (40.8)	0.003
Cardiovascular disease	212 (32.8)	91 (65.9)	<0.001	153 (32.3)	67 (68.4)	<0.001
Coronary heart disease	118 (18.3)	65 (47.1)	<0.001	88 (18.6)	50 (51.0)	<0.001
Congestive heart failure	59 (9.1)	16 (11.6)	0.36	49 (10.3)	14 (14.3)	0.25
Peripheral vascular disease	35 (5.4)	15 (10.9)	0.016	30 (6.3)	18 (18.4)	<0.001
Cerebral vascular disease	77 (11.9)	32 (23.2)	<0.001	52 (11.0)	18 (18.4)	0.041
Chronic liver disease	10 (1.5)	1 (0.7)	0.54	14 (3.0)	1 (1.0)	0.31
Chronic pulmonary disease	94 (14.6)	24 (17.4)	0.41	78 (16.5)	16 (16.3)	0.99
Rheumatological/immunological disease	55 (8.5)	13 (9.4)	0.72	44 (9.3)	11 (11.2)	0.56
Charlson comorbidity index			0.014			<0.001
Low (0)	187 (28.9)	23 (16.7)		144 (30.4)	10 (10.2)	
Medium (1–2)	278 (43.0)	69 (50.0)		209 (44.1)	43 (43.9)	
High (>2)	181 (28.0)	46 (33.3)		121 (25.5)	45 (45.9)	
Nursing home resident	85 (13.2)	8 (5.8)	0.021	47 (9.9)	2 (2.0)	0.017
Functional status prior to the present BSI			0.050*			0.035*
Independent	365 (56.5)	87 (63.0)		307 (64.8)	70 (71.4)	
Partly independent	178 (27.6)	38 (27.5)		108 (22.8)	26 (26.5)	
Dependent	97 (15.0)	11 (8.0)		52 (11.0)	2 (2.1)	
Unknown	6 (0.9)	2 (1.4)		7 (1.5)	0	
Immunosuppressive therapy	86 (13.3)	19 (13.8)	0.79	62 (13.1)	13 (13.3)	0.93
Alcohol abuse	30 (4.6)	4 (2.9)	0.45	20 (4.2)	2 (2.0)	0.38
Smoking						
Non-smoker	416 (64.4)	74 (53.6)	0.017	278 (58.6)	49 (50.0)	0.14
Former smoker	110 (17.0)	38 (27.5)	0.004	105 (22.2)	33 (33.7)	0.026
Present smoker	120 (18.6)	26 (18.8)	0.92	91 (19.2)	16 (16.3)	0.65
Focus of infection						
Urinary tract	358 (55.4)	76 (55.1)	0.98	45 (9.5)	10 (10.2)	0.83
Lungs	46 (7.1)	5 (3.6)	0.15	164 (34.6)	31 (31.6)	0.67
Biliary tract	95 (14.7)	26 (18.8)	0.21	14 (3.0)	5 (5.1)	0.32
Gastrointestinal tract	49 (7.6)	8 (5.8)	0.38	11 (2.3)	2 (2.0)	0.81
Skin or soft tissue	17 (2.6)	2 (1.4)	0.45	66 (13.9)	13 (13.3)	0.71
Other	25 (3.9)	4 (2.9)	0.69	105 (22.2)	28 (28.6)	0.16
Unknown	56 (8.7)	17 (12.3)	0.17	69 (14.6)	9 (9.2)	0.16
Systemic antibiotic therapy before admission	93 (14.4)	20 (14.5)	0.99	49 (10.3)	4 (4.1)	0.076

Table 1 (continued)

Variable	Gram-negative BSI (n=784)			Gram-positive BSI (n=572)		
	No statin use (n=646)	Statin use (n=138)	p-Value	No statin use (n=474)	Statin use (n=98)	p-Value
Place of acquisition						
Community-acquired	335 (51.9)	76 (55.1)	0.52	262 (55.3)	49 (50.0)	0.44
Acquired in hospital	76 (11.8)	21 (15.2)	0.28	73 (15.4)	16 (16.3)	0.81
Healthcare-associated	235 (36.4)	41 (29.7)	0.15	139 (29.3)	33 (33.7)	0.51
Variables expressing the severity of infection						
Severe sepsis or septic shock at the time of diagnosis	149 (23.1)	25 (18.1)	0.22	95 (20.0)	25 (25.5)	0.22
Severe organ failure (SOFA score >2 in any organ) at the time of diagnosis	90 (13.9)	19 (13.8)	>0.99	75 (15.8)	21 (21.4)	0.18
Stay in intensive care unit (ICU)	117 (18.1)	26 (18.8)	0.81	107 (22.6)	36 (36.7)	0.005
Appropriate initial antibiotic therapy	548 (84.8)	122 (88.4)	0.35	397 (83.8)	88 (89.8)	0.16

Data are presented as number of patients (%)

*Excluding unknowns

organ [15, 21]), and admission to an intensive care unit (ICU) were recorded, but not considered confounders. Instead, they may be *mediators* in the pathway between prior statin use and mortality and, therefore, should not be adjusted for in the analyses [7, 22, 23].

Appropriate initial antibiotic therapy was defined as correctly dosed intravenous antibiotic therapy given within 24 h of the time that the blood culture specimen was obtained, with a regimen that was active in vitro against the microbe(s) isolated from blood culture(s) [24]. This variable was not adjusted for in the main analyses, as it was not considered a confounder. Appropriate initial antibiotic therapy is, indeed, strongly associated with outcome of BSI, but it is not associated with prior statin use in such a way that it influences whether a person has been prescribed statin medication. Prior statin use may be associated with the initial antibiotic therapy if statin use mitigates the inflammatory response so that symptoms are masked and, therefore, appropriate initial antibiotic therapy, is delayed. In this case, appropriate initial antibiotic therapy is a mediator in the pathway between statin use and death, and not a confounder. On the other hand, one might postulate some unknown variable that influences whether people are prescribed statin medication and also influences whether they receive appropriate initial antibiotic therapy (e.g., some underlying condition that is not included in the Charlson comorbidity index). To reduce the influence of such an unknown confounder, we also performed an analysis including adjustment for appropriate initial antibiotic therapy.

Ethics

The Regional Committee for Ethics in Medical Research, Health Region IV, Norway approved the study. The Ethics Committee waived the need for informed consent because this was an observational study, the treatment of the patients was standard, and no samples were taken for the purposes of the research.

Statistical analyses

Proportions were compared using the unconditional z-pooled test, which is the unconditional version of the Pearson Chi-squared test [25]. Unordered r × c tables were analyzed using the exact version of the Pearson Chi-squared test. The exact Cochran–Armitage test was used to test for trends in proportions. Univariable analysis of mortality curves was done with Kaplan–Meier analysis. The relation between prior statin use and 90-day total mortality was analyzed using logistic regression. Estimates were accompanied by 95 % confidence intervals (CIs). Two-sided p-values<0.05 were considered significant. Statistical analyses were performed with SPSS 18 and StatXact 9.

Results

During the 10-year period, 784 patients with Gram-negative BSI and 572 with Gram-positive BSI were identified. 17.6 % of those with Gram-negative and 17.1 % of those with Gram-positive BSI were statin users. The patient characteristics are shown in Table 1. In both Gram-negative and Gram-positive BSI, statin users had a higher burden of comorbid diseases, such as diabetes, hypertension, and cardiovascular disease, and they also had a higher Charlson comorbidity index. Variables expressing the severity of infection at the time of diagnosis were not significantly different in statin users and non-users. In Gram-positive BSI, the proportion of patients admitted to an ICU was higher in statin users.

Table 2 Microbial agents in 784 episodes of Gram-negative BSI and 572 episodes of Gram-positive BSI, total and by statin use

Microbial agent(s)	No statin	Statin	Total	*p*-Value
Gram-negative microorganisms				
Escherichia coli	400 (61.9)	95 (68.8)	495 (63.1)	0.13
Klebsiella spp.	54 (8.4)	20 (14.5)	74 (9.4)	0.025
Proteus spp.	29 (4.5)	3 (2.2)	32 (4.1)	0.21
Enterobacter spp.	20 (3.1)	3 (2.2)	23 (2.9)	0.69
Other *Enterobacteriaceae*	19 (2.9)	6 (4.3)	25 (3.2)	0.44
Pseudomonas spp.	35 (5.4)	5 (3.6)	40 (5.1)	0.44
Haemophilus influenzae	15 (2.3)	0	15 (1.9)	0.079
Neisseria meningitidis	8 (1.2)	0	8 (1.0)	0.20
Other aerobic Gram-negative organisms	10 (1.5)	0	10 (1.3)	0.14
Anaerobic Gram-negative rods	28 (4.3)	5 (3.6)	33 (4.2)	0.88
Mixed Gram-negative aerobic or anaerobic rods	28 (4.3)	1 (0.7)	29 (3.7)	0.079
Total Gram-negative microorganisms	646 (100)	138 (100)	784 (100)	
Gram-positive microorganisms				
Streptococcus pneumoniae	162 (34.2)	27 (27.6)	189 (33.0)	0.23
Staphylococcus aureus	120 (25.3)	32 (32.7)	152 (26.6)	0.12
Beta-hemolytic streptococci	70 (14.8)	13 (13.3)	83 (14.5)	0.74
Enterococcus spp.	42 (8.9)	10 (10.2)	52 (9.1)	0.66
Viridans group streptococci	33 (7.0)	7 (7.1)	40 (7.0)	0.94
Coagulase-negative staphylococci	33 (7.0)	6 (6.1)	39 (6.8)	0.88
Listeria monocytogenes	3 (0.6)	1 (1.0)	4 (0.7)	0.88
Anaerobic Gram-positive microorganisms	6 (1.3)	1 (1.0)	7 (1.2)	0.89
Mixed Gram-positive aerobic or anaerobic BSI	5 (1.1)	1 (1.0)	6 (1.0)	0.68
Total Gram-positive microorganisms	474 (100)	98 (100)	572 (100)	

Data are presented as number of patients (%)

The distribution of the microbial agents in relation to statin use is shown in Table 2. The distribution of microbes was essentially similar among statin users and non-users, except that *Klebsiella* spp. were more common among statin users and that some Gram-negative microbes (*Haemophilus influenzae*, *Neisseria meningitidis* and "Other aerobic Gram-negative organisms") did not occur in statin users.

Figure 1 shows the mortality curves for patients with Gram-negative and Gram-positive BSI. In Gram-negative BSI, the total 90-day mortality was 10.1 % (14/138) in statin users and 21.4 % (138/645) in non-statin users, $p=0.002$. In Gram-positive BSI, the total 90-day mortality was 28.6 % (28/98) in statin users and 27.0 % (128/474) in non-statin users, $p=0.90$.

The results of logistic regression analysis of the relation between statin use and 90-day total mortality are shown in Table 3. For the total BSI cohort, we found a negative association between prior statin use and 90-day mortality [odds ratio (OR) 0.69, 95 % CI 0.49–0.98, $p=0.040$). The association was not mitigated by adjustment for possible confounding factors. Among patients with Gram-negative BSI, the mortality was significantly lower in statin users (OR 0.42, 95 % CI 0.23–0.75, $p=0.003$). In Gram-positive BSI, there was no association between statin use and mortality. Adjustment for variables considered confounders did not weaken the association between statin use and 90-day mortality in Gram-

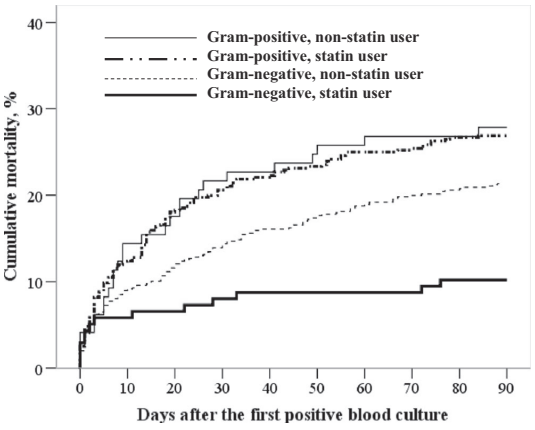

Fig. 1 Mortality curves for patients with Gram-negative and Gram-positive bloodstream infection (BSI) stratified by statin use

Table 3 Logistic regression analysis of the relation between prior statin use and 90-day total mortality

	No. of deaths/patients (%)	Unadjusted OR (95 % CI)	*p*-Value	Adjusted* OR (95 % CI)	*p*-Value
Any BSI					
No statin	285/1,164 (24.5)	1 (reference)		1 (reference)	
Statin	45/245 (18.4)	0.69 (0.49–0.98)	0.040	0.63 (0.43–0.95)	0.025
Gram-negative BSI					
No statin	138/646 (21.4)	1 (reference)		1 (reference)	
Statin	14/138 (10.1)	0.42 (0.23–0.75)	0.003	0.38 (0.20–0.72)	0.003
Gram-positive BSI					
No statin	128/474 (27.0)	1 (reference)		1 (reference)	
Statin	28/98 (28.6)	1.08 (0.67–1.75)	0.75	1.22 (0.69–2.17)	0.49

*Adjusted for age (<65 years, 65–79 years, ≥80 years); sex; Charlson comorbidity index; nursing home resident; functional status (independent, partly independent, dependent, unknown); immunosuppressive therapy; alcohol abuse; smoking (no smoking, former smoker, present smoker); focus of infection (urinary tract, lungs, biliary tract, gastrointestinal tract, other, unknown); use of antibiotics before admission; and place of acquisition (community, healthcare, hospital)

negative BSI (adjusted OR 0.38, 95 % CI 0.20–0.72, p=0.003). The relations between prior statin use and 90-day mortality in Gram-negative and Gram-positive BSI were significantly different; the adjusted OR for the interaction term was 2.95 (95 % CI 1.26–6.89, p=0.012).

In an analysis including adjustment for appropriate initial antibiotic therapy, the effect estimates did not change (adjusted OR 0.38, 95 % CI 0.20–0.73, p=0.004 and OR 1.21, 95 % CI 0.68–2.14, p=0.52 for Gram-negative and Gram-positive BSI, respectively).

Adjusted ORs for the relation between statin use and 90-day mortality were similar when disease categories (e.g., diabetes, cardiovascular disease) were used instead of the Charlson comorbidity index for the total cohort (OR 0.62, 95 % CI 0.41–0.94, p=0.026), Gram-negative BSI (OR 0.37, 95 % CI 0.18–0.74, p=0.005), and Gram-positive BSI (OR 1.13, 95 % CI 0.62–2.07).

After excluding bacterial subgroups with zero statin users (n=33), the association between statin use and 90-day mortality in Gram-negative BSI did not change, either unadjusted (OR 0.44, 95 % CI 0.24–0.78, p=0.006) or after adjustment for confounders (OR 0.41, 95 % CI 0.21–0.78, p=0.007).

For some variables included in the main analysis (focus of infection, use of antibiotics before admission, and place of acquisition), the association with prior statin use is unclear, and it is uncertain whether these variables fulfil the criteria for being a confounder. Additionally, we repeated the analysis after excluding these variables from the model, but the relations between prior statin use and 90-day mortality in Gram-negative (OR 0.37, 95 % CI 0.20–0.70, p=0.002) and Gram-positive BSI (OR 1.00, 95 % CI 0.58–1.75) remained unchanged.

Discussion

In this cohort study of patients with BSI, prior statin use was associated with reduced 90-day mortality in Gram-negative, but not in Gram-positive, BSI. To our best knowledge, no previous research has reported this finding. The effect measures were not changed by adjustment for variables that were considered confounding factors.

In the total cohort of BSI, the unadjusted OR for mortality among statin users compared with non-users did not differ much from what has been found by other investigators [7, 9, 11]. In one study, adjustment for possible confounding factors attenuated the effect measure to a value above 1 [11]. A population-based prospective cohort study on statin use and community-acquired pneumonia [26] raised concerns that previous studies indicating benefits of statins in patients with sepsis had been measuring and reporting a healthy user effect. In that study, statin users were less likely to die or to be admitted to an ICU. After adjusting for confounding factors that reflect patient frailty or healthy user behavior, no reduction in either mortality or the need for admission to an ICU in statin users was found.

In the present study, a significantly lower proportion of statin users were functionally dependent patients or nursing home residents compared with non-users. The proportion of former smokers was significantly higher among statin users. However, these associations of statin use were essentially similar in Gram-negative and Gram-positive BSI. Therefore, it seems unlikely that the observed difference in the possible preventive effect of statins between Gram-negative BSI and Gram-positive BSI in our study could be explained by a healthy user effect.

Most previous investigators have dealt with infection, sepsis, or BSI as if these categories represent homogenous groups with regard to pathogenesis or assumed preventive effect of statins. A few articles reported some data on the relations between statin use and death in Gram-positive and Gram-negative BSI separately. Liappis et al. [4] reported trends of reduced mortality rates in statin users with Gram-negative infection (1/19 vs. 48/223, p=0.13) or *Staphylococcus aureus* infection (0/15 vs. 22/130, p=0.13). Yang et al. [10] found a trend of lower mortality in statin users with Gram-negative bacteremia (10.3 % vs. 16.4 %, p=0.25), but not in Gram-positive bacteremia (33.3 % vs. 27.8 %, p=0.50). Thomsen et al. [7] did not find such a trend in the enterobacterial group of Gram-negative bacteria (adjusted 30-day mortality rate ratio 1.07, 95 % CI 0.61–1.86, p=0.82), nor did Leung et al. find a negative association between statin use and death in Gram-negative BSI (adjusted hazard ratio 1.10, 95 % CI 0.87–1.39) [11]. However, the latter had adjusted for variables expressing the severity of the current infection, i.e., factors that should be considered as mediators rather than confounders, and such an adjustment may attenuate the true associations [22]. Recently, López-Cortés et al. found a negative association between statin use and death within 14 days from *Staphylococcus aureus* BSI (adjusted OR 0.08, 95 % CI 0.01–0.66, p=0.02) [8].

Statins are renowned for their anti-inflammatory and pleiotropic lipid-lowering independent effects. They exert their anti-inflammatory effects through their implication on a variety of molecular pathways of the innate and adaptive immune system, such as their impact on the circulating levels of inflammatory cytokines, as well as anti-coagulant and anti-proliferative effects [27, 28]. With regards to sepsis, many in vitro and animal studies have demonstrated that statins attenuate endotoxin-induced inflammatory responses [29]. Similarly, statins have been found to diminish inflammatory responses induced by *Staphylococcus aureus* lipoteichoic acid [30]. However, there are few studies that have explored the mechanisms for differential effects of statins in Gram-positive and Gram-negative BSI, as observed in the present study. Gram-positive and Gram-negative bacteria have different cell wall components and may stimulate different toll-like receptors (TLRs) on cells of the innate immune system, initiating the transcription of different inflammatory mediator genes. Interestingly, there are several studies showing that statins decrease lipopolysaccharide (LPS) signaling through the downregulation of TLR4 expression on monocytes [31]. Although some studies suggest that statins may also suppress TLR2 signaling in Gram-positive infection, statins may not inhibit the lipoprotein-induced pathway via TLR2 and the LPS-induced pathway via TLR4 to the same extent [32]. Indeed, there are some studies showing that statins inhibit TLR4-mediated activation of interferon regulatory factor 3 (IRF3) and interferon-beta production in macrophages stimulated by LPS, while no such effects were observed for TLR2 stimulation [33]. The clinical relevance of these findings is, however, unknown and require further examination. Nevertheless, they illustrate that there may be molecular mechanisms supporting the findings of different effects of statins in Gram-positive and Gram-negative BSI.

Our findings indicate that the possible preventive effect of statins in BSI may be a subgroup effect seen in Gram-negative, but not in Gram-positive, infection. Whether a negative association between statin use and mortality in Gram-negative BSI really exists as a biological interaction should be further investigated, preferably by randomized controlled studies (RCTs). As of yet, few RCTs have been performed [34, 35], and though several are presently in progress [36], they are designed to study the effect of statin treatment in ongoing infection, not the prophylactic effect of statin use prior to infections. One RCT has shown improved survival in atorvastatin-treated ICU patients with severe sepsis if they had been prior statin users, but no effect was found in patients who received de novo statin therapy [35]. RCTs, so far, have not assessed whether different etiologic agents influence the relation between prior statin use and the outcome of infections.

Strengths and weaknesses of the study

The strength of our study is that our bacteremia database contains confirmed diagnoses and variables collected from the patients' hospital data, which include medical records. Thus, we have more reliable data than diagnostic information from only discharge databases. Our cohort has a sufficient number of patients in the different groups, which makes it possible to assess the relation between statin use and 90-day mortality, an important endpoint in prior studies.

The study has the weakness of being an observational study, burdened with the risk of confounding. Socioeconomic factors have not been adjusted for, but severe confounding by socioeconomic differences seems unlikely because statin prescriptions are reimbursed, and access to hospital treatment is the same for all citizens in Norway. In addition, we have not adjusted for vaccinations, e.g., pneumococcus vaccine or influenza vaccine, which some previous authors have regarded as "healthy user" markers. Nonetheless, we are not aware of confounders that would be likely to cause a strong association of statin use with reduced mortality in Gram-negative, but not in Gram-positive, BSI.

Conclusion

In the present study, the relation between statin use and 90-day total mortality was different in Gram-positive and Gram-negative bloodstream infection (BSI). In Gram-negative BSI, statin use was associated with lower 90-day mortality, which was not the case in Gram-positive BSI.

Acknowledgments This work is supported by the Unit for Applied Clinical Research, Norwegian University of Science and Technology (NTNU); the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU); the Norwegian Surveillance Programme for Antimicrobial Resistance; and Nord-Trøndelag Hospital Trust's Fund for Research and Improvement.

We would like to thank our research nurses for their diligent and accurate work in the data collection process.

Conflict of interest The authors declare that they have no competing interests.

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