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IMPROVED DIAGNOSIS AND MANAGEMENT OF SEPSIS AND BLOODSTREAM INFECTION

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IMPROVED DIAGNOSIS AND MANAGEMENT OF SEPSIS AND BLOODSTREAM INFECTION

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To Elin, Edvin and Lou,

and Janna

POPULAR SCIENCE SUMMARY OF THE THESIS

Sepsis is an infectious disease that kills 11 million people globally every year. The condition, also referred to as blood poisoning, results in life-threatening organ damage due to a misguided immune system reaction. In sepsis, prompt diagnosis is crucial; delays in initiating treatment increases the risk of dying. Unfortunately, the signs of sepsis can be vague and unspecific, making it difficult to detect in time.

A common cause of sepsis is bacterial infections that spreads through the bloodstream. Of particular concern is also the growing threat of bacteria with abilities to withstand commonly used antibiotic treatments, also known as antimicrobial resistance. As a result, the World Health Organization has provided a list of bacteria to prioritize for research, and has specifically pointed out *Pseudomonas aeruginosa*. These bacteria are commonly found in hospitals and mainly affect vulnerable patients with lung disease, ongoing cancer treatment, or patients in intensive care unit wards.

The aim of this thesis was to study diagnosis and treatment of sepsis and bloodstream infections, partly using *P. aeruginosa* as an example. The thesis is based on data from more than 100 000 hospital encounters. In addition, comprehensive genomic data from approximately 800 *P. aeruginosa* from several hospitals in Europe and Australia has been collected through laboratory work. The results are summarized into five studies

In a first study, we demonstrated an objective and scalable approach to automatically diagnose sepsis using electronic health records, with examples of how to continuously monitor the occurrence of sepsis in hospital wards. This information can be used for directing resources and evaluating quality-of-care interventions. We showed that breathing failure is common in sepsis and that this can be accurately measured with a simple and cheap method that calculates the percentage of oxygenated blood via a skin sensor (SpO2). This simplifies sepsis diagnosis, and becomes a lenient alternative to the standard method of performing an arterial blood test. To improve detection of sepsis, an advanced mathematical model based on machine learning was developed to forecast sepsis in patients admitted to the hospital. The model detected sepsis earlier, and with higher accuracy, compared to currently used methods, and was most useful in the first days of the hospital stay. Furthermore, the impact of delayed antibiotic treatment in bloodstream infection was studied, defined as treatment with proven activity against the recovered bacteria. The results demonstrated that delaying antibiotic treatment beyond 12 hours was associated with increased risk of death, providing a timeframe for doctors to act on. Finally, specific bacterial traits of *P. aeruginosa*, determined using genomic data, seemed to affect disease severity and the risk of dying in bloodstream infection, but the added value of this prognostic information was limited.

Together, the studies presented in this thesis contribute to a better understanding of sepsis and of BSI patients, and provide several suggestions aimed at improving diagnosis and treatment.

ABSTRACT

Sepsis is a severe organ dysfunction triggered by infections, and a leading cause of hospitalization and death. Concurrent bloodstream infection (BSI) is common and around one third of sepsis patients have positive blood cultures. Prompt diagnosis and treatment is crucial, but there is a trade-off between the negative effects of over diagnosis and failure to recognize sepsis in time. The emerging crisis of antimicrobial resistance has made bacterial infections more difficult to treat, especially gram-negative pathogens such as *Pseudomonas aeruginosa*.

The overall aim with this thesis was to improve diagnosis, assess the influence of time to antimicrobial treatment and explore prognostic bacterial virulence markers in sepsis and BSI. The papers are based on observational data from 7 cohorts of more than 100 000 hospital episodes. In addition, whole genome sequencing has been performed on approximately 800 invasive *P. aeruginosa* isolates collected from centers in Europe and Australia.

Paper I showed that automated surveillance of sepsis incidence using the Sepsis-3 criteria is feasible in the non-ICU setting, with examples of how implementing this model generates continuous epidemiological data down to the ward level. This information can be used for directing resources and evaluating quality-of-care interventions. In **Paper II**, evidence is provided for using peripheral oxygen saturation (SpO2) to diagnose respiratory dysfunction in sepsis, proposing the novel thresholds 94% and 90% to get 1 and 2 SOFA points, respectively. This has important implications for improving sepsis diagnosis, especially when conventional arterial blood gas measurements are unavailable. **Paper III** verified that sepsis surveillance data can be utilized to develop machine learning screening tools to improve early identification of sepsis. A Bayesian network algorithm trained on routine electronic health record data predicted sepsis onset within 48 hours with better discrimination and earlier than conventional NEWS2 outside the ICU. The results suggested that screening may primarily be suited for the early admission period, which have broader implications also for other sepsis screening tools. **Paper IV** demonstrated that delays in antimicrobial treatment with *in vitro* pathogen coverage in BSI were associated with increased mortality after 12 hours from blood culture collection, but not at 1, 3, and 6 hours. This indicates a time window where clinicians should focus on the diagnostic workup, and proposes a target for rapid diagnostics of blood cultures. Finally, **Paper V** showed that the virulence genotype had some influence on mortality and septic shock in *P. aeruginosa* BSI, however, it was not a major prognostic determinant.

Together these studies contribute to better understanding of the sepsis and BSI populations, and provide several suggestions to improve diagnosis and timing of treatment, with implications for clinical practice. Future works should focus on the implementation of sepsis surveillance, clinical trials of time to antimicrobial treatment and evaluating the prognostic importance of bacterial genotype data in larger populations from diverse infection sources and pathogens.

LIST OF SCIENTIFIC PAPERS

The thesis is based on the following papers, referred to in the text by their corresponding roman numerals:

I. **John Karlsson Valik**, Logan Ward, Hideyuki Tanushi, Kajsa Müllersdorf, Anders Ternhag, Ewa Aufwerber, Anna Färnert, Anders F Johansson, Mads Lause Mogensen, Brian Pickering, Hercules Dalianis, Aron Henriksson, Vitaly Herasevich, Pontus Nauclér
Validation of automated sepsis surveillance based on the Sepsis-3 clinical criteria against physician record review in a general hospital population: observational study using electronic health records data
BMJ Quality & Safety, 2020, vol 29, 735-745

II. **John Karlsson Valik**, Lisa Mellhammar, Jonas Sundén-Cullberg, Logan Ward, Christian Unge, Hercules Dalianis, Aron Henriksson, Kristoffer Strålin, Adam Linder, Pontus Nauclér
Peripheral Oxygen Saturation Facilitates Assessment of Respiratory Dysfunction in the Sequential Organ Failure Assessment Score With Implications for the Sepsis-3 Criteria
Critical Care Medicine, 2022, vol 50, e272-e283

III. **John Karlsson Valik***, Logan Ward*, Hideyuki Tanushi, Anders F Johansson, Anna Färnert, Mads Lause Mogensen, Brian W Pickering, Vitaly Herasevich, Hercules Dalianis, Aron Henriksson, Pontus Nauclér
Predicting onset of sepsis in the emergency department and non-intensive care unit wards using a machine learned Bayesian network model in electronic health records: a cohort study
Manuscript

IV. Jasper Van Heuverswyn*, **John Karlsson Valik***, Pontus Hedberg, Suzanne Desirée van der Werff, Christian Giske, Pontus Nauclér.
Association between time to appropriate antimicrobial treatment and 30-day mortality in patients with bloodstream infections: a retrospective cohort study
Clinical Infectious Diseases; 2022, Sep, Epub ahead of print

V. **John Karlsson Valik**, Christian Giske, Badrul Hasan, Mónica Gozalo Margüello, Luis Martínez Martínez, Manica Mueller Premru, Žiga Martinčič, Bojana Beović, Sofia Maraki, Maria Zacharioudaki, Diamantis Kofteridis, Kate McCarthy, David Paterson, Maria de Cueto, Isabel Morales, Leonard Leibovici, Tanya Babich, Fredrik Granath, Jesús Rodríguez-Baño, Antonio Oliver, Dafna Yahav, Pontus Nauclér
Association of Pseudomonas aeruginosa virulence genotype with patient characteristics, septic shock and mortality in bloodstream infection
Manuscript

*Shared first authorship

RELATED PUBLICATIONS

The following papers were authored or co-authored during the course of the PhD education, but were not in the scope of the thesis:

I. **Valik JK**, Hedberg P, Holmberg F, van der Werff SD, Nauclér P. *Impact of the COVID-19 pandemic on the incidence and mortality of hospital-onset bloodstream infection: a cohort study* **BMJ Quality & Safety**, 2022, vol 31, 379–82

II. Babich T, Naucler P, **Valik JK**, Giske CG, Benito N, Cardona R, et al. *Duration of Treatment for Pseudomonas aeruginosa Bacteremia: a Retrospective Study* **Infectious Diseases and Therapy**, 2022, vol 11, 1505–19

III. Behnke M, **Valik JK**, Gubbels S, Teixeira D, Kristensen B, Abbas M, et al. *Information technology aspects of large-scale implementation of automated surveillance of healthcare-associated infections* **Clinical Microbiology and Infection**, 2021, vol 27, 29–39

IV. Babich T, Naucler P, **Valik JK**, Giske CG, Benito N, Cardona R, et al. *Combination versus monotherapy as definitive treatment for Pseudomonas aeruginosa bacteraemia: a multicentre retrospective observational cohort study* **Journal of Antimicrobial Chemotherapy**, 2021, vol 76, 2172–81

V. Babich T, Naucler P, **Valik JK**, Giske CG, Benito N, Cardona R, et al. *Ceftazidime, Carbapenems, or Piperacillin-tazobactam as Single Definitive Therapy for Pseudomonas aeruginosa Bloodstream Infection: A Multisite Retrospective Study* **Clinical Infectious Diseases**, 2020, vol 70, 2270–80

VI. Alam MU, Henriksson A, **Karlsson Valik J**, Ward L, Naucler P, Dalianis H. *Deep Learning from Heterogeneous Sequences of Sparse Medical Data for Early Prediction of Sepsis* **The 13th International Joint Conference on Biomedical Engineering Systems and Technologies, Valletta, Malta**, 2020, vol 5, 45–55

VII. Babich T, Naucler P, **Valik JK**, Giske CG, Benito N, Cardona R, et al. *Risk factors for mortality among patients with Pseudomonas aeruginosa bacteraemia: a retrospective multicentre study* **International Journal of Antimicrobial Agents**, 2020, vol 55, 105847

VIII. Fröding I, **Valik JK**, Bolinder L, Nauclér P, Giske CG. *Prediction of bloodstream infection caused by extended-spectrum β-lactamase-producing Enterobacterales in patients with suspected community-onset sepsis* **International Journal of Antimicrobial Agents**, 2019, vol 53, 820–9

CONTENTS

LIST OF ABBREVIATIONS

AIC	Akaike information criterion
AMR	Antimicrobial resistance
APR	Area under precision-recall curve
ARDS	Acute respiratory distress syndrome
AST	Antimicrobial suceptibility testing
AUROC	Area under receiver operating characteristic curve
BP	Base pair
BSI	Bloodstream infection
DAMPs	Damage-associated molecular patterns
CCI	Charlson comorbidity index
CDC	Centers for disease control and prevention
CI	Confidence interval
CIF	Cumulative incidence function
CLABSI	Central line-associated bloodstream infection
CLED	Cystine-lactose-electrolyte-deficient
CPN	Causal probabilistic network
CRRT	Continuous renal replacement treatment
dsDNA	Double-stranded DNA
EARS-Net	European antimicrobial resistance surveillance network
eCRF	Electronic case report form
ECDC	European centre for disease prevention and control
ED	Emergency department
EHR	Electronic health records
ESBL	Extended-spectrum β-lactamase
EUCAST	European committee on antimicrobial susceptibility testing
FiO2	Fraction of inspired oxygen
GCS	Glasgow coma scale
HAI	Healthcare-associated infection

HAIBA	Healthcare-Associated Infections Database
HIV	Human immunodeficiency virus
ICD	International classification of diseases
ICU	Intensive care unit
IHD	Intermittent hemodialysis
IMP	Active-on-imipenem metallo-β-lactamase
IQR	Interquartile range
LCBI	Laboratory confirmed bloodstream infection
LPS	Lipopolysaccaride
MDR	Multidrug-resistant
MEWS	Modified early warning score
MLST	Multi locus sequence types
NDM	New Delhi metallo-β-lactamase
NGS	Next-generation genome sequencing
MIMIC-III	Medical information mart for intensive care-III
NEWS2	National early warning score 2
NPV	Negative predictive value
OOB	Out-of-bag
OR	Odds ratio
ORF	Open reading frames
OXA	Active on oxacillin β-lactamase
P3S	Patient Safety Surveillance System
PAGI	*P. aeruginosa* genomic islands
PaO2	Partial pressure of oxygen in arterial blood
PAPI	*P. aeruginosa* pathogenicity islands PAPI
PCoA	Principal coordinate analysis
PER-1	Pseudomonas extended resistant β-lactamase
PPS	Point prevalence surveys
PPV	Positive predictive value
qSOFA	Quick sequential organ failure assessment
RCT	Randomized control trial

RETTS	Swedish rapid emergency triage and treatment system
ROC	Receiver operating characteristics curve
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
SMOTE	Synthetic minority oversampling technique
SOFA	Sequential organ failure assessment
SpO2	Peripheral oxygen saturation
ST	Sequence type
T3SS	Type-III secretion system
TufA	Elongation factor Tu
VFDB	Virulence factor database
VIM	Verona integron-encoded metallo-β-lactamase
WGS	Whole genome sequencing
WHO	World Health Organization
XDR	Extensively drug-resistant

1 INTRODUCTION

Sepsis is a severe organ dysfunction triggered by infections, and a leading cause of hospital admission and death. It is estimated to affect approximately 50 million patients and result in 11 million deaths globally per year [1]. Concurrent bloodstream infection (BSI) is common in sepsis and around one third of sepsis patients have positive blood cultures [2,3]. Several studies have shown that prompt identification and treatment are important factors to increase survival, but there is a difficult trade-off between over diagnosis, leading to unnecessary interventions, and failure to recognize sepsis in time.

Sepsis and BSI can affect previously healthy individuals, but the vast majority of patients suffers from known risk factors such as underlying chronic illness, advanced age or ongoing medical treatment. Recently, Torisson et al. showed that, while hospitalization rates for non-infectious diagnoses declined between 1998 to 2019 in Sweden, there was a concurrent increase in hospitalizations due to infections [4]. Another population based study including approximately 80 000 persons from the HUNT cohort in Norway showed that 22% of the study participants were admitted to the hospital at least once due to bacterial infections [5]. Taken together, these studies highlights the large burden of severe bacterial infections, which is likely reflecting advances in modern healthcare, as well as a growing population of elderly patients [4]. Increasing attention is also being drawn to the deleterious consequences of sepsis for advances in other medical fields where patients are made more susceptible to bacterial infections [6]. Without the ability to rapidly detect and treat sepsis and BSI, much of modern intensive care, advanced surgery and novel treatments of cancer or autoimmune disease, would not be feasible.

Another important aspect is the rapidly emerging epidemic of multidrug-resistant (MDR) bacteria, in particular gram-negative bacteria. Antimicrobial resistant strains of staphylococci, enterococci, *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter,* have spread fast in hospitals worldwide calling for immediate action. In 2017, sepsis was declared a global health priority by the World Health Organization (WHO) [7]. The same year WHO published its first ever list of bacteria that poses great threat to human health, for which further research is urgently needed [8]. On this list, *P. aeruginosa* was given highest priority, alongside with other gram-negative bacteria such as resistant *Acinetobacter baumannii* and *Escherichia coli*.

Collectively, this warrants research focused at advancing the knowledge of severe bacterial infections, in particular due to the increasing patient population at risk and the rapidly emerging epidemic of MDR pathogens. The overarching aim of this thesis was to improve diagnosis, assess the influence of time to antimicrobial treatment and explore prognostic bacterial virulence markers in sepsis and BSI. Five studies are presented, conducted during the period 2016 to 2022 at the Department of Medicine, Solna at Karolinska Institutet and the Karolinska University Hospital in Stockholm, Sweden.

1.1 SEPSIS AND BLOODSTREAM INFECTIONS IN ADULTS

Sepsis is defined as "*life-threatening organ dysfunction caused by a dysregulated host response to infection*" [9]. Although sepsis traditionally was considered equivalent to BSI, this viewpoint was challenged with the introduction of the consensus sepsis definition in early 1990s [3]. The definition centers around the pathophysiological response to an infection, and is not restricted to any type of infection or microbiological confirmation [9]. BSI is still common in sepsis, but it can also occur in patients without the immunological response or organ dysfunction corresponding to sepsis (Figure 1). To this date, no single biomarker exists that correctly identifies sepsis, and the clinical definition is based on a syndrome, inevitably creating a more heterogeneous case definition.

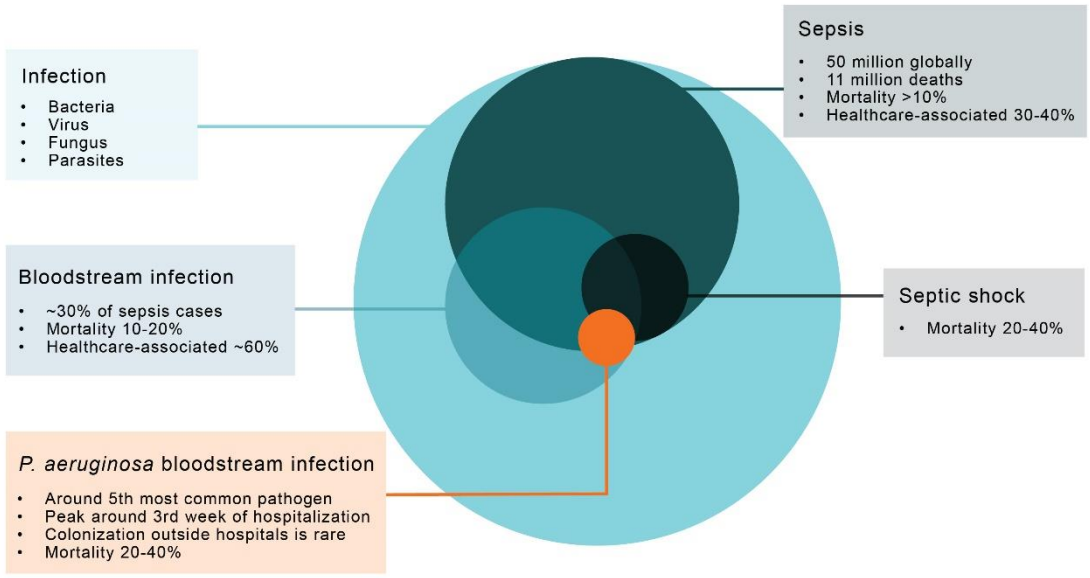

Figure 1. The relationships between infection, sepsis, septic shock and bloodstream infections.

1.1.1 Microbiology of sepsis and bloodstream infections

Although bacterial sepsis is by far the most frequent cause, also viral, fungal and parasitic infection can cause sepsis [10]. The most common source of infection in sepsis is pneumonia, followed by urinary tract and intra-abdominal infections [11–14]. In the large EPIC II study based on point prevalence surveys (PPS) performed in 1265 intensive care units (ICU) worldwide in 2007, 51% of patients in the ICU were infected and among these microbiological cultures were positive in 70% [15]. While not all patients had sepsis, it gives a general idea of the microbiology of the most severe cases of sepsis. The distribution between gram-positives, gram-negatives and fungi (mainly *Candida* spp.) in infected patients were 62%, 47% and 19% respectively. More recently, Rhee C. et al. considered all positive cultures in 17 430 community-onset sepsis episodes and reported the top three pathogens as *Escherichia coli* (33.7%), *Staphylococcus*

aureus (21.3%), and *Streptococcus* spp. (pneumococcus, beta-haemolytic streptococci and viridans streptococci) (13.5%), while *P. aeruginosa* was the sixth most common pathogen [2]. Overall, BSI was common in sepsis and 40% of sepsis patients had positive blood cultures [2]. A recent population-based study in Norway confirmed the findings from Rhee. et al and found that approximately 40% of sepsis patients had positive blood cultures during the 22-year study period [5]. In contrast, a large portion of episodes classified as sepsis are also culture negative, some of which has no clear evidence of infection. One study showed that as many as 30% of patients did not have any positive culture from any culture site [16]. Furthermore, Klouwenberg at al. studied 2579 patients admitted to ICU with sepsis, and found that the post-hoc likelihood of infection was "none" in 13% and only "possible" in 30% [17]. In their study, mortality was not affected by the presence of infection. These findings need to be taken into consideration when interpreting studies of sepsis.

The number of reported BSI in sepsis is affected by several factors such as if blood cultures were collected, type of blood culture (e.g. bacterial, fungal, mycobacterial), if patients received antimicrobial treatment before blood culture collection or if the infection caused invasion of the bloodstream. The most common pathogens in BSI reported from high-income countries are similar to the pathogens reported in sepsis: *S. aureus*, *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Enterococcus* spp, *Streptococcus* spp. (pneumococcus, beta-haemolytic streptococci and viridans streptococci) and coagulase- negative staphylococci, while *Salmonella* spp. is a key pathogen in low-resource settings [18].

1.1.2 Pathophysiology of sepsis

Risk factors for sepsis includes age (very high and low), male sex, immunosuppressive treatment and comorbidities such as cancer, human immunodeficiency virus (HIV) infection and chronic obstructive pulmonary disease [3,10]. Host response in sepsis is based on a complex interplay between inflammatory and anti-inflammatory components (Figure 2) [3]. The magnitude and duration of this response is determined both by pathogen factors (microbial load and virulence) and host factors (genotype, age, comorbidity, and medications). The inflammatory response classically starts with pathogen activation of innate immune cells via cell surface receptors (e.g. toll-like receptors and C-type lectin receptors) and intracellular receptors (e.g. NOD-like receptors, RIG-1-like receptors) [10]. Upon activation, leukocytes (via cytokines, proteases, reactive oxygen species), the complement system and the coagulation cascade elicits cell apoptosis and tissue damage. This in turn feeds the inflammatory loop via damage-associated molecular patterns (DAMPs) and maintains the response [3]. Organ dysfunction is believed to be the end result of impaired tissue oxygenation caused by hypotension (vasodilatation, endothelial damage/oedema and myocardial depression), microvascular thrombosis and mitochondrial damage [10,19]. Most frequent organ dysfunctions occur in the lungs (acute respiratory distress syndrome, ARDS), cardiovascular system (shock), kidneys (oliguria or anuria, acute renal injury) and central nervous system (encephalopathy and delirium). Other common manifestations include impairment of hepatic function, coagulation

system (thrombocytopenia and disseminated intravascular coagulation), gastrointestinal tract (paralytic ileus and diminished intestinal barrier), bone marrow (cytopenias), endocrine functions (adrenal gland suppression, euthyroid sick syndrome) and critical illness polyneuropathy and myopathy [3,10].

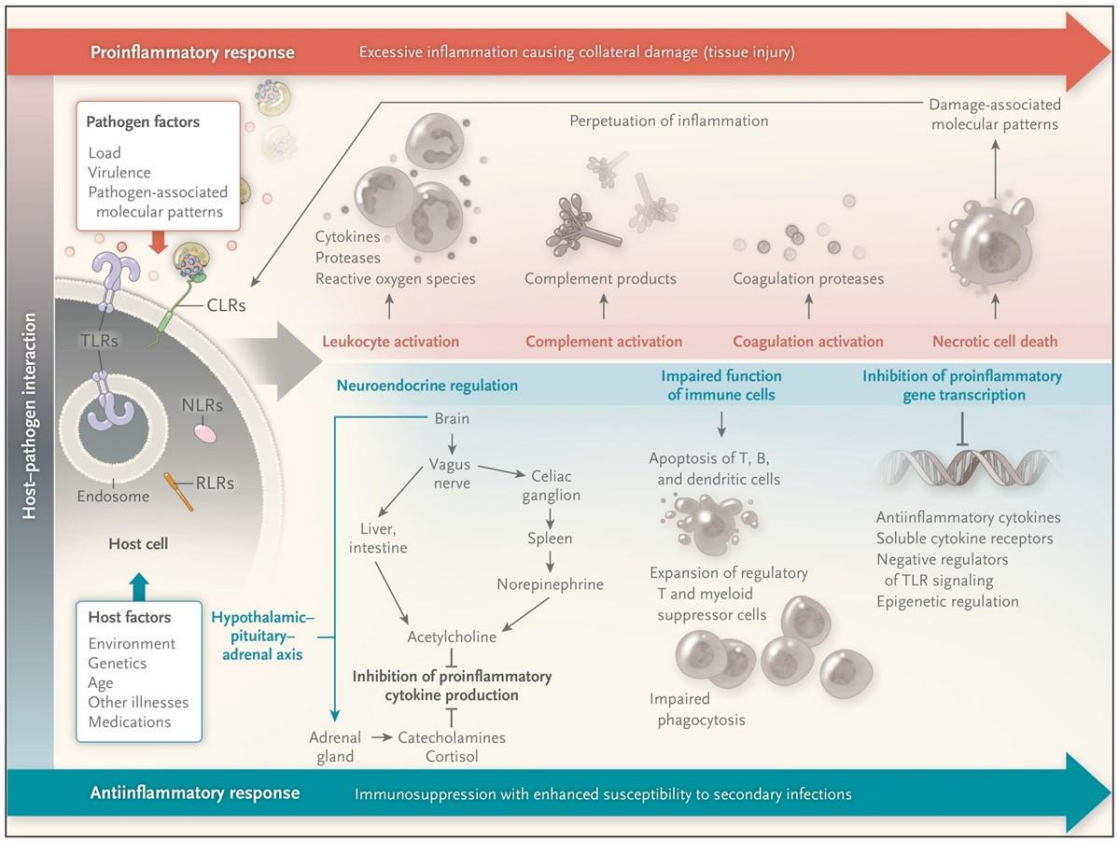

Figure 2. The host response in sepsis. Reproduced with permission from (Angus DC, van der Poll T. N Engl J Med 2013;369:840-851), Copyright Massachusetts Medical Society.

The anti-inflammatory response is promoted by humoral regulation (neuroendocrine and adrenal axis inhibition of proinflammatory cytokines), impaired immune cell function (apoptosis of T- and B-cells, expansion of regulatory T-cells and reduced phagocytosis) and inhibition of proinflammatory gene transcription [3,10]. When dominating, the anti-inflammatory response enhances susceptibility to secondary infections, especially in patients surviving the initial phase of sepsis [3].

1.1.3 Clinical sepsis criteria

1.1.3.1 Sepsis-1 and 2

Although sepsis had been well known for decades, the first attempt to phrase a clinical consensus definition was done in 1991 [20]. The main purpose of the definition was to introduce

a similar terminology when assessing and comparing clinical trial results and novel treatments [21]. The Sepsis-1 definition introduced the term systemic inflammatory response syndrome (SIRS) which was based on: (1) Body temperature higher than 38°C or lower than 36°C, (2) Heart rate higher than 90/min, (3) Hyperventilation evidenced by respiratory rate higher than 20/min or PaCO2 lower than 32 mmHg, and (4) White blood cell counts higher than 12,000 cells/µl or lower than 4,000/µl [20]. The definition included: "Sepsis" - infection with SIRS $\geq$2 points, "severe sepsis"- sepsis associated with organ dysfunction, hypoperfusion or hypotension, and "septic shock" - sepsis with hypotension despite adequate fluid resuscitation [20]. In 2001, the sepsis definition was updated with adding additional signs and symptoms of sepsis, known as Sepsis-2, but the general concept was kept [22]. Over the years, SIRS attracted a lot of criticism, mainly due to poor specificity [23]. One study showed that half of admitted patients developed SIRS sometime during their hospital stay [24]. Similar results were reported from ICUs where Sprung et al. noted that 93% of patients reached the 2 points threshold sometime during their stay [25].

1.1.3.2 Sepsis-3

In 2016 the clinical sepsis definition was revised to overcome the inaccuracy of SIRS and the subjectivity of organ dysfunctions assessment that was evident in prior definitions. A data driven approach was used to produce generalizable criteria for both "suspected infection" and "organ dysfunction" [26]. A main goal was to create operational clinical criteria that could offer consistency in epidemiological studies, clinical trials, as well as be used for patient recognition and management [9]. In Sepsis-3, the SIRS criteria were omitted, and the definition was simplified to include only "sepsis" and the subgroup "septic shock" [9].

- *Sepsis:* Suspected infection was defined as having any microbiologically sample taken and receiving antimicrobial therapy within a predefined time frame. Organ dysfunction was measured as maximum sequential organ failure assessment (SOFA) score 48 hours before to 24 hours after onset of infection and compared to a baseline SOFA score before this time window. An increase in SOFA score by 2 or more points was set as the threshold for sepsis and was associated with an in-hospital mortality of approximately 10% [26]. The SOFA score had been developed previously and is based on a structured assessment of 6 different organ systems (cardiovascular, respiratory, coagulation, central nervous system, liver and renal) [27].

- *Septic shock:* Defined as "*a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than sepsis alone*" [28]. In clinical practice, this was defined as patients that require vasopressors to maintain a mean arterial pressure above 65 mmHg and have a serum lactate level greater than 2 mmol/L in the absence of hypovolemia. Using this definition, septic shock was shown to be associated with an in-hospital mortality of approximately 40% [28].

After the Sepsis-3 definition was presented, validation studies in both retrospective and prospective patient populations have supported the use of SOFA score for assessing organ dysfunction in infected patients to predict in-hospital mortality [29–32]. However, the SOFA-score was originally developed for the ICU-setting, but with the introduction of the Sepsis-3 clinical criteria, the target population had now broadened and included more diverse settings. This becomes problematic when evaluating patients for sepsis in non-ICU or resource-limited settings. One of the primary concerns is how respiratory dysfunction is calculated using the SOFA score [33]. The SOFA respiratory score is based on the ratio between PaO2 and FiO2, as well as data on ventilator support. This requires arterial blood gas analysis and reliable measurements of FiO2. Blood gas analysis is an invasive test which necessitates specific training and is associated with potential severe complications [34]. Furthermore, blood gas analysis requires a laboratory infrastructure and cannot be readily used for continuous respiratory monitoring. In the large multihospital data set used to develop the Sepsis-3 criteria, less than 30% of non-ICU patients had arterial blood gas measurements available, meaning that the respiratory function was not possible to evaluate in the majority of these patients [26].

1.1.4 Epidemiology of sepsis

In population-based studies from Europe and the US, the incidence of bloodstream infection ranges between 113-220 per 100,000 population, of which approximately 30-50% are nosocomial [35–37]. Data from large Scandinavian cohorts have shown increasing incidences of BSI during recent years, and approximately one third of these were hospital-onset [38,39]. The global incidence of sepsis has been more difficult to assess, mainly because of limitations of the Sepsis-1 and -2 definitions [40]. The current estimates ranges from 30-50 million episodes and 6-11 million deaths globally and 1.7 million adult episodes and 250,000 deaths in the United States (U.S) per year [41–43]. A population-based Swedish study by Mellhammar et al. assessed all admitted patients in two healthcare regions receiving intravenous antimicrobial treatment and classified them according to both Sepsis-2 (severe sepsis and septic shock) and Sepsis-3 definition [44]. They estimated the annual incidence according to Sepsis-2 as 687 per 100,000 persons and according to Sepsis-3 as 780 per 100,000 persons, with an in-hospital mortality of 19.8% and 17.4% respectively.

In a 2016 meta-analysis by Fleischmann et al., data from 27 population-based studies between 1979 to 2005 were used to estimate a global hospital-treated population incidence rate of 288 sepsis cases per 100,000 person-years and 148 severe sepsis cases per 100,000 person-years [42]. When restricting the inclusion to only the last 10-years, the incidence rate increased to 437 for sepsis and 270 for severe sepsis per 100,000 person-years respectively. In-hospital mortality was 17% for sepsis and 26% for severe sepsis. The included studies had predominately used

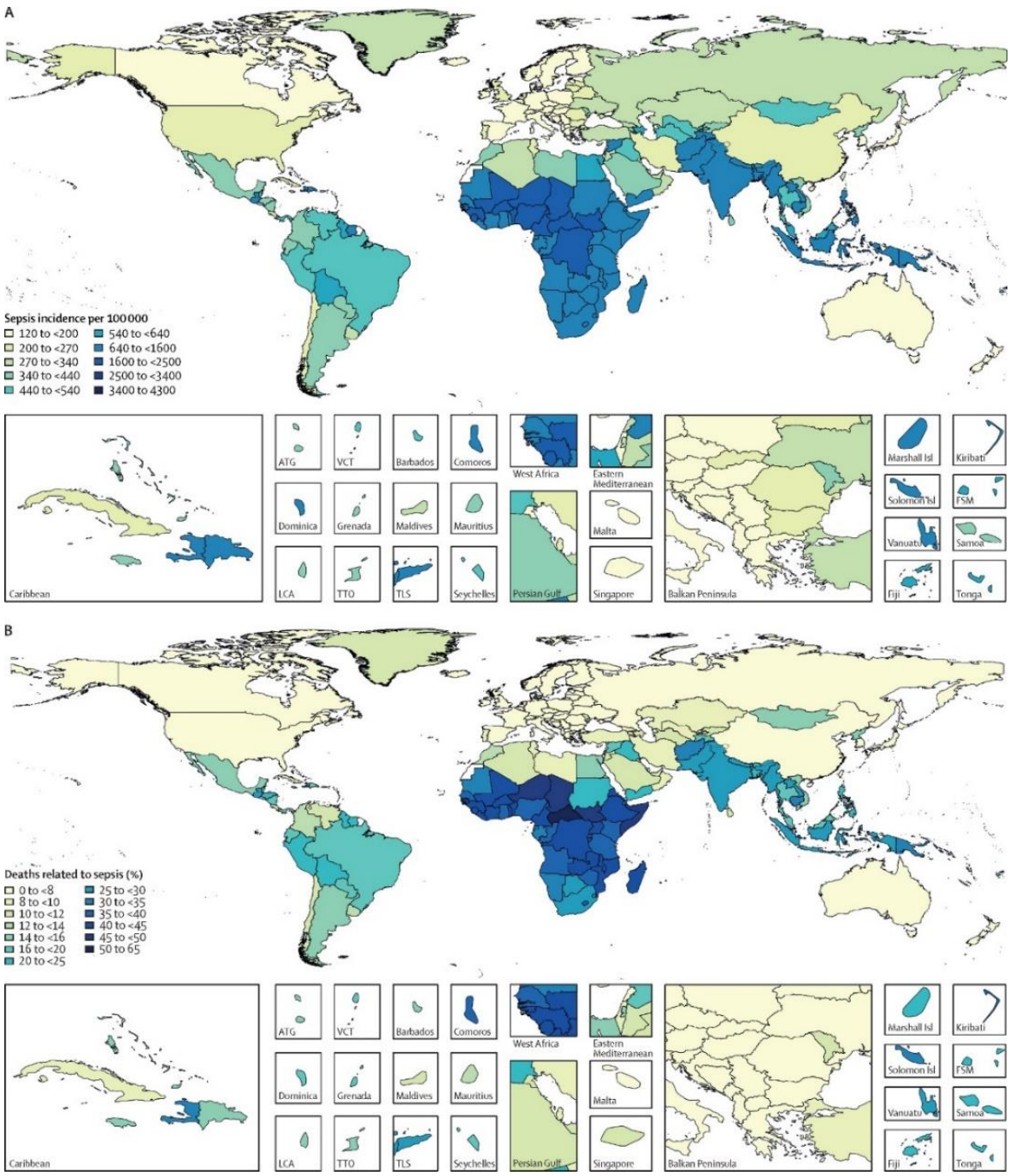

Figure 3. The global burden of sepsis. The figure is reproduced from (Rudd KE, Johnson SC, Agesa KM, et al. Lancet. 2020 Jan 18;395(10219):200-211), which is published under a Creative Commons Attribution 4.0 International (CC BY 4.0) license.

International Classification of Diseases (ICD) codes to classify sepsis cases. A recognized major limitation of the Fleischmann study is that it did not include estimates from low- or middle-income countries. In 2020 Rudd et al. published the most comprehensive sepsis incidence study to date based on the Global Burden of Disease Study of more than 100 million individuals, addressing the previous limitations of the Fleischmann study [43]. They reported an almost doubled estimate of incident sepsis cases compared to previous estimations, largely attributed to people living in areas with lower Socio-demographic index, and estimated that

approximately 20% of all global deaths may be sepsis-related (Figure 3). Another prospective study focusing on a multinational ICU population found that 29.5% of patients hospitalized in the ICU for longer than 24 hours, suffered from sepsis with an in-hospital mortality rate of 25.8% [45]. Mortality in the ICU population increased substantially with lower national income, suggesting association of ICU resources and sepsis outcome.

1.1.5 Healthcare-associated infections: providing a case for surveillance

WHO defines healthcare-associated infections (HAI) as "*An infection occurring in a patient during the process of care in a hospital or other health care facility, which was not present or incubating at the time of admission. Health care-associated infections can also appear after discharge*" [46]. In older literature, the term "nosocomial" is commonly used to describe what today is known as healthcare-associated, hospital-acquired, or hospital-onset infection. In contrast, infections that do not fulfil the HAI definition are typically described as present on admission, community-acquired or community-onset. Unfortunately, all of these are terms are overlapping and have been used to describe different patient populations in the literature.

A rational for considering HAI as a separate entity is the spectrum of pathogens. Distribution of microorganisms differ according to source of infection, but overall the most frequently isolated pathogens reported from the latest European center for disease prevention and control (ECDC) point prevalence surveys (PPS) were: *E. coli* (16.1%), *S. aureus* (11.6%), *Klebsiella spp.* (10.4%), *Enterococcus spp.* (9.7%), *P. aeruginosa* (8.0%) and *Clostridoides difficile* (7.3%) [47]. In U.S, similar microorganisms are reported but notably, the ratio of *C. difficile* (12.1%) is higher and the ratio of *E. coli* (9.3%) is lower [48]. In several of these microorganisms, emerging resistance is a critical problem, especially carbapenem-resistant *P. aeruginosa*, carbapenem-resistant and third-generation cephalosporin-resistant *Enterobacterales*, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *S. aureus* [8,49,50].

The risk of HAI is dependent on factors associated either with the risk of colonization or impaired host defense [51]. A large part of HAIs are also considered preventable if compliance to evidence based guidelines are good, especially for catheter-associated BSI [52–55]. HAI is associated with substantial individual and economic cost attributed to increased length of stay, re-admissions, increased morbidity and increased mortality [56]. In Sweden the cost of HAI has been estimated to SEK 6.5 billion [57]. According to a study from the U.S, the annual costs for the 5 major HAIs were estimated to approximately $9.8 billion between 2011 to 2013 [58]. Surgical site infections contributed to the largest overall cost, but catheter-associated BSI had the highest cost per-case, approximately $45,814 [58]. Similar data from Europe, based on the ECDC PPS in 2011 to 2012, showed that pneumonia and primary BSI accounted for the highest burden of HAI (approximately 60% of the total burden) [59]. This was mainly due to the severity of pneumonia and BSI compared to the other infections. In U.S hospitals, healthcare-associated sepsis after invasive surgery had an attributable length of stay of 10.9 days and costs of $32,900 [60]. For healthcare-associated sepsis not related with surgery the attributable length of stay was

1.9 to 6.0 days and the cost was $5,800 to $12,700. Recent data have also reported an approximately doubled mortality in hospital-onset sepsis compared with community-onset sepsis [61].

When calculating the burden of HAI, there is also a need to account for the cost of antimicrobial resistance. In 2018, Cassini et al. estimated the incidence of infections with 16 bacteria with antimicrobial resistance (AMR) based on data from European Antimicrobial Resistance Surveillance Network (EARS-Net), and ECDC PPS and a systematic review of the literature [62]. They estimated that in 2015, a total number of 671,689 infections due to AMR bacteria occurred in Europe of which 63.5% were healthcare-associated, and resulted in 33,110 attributable deaths. In 2022, a comprehensive study of the global burden of antimicrobial resistance (AMR) was published [63]. The study estimated that, in 2019, almost 5 million deaths was associated with AMR, and approximately 1.3 million deaths was attributed to AMR, with the highest burden in low-resource settings.

1.1.5.1 Epidemiological surveillance of healthcare-associated infections

Surveillance of HAI, combined with feedback to healthcare personnel and policy makers, is central to identify areas of improvement and to guide interventions in infection control programs [64]. In general, incidence surveillance is preferred over prevalence data to enable better comparisons of infection rates between hospitals. Incidence surveillance requires classification of both the HAI (numerator), usually as binary classifier, and relevant denominator data from the entire screening population [65]. For surveillance purposes HAIs have traditionally been defined according to the infectious source. The Both the CDC/National healthcare safety network and ECDC provides detailed HAI definitions [66–68]. In Sweden, similar definitions have been developed by the Swedish association of local authorities and regions [69]. A general concept in all definitions is a time criterion requiring that onset of infection must occur at calendar day 3 or later after admission to hospital. However, as more advanced care is moved from the hospital setting to the patients' home, the concept of HAI gets more complicated [70,71]. As a consequence, all HAI criteria include predefined exceptions to the time criterion and present on admission infections can often be categorized as HAI. Such exceptions are patients that where discharged from acute care hospitals in the preceding 48 hours, patients that had a relevant device inserted just before onset of infection or patients with infections clearly related to prior surgery. Data from PPS in acute care hospitals has shown an overall HAI prevalence of 5.9% in Europe, 4% in the U.S. and 8.9% in Sweden [47,48,72]. In EU/EES, the yearly HAI incidence was estimated to 3,758,014 patients during the period 2016 to 2017 [47]. The prevalence was higher in European tertiary care hospitals (7.1%) compared to primary care hospitals (4.4%) and the prevalence was highest among patients admitted to intensive care units (19.2% of patients). BSI accounted for 10.8% of HAIs in Europe and 9.9% of HAIs in the U.S. Sweden lacks a specific definition of BSI, but includes primary sepsis as source of infections, which during 2018 accounted for 6% of all HAIs [72].

1.1.5.2 Definitions of healthcare-associated sepsis and bloodstream infections

Healthcare-associated sepsis is poorly defined since the HAI criteria focus mostly on specific sites of infections or microbiologically confirmed infections. The ECDC has defined criteria for treated unidentified severe infection in adults and children, but refer to it as a last-resort definition not intended for use unless absolutely needed [67]. The CDC/NHSN, on the other hand, has not issued a distinct HAI definition for sepsis at all [68]. In contrast, the concept of BSI is well defined in both the CDC/NHSN and ECDC documents, but contains some key differences necessary to acknowledge. The CDC/HNSN labels infections of the bloodstream as laboratory confirmed BSI (LCBI), while ECDC simply uses the label BSI. In both definitions, a LCBI or BSI is evident if a recognized pathogen is isolated from a blood culture, or if a predefined skin contaminant is isolated from 2 or more blood cultures collected within a certain time frame. In CDC/NHSN the criteria of LCBI is fulfilled only if no other source of infection was identified. LCBI are further categorized as either CLABSI (Central line-associated BSI) or non-CLABSI [73]. On the contrary, the ECDC BSI definition includes also BSI secondary to other sites of infections as well as BSI related to vascular catheters. In addition, the ECDC definitions includes a separate category for microbiologically confirmed central-venous catheter and peripheral-venous catheter related BSI, which is not present in the CDC/NHSN document. The differences between the definitions have to be considered when comparing surveillance data from different countries, as well as within countries that have switched between the definitions. This was demonstrated by Djuric et al. who compared the CDC/NHSN and ECDC criteria for BSI and found perfect agreement (kappa=1) if studying BSI with no other source of infection, but only substantial agreement when considering "overall BSI" (kappa=0.79) [74].

1.1.5.3 Clinical concepts of healthcare-associated infections

In parallel with the definitions of healthcare-associated BSI developed by CDC/NHSN and ECDC for surveillance, other simpler classifications have been proposed for clinical purposes, such as guiding treatment decisions. Traditionally, BSI had only been distinguished as community-acquired (onset of BSI <48 hours after hospital admission) or nosocomial (onset of BSI >48 hours after hospital admission), based on pathogen distribution. A study by Weinstein et al. in 1997 noted a change in the epidemiology of BSI in their own hospital during a 20-year period [75]. Compared to the mid-1970s, the proportion of BSI classified as nosocomial changed from two thirds to approximately half in the mid-1990s [75,76]. In 2002, first Siegman-Igra et al. and then Friedman et al. suspected that changes in healthcare utility required new BSI definitions [77,78]. Friedman et al. performed a prospective observational study including all patients admitted with BSI and introduced a new classification scheme that distinguished between community-acquired, healthcare-associated, and hospital-acquired BSI. The new concept of healthcare-associated BSI included patients with BSI present on admission, but who had: (1) received intravenous therapy, wound care, or specialized nursing care within 30 days,

(2) received hemodialysis within 30 days, (3) had been hospitalized in an acute care hospital for 2 days or more in the last 90 days, or (4) resided in a nursing home or long-term care facility [78]. Their results showed a clear similarity between healthcare-associated BSI and nosocomial BSI, with regards to causing pathogens, antimicrobial resistance, and mortality. The definition has had a substantial impact on classification of BSI in epidemiological studies, clinical care of BSI patients and affected recommendations for empirical antimicrobial therapy [79–84]. In the study by Friedman et al., 28% of BSI was classified as community-acquired, 37% as health care–associated and 35% as hospital-acquired. However, in sepsis, the spectrum is different. In a study of 307,491 sepsis cases, 62.8% were community-acquired, 25.9% were healthcare-associated and 11.3% were hospital-acquired severe sepsis [6].

1.1.6 Methods to monitor sepsis incidence

The most common data source in sepsis surveillance has been administrative hospital data, that is discharge diagnosis, or mandatory reporting to specific databases [85,86]. However, this approach carries risk of bias and make comparison between hospitals difficult [41,87]. It has been shown both in Swedish and international reports, that the use of diagnosis codes was associated with considerable variability in reporting [88,89]. Studies have suggested that reports of increased sepsis incidence during later years can partly be attributed to changes in coding rather than a true rise in incidence [90–95]. For sepsis mortality trends, on the other hand, claims data seems to better reflect a true decline [96].

As seen by the differences in sepsis incidence by Fleischmann et al. and Mellhammar et al., ICD-coding tend to underestimates the incidence of sepsis in comparison to using clinical data [42,44]. Similar results have been seen in other studies where medical record review was compared to ICD-codes [97,98]. However, medical record review to classify if patients have sepsis or not is both resource intensive and associated with high levels of subjectivity and variations between different clinicians [99,100]. With the increasing use of electronic health records (EHR), the new Sepsis-3 definition has enabled a more objective sepsis incidence surveillance based on clinical data. Recently, a case definition, denoted Adult Sepsis Event, was developed by the CDC to simplify automatic sepsis surveillance using EHR data [101]. Rhee et al. used this method to study incidence trends in 409 U.S hospitals and compared the results to claims data based on ICD-codes [102]. They found that sepsis occurred in 6% of all adult hospital admission and in-hospital mortality in these patients was 15%. Although ICD-codes indicated a rising trend in sepsis incidence during the study period, objective classification of clinical data failed to demonstrate any changes in neither incidence nor in-hospital death. In addition, the EHR-based classification was more sensitive in diagnosing sepsis (69.7% for EHR-based vs 32.3% for ICD-codes), when using physician review as the reference standard. Furthermore, Page et al. showed that the criteria identified several serious nosocomial infections missed by currently reportable HAIs in a U.S setting [103]. The major limitation of the CDC-definition is that it varies form Sepsis-3 clinical criteria by not assessing organ dysfunction according to SOFA-score. The definition is dependent on treatment interventions for 2 organ dysfunction criteria

(cardiovascular and respiratory). This biases sepsis surveillance based on access to ICU care and towards patients qualifying for aggressive treatment, which limits generalizability to all hospitalized patients.

1.1.7 Early sepsis recognition

In sepsis, timely recognition and treatment is key for survival, warranting structured approaches to guarantee early identification [104]. The 2016 Surviving Sepsis Campaign Guidelines recommend hospitals to have sepsis screening for all acutely ill, high risk patients, but it does not specify which method to use [105]. To succeed with sepsis screening programs, it is necessary to begin with educational efforts, building new routines and generating behavioral changes [106–109]. Such programs require multi-professional involvement of nurses, clinicians, administrative staff and policy makers [105]. Most acute care hospitals have implemented triage systems at admission, followed by continuously or intermittent monitoring of patients during the remaining of the care episode until discharge [110]. Both triage and monitoring systems are built on clinical decision rules, such as National Early Warning Score (NEWS2), Modified Early Warning Score (MEWS) and the Swedish Rapid Emergency Triage and Treatment System (RETTS) [110–113]. These scores are general in scope and developed to detect deteriorating patients independently of cause. The major scores aimed specifically for sepsis in clinical use are based on SIRS or the quickSOFA (qSOFA) [114–116]. In addition, the surviving sepsis campaign website have collected locally developed sepsis screening scores, most of them based on SIRS [117].

The effectiveness of automated alerting systems compared to standard of care for sepsis management was evaluated in a meta-analysis from 2022 [118]. A total of 36 studies were included of which only 6 were randomized control trials (RCTs) [118]. The meta-analysis concluded a favorable effect of using automated alerts for identifying sepsis and suggested that machine learning monitoring systems combined with clinical interventions may be a way forward, especially for the non-ICU setting.

1.1.7.1 Basic principles of screening tools

The demands of a sepsis screening tools differ depending on the screening population, both with regards to data availability (high-resolution or low-resolution) and screening frequency (single, intermittent, or continuously). In hospitals, the three major screening populations are located at the Emergency department (ED), the wards and the ICU [119]. The majority of published sepsis screening tools are based on simple heuristic scoring systems, which are either calculated manually on paper or automatically from EHR data. Harrison et al. has listed key features that needs to be addressed before successfully implementing automated sepsis alert systems [115]. Most important, screening algorithms needs availability of real-time patient data. Second, screening algorithms must perform well, with a particular focus on the positive

predictive value (PPV) to decrease information overload and alert fatigue. Third, the alert system must be integrated in the clinical workflow and elicit a meaningful response to the alert that improves patient care.

Evolution of wearable patient monitoring systems, machine learning models and novel alert delivery systems all have the potential to improve early sepsis recognition further [115]. In particular, machine learning methods have gained increasing interest due to their ability to classify (diagnosing) and predict (prognosis) [120–123]. Although the models per se are not novel, the advances in processor speed and digitalization of healthcare data has boosted the field [124]. Chen et al. have described some key concepts of machine learning in healthcare, which are important to consider also for sepsis screening tools based on these techniques (Table 1) [125]. It is worth mentioning that the well-established practice of dividing data into development and test data sets have been challenged and Riley et al. argued for resampling methods such as bootstrapping or repeated cross-validation instead, especially when using regression methods and smaller data sets [126].

Table 1. Development of machine learning models in healthcare based on Chen et al.[125]

Development steps	Key concepts
Problem	Choose a suitable task where high quality data is available
Data processing	If an external validation data set is not available, divide the data into two separated datasets, a development set (for training and tuning parameters) and a hold-out validation set (for evaluating model performance). Assure good label quality. Acknowledge that a random split can have impact on model performance, especially in small data sets [126]
Model building	Use only the development set for model building. Choose between supervised or unsupervised models and train it with sufficient amounts of data. Decide on a strategy for feature selection, pre-processing of variables and how to handle missing data and class imbalance. Control hyper parameter optimization. Avoid underfitting by choosing a model with appropriate capacity in relation to the data complexity. Avoid overfitting by using a tuning set (or cross validation) within the development set and/or different regularization techniques
Evaluation	Assess performance in one or several external validations set with discrimination metrics (threshold-free: area under the receiver operating characteristic curve, area under the precision recall curve, c-statistics, and threshold-dependent: sensitivity, specificity, positive predictive value) and/or calibration metrics (matching between predicted probabilities and actual probabilities, such as Hosmer-Lemeshow statistics). Acknowledge that the generalizability of the model is dependent on the heterogeneity of the validation set. Compare the model with a relevant baseline
Pre-implementation	Evaluate user interaction, workflow integration, alert burden and potential clinical impact in real world scenarios

1.1.7.2 SIRS-based screening

SIRS was initially developed as part of the sepsis definition, but has been evaluated as a screening score in several studies [116]. When applied as such, it has usually been modified, such as adding measures of lactate, blood pressure, signs of infection or organ dysfunction [127–129]. Several studies have evaluated models based on automated scoring with alerts transmitted via paging or the EHR system [130–137], but paper based nurse-driven screening tools have also been reported [138,139]. Most of these screening systems have been combined with other interventions such as educational efforts and sepsis response teams, hence, the direct effect of the sepsis screening tool is difficult to evaluate. Many studies have showed improved adherence to sepsis process measurements, but data on mortality improvement is elusive [116,140]. A study by Torsvik et al. showed that development of organ failure and mortality decreased after implementing a structured nurse-led sepsis identification tool based on SIRS in a Norwegian hospital [141]. However, this was a before-and-after study of a defined BSI population, but data from RCTs including general hospital populations have failed to show a similar impact of specific sepsis screening. Three RCTs evaluating automated sepsis screening systems compared to standard care (2 from ICU, 1 from ward setting) have been published [133,136,137,142]. None of them showed significant differences in time to antimicrobial treatment, length of stay or in-hospital mortality.

1.1.7.3 qSOFA-based screening

In the Sepsis-3 definition, a new simpler scoring system was proposed called qSOFA [26]. The score is based on assessment of 3 organ system: altered mental status, respiratory rate more than 22 breaths per minute and low systolic blood pressure of 100 mmHg or less [26]. It is important to acknowledge that qSOFA was not developed as a screening score for sepsis, but rather as a prognostic score in patients with sepsis [140,143]. When assessed in patients outside of the ICU, a qSOFA score of 2 or more points performed well in predicting poorer sepsis outcomes such as in-hospital death and organ failure in several different patient populations [26,144–147]. However, qSOFA has several limitations when assessed as a sepsis screening score, such as long time to trigger and low sensitivity [144,148–153]. qSOFA also performed generally worse when compared to existing systems, such as NEWS or MEWS [154]. Based on these data, the qSOFA is considered insufficient to use as alone for sepsis screening and was not recommended in the 2021 Surviving Sepsis Campaign guidelines [23,105,155].

1.1.7.4 NEWS2-based screening

The NEWS score was first presented in 2012 by the Royal College of Physicians in United Kingdom to standardize clinical monitoring [110]. Since then the score has been implemented in many hospitals around the world, including Sweden. In 2017 an updated version was released, NEWS2, which had been optimized specifically for sepsis recognition. The NEWS score is based on measurements of respiratory rate, peripheral oxygen saturation (SpO2), oxygen

treatment, systolic blood pressure, pulse, consciousness, and temperature. NEWS have been applied as an automated score for sepsis screening [156]. In a recent study NEWS outperformed both SIRS and qSOFA in predicting meaningful outcomes such as in-hospital mortality and ICU-admission [116,157]. These results were later confirmed by Usman et al. who showed better accuracy for sepsis detection (area under receiver operating characteristics curves [AUROC] for NEWS=0.91, SIRS=0.88 and qSOFA=0.81) and sepsis-related mortality (AUROC for NEWS=0.95, SIRS=0.89 and qSOFA=0.87) when applied in the ED [158]. For sepsis detection NEWS had sensitivity 84.2% and specificity 85.0%, compared to SIRS (sensitivity 86.1% and specificity 79.1%) and qSOFA (sensitivity 28.5% and specificity 98.9%).

1.1.7.5 Screening based on machine-learning models

As indicated by several systematic reviews published recently, the field of machine learning based models to predict sepsis has become increasingly popular [159–163]. Studies have primarily used large EHR databases or the publicly available Medical Information Mart for Intensive Care-III (MIMIC-III) ICU database from a single center in the U.S for either development, validation or both [164]. The latter is restricted to ICU patients. There is also substantial inconsistency between sepsis definitions, data pre-processing methods, feature engineering, models and evaluation metrics, which makes direct comparison of studies complicated [165]. The machine learning models generally show good ability of predicting sepsis onset in retrospective data (AUROC between 0.80 to >0.90), and when assessed with a comparator they always performed better than scores such as qSOFA and MEWS. However, few studies clearly presented data on PPV. A number of scores have been published such as: Thiel et al. (Recursive Partitioning And Regression Tree, non-ICU population) [166], Risk of Sepsis score (gradient tree boosting, ED-population) [167], Artificial Intelligence Sepsis Expert (cox proportional hazards, ICU-population) [168], InSight (gradient tree boosting, ICU-population) [169–171], EWS2.0 (random-forest, non-ICU population) [172], TREWScore (cox proportional hazards, ICU-population) [173], and the Epic Sepsis Model (ESM) developed by the EHR-vendor Epic (logistic regression, non-ICU population) [174]. In addition, two studies have used Bayesian network models to predict sepsis and organ dysfunction resulting from sepsis [175,176].

Several of these models have also been implemented in clinical practice, all in the US, with mixed results (Table 2). In general, the quality of these studies is low and evaluation is mostly based on pre- and post-implementation designs. As an example, two studies evaluating the Insight algorithm only reported differences in sepsis-related outcomes between the periods and did not assess the direct impact of the screening, precluding interpretation of its effectiveness [177,178]. This is usually problematic since most of these implementations are coupled with educational efforts raising awareness of sepsis among healthcare works. Only two small single center RCTs have been reported, one of Insight in an ICU-setting, and one of ESM (Epic) in an ED-setting [174,179]. The Insight study enrolled all patients admitted to the ICU and randomized them to surveillance with the algorithm and telephone delivered alerts, or standard of care. In the intervention group, there was a significant 12.4% relative risk reduction of in-hospital

mortality, as well as decreased length of stay [179]. However, the number of sepsis cases was not clearly reported, and it is difficult to rule out that the effect of the algorithm came from increasing clinician awareness of high-risk patients rather than by predicting sepsis. The ESM alert was evaluated in a single ED and included an EHR-based alert combined with EHR-based pharmacist notification, or standard of care [174]. All ED patients were screened, but only patients who were flagged by the algorithm were enrolled, except for patients evaluated primarily for trauma, stroke, cardiac ischemia rule out or acute blood loss. The intervention was only associated with a modestly shortened time to antibiotic administration, but no increase in other clinical interventions [174]. However, it was not clear from the study if the patients actually had sepsis, or how many sepsis episodes the ESM missed, and without this information the study results are difficult to interpret. This is especially important since external validation of the EMS score previously have shown that it missed 67% of patients with sepsis and generated a large burden of false positive alarms in 18% of all hospitalized patients [180].

1.1.7.6 Screening scores for bloodstream infection

Most screening tools for BSI are also based on heuristic scores derived from regression models to predict blood culture positivity [181–186]. Much focus has been on developing scores for specific pathogens in BSI, such as extended-spectrum β-lactamase (ESBL)-producing *Enterobacterales* [187,188]. Few more advanced screening tools for BSI prediction have been published, but one model based on a Bayesian network (TREAT) have shown good ability to stratify patients according to risk of BSI as well as predicting specific BSI pathogen [189–191]. The TREAT model was also evaluated in a multicenter cluster randomized trial and showed improved rates of appropriate empirical antimicrobial treatment while reducing the use of broad-spectrum therapy [192]. Studies based on other machine learning methods, such as deep neural networks or decision trees, have been reported with high AUROC, but low area under precision-recall curve (APR) and insufficient performance in external data sets [193–195]. A research group in the Netherlands used an opposite approach and aimed at developing a machine learned prediction tool integrated in the EHR to identify patients with low risk of having positive blood cultures [196]. Their model performed well (AUROC 0.76 [95% CI, 0.71-0.81]) in a multicenter prospective validation cohort and the authors suggested that blood cultures could be safely withhold in 30% of patients in the ED when using their model [197]. For prediction of mortality in BSI, the Pitt bacteremia score was developed to predict mortality in gram-negative bacteremia [198]. A quick Pitt (qPitt) score has also been developed, showing better discriminative performance for mortality than both qSOFA and SIRS [199].

Table 2. Publications of implemented machine-learning based sepsis screening tools

Algorithm	Year	Method	Study design	Setting (Country)	Sample size	Conclusion
Thiel et al.[200]	2011	RPART	Prospective trial, single center, of 2 intervention wards and 4 control wards. Patients were screened and included if a positive alert was triggered	Non-ICU (US)	300	Increase in sepsis process measures in the intervention group, but no difference in ICU-admission, LOS or in-hospital mortality compared to the non-intervention group
Insight[177]	2020	Gradient tree boosting	Pre-post study, multi-site, of a telephone delivered alert. Patients were included if SIRS >2p and ≥1 organ dysfunction criteria	All wards (US)	17 758	Reduction in sepsis-related in-hospital mortality, LOS and 30-day readmission rate. No data on the direct impact of the alerts
Insight[178]	2017	Gradient tree boosting	Pre-post study, single center, step-wise tuning of a telephone delivered alert. Patients were included if they fulfilled sepsis criteria	All wards (US)	1328	Reduction in sepsis-related in-hospital mortality, LOS and 30-day readmission rate. No data on the direct impact of the alerts
Insight[179]	2017	Gradient tree boosting	RCT, single center. All patients admitted to the ICU were enrolled and randomized to surveillance with the algorithm and telephone delivered alerts or standard of care	ICU (US)	142	Reduction in in-hospital mortality (1.25% absolute reduction) and LOS
EWS2.0[172]	2019	Random-forest	Pre-post study, single center, comparing a period of silenced alerts with a period of combined EHR and text messages based alerts	Non-ICU (US)	54 464	The alert had a real-world sensitivity of 26%, specificity of 98% and PPV of 29%. It resulted in a small increase in lactate testing and intravenous fluid administration, but no difference in mortality, discharge disposition, or transfer to ICU
TREWScore[201]	2022	Cox proportional hazards	Prospective, multi-site. Only patients with sepsis who were identified before initiation of antimicrobial treatment were included in the primary analysis	All wards (US)	6877	Patients with alerts confirmed by a clinician within 3h had reduced in-hospital mortality (3.3% [CI 1.7-5.1%] absolute reduction), organ failure and LOS
ESM (Epic)[174]	2021	Logistic regression	RCT, single center. All patients admitted to the ED were screened and randomized to an EHR-based alert combined with EHR-based pharmacist notification or standard of care if an alert was triggered	ED (US)	598	The intervention was associated with a modestly shortened time to antibiotic administration. Patients evaluated primarily for trauma, stroke, cardiac ischemia rule out or acute blood loss were excluded

Abbreviations: recursive partitioning and regression tree (RPART), systemic inflammatory response syndrome (SIRS), randomized controlled trial (RCT), electronic heath record (EHR), intensive care unit (ICU), length of stay (LOS), positive predictive value (PPV) and confidence interval (CI)

1.2 CLINICAL SEPSIS MANAGEMENT

The Surviving Sepsis Campaign Guidelines have published evidence-based recommendations since 2004 [105]. As opposed to the 2016 version of the guidelines, the updated 2021 version have also been endorsed by the Infectious Diseases Society of America (IDSA) [202]. In addition, local sepsis guidelines are published by Swedish Infectious Diseases Society [203]. Based on the studies supporting these guidelines, a brief summary of the key aspects in clinical management of sepsis is presented below.

1.2.1 Sepsis bundles

Educational efforts and the introduction of care bundles in sepsis have previously shown to increase both guideline compliance and reduce in-hospital mortality [107,204–206]. Stakeholders such as the Surviving Sepsis Campaign Guidelines and the US Centers for Medicare & Medicaid Services, have issued sepsis bundles to be performed within 1 to 3 hours after sepsis recognition [105,207,208]. They require clinicians to collect blood cultures, measure lactate, initiate broad-spectrum intravenous antimicrobials and administer 30 mL/kg of intravenous crystalloid fluid for hypotension or lactate ≥ 4 mmol/L. Additionally, if hypotension persists during or after fluid resuscitation, initiation of vasopressors are recommended to maintain mean arterial pressure ≥ 65 mm Hg. Similar bundles have also been adopted by the national Swedish sepsis guidelines [203]. Reporting adherence to the SEP-1 bundle became mandatory for US hospitals in 2015, however, the beneficial impact of this mandate on the quality of sepsis care has been an area of debate [209]. In a cohort study evaluating 117 510 patients with suspected sepsis from 114 hospitals in the US, the SEP-1 implementation was associated with increased lactate testing rates, but no change in broad-spectrum antibiotic use or mortality rates [210]. Conversely, a sepsis triage system in a Swedish ED which include mandatory support of an Infectious Diseases (ID) physician, reduced time to antimicrobial treatment and length of stay, as well as improved diagnostic procedures and supportive care [211,212]. The beneficial effect of an ID-physician attending sepsis patients in the ED was confirmed by an Italian study that showed both increased guideline adherence and decreased mortality [213]. Furthermore, studies of both sepsis and BSI in general, and *S. aureus* bacteremia in particular, have shown decreased mortality and healthcare cost when involving ID-physicians in the patient care [214–216].

1.2.2 Anti-infectious treatment

There is broad consensus that treatment in sepsis and suspected BSI should be initiated empirically before culture results are available. Estimations are that inappropriate empirical treatment, defined as treatment without *in vitro* activity against the causing bacteria or fungus, occurs in approximately 20-30% of severe infections [217]. Retrospective studies have also shown an association between discordant treatment and higher mortality, in particularly in

hospital-onset BSI [2,218,219]. In 2021, Kadri et al. included 21,608 BSI episodes from 131 hospitals in the US, which is the largest retrospective cohort study to date [220]. They showed an increased mortality risk with inappropriate treatment at 24 hours, irrespectively of antimicrobial resistance or critical illness, but did not assess hourly estimates.

The timing of empirical antimicrobial treatment has been an area of debate for many years[221–223]. Several retrospective studies and meta-analysis have shown better survival with early treatment within 1-3 hours, especially for septic shock [223–229], while other studies have failed to show a benefit [230–234]. The only randomized trial of early antimicrobial treatment in a pre-hospital setting showed no difference in mortality between groups despite a time gain of 96 minutes [235]. A secondary analysis of another RCT evaluating different resuscitation protocols in three US EDs also failed to show an increase in mortality with hourly delays of antibiotics after triage [236]. However, the risk associated with mortality increased if antibiotics were delayed to the time period after shock was recognized. Hranjec et al. performed a before and after investigation of patients with suspected infection and blood cultures collected in a surgical ICU, comparing a strategy consisting of aggressive early antimicrobial treatment, with a conservative policy where antimicrobial treatment was withheld until there was microbiological evidence of infection [237]. They found that changing to a conservative policy was associated with more favorable patient outcomes without increased mortality. The primary argument for not showing an effect of giving prompt antimicrobial treatment is that onset time zero is a vague measure, which may vary greatly even among patients in the same setting, especially in the ED [222]. Naturally, the effect of these differences weakens the longer patients are followed after admission. Another important limitation is that studies of sepsis in general have not accounted for appropriateness of treatment based on *in vitro* pathogen-drug coverage which may have affected the results.

Empirical combination therapy with antibiotics is generally not recommended in sepsis, unless the risk of MDR pathogens are high [105,238,239]. Studies have shown that antimicrobial therapy safely can be narrowed when culture results are known [240,241], and that combination therapy is not warranted as definitive therapy except for specific situations with highly resistant organisms [105,242,243]. Routine use of empirical anti-fungal treatment is probably not necessary in sepsis acquired at the ICU, unless patients have higher risk for fungal infection [105,244]. Furthermore, most guidelines highlight adequate source control as an important intervention in sepsis management [105]. This was supported by a retrospective cohort study by Reitz, et al. which showed that source control within 6 hours of sepsis onset was associated with an approximately 30% reduction in the mortality risk [245]. Other studies, especially in *S. aureus* BSI, have shown similar benefit of adequate source control [246,247].

1.2.3 Treatment of organ dysfunction

A recent meta-analysis including patients from three RTCs (ProCESS, ARISE, and ProMISe) concluded that patients with septic shock should receive standard intensive care, without the need for sepsis specific early goal-directed treatment [11,12,248,249]. Treatment of circulatory failure with balanced crystalloid fluids are associated with a mortality benefit compared to normal saline and HES-solutions [250]. In a large RCT (CLASSIC), restrictions in intravenous fluid volumes did not affect patient outcome [251]. If colloids are considered, albumin is the preferred choice [250]. Among vasopressors, norepinephrine is the most beneficial and should be preferred as first line treatment [252,253]. Vasopressin can be added if insufficient hemodynamic treatment response despite norepinephrine [105]. In the most recent Surviving Sepsis Campaign Guidelines, intravenous corticosteroid treatment is now recommended for all adults with septic shock and ongoing vasopressor treatment based on three recent RCTs (ADRENAL, APROCCHSS and VANISH) [254–257]. Corticosteroids has primarily been associated with faster shock resolution and increased number of vasopressor free days, but no clear effect on mortality has been shown [105]. For red blood cell transfusion, results from the TRISS-trial found no difference between a restrictive compared to a liberal transfusion strategy [258,259].

In non-hypercapnic hypoxic respiratory failure, high-flow nasal oxygen is recommended, but there is insufficient evidence to state any oxygen targets or recommend non-invasive ventilation [260–263]. These recommendations are mainly based on heterogeneous ICU-populations and large studies of specific sepsis populations are sparse. If patients require mechanical ventilations due to sepsis induced ARDS, protective ventilation strategies with low tidal volumes of 6 mL/kg predicted body weight has been show beneficial and are strongly recommended [105]. Prone positioning is recommended in moderate to severe ARDS. In sepsis induced acute kidney injury requiring renal-replacement therapy, there is no difference between continuous (CRRT) versus intermittent hemodialysis (IHD) [264,265]. More recently, the IDEAL-ICU and STARRT-AKI trials did not find any evidence in favor of an early renal-replacement strategy compared to standard or delayed strategy [266,267]. Blood purification strategies, vitamin C, immunoglobulins or sodium bicarbonate therapy are not recommended, except for sodium bicarbonate therapy in severe metabolic academia and acute kidney injury [105].

1.3 CHALLENGING PATHOGENS IN SEPSIS AND BLOODSTREAM INFECTION

Some pathogens causing sepsis have been shown to be particularly challenging. Data from the SENTRY program, which performs global surveillance and monitor pathogens from consecutive BSI episodes, has shown a decreasing trend of *S. aureus* and increasing trend of *E. coli* during the last two decades [268]. *S. pneumoniae* has decreased from 4% during the years 1997-2000 to 1.9% during the years 2013-2016. Importantly, the SENTRY program noticed a stable or declining trend in resistance among gram-positive bacteria, whereas the prevalence of multi-drug resistant (MDR) gram-negative bacteria increased continuously during the 20-year period. This fast spread of MDR gram-negative bacteria in hospitals worldwide has triggered a call for action [49]. Especially infections caused by ESBL-producing *Enterobacterales*, MDR *P. aeruginosa* and carbapenem-resistant *Acinetobacter* spp. have been linked to longer hospital stay, increased costs and higher mortality [269]. Mauldin et al. further quantified the attributable length of stay and hospital cost for HAI caused by AMR gram-negative infections compared to HAI caused by susceptible gram-negatives as an additional 23.8% and 29.3% respectively [270]. Due to this emerging crisis, WHO issued a statement in 2017 calling for more research on problematic bacteria, giving highest priority to resistant *P. aeruginosa*, *A. baumannii* and *E. coli* [8]. In this thesis, we focused on *P. aeruginosa* as model since it is one of the most challenging pathogen in the clinic, both due to high virulence and antibiotic resistance.

1.3.1 *Pseudomonas aeruginosa* bloodstream infection

Population-based studies have shown a *P. aeruginosa* BSI incidence rate of 2.3-6.6/100,000 depending on study [271–273]. Although *P. aeruginosa* is among the top pathogens in gram-negative BSI, it is still an uncommon cause of BSI with reported incidence of roughly 5 cases per 10,000 hospital admissions [274,275]. Mortality is high ranging between 20-40% [271,276]. *P. aeruginosa* has traditionally been associated with hospital-onset BSI, with a peak incidence around the third week of hospitalization [277]. Strictly community-acquired *P. aeruginosa* BSI is rare, but among community-onset healthcare-associated BSI, *P. aeruginosa* is recognized as an important pathogen [84,278]. Main risk factors derived from population-based studies are: increasing age, male sex, diabetes, solid organ transplantation, cancer, hemodialysis, HIV-infection and underlying chronic lung disease [271–273]. MDR *P. aeruginosa* is defined as resistance to at least one antibiotic in at least three of the following antibiotic categories: anti-pseudomonal penicillins + β-lactamase inhibitors, anti-pseudomonal cephalosporins, anti-pseudomonal fluoroquinolones, anti-pseudomonal carbapenems, aminoglycosides, monobactams, phosphonic acids or polymyxins [279]. Extensively drug-resistant (XDR) *P. aeruginosa* is defined as resistance to at least one agent in all but two or fewer of the antimicrobial categories above [279]. It is worth noting that these definitions are currently undergoing revision by the Transatlantic Task Force on Antimicrobial Resistance (TATFAR) (personal communication with Christian Giske). Studies addressing risk factors for

MDR *P. aeruginosa* BSI have recognized important features such as locally emerging bacterial clones, prior hospital/ICU-stay and prior antibiotic use, especially fluoroquinolones and carbapenems [280–283]. Among healthy individuals, colonization of *P. aeruginosa* is rare, but increases with length of hospital stay [284–286]. Severe *P. aeruginosa* infections are most often associated with vulnerable hosts such as neutropenic, cystic fibrosis, bronchiectasis and mechanically ventilated patients (ventilator associated pneumonia) [287]. In addition, patients with extensive skin barrier disruption such as burn or pressure wounds are at increased risk of *P. aeruginosa* infection, as well as infections of the eye (keratitis) and ears (external otitis). An unusual, but important clinical presentation is ecthyma gangrenosum, a cutaneous manifestation associated with *P. aeruginosa* BSI [288].

1.3.2 Treatment of *Pseudomonas aeruginosa*

P. aeruginosa is a difficult to treat pathogen with intrinsic resistance to many broad-spectrum antimicrobials. Inappropriate empirical treatment has been shown to correlate to increased mortality, particularly if patients are presenting with more severe disease, but the evidence is not overwhelming and generally of low quality [276,289–291]. Commonly active agents are shown in Table 3 [279]. For MDR isolates, a few novel as β-lactam/β-lactamase inhibitors and a new siderophore cephalosporin agent have recently been introduced [292,293]. Available data suggest that among the novel agents, meropenem-vaborbactam has limited activity against meropenem resistant *P. aeruginosa* [294]. Even though fosfomycin has been suggested as single treatment for uncomplicated urinary tract infection or combination treatment in more severe MDR *P. aeruginosa* infections [295], this is generally not recommended due to widespread prevalence of the *fosA* gene and an unfavorable relation between minimal inhibitory concentrations and drug exposure [296,297]. For most antimicrobials in *P. aeruginosa* infection, higher dosing is usually required to reach adequate effect [298]. In uncomplicated *P. aeruginosa* BSI, recent studies suggest short course treatment (7-11 days) to be safe [299,300].

Although commonly used, there is no agreement regarding the superiority of any specific monotherapy [301]. One larger study from the PA BSI cohort, and three smaller observational studies from other cohorts, have compared different monotherapies (manly different β-lactams) as definite treatment for *P. aeruginosa* BSI. None of the studies reported any differences between treatments [302–305]. An old controversy is the use of combination- versus monotherapy. Rationale for combination therapy includes increased probability of appropriate empirical coverage, possible synergistic activity, and the possibility to prevent emerging antimicrobial resistance. Disadvantages of combination therapy are mainly nephrotoxicity, adverse events, drug-interactions and C. difficile infection [306]. Combination therapy usually involves a β-lactam and either an aminoglycoside or a fluoroquinolone [306]. However, the evidence is scattered and several observational studies have failed to present convincing proof that using two active agents offers a survival benefit [307–309], while others have shown effect on mortality [310,311]. Evidence supporting effect on combination therapy on emerging resistance is also scarce, although this is difficult to study in the clinical setting and systematic studies of

especially combinations of different β-lactams are missing [312]. In summary, based on available data, combination therapy should only be considered for *P. aeruginosa* BSI in specific situations, such as empirical therapy in septic shock [301,306]. Also selected cases infected with MDR isolates, in particular when colistin is used, can be considered for combination therapy[313].

Table 3. Overview of common antimicrobial agents with activity against *P. aeruginosa*, modified from Magiorakos et al. [279]

Antimicrobial category	Mode of action	Antimicrobial agent
Antipseudomonal cephalosporins	Cell membrane synthesis inhibitor via penicillin-binding proteins	Ceftazidime Cefepime
Antipseudomonal penicillins + β-lactamase inhibitors	Cell membrane synthesis inhibitor via penicillin-binding proteins	Ticarcillin-clavulanic acid Piperacillin-tazobactam
Antipseudomonal carbapenems	Cell membrane synthesis inhibitor via penicillin-binding proteins	Meropenem Imipenem Doripenem
Monobactams	Cell membrane synthesis inhibitor via penicillin-binding proteins	Aztreonam
Aminoglycosides	Protein synthesis inhibitors	Tobramycin Amikacin
Polymyxins	Disrupts cell membrane integrity via lipid A	Colistin Polymyxin B
Antipseudomonal fluoroquinolones	Inhibits bacterial DNA replication and transcription	Ciprofloxacin Levofloxacin
Novel β-lactam + β-lactamase inhibitors	Cell membrane synthesis inhibitor via penicillin-binding proteins	Ceftazidime-avibactam Ceftolozane-tazobactam Imipenem-cilastatin-relebactam Meropenem-vaborbactam*
Siderophore cephalosporin	Cell membrane synthesis inhibitor via penicillin-binding proteins	Cefiderocol

*Only in certain circumstances with carbapenemases

1.3.2.1 Future treatments and vaccinations

The clinical development pipeline for treatment of *P. aeruginosa* infections includes monoclonal antibodies, phages, iron metabolism disruptors, antibiotics with new modes of actions, polymyxin derivates and new combinations of β-lactam/β-lactamase inhibitors [314]. In addition, treatments aimed at virulence factors, such as biofilm formation or the Type-III secretion system (T3SS), could be promising as adjuvant therapy in *P. aeruginosa* infections, but their role in BSI is elusive [315–317]. Since the 2017 WHO alert, few new treatments have reached late stages of the clinical development [8,314]. Of the 32 novel agents reported in December 2021, 5 had reached phase III stage and 9 had been terminated due to not meeting study endpoints or safety concerns. In particular, trials of *P. aeruginosa*-specific monoclonal antibodies have so far been disappointing, but also one trial of a topical bacteriophage cocktail in burn patients failed to meet its endpoint in phase I/II [314,318]. There are currently ongoing trials of bacteriophages for chronic otitis media (topical treatment), burns (topical treatment), pressure wounds (topical treatment) and pneumonia (inhaled therapy) [314,319]. In addition, one phase II trial of Ftortiazinon, a T3SS inhibitor, in combination with cefepime is currently recruiting patients in Russia (ClinicalTrials.gov Identifier: NCT03638830) [320]. Preventive strategies such as vaccines have been tested, mainly in cystic fibrosis patient, with so far disappointing results [321,322]. However, as pointed out by Hart et al., vaccine development has mainly been directed towards pulmonary infections and only 11 out of 159 vaccine studies evaluated protections against *P. aeruginosa* BSI, which may hold better promise [323].

1.3.3 Microbiological aspects of *Pseudomonas aeruginosa*

P. aeruginosa is an aerobic gram negative rod and a common opportunistic pathogen particularly found in the hospital environment [324]. In the laboratory, *P. aeruginosa* is characterized by a sweet fruity odour, pearlescent appearance, distinct green-blue color (due to pyocyanin and pyoverdin) and of being oxidase positive and lactose non-fermenting. The *P. aeruginosa* genome consists of a large circular chromosome with 5.5-7 million base pairs (bp) and a high G+C content of 65-67%, as well as a shifting number of plasmids [325]. Approximately 90% of its chromosome is highly conserved between strains (core genome), and 10% is made up of an accessory gene pool containing elements that can be horizontally transferred and thus vary among strains (accessory genome) [326]. These accessory genomic elements, also known as *P. aeruginosa* genomic islands (PAGI) or *P. aeruginosa* pathogenicity islands (PAPI), offers a unique phenotypic plasticity and ability to adapt to different environments. PAGI/PAPI can contain both virulence factors and resistance mechanisms [326]. To date, 42 different genomic islands have been described in *P. aeruginosa* [327]. The accessory genome appears as blocks of at least four adjacent open reading frames (ORFs) scattered in regions of genome plasticity (RPGs) within the conserved core genome in a mosaic structure [325]. The core genome consists of approximately 5000 protein-coding genes, of which 321 has been suggested as essential, while the accessory gene pole ranges between 600-1400 protein-coding genes [328].

P. aeruginosa demonstrates a non-clonal epidemic population structure, sometimes disrupted by emerging successful clones [329–331]. In addition, Ozer et al. found that majority of *P. aeruginosa* isolates segregate into two genetically distinctive groups with little intergroup recombination in the core genome, and more accessory gene flow within than between groups [332]. The two groups were highly separated by the *exoS* and *exoU* genes of the T3SS. There is a global surge of healthcare-associated infections caused by MDR or XDR *P. aeruginosa* often belonging to one of the high risk-clones, commonly ST111, ST175 and ST235 (Figure 4) [282,331,333]. In addition, MDR/XDR clonal complexes have been shown to share similar biological traits such as increased biofilm formation and mutant frequency but also reduced motility, fitness and virulence [334–336].

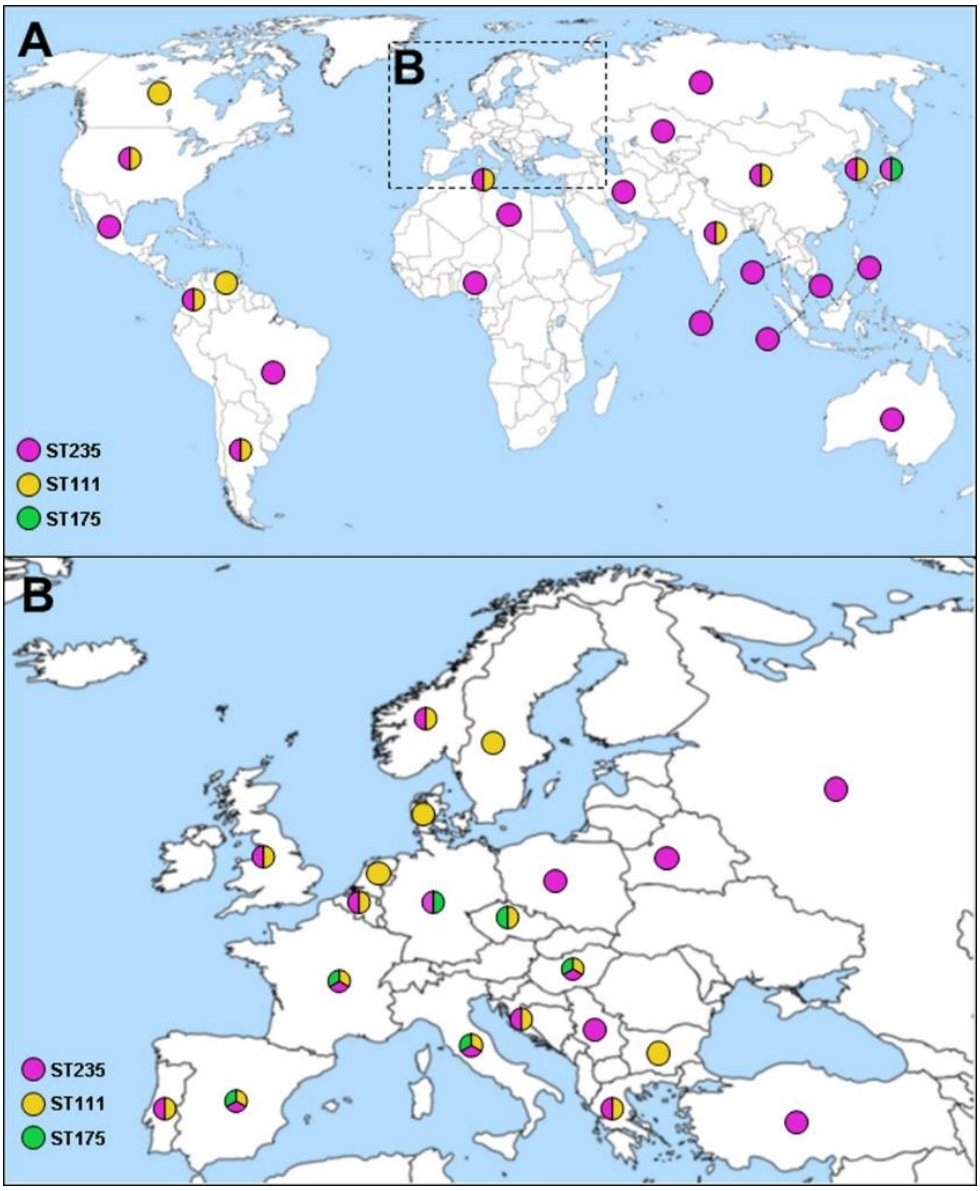

Figure 4. Global spread of epidemic *P. aeruginosa* high-risk clones. Reproduced with permission from (Oliver A, et al. Drug Resist Updat. 2015 Jul-Aug;21-22:41-59), Copyright Elsevier.

1.3.3.1 Resistance mechanisms

Intrinsic resistance mechanisms in *P. aeruginosa* is mainly due to efflux pumps, such as MexAB-OprM, and an outer membrane that has lower permeability than in other gram-negative bacteria [287,337]. Most *P. aeruginosa* carries the chromosomally coded inducible β-lactamase AmpC, affecting resistance against aminopenicillins and cephalosporins through selection of AmpC-depressed mutants [333]. Like any gram-negative bacteria, *P. aeruginosa* can acquire additional resistance mechanisms through horizontal gene transfer or chromosomal mutations. Acquired ESBL such as PER-1, OXA and metallo-β-lactamases IMP, NDM and VIM confer resistance through hydrolysis to many of the β-lactams, including carbapenems (IMP, NDM and VIM) [337,338]. Loss of the porin OprD decreases permeability and is the most common mechanism of carbapenem resistance in *P. aeruginosa*. Rare phenotypes include imipenem resistant but meropenem susceptible strains, which is primarily caused by mutations in *oprD*, and meropenem resistant but imipenem susceptible strains, which is caused by over expression of MexAB-OprM efflux pumps [339]. Aminoglycoside resistance is generally caused by aminoglycoside-modifying enzymes, rRNA methylases and efflux [338]. Fluoroquinolone resistance is mediated through target site mutations [338]. Upregulation of efflux pumps is a common mechanism and mediates resistance to both β-lactams, aminoglycosides and fluoroquinolones [337,338].

1.3.3.2 Virulence factors

P. aeruginosa usually grows in the extracellular space and bacterial clearance depends mainly on neutrophils [340]. The bacteria have developed several mechanisms to evade the host and obtain nutrients, and evolution of specific virulence traits seems to be associated with clinical infection. Fenner et al. showed that *P. aeruginosa* collected from clinical respiratory samples was more virulent than environmental strains [341]. Ledizet et al. also showed that the T3SS were more common in clinical samples collected from infections than samples deemed to be colonization [342]. Main virulence traits of *P. aeruginosa* are single flagella (motility and attachment), type IV cell surface pilli (adherence), lipopolysaccaride (LPS), polysaccharide alginate secretion (mucoid), planktonic growth (biofilm), quorum sensing (communication/biofilm), T3SS (contact dependent exotoxins) and other secreted exotoxins (ExoA, proteases, pyocyanin, pyoverdin, hemolysins, elastase) (Table 4) [287,343,344]. In particular quorum sensing, a sophisticated way of communication with signal molecules to control bacterial population densities, has been acknowledged as an important mechanism for retained virulence [345,346]. It is likely that some virulence features require a complex interplay between several genomic islands and it has also been shown that more virulent *P. aeruginosa* strains carries PAGIs that are not found in less virulent counterpart [347].

In particular, secretion systems are important virulence determinants in gram-negative bacteria and 6 different types have been identified in *P. aeruginosa* [343]. Thought to have evolved as a protection against environmental predators, the T3SS has attracted much attention because of its association with human infection and increased resistance [344,348]. When activated, the T3SS

secrets any of the effector proteins *exoS, exoT, exoU* and *exoY,* which facilitates invading of epithelial cells and provokes an immune response. It is important to note that all *P. aeruginosa* strains carrying the T3SS does not necessarily have genes coding for all the effector proteins. Studies have shown prevalence of *exoS* (poly-substrate toxin) in 2/3 and *exoU* (phospholipase) in 1/3 of isolates from clinical infections, but never both of them in the same isolate which may indicate different subspecies [332,344]. *P. aeruginosa* strains shown to secrete any of the T3SS effector proteins (mainly *exoS* and *exoU*) have been associated with worse clinical outcome in ventilator associated pneumonia and BSI [349,350]. The *exoU* genotype has also been linked to increased exacerbations in patients with bronchiectasis [351]. A study by Peña et al. characterized a large number of Spanish *P. aeruginosa* BSI cases and found an association between the *exoU* genotype with mortality within 5 days [352]. Building on the same cohort, Sánchez-Diener et al. further classified the *P. aeruginosa* isolates according to virulence phenotype in a *Caenorhabditis elegans* infection model, but found that it was a poor predictor of mortality in BSI, despite being well correlated with T3SS genotype [353]. Using genotype to predict pathogenicity in *P. aeruginosa* has recently been questioned by Panayidou et al. whom argued that strain-to-strain virulence variation could not be correctly determined at the genome level, but rather by using functional transcriptomics at the pathway level [354]. Pincus et al., on the other hand, showed that a genome-based machine learning model was able to predict *P. aeruginosa* virulence in 115 clinical isolates (mainly from BSI), but only via a diffuse genomic signature and not based on individual genes [355].

Table 4. Virulence features in *P. aeruginosa* described in the Virulence Factor Database [356]

Pathogenicity	**Virulence factor**
Adherens and motility	Flagella, lipopolysaccarid (LPS), type IV pili, hemagglutinin, elongation factor-Tu
Antiphagocytosis	Alginate (mucus)
Biosurfactant	Rhamnolipid (*rhlA*, *rhlB*, r*hlL*)
Siderophores - Iron uptake	Pyochelin, pyoverdine
Pigment	Pyocyanin, pyoverdine
Protease	Alkaline protease, LasA, LasB (elastase)
Quorum sensing	*lasI, lasR, rhlI, rhlR*, phenazines
Secretion system	One-step secretion system: T1SS, T3SS, T6SS (also known as HSI-I). Two-step secretion systems: T2SS, T5SS.
Toxin	*exoA, exoS, exoT, exoU, exoU*, exolysin (*exlA, exlB*), phospholipase C (hemolysin)

1.3.3.3 Whole genome sequencing

In 2000, the complete *P. aeruginosa* (PAO1) genome was sequenced for the first time [357]. Recently high-throughput whole genome sequencing (WGS) techniques have become economically feasible as a rapid routine tool for molecular characterization [358,359]. Most established next-generation genome sequencing (NGS) platforms, such as the HiSeq 3000 (Illumina), uses short reads (2x150 bp), but third generation sequencers (Oxford Nanopore) can generate long reads up to 300,000 bp. By using open source international databases, information on clones, resistance- and virulence genes can be readily extracted from genomic data [360]. In the clinical setting, the main usage area for WGS is outbreak management and pathogen surveillance. However, as knowledge on the importance of specific bacterial genotypes in human infection increases, the method has potential to be used as detailed molecular diagnosis tool in daily practice [361,362].

2 RESEARCH AIMS

Overall aim:

To improve diagnosis, assess the influence of time to antimicrobial treatment and explore prognostic bacterial virulence markers in sepsis and bloodstream infection.

Specific aims were to:

Paper I
Develop and validate an automated sepsis surveillance algorithm using electronic health record data, and demonstrate utility by determining the burden of hospital-onset sepsis and variations between wards.

Paper II
Evaluate the use of non-invasive respiratory assessement in the Sepsis-3 cirteria by studying the association between worst SpO2 during onset of suspected infection and mortality.

Paper III
Develop a machine learning sepsis prediction model based on electronic health record data, and evaluate the score in a clinically realistic use-case with comparison to conventional screening methods outside the ICU setting.

Paper IV
Study the association of time to appropriate antimicrobial treatment and 30-day mortality in bloodstream infection.

Paper V
Assess the association of *Pseudomonas aeruginosa* virulence genotype with patient characteristics, septic shock and mortality in bloodstream infection.

3 MATERIALS AND METHODS

3.1 STUDY DESIGN, SETTING AND PARTICIPANTS

This thesis is based on observational data from 7 different cohorts of patients presenting to hospitals in Europe, Australia, and Canada (Table 5). All papers are cohort studies, with participants entering the study at either a hospital encounter (**Paper I-III**), or at blood culture collection (**Paper IV-V**), and then followed for a specified time frame. The cohorts are analyzed per episode basis, except for **Paper V**, where participants could be included only once. The majority of participant were collected from the Karolinska University Hospital, which is a large academic center divided between two hospitals and has a catchment area of approximately 2.3 million inhabitants.

The Health Bank, used in **Paper I-III**, is a big data archive consisting of all EHRs from patients at Karolinska University Hospital between 2006 and 2014. The database is located at the Department of Computer and Systems Sciences at Stockholm University. Due to better recordings in the EHR system during the later time period, we restricted our analyses to July 2012 until December 2013, with the exception of prior ICD-codes to estimate co-morbidity which were retrieved up to 5 years before inclusion. In the Health Bank cohort, included patients were followed until discharge or death, but in **Paper III**, episodes were also truncated at ICU-admission. The Health Bank only included data on in-hospital mortality. Four external validation cohorts (KH, HERO, Impressed and SepsisAlarm), originally collected for other studies, were also analyzed in **Paper II**. In these validation cohorts, included patients were followed for up to 30 days, except for 36 patients where only data on in-hospital mortality was available.

The 2SPARE database is another big data archive located at the Division of Clinical Epidemiology, Karolinska University Hospital. The database includes EHRs from all patients at Karolinska University Hospital between January 2010 until August 2021. In **Paper IV**, all significant BSI episodes from January 2012 until December 2019 were included. We did not include patients from 2020 or 2021 to avoid substantial population differences due to the SARS-CoV-2 pandemic. All included patients were followed for up to 30 days.

The PA BSI network is an international research network including consecutive adult patients with monobacterial *P. aeruginosa* BSI between years 2009-2015 [305,363,364]. The database consists of a retrospective cohort of 2396 patients collected at 25 centers from 9 Countries in Europe and Australia. Centers who routinely saved their *P. aeruginosa* isolates were eligible for participation in the **Paper V**, and 6 sites were finally included (Seville, Santander, Heraklion, Ljubljana, Stockholm and Brisbane). In Stockholm, only patients presenting to the Karolinska University Hospital were included.

Table 5. Included cohorts

Cohort	Setting	Inclusion criteria	Exclusion criteria	Sample size	Study
Health Bank[365]	Karolinska University Hospital	All patients ≥18 years admitted for 24h or longer between July 2012 until December 2013	Patients were excluded if admitted to an obstetric ward and censored during ICU-care, due to lack of data on vital parameters and medications for these wards	82 852 (19479 with suspected infection)	Paper I-III
KH	Karolinska University Hospital	All patients ≥18 years with suspected infection at the ED between October 2015 and November 2018	None	9190	Paper II
HERO	Skåne University Hospital in Lund, Helsingborg Hospital and St Paul's Hospital Vancouver (Canada)	ED patients ≥18 years with suspected infection and at least one of: respiratory rate >25 breaths/minute, heart rate >120 beats/minute, altered mental awareness, systolic blood pressure below 100 mmHg, SpO2 <90%, or <93% if ongoing oxygen treatment. Patients were enrolled between February 2015 and March 2016	No informed consent	241	Paper II
Impressed	Skåne University Hospital in Lund and Malmö, Örebro University Hospital and Linköping University Hospital	ED patients ≥18 years with suspected infection and at least one of Systemic Inflammatory Response Syndrome criteria or self-reported fever or chills. Suspected infection was determined at inclusion by the attending physician. Patients were enrolled between March and November 2011	No informed consent	649	Paper II
SepsisAlarm	Skåne University Hospital in Lund	ED patients ≥18 years with fever or history of fever and highest priority according to Rapid Emergency Triage System or lactate >3.5 mmol. Patients were enrolled consecutively as part of a prospective study between April 2017 and February 2018	Opt-out	506	Paper II
2SPARE	Karolinska University Hospital	All patients ≥18 years with significant BSI admitted between January 2012 to December 2019	BSI onset at the ICU or obstetrical wards, or if patients received appropriate treatment prior to BSI, or if not receiving any antibiotics within 24h	10 628	Paper IV
PA BSI network [305,363,364]	Stockholm (Karolinska), Seville, Santander, Heraklion, Ljubljana, Brisbane	Consecutive patients ≥18 years with monobacterial *P. aeruginosa* BSI between January 2009 to October 2015. Centers whom routinely saved their isolates were eligible for participation	Polymicrobial BSI or no available *P. aeruginosa* isolate	773	Paper V

Abbreviations: emergency department (ED), electronic health records (EHR), intensive care unit (ICU), peripheral oxygen saturation (SpO2), *Pseudomonas aeruginosa* (PA), bloodstream infection (BSI)

3.2 DATA COLLECTION AND DEFINITIONS

3.2.1 Clinical data

The definitions used throughout this thesis are presented in Table 6. **Paper I-IV** are mainly based on structured clinical data collected retrospectively from EHRs, except for 3 of the validation cohorts in **Paper II** where data had previously been collected prospectively by study investigators [366,367]. Data collection included demographics, hospital administrative data, vital parameters, laboratory findings, microbiological data, medications, and mortality. In **Paper I** and **Paper IV**, manual medical record review was also performed to collect specific information from unstructured medical free text notes. This had two main purposes: In **Paper I**, the sepsis surveillance algorithm was evaluated using a physician reviewed reference standard where patients were classified as either fulfilling the Sepsis-3 criteria or not. This involved a stratified sampling of 2 validation sets including 1000 hospital admissions from the entire hospital cohort. The first validation set included 674 hospital episodes sampled randomly form patients with suspected infections, where the likelihood of sepsis was greater. The second validation set included 326 hospital episodes sampled randomly from patients without suspected infections, where sepsis was less likely. In **Paper IV**, medical record review was performed for patients that were admitted to the ICU without appropriate therapy within 72 hours of BSI onset (n=181). This was done to avoid misclassification of antimicrobial treatment because of missing structured data on medications from ICU. The clinical data collected in **Paper V** is entirely based on review of medical records at each participating center according to a common electronic case report form (eCRF). The collected parameters included patient characteristics, details of the infection, treatment data, and outcomes. Data on septic shock was not available from Seville (n=134).

3.2.2 Laboratory data

In **Paper V**, available bacterial isolates were identified from the clinical microbiological laboratories at each participating center. Retrieved isolates were sent to Karolinska University Hospital, using Amies transport medium in room temperature for European samples and glycerol transport medium in frozen temperature (-80 degree Celsius) for the Australian samples. Directly upon arrival, bacterial samples were incubated overnight on Cystine-Lactose-Electrolyte-Deficient (CLED) agar, and fresh colonies were collected and stored in a biobank at -80 degree Celsius. Antimicrobial susceptibility testing (AST) was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion method using Mueller-Hinton agar plates and disks from Oxoid (Basingstoke, UK). Interpretation of susceptibility was based on EUCAST clinical breakpoint tables version 10.0.

Table 6. Definitions used in the thesis and papers

Subject	Definition
Suspected infection	Having any culture taken and at least 2 doses of antimicrobials administered. If the patient was admitted to the ICU prior to 24h, or died prior to 48h from the first dose of antimicrobials, they were deemed to have a suspected infection despite only being given 1 dose. Cultures had to be performed within 24h after the start of antimicrobial treatment. Antimicrobial treatment had to be started within 72h after culture. Onset of infection was determined based on which of these events occurred first
Sepsis	A suspected infection in combination with an increase in SOFA score by $\geq$2 points compared to the baseline. Organ dysfunction was measured as the maximum SOFA score 48h before to 24h after onset of infection and compared to a baseline SOFA score measured separately. Some adaptations to the original SOFA score were made in order to deal with common missing data outside of the ICU-setting, which are further described in detail in **Paper I**. Onset of sepsis was when the patient fulfilled the organ dysfunction criteria
Septic shock	Defined in **Paper IV** as, within 24h of BSI onset, receiving vasopressor treatment or ICU admission with septic shock as the main reason for admission. In **Paper V**, septic shock was defined as sustained hypotension despite adequate fluid replacement and need for starting or increasing dosing of vasopressor drugs
Hospital-onset sepsis	Suspected infection and organ dysfunction 48h after admission, or readmission with sepsis within 48h of discharge
Hospital-onset bloodstream	Blood culture collection 48h after admission
Significant bloodstream infection	Defined as positive blood cultures. This excluded pre-defined contaminants isolated in only one culture bottle or only one set (1 anaerobe and 1 aerobe blood culture bottle) if more than one set of blood cultures were collected within 24h. Identification of contaminants were based on the CDC/National Healthcare Safety Network Patient Safety Component Manual
Appropriate antimicrobial therapy	Receiving at least one antimicrobial agent for which the identified pathogen was found to be susceptible *in vitro*. In polymicrobial infection, all identified pathogens needed to be covered by at least one antimicrobial agent to be classified as appropriate therapy. In all studies, antimicrobial susceptibility was inferred from disk diffusion methods. In **Paper IV**, surrogate antibiograms with imputed susceptibilities were created for drugs not directly registered in the susceptibility report based on: reported susceptibilities, known intrinsic resistance, expert rules and breakpoint tables from the EUCAST, similar to previously described methods [2,220]
Inappropriate antimicrobial therapy	Receiving treatment without *in vitro* pathogen coverage or no treatment
Source of infection	Defined in **Paper I** during medical record review according to previously validated criteria based on CDC and The International Sepsis Forum definitions [66,368,369]. In addition, episodes were divided by source and classified on a 4-graded scale according to likelihood of infection as: no infection, possible infection, probable infection and definite infection [17]. In **Paper II** and **IV**, source of infection was defined according to ICD-10 codes registered during the admission. In **Paper V**, source of infection was determined by the reviewing physician guided by the CDC criteria [66]
Immunosuppression	Defined in **Paper IV** based on ICD-10 codes registered in the year prior to admission until 24h after admission. In **Paper V**, immunosuppression was defined as either chemotherapy during the last 30 days, systemic corticosteroid treatment (>10 milligrams of prednisone for >29 days), neutropenia (absolute neutrophil count <0.5x109/liter), solid organ transplant, bone marrow transplant and/or chronic dialysis treatment
Multidrug-resistance	Phenotypic resistance to three or more antimicrobial drugs from different drug classes [279]. In **Paper IV**, findings of Methicillin-resistant *S. aureus*, *Enterobacterales* with extended-spectrum beta-lactamases production or vancomycin-resistant enterococcus were considered antimicrobial-resistant phenotypes
Charlson comorbidity index	Generally defined based on ICD-10 codes available from 5 years before admission [370]

Abbreviations: Centers for Disease Control and Prevention (CDC), intensive care unit (ICU), International classification of diseases (ICD), bloodstream infection (BSI) and European committee on antimicrobial susceptibility testing (EUCAST)

To perform WGS, bacterial colonies were collected from overnight cultures for DNA extraction with the EZ1 Advanced XL system (Qiagen). The quantity of the extracted DNA was measured using a Qubit double-stranded DNA (dsDNA) assay kit (Life Technologies Europe). Extracted DNA was diluted to an approximate target sample concentration of 10 nanogram/microliters and a target sample volume of 50 microliters before sequenced on Illumina HiSeq sequencer (San Diego, CA, USA) at Science for Life laboratory (SciLifeLab, Solna, Sweden), producing 2 × 150 bp paired-end sequences.

WGS data were then processed through an in-house bioinformatics pipeline which was set up specifically for the study. In short, the quality of the reads was first assessed using FastQC and the short reads were trimmed and filtered using Trim Galore. Reads were then assembled *de novo* into longer contigs using SPAdes. Multi Locus Sequence types (MLST) were determined *in silico* using the BLAST+ package and reference sequences from the pubMLST database. The phylogenetic tree was constructed using MAFFT and the maximum-likelihood algorithm in FastTree. Open reading frames (ORFs) on contigs were predicted using Prodigal, and Diamond BLASTx was used to searching ORFs against reference proteins in the Virulence Factor Database (VFDB) and Victor database [356,371]. The coverage and identity thresholds were set at 80% to be considered a match.

3.3 STATISTICAL ANALYSES

In general, results were presented as frequencies and percentages, mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate. The difference between independent categorical variables was assessed using Chi-square test if the expected cell count was above 5, otherwise the Fisher exact test was used. The difference between non-normally distributed continuous variables was assessed with Mann-Whitney U or Kruskal-Wallis tests, depending on the number of groups compared. Confidence intervals (CI) were presented as the interval between the 2.5th and 97.5th confidence levels. Two-sided P-values $\leq$0.05 were considered statistically significant. The analyses were performed in STATA, R and Python.

3.3.1 Application of statistical methods in each paper

3.3.1.1 Paper I

A rule-based classification algorithm was developed based on the Sepsis-3 clinical criteria (Table 6). To assess algorithm performance, we used a method previously described by Rhee et al. [102]. A 2x2 confusion matrix was constructed for each of the validation sets with the medical record review as reference standard. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were then calculated by generalizing the proportions from the validation set to the entire source population. CIs were obtained from point estimates of the 2.5th and the 97.5th percentiles of bootstrap sampling of the 2x2 confusion

matrixes derived from the validation. The probability of hospital-onset sepsis was calculated using the cumulative incidence function (CIF), accounting for competing risks: ICU admission, discharge or death. The CIF provides the accumulated risk of the event of interest (or the competing event) up to a given time *t*. The comparison of CIF between wards was estimated based on the subdistribution hazard model by Fine and Gray.

3.3.1.2 Paper II

SpO2 was categorized a priori as: 100-97% (reference), 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89-88%, 87-86%, 85-84%, 83-82% and 81-50%. We then used multivariable logistic regression to evaluate the association between our predictor of interest, SpO2 category, and mortality. The exponentiated β-coefficients of SpO2 estimates the odds ratios (OR), which can be interpreted as the relative risk of death compared to the reference category SpO2 100-97%. The model was adjusted for possible confounders, which were determined a priori as: age, sex, Charlson comorbidity index (CCI) group and SOFA score (respiration component omitted). Analyses were performed both in the main cohort and in the validation data sets. To account for dependency of data when pooling episodes in the validation set, cohort was added as random effect variable. Furthermore, the SOFA score was originally developed for mortality prediction, but the impact of including SpO2 in the SOFA score had not been systematically assessed. Using main cohort data, we fitted several logistic regression models including baseline predictors (age, sex and comorbidities) alone and baseline predictors plus SOFA score with and without adding SpO2 measurements. Model fit was assessed with the Akaike Information Criterion (AIC). Based on these models, the probability of death was predicted in both the main and pooled validation cohorts, respectively. Model predictions were compared with AUROC using the method by Delong [372].

3.3.1.3 Paper III

The cohort was divided into a training and tuning data set (July 2012 – June 2013) and a validation data set (July 2013 – December 2013). The split was based on calendar time to ensure algorithm performance over time. Sepsis onset was determined according to the validated rule-based Sepsis-3 classification algorithm developed in **Paper I**. A machine learning model based on a Bayesian network (SepsisFinder) was trained to predict sepsis onset within 48 hours using routine measurements of vital parameters, laboratory variables and hospital administrative data. The Bayesian network model, also known as a causal probabilistic network (CPN)/graphical model, is constructed of predefined random variables (nodes) linked together with arrows representing conditional probabilities [373]. Furthermore, it is possible to state variables that are conditionally independent from each other and to add hidden variables – i.e. variables that are not observed but encode a concept such as severity of disease. This creates a web of a cause and effect relationships between variables, e.g. vital parameters and laboratory test results, but also concepts like SIRS. In **Paper III**, both the structure of the CPN and the probabilities were specified using a combination of manual curation and automated learning [191]. The joint probability of sepsis was estimated based on all variables of the CPN, including information from missing variables [191].

To simulate a clinically realistic use-case, a score was generated hourly on all admission, providing a new variable was registered. In addition, the alarm was silenced for 48 hours after each positive trigger, to mimic a situation where healthcare providers are thought to act on a threshold-based warning system. We used a "soft window" approach where we allowed a trigger to happen at any time during the 48-hour window prior to sepsis, but assumed that a negative screen was a true negative because the patient had not yet started to show signs of sepsis (Figure 5). This was done to prevent bias in model training, by punishing the model for what in many instances would be a correct negative screen. Hence, each sepsis patient was only considered as false negative if they were never detected during the 48-hour time window.

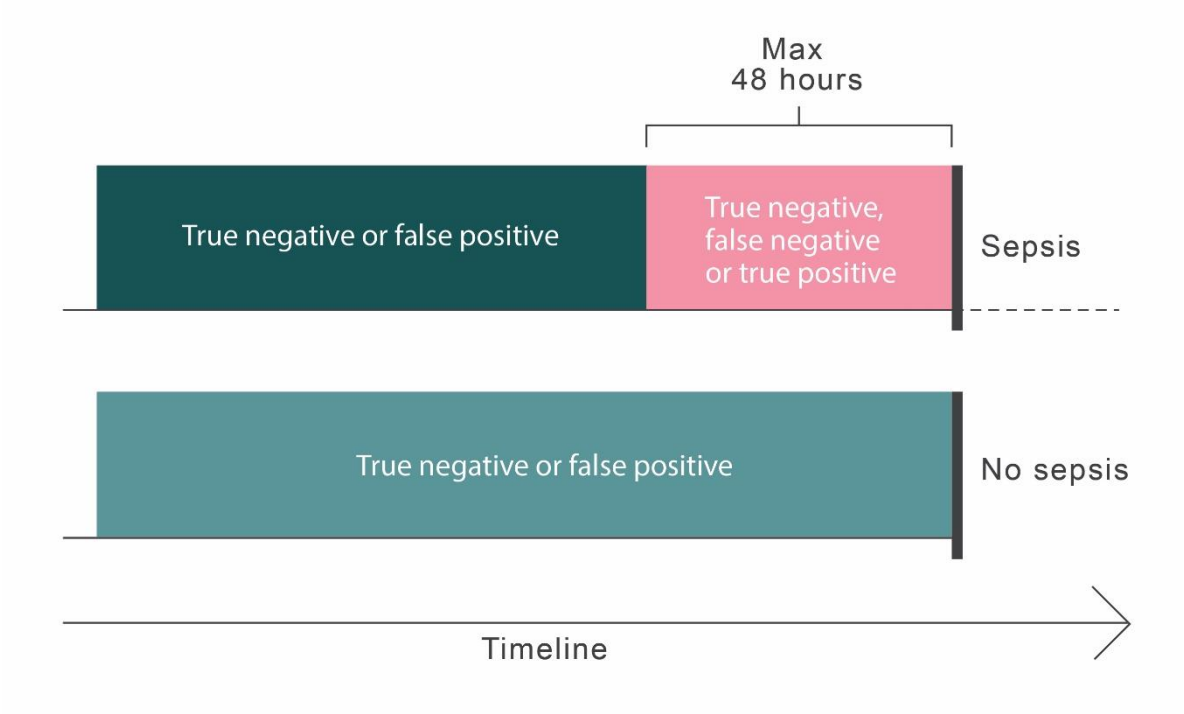

Figure 5. Schematic overview of the method used when calculating the discriminatory performance of SepsisFinder and NEWS2. The colors represent the different screening windows.

Discrimination was assessed by summary metrics AUROC and APR based on individual screens, with bootstrapped CIs. The ROC curve describes the relationship between true positive subjects (sensitivity or recall) and false positive subjects (1-specificity) for all discriminatory thresholds of the algorithm predictions. The PR curve is based on the same logic as ROC, but describes the relationship between sensitivity and the PPV. The APR is especially informative in data sets with high class-imbalance. Model performance was compared to the discriminatory performance of NEWS2, and assessed in subgroups in the validation set to identify areas for potential applicability. Finally, 3 operating points for SepsisFinder were chosen to match that of the standard clinical decision-making thresholds for NEWS2: NEWS2=5 and NEWS2=7, and the threshold that gave closest to 85% sensitivity. Timeliness of the true positive alert, defined as hours before sepsis onset, was assessed for each threshold in the true positive cases, and compared to NEWS2.

3.3.1.4 Paper IV

We evaluated the association between inappropriate therapy and 30-day mortality using multivariable logistic regression, with and without stratification based on disease severity (high SOFA score ≥2 points or low SOFA score <2 points). The analyses were adjusted for possible confounders identified a priori as: age, sex, CCI, immunosuppression, SOFA score, polymicrobial BSI, source of infection, calendar year and hospital-onset of BSI. Since we studied a time dependent exposure, patients with inappropriate treatment had to be alive to be switched to appropriate treatment, leading to immortal time bias. To handle this in the analyses, we used the landmark method where deceased patients, as well as those with undetermined drug-bug combinations, were excluded at each pre-defined time point (1, 3, 6, 12, 24, 48 and 72 hours). As a consequence, results could only be generalized to patients surviving to each landmark. The subgroup of patients with septic shock was analyzed separately.

3.3.1.5 Paper V

The association of virulence genotype with mortality and septic shock was assessed using 3 different data driven approaches to define exposure, all accounting for different levels of aggregation of virulence genotype data: (I) based on common bacterial clones, (II) based on distinguishable virulence gene clusters, and (III) based on individual virulence genes. Epidemic clones were categorized as sequence types (STs) occurring >10 times in the cohort (n=11 different STs). After annotation of virulence genes, there were 247 sequences matching reference proteins in the VFDB and 91 sequences matching reference proteins in the Victors database, but several genes were overlapping between the databases. Since a large portion of virulence genes were either match or no match in most of the bacterial isolates, they were filtered based on frequency to enable meaningful downstream analyses of individual genes. A threshold of virulence gene match in between 2% to 98% of the isolates was chosen, and genes with high collinearity were also grouped. The final dataset consisted of 26 gene variables.

To explore clusters of virulence genotypes in the cohort, Principal Coordinate Analysis (PCoA) was performed. PCoA is a method of visualizing (dis)similarities between subjects based on large sets of variables in a spatial representation [374]. The PCoA requires transformation of data into a distance matrix in a Euclidean space with Cartesian Coordinates. In **Paper V**, the PCoA was based on a Euclidean distance matrix of the binary match/no match of all annotated virulence genes from the VFDB. The spatial matrix was then rotated so that a new axis could be drawn without changing the relative distance between each bacterial isolate. A second axis was drawn orthogonal to the first. The axes were ordered hierarchically and display the maximum variation in the data using two dimensions. Hence, most variation was described by the first axis and second most variation was described by the second axis.

Furthermore, we assessed the relationship between patient characteristics and infection by the epidemic clones, as well as the virulence clusters derived from the PCoA. The purpose of these analyses was to explore indirect signs of invasive potential of specific clones. In the next step, we evaluated the association of ST, virulence clusters, T3SS and individual virulence genes

with patient outcome. Each virulence trait was first assessed in a univariable logistic regression model, and then selected variables were further assessed in a multivariable logistic regression model. Adjustments were made for: geographical site, age group, gender, CCI group, immunosuppression, department of hospitalization and hospital-onset BSI. Finally, to evaluate if virulence genotype carried important information to predict patient outcome, we trained and tested several prediction models based on a random forest classifier, where each model included baseline patient variables and a different set of virulence genotype data (Figure 6). The random forest method was chosen since there was a large number of predictors in relation to the sample size, as well as to account for complex interactions between variables. The cohort was randomly split 80/20 into a training set for model development and tuning, and a test set for model evaluation. To account for variability of the random 80/20 data set split, this process was repeated 20 times using different seeds generated from random integer numbers. Discrimination was compared using the distribution of AUROC for the 20 different splits. In addition, the difference in AUROC between the models including virulence data and the reference model for each of the 20 splits were calculated.

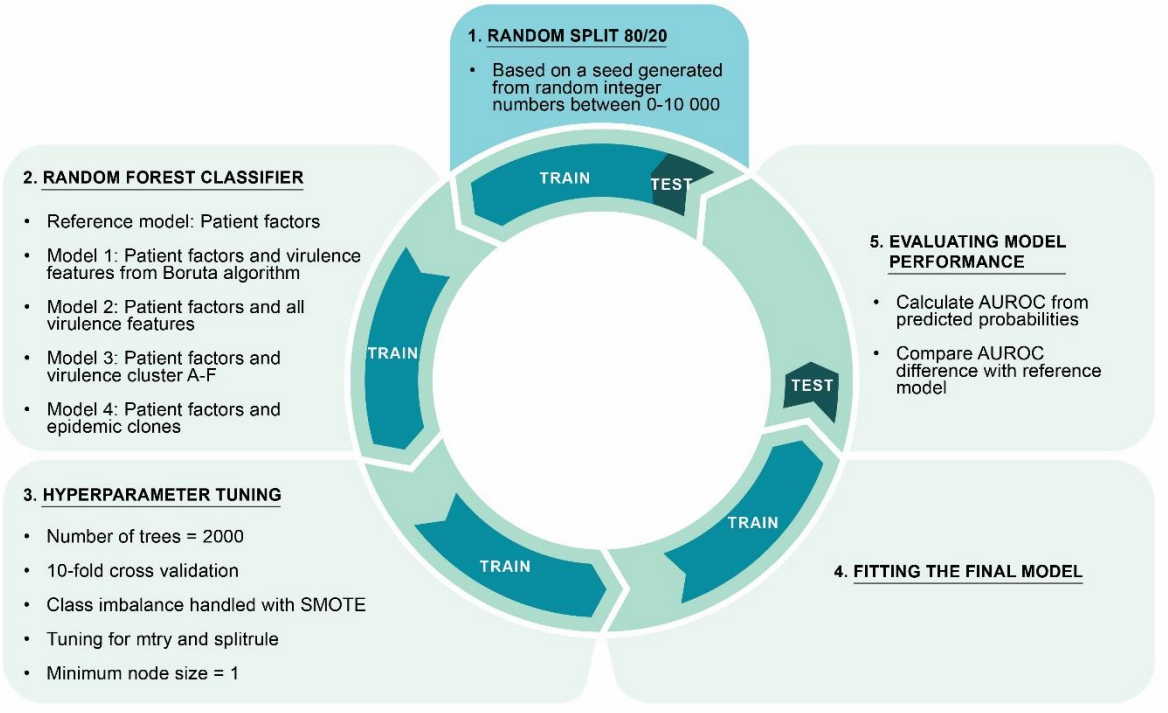

Figure 6. Schematic overview of the method used when fitting the random forest model. To account for variability of the random 80/20 data set split, this process was repeated 20 times. Patient factors were: age, gender, comorbidity, department of hospitalization, immunosuppressed state and nosocomial infection. Model 2 included all filtered virulence genes (n=26). Mtry means number of variables randomly sampled at each split.
Abbreviations: synthetic minority oversampling technique (SMOTE).

The Random forest model is based on multiple independently grown decision trees, but generates the final classification based on the majority vote, hence, the forest [375]. The model is able to pick up complex non-linear interactions between predictors, and handles situations where predictors dominates the number of samples, since not all predictors are used simultaneously. To prevent from overfitting, each tree is built on a subset bootstrap sample from the training data, which randomly leaves out approximately 1/3 of the observations, known as the out-of-bag (OOB) sample [376]. The OOB sample is used for model predictions during training to improve the learning. Each decision tree is built by splitting observations into nodes until a decision on class membership has been made for all observations. The node size determines the minimum number of cases allowed in each terminal node. At each node split, a number of randomly selected predictors (defined as *mtry* in Figure 6) are considered, and the one that produces the most separation between the observations is picked. The split rule determines how the value of the predictor is separated at each split. This process is then repeated for *n* trees.

In addition, one of the models included a feature selection step using a Boruta algorithm in the training set [377]. The Boruta is designed to create a permuted copy of each predictor variable called a "random shadow feature". To determine which predictors are more associated with the outcome than chance, it then fits a random forest classifier and iteratively removes independent variables which are less important than the random features based on statistical testing.

3.4 ETHICAL CONSIDERATIONS

All studies were approved by the regional ethical review boards at each center where data was collected. Since the studies are based on retrospective data from a large number of participants, with incidence or mortality as an outcome of interest, informed consent was not possible to collect without biasing the results. Hence, the ethical review boards gave their approval to the studies with a waiver of consent from participants. The primary ethical consideration was lack of autonomy, and breach of privacy, since we had to collect data from patients' medical records. To minimize the impact on individual participants, several measures were taken. First, the majority of data was collected from pseudo-anonymized research databases with no available key for the researchers and, hence, no possibility to identify individual patients. Second, only the minimal necessity of data was collected and all data was kept in secure storage. Only responsible researchers had access to the data. Finally, results were presented on an aggregated level to further avoid the possibility to identify any individual participants. None of the studies had direct impact on the care of the individual patients. Collectively, it was judged that the beneficial impact of these studies would outweigh the potential harm subjected to any participant.

RESULTS

3.5 AUTOMATED SEPSIS DIAGNOSIS TO ENABLE EPIDEMIOLOGICAL SURVEILLANCE

The rule-based sepsis algorithm was developed to classify onset of sepsis in non-ICU wards using EHR data (see **Paper I** for detailed description of the algorithm). To validate the algorithm performance and estimate the burden of sepsis in the hospital, we included 82 653 hospital episodes (54 884 patients). Among these episodes, 19 479 (23.6%) contained a suspected infection. The median patient age was 64 years and 50.9% were women. After physician medical record review, 343 out of 1000 subjects fulfilled the organ dysfunction criteria in Sepsis-3. Among these episodes, 109/343 (31.8%) had possible infection, 87/343 (25.4%) had probable infection, 117/343 (34.1%) had definite infection and 30/343 (8.7%) had no infection. After excluding patients with no infection, a total of 313 out of 1000 reviewed subjects were finally considered as true sepsis by reviewers. Ranking the source of infection in confirmed sepsis episodes gave the following result: respiratory (n=119/313, 38.0%), urogenital (n=54/313, 17.3%), unknown source (n=42/313, 13.4%), bloodstream (35/313, 11.2%), skin, bone and joint (30/313, 9.6%), abdominal (n=26/313, 8.3%) and other infectious sources (7/313, 2.2%).

Algorithm performance against the physician reference is shown in Table 7. Reduced algorithm sensitivity was mainly caused by respiratory dysfunction or altered mental status only being mentioned in free text, overestimation of pre-existing organ dysfunction or sepsis-related organ failure not captured within the stated timeframe. Imperfect algorithm specificity was usually due patients being judged as not having an infection by reviewers, misclassification of baseline SOFA score or obvious measurement errors of vital parameters in the EHR.

Table 7. Algorithm performance against the medical record reviewed reference in the entire hospital cohort (n=82 653)

Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
0.887 (95% CI: 0.799-0.964)	0.985 (95% CI: 0.978-0.991)	0.881 (95% CI: 0.833-0.926)	0.986 (95% CI: 0.973-0.996)

Abbreviations: confidence interval (CI), positive predictive value (PPV) and negative predictive value (NPV)

When applying the surveillance algorithm in the entire study population, 8599 sepsis episodes were identified, and only 13.4% of these had a corresponding ICD-code indicative of sepsis. Among the sepsis episodes, 7493 (87.1%) and 1106 (12.9%) were classified as community-onset and hospital-onset sepsis, respectively. The most frequent organ dysfunction registered

by the algorithm in sepsis episodes were respiratory and renal dysfunction (Figure 7). The probability of acquiring hospital-onset sepsis with longer hospital stay differed significantly depending on type of hospital ward. Transplant (CIF 0.078 at day 30) and hematology (CIF 0.061 at day 30) wards were associated with the highest risk, while orthopedic (CIF 0.004 at day 30) wards were associated with the lowest risk.

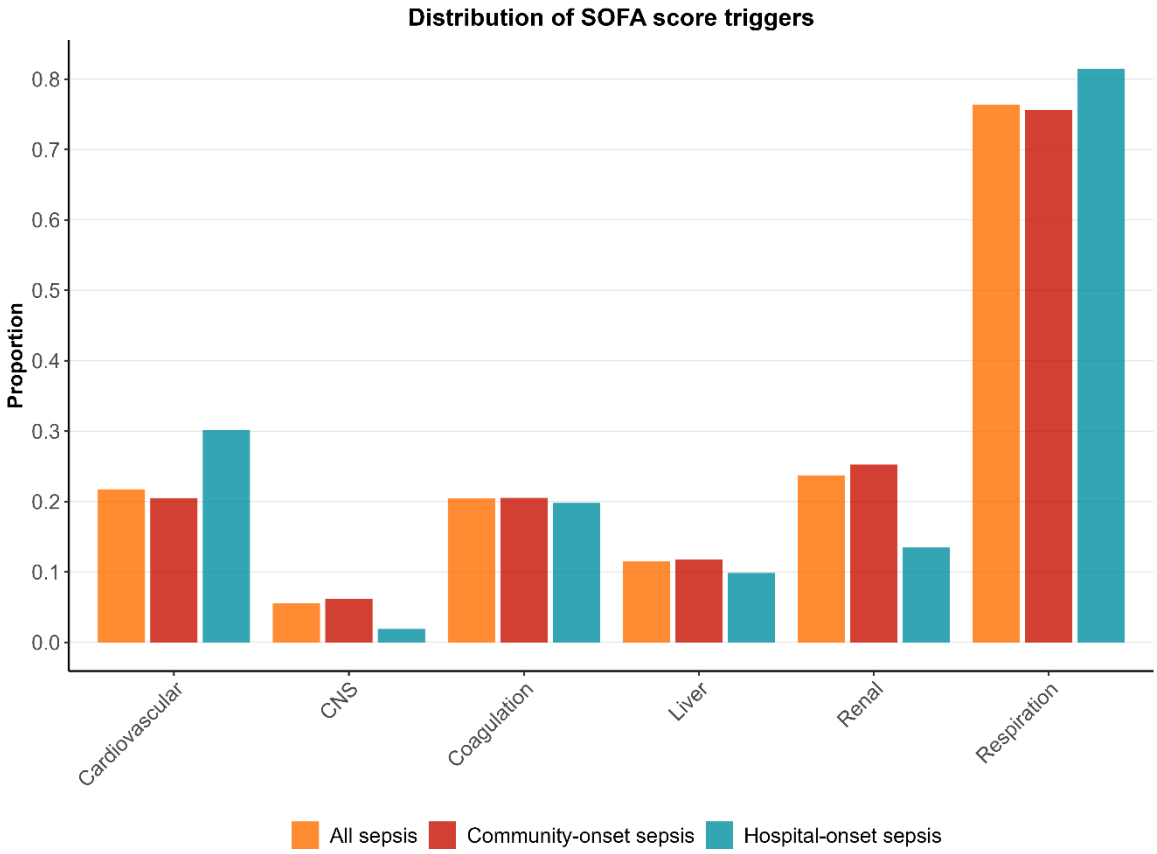

Figure 7. The proportion of sepsis episodes where the SOFA score component contributed to the sepsis classification.

3.6 PERIPHERAL OXYGEN SATURATION TO DIAGNOSE RESPIRATORY DYSFUNCTION IN SEPSIS

The findings in **Paper I** showed that the majority of sepsis diagnoses were triggered by respiratory dysfunction (Figure 7). However, to adapt the SOFA score to a non-ICU setting, the respiratory assessment was based on peripheral oxygen saturation (SpO2), which had previously not been validated in the Sepsis-3 criteria. To assess the use of SpO2 to diagnose sepsis, we revisited the group of suspected infections which also had an SpO2 measurement (17 738 episodes) from the Health Bank cohort (median age 67.0 [53.0-77.0]; 9007 [46.4%] women; 1044 [5.4%] died), as well as including 4 external validation cohorts: KH, HERO, Impressed and SepsisAlarm (n=10 486 with SpO2 measurements). The validation cohorts had a range of; median age 61.0-76.0; proportion of women 42.1-50.2%; and mortality rate 2.3-13.3%. Compared to reference SpO2 100-97%, SpO2 96% or 95% were not significantly

associated with increased mortality, but the risk of death increased gradually starting from SpO2 94% (Figure 8). In the validation cohorts, the odds ratios (ORs) were smaller and displayed more variation, but followed a similar trend with a significantly increased mortality risk at SpO2 94% (Figure 8).

Figure 8. Association of SpO2 at onset of suspected infection and mortality. The odds ratios are adjusted for age, sex, comorbidity and organ dysfunction other than respiratory failure. Cohort was also added as random effect when analyzing the pooled validation cohorts (orange line).

When adding SpO2 to the SOFA score in the main cohort, the discrimination of mortality improved from AUROC 0.75 (95% CI, 0.74-0.77) without respiratory assessment, to AUROC 0.78 (95% CI, 0.77-0.80, *P*<0.001) with respiratory assessment. Since decreasing SpO2 was clearly associated with mortality in patients with suspected infections, we also wanted to apply operational thresholds of SpO2 in the SOFA score. The original SOFA score is based on arterial blood gas measurements of PaO2/FiO2 ratio to assess respiratory function. Using validated converting tables, SpO2 can be transformed to the equivalent PaO2 value. Based on this conversion, SpO2 levels of 95% and 91% without oxygen treatment equals 1 and 2 SOFA points respectively [378]. As seen from Figure 8, the mortality increased significantly at SpO2 94%, and at SpO2 90% the CIs did not overlap in the main cohort. A post-hoc explorative

analysis was performed where SpO2 94% and 90% were operationalized to generate 1 and 2 SOFA points respectively. A logistic regression model based on the new thresholds showed similar predictive performance as the original thresholds (SpO2 95% and 91%). However, with the new thresholds, the number of patients classified according to the Sepsis-3 criteria decreased by 7-10% in both the main and pooled validation cohorts, with similar or slightly increased mortality (Table 8).

Table 8. Changes of sepsis classification depending on SpO2 threshold in the SOFA score

	Main cohort		**Validation cohorts (pooled)**	
Sepsis-3 classification	**Original thresholds (SpO2 95% and 91%)**	**New thresholds (SpO2 94% and 90%)**	**Original thresholds (SpO2 95% and 91%)**	**New thresholds (SpO2 94% and 90%)**
Number of episodes	7267	6788	4160	3735
Mortality (%)	8.5	9.2	12.0	12.6

Abbreviations: peripheral oxygen saturation (SpO2) and sequential organ failure assessment (SOFA)

3.7 EARLY IDENTIFICATION OF SEPSIS USING A MACHINE LEARNED BAYESIAN NETWORK MODEL

Using the sepsis algorithm developed in **Paper I**, we were now able to generate an objective assessment of sepsis onset in a large set of hospitalized patients. This enabled further studies of early sepsis identification using a machine learned prediction model, which generally requires large data sets to make meaningful evaluations. Using the Health Bank cohort, 8038 (9.7%) sepsis episodes, were included in **Paper III**. Since hospital episodes were truncated at ICU-admission, the number of sepsis cases are slightly lower than in **Paper I** (n=8599). Among the sepsis episodes, 6889 (8.3%) were classified as community-onset and 1149 (1.4%) were classified as hospital-onset. After dividing the data into two time periods, the training set (July 2012 – June 2013) comprised 56 302 (67.9%) admissions with 5436 (9.7%) sepsis episodes, and the validation set (July 2013 – December 2013) comprised 26 550 (32.2%) admissions with 2602 (9.8%) sepsis episodes. A Bayesian network model (SepsisFinder) was trained according to a process described in **Paper III**. Algorithm performance and timeliness of true positive alarms before sepsis onset are shown in Table 9. SepsisFinder had good discriminative ability and outperformed NEWS2 in terms of AUROC and APR. In addition, SepsisFinder predicted sepsis onset (organ dysfunction) significantly earlier than NEWS2 for all tested alarm thresholds. Antibiotic administration usually occurred after onset of sepsis related organ dysfunction. With a sensitivity threshold close to 85%, SepsisFinder triggered median 5.5h (IQR 1.9-22.8h) before antibiotic administration in sepsis patients.

In subgroup analyses of SepsisFinder performance, the AUROC was robust to changes in the population screened and timing of screening, except for patients who died (AUROC 0.872).

On the contrary, the APR changed substantially in different subgroups of patients, with higher APR of 0.595 in 2-day long episodes and lower APR of 0.021 in hospital-onset sepsis episodes. The APR also decreased with more hospital days screened. These results indicate better clinical applicability of the SepsisFinder earlier on during the hospitalization period, as well as less pronounced applicability for longer hospital episodes or predictions of very rare events such as hospital-onset sepsis. The APR was high in episodes with confirmed BSI (APR 0.350), i.e. culture positive sepsis, compared to episodes with no BSI (APR 0.164), i.e. culture negative sepsis. In addition, APR was higher in risk periods prior to surgery (APR 0.231) compared to risk periods post-surgery (APR 0.126), indicating superior performance in non-surgical episodes or in episodes before surgery.

Table 9. Comparison of the predictive performance between SepsisFinder and NEWS2

Variable	SepsisFinder	NEWS2
AUROC (95% CI)	0.950 (0.946 – 0.954)	0.871 (0.858 – 0.877)
APR (95% CI)	0.189 (0.173 – 0.201)	0.149 (0.138 – 0.161)
Timeliness at sensitivity 20%, median (IQR)[a]	1.0 (0.0-8.0)[b]	0.0 (0.0-2.0)[b]
Timeliness at sensitivity 42%, median (IQR)[a]	1.0 (0.0-8.0)[c]	0.0 (0.0-4.0)[c]
Timeliness at sensitivity 85%, median (IQR)[a]	2.0 (0.0-11.0)	Not avaliable

[a]Timeliness defined as hours between alarm trigger and sepsis onset in true positive patients. Sensitivity 20% matches that of NEWS=7 points. Sensitivity 42% matches that of NEWS=5 points.
[b]P<0.0001 for comparison
[c]P<0.0001 for comparison

3.8 IMPACT OF TIME TO APPROPRIATE ANTIMICROBIAL TREATMENT IN BLOODSTREAM INFECTION

The main reason to identify sepsis early is to enable initiation of prompt antimicrobial treatment. However, the urgency of antimicrobials is an area of debate and the impact of timing of appropriate antimicrobial therapy with *in vitro* coverage of the cultured pathogens remains unclear. To study this question, we used the 2SPARE database and focused on bloodstream infections (**Paper IV**). After applying inclusion and exclusion criteria, the cohort comprised 10 628 BSI-episodes, occurring in 9192 unique patients. The study population had a median age of 69 years, 56.8% were females and 30-day mortality was 11.8%. The vast majority of BSIs were community-onset (85.3%), and the most prevalent pathogens were *E. coli*, *S. aureus* and viridans streptococci. Polymicrobial BSI was uncommon (11.5%), and few episodes included possible skin contaminants (9.9%) or antimicrobial-resistant strains (4.0%). Based on high SOFA-scores and a high proportion of combination therapy, the patients who received appropriate therapy within 1 hour seemed to suffer from more severe illness. Furthermore, in

the group of patients receiving appropriate therapy after 24 hours, the proportions of antimicrobial-resistant pathogens, polymicrobial infections and potential contaminants were high. Septic shock at blood culture collection occurred in 608 of 10 628 (5.7%) episodes, and the majority of these had received appropriate treatment within the first hours. At the 12-hour landmark time, only 77 of the 608 (12.6%) septic shock episodes still had inappropriate treatment.

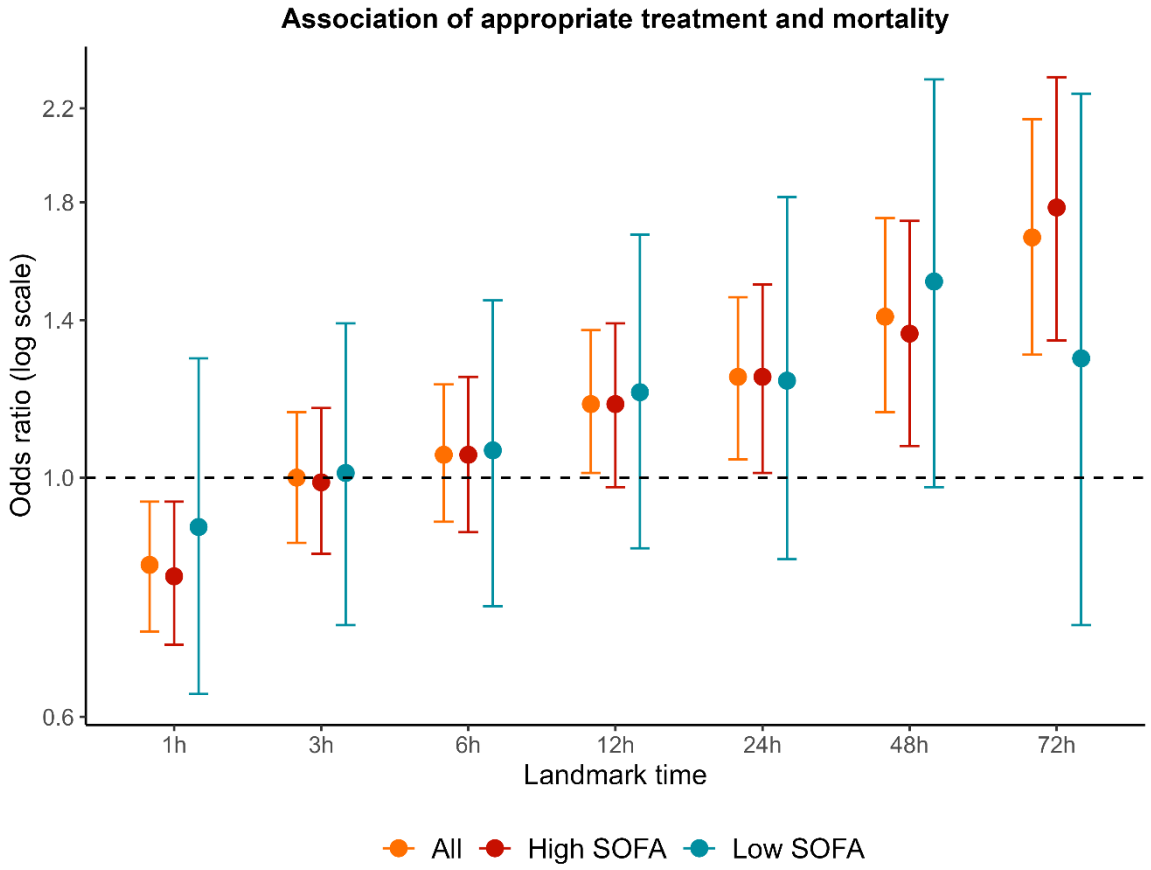

Figure 9. Association of inappropriate therapy and mortality stratified by all episodes, episodes with low SOFA score <2 and episodes with high SOFA score ≥2. The odds ratios are adjusted for age, sex, CCI, immunosuppression, SOFA-score, polymicrobial bloodstream infection, source of infection, admission year and community vs. hospital-onset.

In Figure 9, the association between appropriate therapy and 30-day mortality is presented for each landmark time. At the 1-hour landmark, inappropriate treatment was associated with lower risk of death (OR 0.83 [95% CI, 0.72-0.95]), indicating some residual indication bias despite controlling for several factors associated with disease severity. At the 12-hour landmark, inappropriate treatment was associated with significantly increased mortality (OR 1.17 [95% CI, 1.01-1.37)]), and the risk was increased also at the later time points. The point estimates were not affected by stratifying on disease severity to any large degree, but the precision decreased. In episodes with septic shock, we did not have sufficient power to make any conclusions, and there was no clear trend in any direction.

3.9 ASSOCIATION OF BACTERIAL VIRULENCE GENOTYPE WITH PATIENT CHARACTERISTICS, SEPTIC SHOCK AND MORTALITY IN *P. AERUGINOSA* BLOODSTREAM INFECTION

As shown in **Paper IV**, appropriate antimicrobial treatment is a crucial prognostic factor in BSI. However, we also noticed heterogeneity when looking into subgroups of different BSI pathogens suggesting that other bacterial factors may play a role in determining patient outcomes (**Paper IV**, Supplement Figure 6). In BSI, *P. aeruginosa* is one of the most challenging pathogens, commonly found in hospital-onset infections. Several laboratory studies have suggested that bacterial virulence genotypes may influence infection outcome, and that virulence could be targeted by interventions or novel adjuvant treatments in addition to antibiotics, but data from clinical infections are sparse. From the PA BSI network, we recruited centers which routinely saved their bacterial isolates (**Paper V**). In total, 773 patients were included with median age 68 years (IQR 57-78 years) and 267 of 773 (34.5%) females. Comorbidity was common and 571 of 773 (73.8%) patients had a CCI index of 2 points or more. In total, 138 of 773 (17.9%) patients had chronic lung disease, 577 of 773 (47.1%) patients were immunocompromised and 131 of 773 (16.9%) patients had no underlying comorbidities registered. Overall, 120 of 773 (15.5%) patients died within 7 days and 182 of 773 patients (23.5%) died within 30 days of blood culture. Septic shock at BSI onset occurred in 115 of 639 (18.0%, Seville excluded due to missing data) patients and the mortality rate was 44.4% (51 of 115 patients) day 7 and 53.9% (62 of 115 patients) day 30.

Table 10. Association of virulence cluster with sequence type and antimicrobial resistance

Variable	Virulence cluster					
	A	B	C	D	E	F
Size, No. (% of total cohort)	36 (4.6)	51 (6.6)	72 (9.3)	134 (17.3)	220 (28.5)	237 (30.7)
MDR phenotypes, No. (%)	2 (5.6)	0 (0.0)	2 (2.7)	32 (23.9)	36 (16.4)	39 (16.5)
Dominating sequence type	ST253	ST274	ST244	ST111	ST175, ST313, ST395 and ST446	ST235, ST179 and ST17

Abbreviations: multi-drug resistance (MDR) and numbers (No)

The most common ST was ST244 (n=36), followed by ST111 (n=34), ST235 (n=31) and ST175 (n=28). The proportion of MDR phenotype differed depending on ST (p=0.0001), with 85.3% of ST111, 71.4% of ST175 and 64.5% of ST235 isolates exhibiting such resistance. In total, 83 of 112 (74.1%) MDR isolates belonged to one of the epidemic clones. There was a significant age difference between patients depending on epidemic clone (p=0.009). Patients with ST175 (median 60 years [IQR 50-67 years]) and ST446 (median 55 years [QR 47-58

years]) were younger, while patients with ST313 (median 71 years [IQR 68-84 years]) were older. Chronic lung disease was common in patients infected with ST175 (11 of 28 patients [39%]), but not in patients infected with ST235 (1 of 31 patients [3%]) (p=0.01). None of the patients with ST313 had chronic lung disease. Hosts with immunosuppression or absence of underlying comorbidities were not associated with epidemic clones (p=0.50 and 0.24). Using PCoA on a matrix of all annotated genes in the VFDB, we made a novel discovery of 6 large genotypic virulence clusters including more than 20 isolates (Cluster A-F). Each cluster was present in several geographical locations and was dominated by different STs (Table 10), but was not associated with specific patient phenotypes.

In further analyses, we focused on the association between virulence genotype with mortality and septic shock. ST235 and ST175 were associated with increased 7-day and 30-day mortality after adjusting for confounding factors. None of the STs were significantly associated with septic shock at onset of BSI. Similar findings were seen for virulence clusters, which were neither associated with mortality nor septic shock. Similarity between query nucleotide sequence with reference genes involved in rhamnolipid biosynthesis were negatively associated with 7- and 30-day mortality (OR 0.36 [95% CI, 0.13-0.98], p<0.05; and OR 0.33 [95% CI, 0.13-0.83], p=0.02, respectively). In addition, flagella (*flaG, fleP, flg, fliC, fliD, fliS*) and gene reference homology were negatively associated with 7-day mortality (OR 0.56 [95% CI, 0.35-0.90], p=0.02; and OR 0.36 [95% CI, 0.13-0.98], p=0.05, respectively). Presence of the *exoU* genotype and homology in Elongation factor Tu (*tufA*) with gene reference were associated with septic shock (OR 1.99 [95% CI, 1.08-3.64], p=0.03; and OR 2.96 [95% CI, 1.38-6.34], p=0.005, respectively), but not with mortality. The *exoS* and *exoY* genotypes were negatively associated with septic shock (OR 0.52 [95% CI, 0.29-0.95], p=0.03; and OR 0.41 [95% CI, 0.21-0.81], p=0.01, respectively).

In the next step, we used a random forest classifier to assess if virulence genotype added any predictive information compared to using only information on readily available patient factors. In Model 1, the virulence genotype was based on features selected using the Boruta algorithm, but it is worth noticing that the algorithm does not focus on the direction of the association. Figure 10 shows the distribution of selected variables after the Boruta was run 20 times using different random 80/20 splits of data. For predicting 7-day mortality, the variables most often deemed important by the Boruta were related to pyoverdine (*fpvA, pvdD, pvdI, pvdJ*) and rhamnolipid (*rhlA, rhlB* and *rhlI)* synthesis. For predicting 30-day mortality, rhamnolipid was selected by the algorithm every time. In septic shock, the T3SS related effector proteins *exoS* and *exoU* were most often selected. Predictions of mortality and septic shock using the random forest model generally performed slightly better when adding virulence genotype data compared to using patient variables alone (Table 11). When predicting mortality, the prediction models had higher AUROC than the reference model in 14 to 19 of the 20 algorithm runs (70-95%) depending on model. For prediction of septic shock, Model 1 performed better than the reference model in 15 of 20 (75%) algorithm runs. Overall, the differences in AUROC between the models using virulence data compared to the reference model were small.

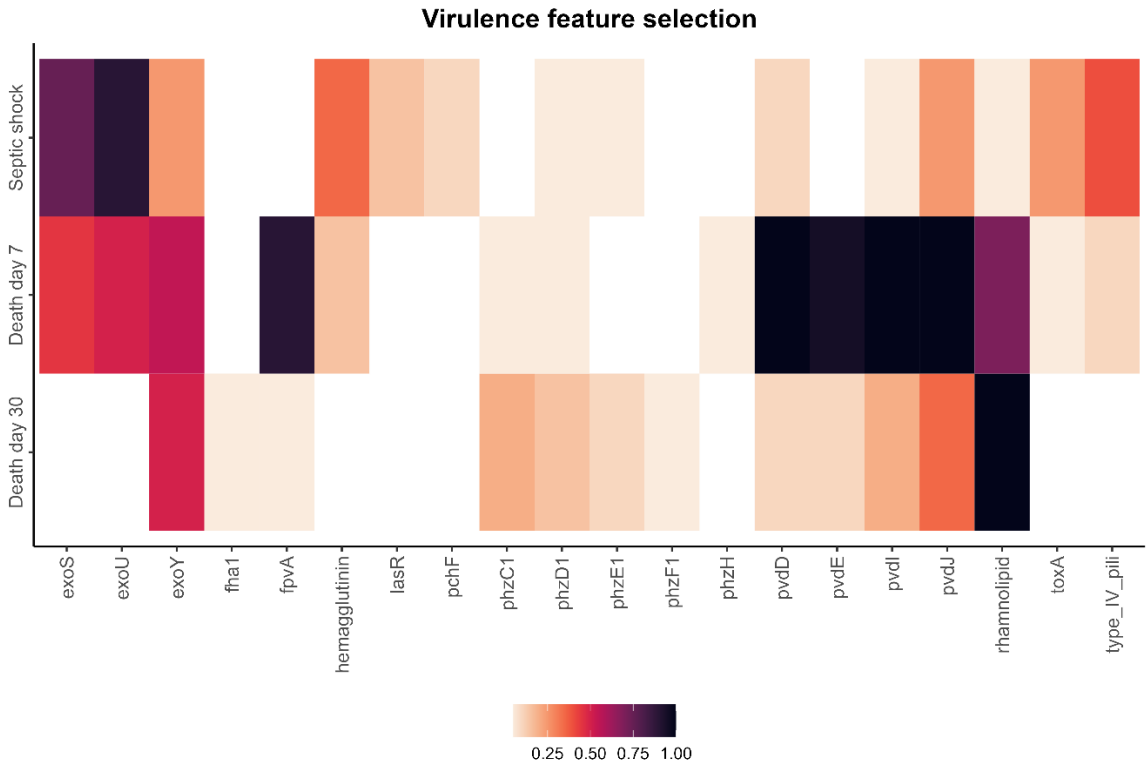

Figure 10. Results from running the Boruta feature selection algorithm on the training set, repeated for each of the 20 random splits of data. The color scale represents the proportion of algorithm runs in which the feature was selected. The figure only includes virulence features selected a minimum of once and white color represents feature that where never selected for that specific outcome.

Table 11. Prediction model outputs with and without virulence genotype data

Model	Mortality day 7 Median AUROC (IQR)	Mortality day 30 Median AUROC (IQR)	Septic Shock* Median AUROC (IQR)
Reference model – Patient factors	0.743 (0.715-0.763)	0.706 (0.681-0.734)	0.803 (0.761-0.832)
Model 1 – Patient factors and Boruta features	0.756 (0.711-0.795)	0.725 (0.697-0.747)	0.816 (0.789-0.842)
Model 2 – Patient factors and all virulence features	0.731 (0.707-0.786)	0.713 (0.685-0.742)	0.791 (0.771-0.821)
Model 3 – Patient factors and virulence clusters	0.757 (0.715-0.789)	0.731 (0.702-0.750)	0.803 (0.758-0.828)
Model 4 – Patient factors and epidemic clones	0.761 (0.728-0.804)	0.736 (0.717-0.768)	0.775 (0.755-0.793)

Abbreviation: area under receiver operating characteristics (AUROC) and interquartile range (IQR)

*Seville was excluded from the training an testing data due to missing data on septic shock

4 DISCUSSION AND PERSPECTIVES

In this thesis, several findings with implications for diagnosing and treating sepsis and BSI are presented. The results are based on observational data from 7 cohorts of more than 100 000 hospital episodes, as well as WGS data from approximately 800 invasive *P. aeruginosa* isolates collected from several centers in Europe and Australia.

In **Paper I,** we demonstrated that fully automatic surveillance of sepsis incidence using the Sepsis-3 case definition is feasible outside an ICU setting, with examples of how implementing this model generates continuous high quality epidemiological data down to individual ward level. In **Paper II**, evidence is provided for using non-invasive measurements of SpO2 to diagnose respiratory dysfunction in the Sepsis-3 criteria, proposing the novel cut-offs SpO2 94% and 90% to generate 1 and 2 SOFA respiratory points, respectively. This has important implications for improving sepsis diagnosis, especially when conventional arterial blood gas measurements are not available in epidemiological surveillance and research, but also in clinical situations such as emergency practice, rapid response teams and resource-limited settings. In **Paper III**, we showed that the sepsis surveillance classification can be utilized to develop machine learning screening tools to improve early identification of sepsis. A Bayesian network algorithm (SepsisFinder) trained on sparse routine EHR data was able to predict sepsis onset within 48h with better discriminatory performance and earlier in the clinical course than conventional NEWS2 outside the ICU-setting. Based on the results, screening may primarily be suited for the period directly following admission when the pre-test probability of sepsis is higher, which have implications also for other sepsis screening tools. In **Paper IV**, we demonstrated that receiving appropriate antimicrobial treatment after 12 hours in BSI were associated with increased mortality, but not if treatment were delayed for only 1, 3, or 6 hours. This indicates a time window where clinicians should focus on the diagnostic workup, and proposes a benchmark for developing rapid diagnostics of blood cultures. Finally, in **Paper V**, we showed that the bacterial virulence genotype has some impact on mortality and septic shock in *P. aeruginosa* BSI, but that the added value of including virulence data in the prognostic assessment of these patients was minor.

Educational efforts and the introduction of sepsis care bundles have been associated with decreased mortality, justifying structured management of sepsis in hospitals [107,204–206]. A cornerstone of most quality improvement programs are disease surveillance with feedback to healthcare providers [64]. Using objective clinical data for surveillance have been shown in several studies to offer an unbiased estimate over administrative diagnosis codes [102]. In **Paper I**, and the related editorial, the first ever report of a validated fully automated surveillance algorithm built on the Sepsis-3 criteria is presented [379]. The algorithm correctly captured almost 90% of sepsis patients, while only 13.4% had ICD-10 codes corresponding to sepsis, confirming findings from previous studies comparing medical record review to ICD-codes [97,98]. By using a physician reviewed gold standard, we could also show that the Sepsis-3 clinical criteria in 91% of cases captured patients where clinicians continue to maintain infection as a

likely diagnosis also after the initial treatment phase, when more diagnostic information is available. This provides an external validation of the Sepsis-3 criteria, and speaks in favor of applying them for epidemiological surveillance in clinical practice.

The major strength of the developed surveillance algorithm is that it is objective, scalable and utilizes readily available EHR data, which enables classification of sepsis onset down to individual ward and patient level. The integration of surveillance data in patients' medical records, may help bridging the gap between healthcare providers, data analysts and policy makers. As an example, the surveillance algorithm is currently being implemented in Region Västerbotten. The algorithm script has been adapted to prospective data and screens all admitted patients every 24 hours and generates daily reports into a web-based interface called Patient Safety Surveillance System (P3S). Between January and September 2022, the algorithm captured 441 sepsis episodes at Umeå University hospital, of whom 14% developed in two specific internal medical wards (personal communication Andreas Winroth). This information is now provided as feedback to wards as part of infection control measures. Similar results were seen in the retrospective data from Karolinska University Hospital presented in **Paper I**, where hospital-onset sepsis differed substantially between different wards, enabling targeted interventions.

The main limitation of fully automated sepsis surveillance is misclassification of episodes. As seen in the validation, the algorithm missed approximately 10% of sepsis cases, and when looking specifically at patients with suspected infections, it also misclassified 10% of non-sepsis cases as being sepsis. If using only aggregated data from longer time periods to interpret trends, this is usually a minor issue. However, if surveillance is integrated more closely in the EHR of individual patients, this may affect the credibility of the classification and such implementation would require close collaboration with healthcare providers to clarify the limitations and avoid misunderstandings. This would be required despite the fact that algorithm performance likely is similar to that of the general clinician. Based on this, surveillance data also needs to be used with caution in pay-for-performance measures, or in similar incentives. It is also important to emphasize that the degree of preventability of sepsis in the setting of surveillance is highly uncertain, which needs to be considered when communicating surveillance data directly to healthcare providers, but also to patients [380].

As shown in Figure 11, surveillance of infections can be organized either centrally or locally [381]. One of the best examples of a surveillance system based on objective clinical data in current use is the national Healthcare-Associated Infections Database (HAIBA) in Denmark [382,383]. HAIBA uses centrally implemented algorithms based on individual patient data to assess incidence of 5 healthcare-associated infections: BSI, urinary tract infections, *Clostridoides difficile* colitis and deep seated post-surgical infections after hip or knee replacements. A major difference between HAIBA and the sepsis surveillance algorithm in **Paper I** is the resolution of data, since HAIBA does not include information on administered medications and vital parameters [382,384]. This has substantial implications for the precision of healthcare-associated infection surveillance using HAIBA [385,386].

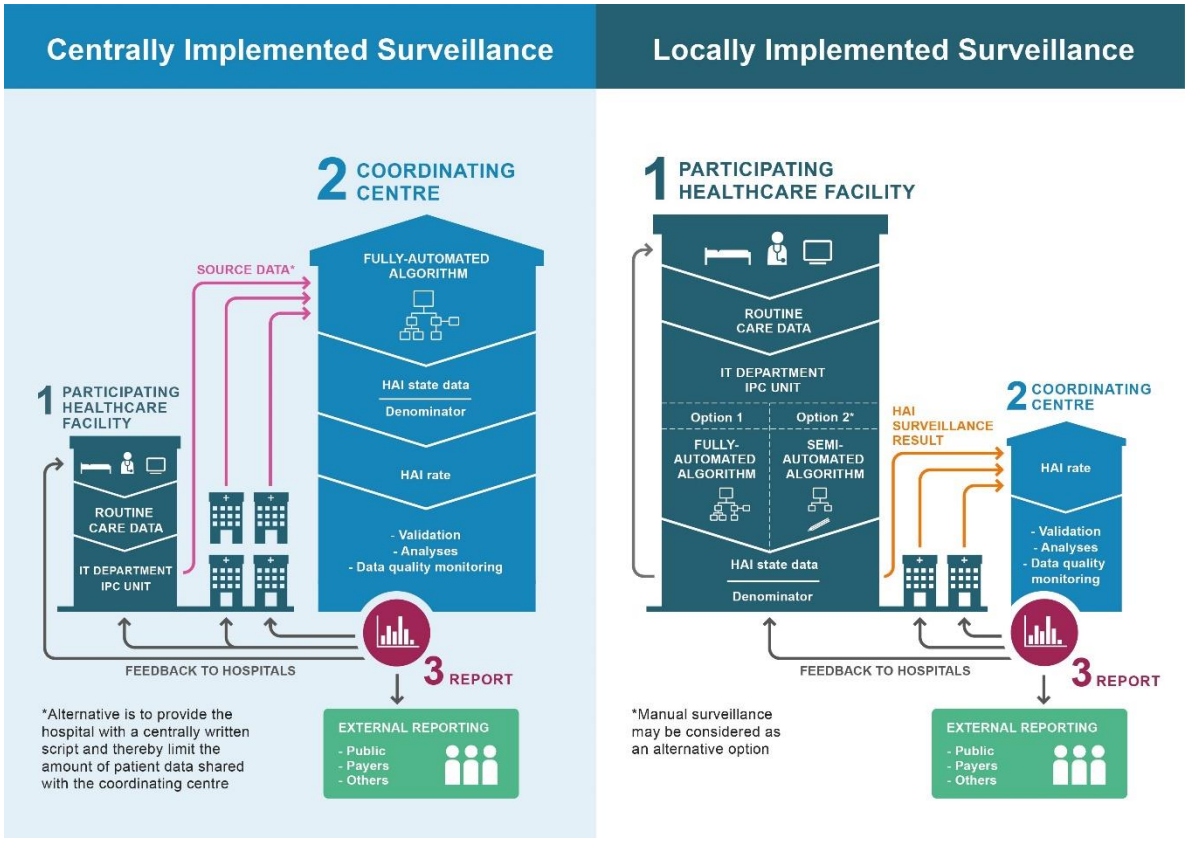

Figure 11. Illustration of approaches to implementation of incidence surveillance of healthcare-associated infections within networks of hospitals. **Abbreviations:** healthcare-associated infection (HAI) and infection prevention and control (IPC). The figure is reproduced from (van Mourik MSM, et al. Clin Microbiol Infect. 2021 Jul, S3-S19), created by John Karlsson Valik and Elin Abbevik, and reused with permission from Elsevier.

The Sepsis-3 criteria are based on the SOFA score, which was originally developed for the ICU-setting [27]. When building the surveillance algorithm in **Paper I**, it required a few adaptations of the Sepsis-3 criteria to better fit data from non-ICU wards. One of the major alterations was including SpO2 measurements in addition to directly measured PaO2 for assessment of the SOFA respiratory score. In the original study by Seymour et al. presenting the operational Sepsis-3 criteria, less than one third of non-ICU patients had PaO2/FiO2 measurements available, but it is unclear how many of the patients classified as sepsis that actually had SOFA respiratory score triggers [26]. In **Paper I**, approximately 75% of sepsis patients had SOFA score triggers involving respiratory dysfunction, with the majority of them based on SpO2. The advantages of using SpO2 over arterial blood gas measurements to assess respiratory function are many: it is cheap, it is widely available, it takes minimal amount of time to register, it can be used for continuous monitoring, it does not require any specific training, and most importantly, it is not an invasive procedure involving the risk of harming patients. However, the strength of being easy to use is also the main problem with SpO2 since it becomes prone to measurement errors, especially when registered widely in the EHR. SpO2 measurements can also be inaccurate in patients with circulatory shock or darker skin colour, especially at Spo2 at lower saturation levels [387]. The use of SpO2 had previously been validated in the Kigali modifications of the Berlin ARDS classification [388], as well as in pure correlation

studies between SpO2 and PaO2 measurements [389,390], but it was unclear if it were appropriate as a measurement of respiratory dysfunction in sepsis.

In **Paper II**, we confirmed in patients with suspected infections, that SpO2 indeed was associated with mortality, except for patients with chronic pulmonary disease. We also showed that the predictive performance of the SOFA score improved by including data on SpO2. These findings have important implications for sepsis classification, since our data suggests that routinely registered SpO2 measurements can be trusted, and that arterial blood gas analysis may be redundant in the non-ICU setting. It is also a step towards facilitating sepsis research, also from an ethical standpoint, since it validates respiratory assessment based on a non-invasive strategy and strengthens the case for using the Sepsis-3 criteria in both observational and clinical studies in resource-limited settings. However, even though comparison of AUROC between SOFA score using SpO2 or PaO2 in the KH cohort showed similar AUROCs, a limitation of the study was that we did not have access to head-to-head comparison of PaO2 and simultaneously measured SpO2. Unfortunately, data on oxygen delivery and mechanical respiratory support was usually lacking, and further studies are needed to better account for these factors.

Another implication of our study was the suggestion to use specifically developed thresholds for SpO2 (SpO2 94% and 90%), and not the PaO2 based thresholds (SpO2 95% and 91%) to generate up to 2 points in the SOFA score. The new thresholds (SpO2 94% and 90%) lead to fewer cases being classified according to the Sepsis-3 criteria, but similar or slightly higher mortality. This suggests increasing specificity in sepsis classification, which are more likely to improve acceptance among clinicians. It was also an illustration of how seemingly small changes in thresholds can have substantial influence on an operationalised classification like the Sepsis-3 criteria, which stresses the need to perform proper validation when adapting prior scores to new settings. During 2021, an automated SOFA score calculator was implemented in the EHR system in Region Stockholm. Using the findings from **Paper II**, this calculator is currently being updated to use the novel SpO2 thresholds for respiratory assessment, if arterial PaO2 is not measured directly. The decision to use the new threshold reflected a general view among the clinicians in the implementation steering group that these cut-offs were better aligned with their conception of a pathological SpO2 value. A next step will be to advocate for the formal inclusion of these SpO2 thresholds in the SOFA score, whenever the score is applied in patients outside the ICU-setting.

The sepsis surveillance algorithm developed in **Paper I** also provided the ability to easily, and with high precision, classify a large number of hospital episodes according to time of sepsis onset. This type of high-throughput classification is well suited to create an objective benchmark for sepsis prediction tools integrated with the EHR system. It is also an approach that captures the entire intended screening population and generates results which are easier to compare and more generalizable to other settings. In **Paper III**, this was demonstrated by using the surveillance data to train and validate a machine learning prediction model. Our score was based on a supervised Bayesian network model, which is especially suited for capturing

dynamic uncertainty relevant for decision-making and has superior learning properties compared to machine learning methods based on merely mimicking historical data [391,392]. The model principle is similar to that of clinical reasoning, providing a more straight forward concept for clinicians [393]. This may improve the users trust in model predictions, which has been acknowledge as a major barrier in implementation [115,125].

In **Paper III**, we aimed to simulate the algorithm as it would be implemented in a clinical setting. Sepsis related organ dysfunction was used as the main outcome to better reflect the pathophysiological onset of sepsis, rather than predicting the time of clinical identification based on cultures or antibiotic administration. In addition, we evaluated the score in all patients admitted to the hospital, which would be the intended screening population. The AUROC of SepsisFinder was within a similar range, or higher, than reports of sepsis prediction models based on other machine learning techniques [160]. Many studies report a cumulative maximum score, meaning no limit on how early sepsis is detected, which has low clinical applicability since the positive alarm can be unrelated in time to the actual sepsis episode [167,173]. In **Paper III**, to ensure correlation with the sepsis event, we only considered alarms within 48 hours of a sepsis case as true positive.

When dealing with unusual outcomes and high-class imbalance in prediction modelling, presenting AUROC alone can be misleading. In **Paper III**, sepsis was uncommon with 9.8% of patients in the validation set experiencing a sepsis event, which is within the similar range of other studies. Despite this, APR curves have not been frequently reported in studies of sepsis screening tools based on machine learning [167–170,173]. In most circumstances, since sepsis is a medical emergency associated with substantial mortality, high sensitivity would likely be preferred. However, this comes at the expense of more false positive alarms. The proportion of false positive alarms among all positive alarms (PPV) are usually considered highly relevant in the clinical setting, especially if healthcare providers are expected to act on the alarms, or to avoid alarm fatigue [115]. The PPV is affected by the prevalence of the outcome. In **Paper III**, the majority of sepsis events developed within the first days of admission and only 1.4% of the total cohort had a sepsis event occurring later during the hospitalization. This resulted in decreasing prevalence of sepsis with longer hospital stay, which partly explains the lower PPV. SepsisFinder, as well as other sepsis screening tools, may thus have better applicability early during hospitalization, when sepsis is much more common. Furthermore, the APRs indicated that the SepsisFinder performed better in culture positive sepsis and risk periods prior to surgery, than in culture negative sepsis and risk periods after surgery. This may be reflective of the complexity of classifying sepsis in patients with an inflammatory response caused by other processes than infections and could indicate misclassification of both the reference surveillance classification and the SepsisFinder prediction in these episodes.

The main limitation in **Paper III** is that our evaluation was based on retrospective data from one center, and although we trained and tested our model using different time periods (rather than just a random split), the SepsisFinder would need validation in external data sets to confirm performance. The predictions generated by SepsisFinder were calculated as a

continuous probability between 0-100% (similar to most prediction models). This type of information can be difficult to interpret in a busy clinical setting, and threshold dependent classifications are usually easier to act on [115]. It is worth noticing that, if implemented in real-world settings, prediction scores may modify the risk of reaching the outcome, making post-implementation evaluations more difficult. To overcome some of these inherent problems of balancing thresholds between sensitivity and PPV, combining screening with other interventions could be a way forward. Identifying a high-risk population automatically based on dynamic patient factors, but accepting a higher number of false positive cases, enables coupled interventions such as increased surveillance, checklists, or selecting patients where more advanced or costly testing is warranted, and in which patients it is not. However, increased testing or other interventions in false positive patients may also inflict more harm and costs, than good. Another appealing approach could be to focus on "rule-out" instead of "rule-in". An example of this strategy is provided by Boerman et al. who showed that using a machine learning model, blood cultures could be safely withhold in 30% of patients in the ED due to low probability of positive growth [196,394]. Finally, further studies on implementing sepsis screening in a real-world clinical scenario are needed to evaluate the integration with clinical workflows and the potential impact on patients' outcomes.

The main argument to focus on early identification of sepsis is to initiate prompt treatment. In particular, early antimicrobial treatment has been shown to decrease mortality in large observational studies of sepsis. This has affected both Swedish and international guidelines to recommend broad-spectrum antibiotics within 1 hour of septic shock and 3 hours of sepsis [105,203,227]. However, the evidence behind these recommendations are not convincing, except for maybe septic shock or bacterial meningitis [223]. Additionally, very few studies have assessed the appropriateness of treatment based on *in vitro* drug-pathogen coverage.

In **Paper IV**, we focused on culture positivity (i.e. BSI). Many of the included patients had sepsis, but the inclusion was not restricted to a sepsis population. The results showed a weaker association for timing of antibiotics than previous studies of sepsis have reported, which may have several explanations [225,226]. Time zero is a rather arbitrary time point and it is not likely that there would be a biologically plausible effect of hourly delays in treatment, but rather the effect of treatment should sum up over longer time periods [222]. Since we did not restrict the analyses to a sepsis population, it is likely that our BSI cohort had less critical illness compared to previous studies. It was a single center cohort from Sweden, where healthcare is easily accessible, which means patients may have sought care earlier in their disease trajectory. Other beneficial care decisions, such as fluid therapy, might also be more equally distributed among the groups of appropriate and inappropriate treatment, resulting in a smaller residual effect attributed to antimicrobials.

Furthermore, as illustrated by the septic shock patients, the absolute majority of patients with critical illness had received treatment within 12 hours, and the levels of antimicrobial resistance was very low. This means, patients receiving the most commonly used antibiotics in our hospital, also has a high likelihood of receiving appropriate treatment. The fact that patients

receiving appropriate treatment within 1 hour of blood culture collection were more likely to die, is an illustration of the well-known paradox, that the sickest patients receive broad-spectrum treatment earlier (indication bias). Despite our efforts to control for this effect, the analyses likely suffered from residual indication bias. Further support of this can be found in Supplement Figure 7 in **Paper IV,** where patients with resistant pathogens had higher odds ratios at every time thresholds, but confidence intervals were large due to the small sample size. On the other hand, we studied a very large cohort (the second largest study of its kind) and would the association between receiving immediate treatment be strong, we would most likely have captured it with our data. Even among patients with septic shock at onset, the groups were evenly distributed at the 1h landmark (n=266 with inappropriate treatment and n=317 with appropriate treatment), and a bit less so at the 3h landmark (n=149 with inappropriate treatment and n=418 with appropriate treatment), but still the risk of death were not higher in those with inappropriate treatment (Supplement Figure 2 in **Paper IV**).

Despite a possible trend towards increased mortality also at earlier thresholds, there was a significantly increased mortality first at the 12-hour threshold. In terms of clinical implications, what does the 12-hour threshold means? The findings in **Paper IV** showed a clear benefit of adequate empirical treatment in BSI, which is similar to the largest study of appropriate treatment in BSI as of today, although that study could only assess adequate treatment on the day of blood culture compared to later treatment [220]. Current practice in clinical microbiological laboratories rarely provides microbiological culture results earlier than after 24 hours. This means that antimicrobial treatment needs to be given before the culture results are available for many patients. Choosing appropriate treatment is not easy and studies have shown that unnecessary broad-spectrum or combination regiments in sepsis is associated with higher mortality [2]. So should clinicians wait for 12 hours before giving treatment? The simple answer is no; there is no end in itself to wait if the suspicion of BSI is high. On the other hand, our findings support a slightly more nuanced approach than the "one size fits all" time limits provided in guidelines and should motivate clinicians to perform a more extensive diagnostic work up, which is often possible to achieve within 12 hours. This enables clinicians to support their suspicion of BSI, as well as the likely source of infection, with objective findings such as laboratory results or radiology. This facilitates giving targeted and individualized treatment, as opposed to just giving broad-spectrum treatment to all and wait for the culture results. Advances in faster microbiological analyses will support this approach even more.

Antimicrobial treatment is one of few modifiable prognostic factors in sepsis and BSI. Other therapeutical interventions, such adjuvant anti-virulence agents have been suggested, but few have reached late stages of clinical development [314,316]. The first anti-toxin drug to be introduced on the market was bezlotoxumab, a monocloncal antibody targeting *Clostridoides difficile* toxin B [316]. Other anti-toxin treatments, targeting the alpha-toxin in *S. aureus* (e.g tosatoxumab) and the T3SS in *P. aeruginosa* (Ftortiazinon) are currently undergoing phase 2 and 3 trials [314,316]. With this ongoing therapeutic development in mind, the aim with **Paper V** was primarily to assess the impact of indirect markers of virulence on patient outcomes in *P. aeruginosa* BSI. One of the main strengths of the study was that isolates were collected

consecutively from all *P. aeruginosa* BSI during the study period. The study also presents one of the world's largest cohorts of whole genome sequenced *P. aeruginosa*, collected from several different geographical sites in Europe and Australia, speaking in favor of generalizability of the findings.

The data presented in **Paper V** provides a unique molecular epidemiological description of the distribution of sequence types and resistance phenotypes in *P. aeruginosa* BSI, which may have implications for vaccine development. As an example, we noted that one third of BSIs were caused by any of 11 most common clones, and these isolates accounted for 75% of all MDR phenotypes. Targeting these strains with an effective vaccine would have a major impact on the prevalence of antimicrobial resistance in *P. aeruginosa* BSI. We were also able to identify 6 major virulence clusters, which were associated with specific STs and differed in the proportion of MDR phenotypes. These results are in line with previous findings that clonal complexes are associated with specific virulence phenotypes [334–336]. The virulence clusters were not associated with patient outcomes in the multivariable logistic regression model, however, the random forest model which combined data on patient factors and virulence cluster generally had a slightly higher AUROC when predicting mortality than the reference model. For predicting septic shock, neither data on epidemic clones, nor virulence cluster added any important information to the model. This indicates that the virulence clusters may have some importance for mortality prediction, but it is likely that this is due to the association between cluster and ST, rather than an independent virulence profile.

Unlike annotation of acquired resistance genes, which are either present or not, interpreting results from virulence gene annotation is more complex. Most genes with a matching frequency of less than 98% in gene annotation (Supplement Figure 1 in **Paper V**) were part of the chromosomal accessory genome (e.g. T3SS), but some of the genes are essential and generally found in the core genome (e.g. Elongation factor-Tu) [326]. For some of the virulence genes, a match/no match can be interpreted as gene presence/absence (e.g. T3SS), while for others, a match/no match means heterogeneity between the query nucleotide sequence and the reference virulence protein [326]. In other words, the gene is "there", but it is different. With this distinction in mind, the findings indicated both factors associated with mortality, such as rhamnolipid synthesis, pyoverdine and flagella function, as well as factors associated with septic shock, such as the T3SS effector proteins. The factors associated with mortality are all connected to biofilm formation, and may thus be associated with chronic infections where host adaptations are better tuned and virulence usually is less [395–397]. The T3SS, on the other hand, has in both laboratory and clinical studies mainly been linked to invasiveness and disease severity, which is well in line with our findings of an association with septic shock [349,350,352].

A limitation of **Paper V** is that we did not assess virulence phenotype and it is unclear if our genotype classification translates into actual virulence traits in all isolates. However, laboratory studies in *P. aeruginosa* has previously shown that virulence genotype is clearly associated with the T3SS virulence phenotype [353]. Another caveat is that we only had access to *P. aeruginosa* recovered from blood cultures, meaning they were already capable of causing

invasive disease. We cannot rule out that all isolates included in this study had a more homologous virulence genotype and that genomic comparison with strains recovered from other infections, colonization, or environmental sources, would have yielded more distinct differences. Indeed, a comparative genomic study of *P. aeruginosa* recovered form ophthalmologic and cystic fibrosis patients generally showed high genomic diversity, but also clustering of specific strains in eye infections [398].

In a previous study from the PA BSI network, risk factors for mortality were mainly unmodifiable patient variables such as age, gender, comorbidity, immunosuppression, hospital-onset infection, and ICU care [364]. In **Paper V** we sought to investigate if adding virulence genotype markers improved prediction of patient outcomes compared with a reference model using only the patient factors. This was a pragmatic evaluation aimed at assessing virulence data which is readily available and would be easy to implement in an analytical pipeline for the clinical setting. We wanted to assess WGS as a routine diagnostic tool in *P. aeruginosa* BSI, and did not take into account other genomic aspects, such as mutations, accessory genome determination or transcriptomics [355]. A random forest model was chosen mainly because of sample size and to account for complex non-linear interactions between predictors. The model performances showed quite large variability depending on the 80/20 random splits, indicating limited power to draw firm conclusions. In general, the models using selected amounts of virulence data performed slightly better than the reference model, but the difference in AUROC between models were small. Based on these findings, collecting virulence genotype information routinely in *P. aeruginosa* BSI is probably not warranted for prediction of outcome in the clinical setting. Yet, our findings indicate that the virulence genotype contains at least some relevant prognostic information, and we cannot rule out a possible effect of adjuvant anti-virulence treatments. It remains unclear if these findings can be extrapolated to other important BSI pathogens, and additional studies would be needed to assess the overall value of WGS in the diagnostics of BSI.

5 CONCLUSIONS

The following conclusions are drawn:

I. Using data from electronic health records, an automated surveillance algorithm based on the Sepsis-3 criteria had good validity compared with medical record review in non-ICU wards. The algorithm revealed differences in the burden of hospital-onset sepsis depending on ward type.

II. Lower peripheral oxygen saturation (SpO2) was associated with gradually increasing mortality in patients with suspected infections presenting to the hospital. Mortality prediction improved if the SOFA score included respiratory assessment based on SpO2 94% to get 1 point and SpO2 90% to get 2 points, supporting its use in the Sepsis-3 criteria as an alternative to arterial blood gas measurement outside the ICU-setting.

III. A machine learned Bayesian network algorithm (SepsisFinder) trained on sparse routine electronic health record data was able to predict sepsis onset within 48 hours with better discrimination and earlier in the clinical course than NEWS2 outside the ICU. Based on the higher positive predictive value earlier during hospital stay, SepsisFinder may primarily be suited for the period directly following admission.

IV. Delays in appropriate antimicrobial treatment for 12 hours and beyond were associated with increased 30-day mortality in bloodstream infection. These findings indicate a tagret for developing rapid diagnostics of blood cultures to guide empirical treatment.

V. The bacterial virulence genotype was associated with increased mortality and septic shock in *P. aeruginosa* bloodstream infection. The added value of virulence genotype data in the prognostic assessment of *P. aeruginosa* bloodstream infection was minor.

In summary, this thesis demonstrates an objective and scalable approach to incidence surveillance and early detection of sepsis outside the ICU-setting, as well as a simpler method to diagnose sepsis-related respiratory failure with SpO2. In BSI, evidence is provided for initiating appropriate antimicrobial treatment within 12 hours after blood culture collection, and the findings suggests that *P. aeruginosa* virulence genotype may affect disease severity and mortality, however, it was not a major prognostic determinant.

6 FUTURE DIRECTIONS

With regards to future research and clinical implementation, this thesis provides an outlook in three main areas: (I) Automated epidemiological surveillance and risk assessment, (II) improving disease severity scores and guidelines, and (III) detailed molecular diagnostics of acute bacterial infections.

6.1 AUTOMATED EPIDEMIOLOGICAL SURVEILLANCE AND RISK ASSESSMENT

The fully automated sepsis surveillance algorithm presented in **Paper I** is a proof-of-concept for using high-resolution patient data from EHRs to monitor the incidence of severe infections. Further studies to apply and validate a similar model also in the ICU-setting is needed. The approach offers a novel, objective and accessible data source for both healthcare providers and policy makers, but also researchers and the public. To implement this using real-world data, there are several barriers to overcome, such as legal requirements, endorsement by stakeholders and availability of source data [381,399]. However, as already mentioned, the algorithm in **Paper I** is implemented prospectively in Region Västerbotten and efforts will be concentrated on including additional hospitals. Future works should focus on better adapting todays surveillance case definitions to structured EHR data, investigate how to implement case-mix adjustments and validate user interfaces and trend-analysis. The PRAISE – providing a roadmap for automated infection surveillance in Europe – network was formed in 2019 to address some of these issues resulting in three guidance documents [65,381,399]. The network is currently working with a consistent surveillance definition of hospital-onset BSI in Europe.

Another benefit of having rule-based algorithms to classify severe infections based on objective clinical data (**Paper I**), or to classify drug-pathogen mismatch (**Paper IV**), is to generate training data sets which are large enough to enable development of better prediction models. **Paper III** provides an example of a model built using surveillance data as basis, but further research is needed on how such models would be integrated in the clinical work flow and if it impacts patient outcomes. Risk assessment tools integrated in the EHR have the potential to enable automated stratification of patients upon hospital admission and aid clinicians in decision making. This could push the development of individualized care in infectious diseases forward, instead of the "one size fits all" provided by guidelines, but needs verification in studies.

6.2 IMPROVING DISEASE SEVERITY SCORES AND GUIDELINES

The study of novel SpO2 thresholds in **Paper II** offers a concrete suggestion on how to adapt the SOFA score to a broader patient population outside the ICU, which would improve the operational Sepsis-3 criteria. Future studies are needed to assess how to include information on oxygen treatment and mechanical ventilation in relation to these SpO2 thresholds, and evaluate if the findings are valid also in the ICU-setting. In addition, the SOFA score was developed in the 90s and has not been updated since. With the introduction of the Sepsis-3 criteria, the score became relevant for a broader setting and future studies should focus on adapting and validating also the other components of the score to match progress in the digitalization of healthcare data and medical advances. This includes, but is not restricted to, improved validation of a baseline SOFA score and the impact of time-varying SOFA score trajectories in hospitalized patients.

Furthermore, the 2021 Surviving Sepsis Campaign Guidelines demonstrated a major paradigm shift in the recommendations of timing of antimicrobial treatment. Compared to the guideline from 2016, a recommendation was included to allow delayed start of antimicrobial treatment up to 3 hours in stable patients with suspected sepsis [105]. The same recommendation was adopted by the national Swedish sepsis guidelines in 2022 [203]. Although the results from **Paper IV** needs to be verified in other cohorts of culture positive sepsis patients before changing guideline recommendations, it provides another piece of the puzzle. However, to finally answer the question of timing of appropriate empirical treatment, a RCT would be advised. By design, this is not possible with the current diagnostic methods, since culture results are unavailable at blood culture collection. An alternative could be a trial of intensified diagnostic workup without empirical treatment compared to standard of care in stable patients without septic shock. Based on the results from **Paper IV**, and other data demonstrating the negative impact of overtreatment, this kind of study would now be ethically feasible [2].

6.3 DETAILED MOLECULAR DIAGNOSTICS OF ACUTE BACTERIAL INFECTIONS

WGS has become accessible and cheap enough to be used in routine diagnostics. Culture-independent sequencing directly from patient samples is challenging, but better techniques are rapidly evolving [400]. In **Paper V**, we take a first step in using virulence genotype data to advance the diagnostic granularity of acute infections, but we are only scratching the surface. Although **Paper V** reports one of the largest cohorts of consecutively collected *P. aeruginosa* BSI to date, sample size needs to increase to be able to assess uncommon virulence genotypes or bacterial clones in a meaningful way. To evaluate the full potential of WGS data in clinical microbiological diagnostics, future studies need to focus on analyzing more complex genomic pipelines using larger and more diverse bacterial cohorts also from other infectious sources and pathogens. This should include analyses of transcriptomics.

Another area for future research is to focus on resistance genotype data, both from a molecular epidemiological perspective and as a diagnostic tool in acute infections. Studies are needed to assess if having information on resistance genotype impacts time to appropriate antimicrobial treatment compared to current phenotypical culture dependent methods. This includes developing better bioinformatics pipelines for assessing chromosomal resistance mechanisms. Other valuable applications would be prediction of phenotypic bacterial traits *in silico*, such as virulence and antimicrobial resistance, based on combinations of WGS data and state-of-the-art machine learning techniques. Finally, with our molecular characterization of consecutively collected *P. aeruginosa* isolates, we provide a rationale for future vaccine development in BSI, which may curb the impact of MDR strains.

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8 REFERENCES

1. Fleischmann-Struzek C, Mellhammar L, Rose N, et al. Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. *Intensive Care Med*. 2020;46(8):1552-1562. doi:10.1007/s00134-020-06151-x

2. Rhee C, Kadri SS, Dekker JP, et al. Prevalence of Antibiotic-Resistant Pathogens in Culture-Proven Sepsis and Outcomes Associated With Inadequate and Broad-Spectrum Empiric Antibiotic Use. *JAMA Netw open*. 2020;3(4):e202899. doi:10.1001/jamanetworkopen.2020.2899

3. Angus DC, van der Poll T. Severe Sepsis and Septic Shock. *N Engl J Med*. 2013;369(9):840-851. doi:10.1056/NEJMra1208623

4. Gustav T, Mari R, Olle M, Fredrik R. Hospitalisations with infectious disease diagnoses in somatic healthcare between 1998 and 2019: A nationwide, register-based study in Swedish adults. *Lancet Reg Heal Eur*. 2022;16:100343. doi:10.1016/j.lanepe.2022.100343

5. Liyanarachi KV, Solligård E, Mohus RM, Åsvold BO, Rogne T, Damås JK. Incidence, recurring admissions and mortality of severe bacterial infections and sepsis over a 22-year period in the population-based HUNT study. *PLoS One*. 2022;17(7):e0271263. doi:10.1371/journal.pone.0271263

6. Page DB, Donnelly JP, Wang HE. Community-, Healthcare-, and Hospital-Acquired Severe Sepsis Hospitalizations in the University HealthSystem Consortium. *Crit Care Med*. 2015;43(9):1945-1951. doi:10.1097/CCM.0000000000001164

7. Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing Sepsis as a Global Health Priority - A WHO Resolution. *N Engl J Med*. 2017;377(5):414-417. doi:10.1056/NEJMp1707170

8. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis*. 2018;18(3):318-327. doi:https://doi.org/10.1016/S1473-3099(17)30753-3

9. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-810. doi:10.1001/jama.2016.0287

10. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *BMJ*. 2016;353:i1585. doi:10.1136/bmj.i1585

11. PRISM Investigators, Rowan KM, Angus DC, et al. Early, Goal-Directed Therapy for Septic Shock - A Patient-Level Meta-Analysis. *N Engl J Med*. 2017;376(23):2223-2234. doi:10.1056/NEJMoa1701380

12. ProCESS Investigators, Yealy DM, Kellum JA, et al. A randomized trial of protocol-based care for early septic shock. *N Engl J Med*. 2014;370(18):1683-1693. doi:10.1056/NEJMoa1401602

13. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med*. 2001;29(7):1303-1310. http://www.ncbi.nlm.nih.gov/pubmed/11445675.

14. Lagu T, Rothberg MB, Shieh M-S, Pekow PS, Steingrub JS, Lindenauer PK. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. *Crit Care Med*. 2012;40(3):754-761. doi:10.1097/CCM.0b013e318232db65

15. Vincent J-L, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA*. 2009;302(21):2323-2329. doi:10.1001/jama.2009.1754

16. Opal SM, Garber GE, LaRosa SP, et al. Systemic host responses in severe sepsis analyzed by causative microorganism and treatment effects of drotrecogin alfa (activated). *Clin Infect Dis*. 2003;37(1):50-58. doi:10.1086/375593

17. Klein Klouwenberg PMC, Cremer OL, van Vught LA, et al. Likelihood of infection in patients with presumed sepsis at the time of intensive care unit admission: a cohort study. *Crit Care*. 2015;19:319. doi:10.1186/s13054-015-1035-1

18. Kern W V., Rieg S. Burden of bacterial bloodstream infection-a brief update on epidemiology and significance of multidrug-resistant pathogens. *Clin Microbiol Infect*. 2020;26(2):151-157. doi:10.1016/j.cmi.2019.10.031

19. Lelubre C, Vincent J-L. Mechanisms and treatment of organ failure in sepsis. *Nat Rev Nephrol*. 2018;14(7):417-427. doi:10.1038/s41581-018-0005-7

20. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*. 1992;101(6):1644-1655. doi:10.1378/chest.101.6.1644

21. Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM consensus conference on sepsis and organ failure. *Chest*. 1992;101(6):1481-1483. doi:10.1378/chest.101.6.1481

22. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Med*. 2003;29(4):530-538. doi:10.1007/s00134-003-1662-x

23. Vincent J, Martin GS, Levy MM. qSOFA does not replace SIRS in the definition of sepsis. *Crit Care*. 2016;20(1):210. doi:10.1186/s13054-016-1389-z

24. Churpek MM, Zadravecz FJ, Winslow C, Howell MD, Edelson DP. Incidence and Prognostic Value of the Systemic Inflammatory Response Syndrome and Organ Dysfunctions in Ward Patients. *Am J Respir Crit Care Med*. 2015;192(8):958-964. doi:10.1164/rccm.201502-0275OC

25. Sprung CL, Vincent J, Gall J Le, Reinhart K, Ranieri VM. An evaluation of systemic inflammatory response syndrome signs in the Sepsis Occurrence in Acutely ill Patients (SOAP) study. 2006:421-427. doi:10.1007/s00134-005-0039-8

26. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):762-774. doi:10.1001/jama.2016.0288

27. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med*. 1996;22(7):707-710. doi:10.1007/BF01709751

28. Shankar-Hari M, GS P, ML L, Al E. Developing a new definition and assessing new clinical criteria for septic shock: For Shankar-Hari, M, Phillips GS, Levy ML, and Et Al. 2016. "Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Co. *JAMA*. 2016;315(8):775-787. http://dx.doi.org/10.1001/jama.2016.0289.

29. Donnelly JP, Safford MM, Shapiro NI, Baddley JW, Wang HE. Application of the Third International Consensus Definitions for Sepsis (Sepsis-3) Classification: a retrospective population-based cohort study. *Lancet Infect Dis*. 2017;17(6):661-670. doi:https://doi.org/10.1016/S1473-3099(17)30117-2

30. Wang J-Y, Chen Y-X, Guo S-B, Mei X, Yang P. Predictive performance of quick Sepsis-related Organ Failure Assessment for mortality and ICU admission in patients with infection at the ED. *Am J Emerg Med*. 2016;34(9):1788-1793. doi:10.1016/j.ajem.2016.06.015

31. Freund Y, Lemachatti N, Krastinova E, Al E. Prognostic accuracy of sepsis-3 criteria for in-hospital mortality among patients with suspected infection presenting to the emergency department. *JAMA*. 2017;317(3):301-308. http://dx.doi.org/10.1001/jama.2016.20329.

32. Raith EP, Udy AA, Bailey M, et al. Prognostic Accuracy of the SOFA Score, SIRS Criteria, and qSOFA Score for In-Hospital Mortality Among Adults With Suspected Infection Admitted to the Intensive Care Unit. *JAMA*. 2017;317(3):290-300. doi:10.1001/jama.2016.20328

33. Lambden S, Laterre PF, Levy MM, Francois B. The SOFA score-development, utility and challenges of accurate assessment in clinical trials. *Crit Care*. 2019;23(1):374. doi:10.1186/s13054-019-2663-7

34. Rowling SC, Fløjstrup M, Henriksen DP, et al. Arterial blood gas analysis: as safe as we think? A multicentre historical cohort study. *ERJ open Res*. 2022;8(1). doi:10.1183/23120541.00535-2021

35. Kern W V, Rieg S. Burden of bacterial bloodstream infection-a brief update on epidemiology and significance of multidrug-resistant pathogens. *Clin Microbiol Infect*. 2020;26(2):151-157. doi:10.1016/j.cmi.2019.10.031

36. Laupland KB, Church DL. Population-based epidemiology and microbiology of community-onset bloodstream infections. *Clin Microbiol Rev*. 2014;27(4):647-664. doi:10.1128/CMR.00002-14

37. Mehl A, Åsvold BO, Lydersen S, et al. Burden of bloodstream infection in an area of Mid-Norway 2002-2013: a prospective population-based observational study. *BMC Infect Dis*. 2017;17(1):205. doi:10.1186/s12879-017-2291-2

38. Holmbom M, Giske CG, Fredrikson M, et al. 14-Year Survey in a Swedish County Reveals a Pronounced Increase in Bloodstream Infections (BSI). Comorbidity - An Independent Risk Factor for Both BSI and Mortality. *PLoS One*. 2016;11(11):e0166527. doi:10.1371/journal.pone.0166527

39. Kontula KSK, Skogberg K, Ollgren J, Järvinen A, Lyytikäinen O. Population-Based Study of Bloodstream Infection Incidence and Mortality Rates, Finland, 2004-2018. *Emerg Infect Dis*. 2021;27(10):2560-2569. doi:10.3201/eid2710.204826

40. Vincent J, Opal SM, Marshall JC, Tracey KJ. Sepsis definitions: time for change. *Lancet (London, England)*. 2013;381(9868):774-775. doi:10.1016/S0140-6736(12)61815-7

41. Rhee C, Dantes RB, Epstein L, Klompas M. Using objective clinical data to track progress on preventing and treating sepsis: CDC's new "Adult Sepsis Event" surveillance strategy. *BMJ Qual Saf*. 2019;28(4):305-309. doi:10.1136/bmjqs-2018-008331

42. Fleischmann C, Scherag A, Adhikari NKJ, et al. Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med*. 2016;193(3):259-272. doi:10.1164/rccm.201504-0781OC

43. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet (London, England)*. 2020;395(10219):200-211. doi:10.1016/S0140-6736(19)32989-7

44. Mellhammar L, Wullt S, Lindberg Å, Lanbeck P, Christensson B, Linder A. Sepsis Incidence: A Population-Based Study. *Open forum Infect Dis*. 2016;3(4):ofw207. doi:10.1093/ofid/ofw207

45. Vincent J-L, Marshall JC, Ñamendys-Silva SA, et al. Assessment of the worldwide burden of critical illness: the Intensive Care Over Nations (ICON) audit. *Lancet Respir Med*. 2014;2(5):380-386. doi:http://doi.org/10.1016/S2213-2600(14)70061-X

46. World health oranization. *Guidelines on Core Components of Infection Prevention and Control Programmes at the National and Acute Health Care Facility Level*.; 2016. http://www.wipo.int/amc/en/mediation/rules.

47. Suetens C, Latour K, Kärki T, et al. Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017. *Eurosurveillance*. 2018;23(46):1-17. doi:10.2807/1560-7917.es.2018.23.46.1800516

48. Magill SS, Edwards JR, Bamberg W, et al. Multistate Point-Prevalence Survey of Health Care–Associated Infections. *N Engl J Med*. 2014;370(13):1198-1208. doi:10.1056/NEJMoa1306801

49. Boucher HW, Scheld M, Bartlett J, et al. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clin Infect Dis*. 2009;48(1):1-12. doi:10.1086/595011

50. Anton Y. Peleg, M.B., B.S., M.P.H. and David C. Hooper M. Hospital-Acquired Infections Due to Gram-Negative Bacteria. *N Engl J Med*. 2010;362(19):1804-1813. doi:10.1056/NEJMra0904124.Hospital-Acquired

51. Vincent J-L. Nosocomial infections in adult intensive-care units. *Lancet (London, England)*. 2003;361(9374):2068-2077. doi:10.1016/S0140-6736(03)13644-6

52. Umscheid CA, Mitchell MD, Doshi JA, Agarwal R, Williams K, Brennan PJ.

Estimating the proportion of healthcare-associated infections that are reasonably preventable and the related mortality and costs. *Infect Control Hosp Epidemiol*. 2011;32(2):101-114. doi:10.1086/657912

53. Marang-van de Mheen PJ, van Bodegom-Vos L. Meta-analysis of the central line bundle for preventing catheter-related infections: a case study in appraising the evidence in quality improvement. *BMJ Qual Saf*. 2016;25(2):118-129. doi:10.1136/bmjqs-2014-003787

54. Blot K, Bergs J, Vogelaers D, Blot S, Vandijck D. Prevention of central line-associated bloodstream infections through quality improvement interventions: a systematic review and meta-analysis. *Clin Infect Dis*. 2014;59(1):96-105. doi:10.1093/cid/ciu239

55. Loveday HP, Wilson JA, Pratt RJ, et al. epic3: national evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. *J Hosp Infect*. 2014;86 Suppl 1(S1):S1-70. doi:10.1016/S0195-6701(13)60012-2

56. Rahmqvist M, Samuelsson A, Bastami S, Rutberg H. Direct health care costs and length of hospital stay related to health care-acquired infections in adult patients based on point prevalence measurements. *Am J Infect Control*. 2016;44(5):500-506. doi:https://doi.org/10.1016/j.ajic.2016.01.035

57. Vårdrelaterade infektioner. 2016.

58. Zimlichman E, Henderson D, Tamir O, et al. Health Care–Associated Infections: A Meta-analysis of Costs and Financial Impact on the US Health Care SystemMeta-analysis of Health Care–Associated InfectionsMeta-analysis of Health Care–Associated Infections. *JAMA Intern Med*. 2013;173(22):2039-2046. doi:10.1001/jamainternmed.2013.9763

59. Cassini A, Plachouras D, Eckmanns T, et al. Burden of Six Healthcare-Associated Infections on European Population Health: Estimating Incidence-Based Disability-Adjusted Life Years through a Population Prevalence-Based Modelling Study. *PLoS Med*. 2016;13(10):e1002150. doi:10.1371/journal.pmed.1002150

60. Eber MR, Laxminarayan R, Perencevich EN, Malani A. Clinical and economic outcomes attributable to health care-associated sepsis and pneumonia. *Arch Intern Med*. 2010;170(4):347-353. doi:10.1001/archinternmed.2009.509

61. Rhee C, Wang R, Zhang Z, et al. Epidemiology of Hospital-Onset Versus Community-Onset Sepsis in U.S. Hospitals and Association With Mortality: A Retrospective Analysis Using Electronic Clinical Data. *Crit Care Med*. May 2019:1. doi:10.1097/CCM.0000000000003817

62. Cassini A, Högberg LD, Plachouras D, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis*. 2018. doi:https://doi.org/10.1016/S1473-3099(18)30605-4

63. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet (London, England)*. 2022;399(10325):629-655. doi:10.1016/S0140-6736(21)02724-0

64. van Mourik MSM, van Rooden SM, Abbas M, et al. PRAISE: providing a roadmap for automated infection surveillance in Europe. *Clin Microbiol Infect*. 2021;27:S3-S19.

doi:https://doi.org/10.1016/j.cmi.2021.02.028

65. Behnke M, Valik JK, Gubbels S, et al. Information technology aspects of large-scale implementation of automated surveillance of healthcare-associated infections. *Clin Microbiol Infect*. 2021;27:S29-S39. doi:https://doi.org/10.1016/j.cmi.2021.02.027

66. Horan TC, Andrus M, Dudeck M a. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*. 2008;36(5):309-332. doi:10.1016/j.ajic.2008.03.002

67. European Centre for Disease Prevention and Control (ECDC). *Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use in European Acute Care Hospitals – Protocol Version 5.3*.; 2016. doi:10.2900/374985

68. CDC. Identifying healthcare-associated infections (HAI) for NHSN surveillance. 2019;2015(April):1-14. https://www.cdc.gov/nhsn/pdfs/pscmanual/2psc_identifyinghais_nhsncurrent.pdf.

69. SKL. Nationell satsning för ökad patientsäkerhet Mätning av vårdrelaterade infektioner inom sluten somatisk. 2019;1(9):1-9.

70. Shepperd S, Gonçalves-Bradley DC, Straus SE, Wee B. Hospital at home: home-based end-of-life care. *Cochrane Database Syst Rev*. 2016;(2). doi:10.1002/14651858.CD009231.pub2

71. Leff B. Defining and disseminating the hospital-at-home model. *Can Med Assoc J*. 2009;180(2):156 LP - 157. doi:10.1503/cmaj.081891

72. SKL. Mätning av vårdrelaterade infektioner i slutenvården. https://skl.se/halsasjukvard/patientsakerhet/matningavskadorivarden/matningvardrelateradeinfektioner.2333.html.

73. CDC. Bloodstream Infection Event (Central Line-Associated Bloodstream Infection and Non-central Line Associated Bloodstream Infection). *Centers Dis Control Prev Natl Healthc Saf Network*. 2018;(January):www.cdc.gov/nhsn/PDFs/pscManual/4PSC_CLABScurrent. https://www.cdc.gov/nhsn/PDFs/pscManual/4PSC_CLABScurrent.pdf.

74. Djuric O, Markovic-Denic L, Jovanovic B, Bumbasirevic V. Agreement between CDC/NHSN surveillance definitions and ECDC criteria in diagnosis of healthcare-associated infections in Serbian trauma patients. *PLoS One*. 2018;13(10):e0204893. https://doi.org/10.1371/journal.pone.0204893.

75. Weinstein MP, Towns ML, Quartey SM, et al. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. *Clin Infect Dis*. 1997;24(4):584-602. doi:10.1093/clind/24.4.584

76. Weinstein MP, Reller LB, Murphy JR, Lichtenstein KA. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. *Rev Infect Dis*. 5(1):35-53. http://www.ncbi.nlm.nih.gov/pubmed/6828811.

77. Boaz F, Yoav G, Aliza N, et al. Reappraisal of Community-Acquired Bacteremia: A Proposal of a New Classification for the Spectrum of Acquisition of Bacteremia. *Clin Infect Dis*. 2002;34(11):1431-1439. doi:10.1086/339809

78. Friedman ND, Kaye KS, Stout JE, et al. Health Care - Associated Bloodstream Infections in Adults: A Reason To Change the Accepted Definition of Community - Acquired Infections. *Ann Fam Med*. 2002:791-798. doi:10.1097/00019048-200206000-00016

79. Cardoso T, Almeida M, Friedman ND, et al. Classification of healthcare-associated infection: a systematic review 10 years after the first proposal. *BMC Med*. 2014;12(1):40. doi:10.1186/1741-7015-12-40

80. Shorr AF, Tabak YP, Killian AD, Gupta V, Liu LZ, Kollef MH. Healthcare-associated bloodstream infection: A distinct entity? Insights from a large U.S. database*. *Crit Care Med*. 2006;34(10). https://journals.lww.com/ccmjournal/Fulltext/2006/10000/Healthcare_associated_bloodstream_infection__A.12.aspx.

81. Rodríguez-Baño J, López-Prieto MD, Portillo MM, et al. Epidemiology and clinical features of community-acquired, healthcare-associated and nosocomial bloodstream infections in tertiary-care and community hospitals. *Clin Microbiol Infect*. 2010;16(9):1408-1413. doi:10.1111/j.1469-0691.2010.03089.x

82. Son JS, Song J-H, Ko KS, et al. Bloodstream Infections and Clinical Significance of Healthcare-associated Bacteremia: A Multicenter Surveillance Study in Korean Hospitals. *J Korean Med Sci*. 2010;25(7):992-998. http://synapse.koreamed.org/DOIx.php?id=10.3346%2Fjkms.2010.25.7.992.

83. Vallés J, Calbo E, Anoro E, et al. Bloodstream infections in adults: Importance of healthcare-associated infections. *J Infect*. 2008;56(1):27-34. doi:10.1016/j.jinf.2007.10.001

84. Kollef MH, Zilberberg MD, Shorr AF, et al. Epidemiology, microbiology and outcomes of healthcare-associated and community-acquired bacteremia: A multicenter cohort study. *J Infect*. 2011;62(2):130-135. doi:10.1016/j.jinf.2010.12.009

85. Paoli CJ, Reynolds MA, Sinha M, Gitlin M, Crouser E. Epidemiology and Costs of Sepsis in the United States-An Analysis Based on Timing of Diagnosis and Severity Level. *Crit Care Med*. 2018;46(12):1889-1897. doi:10.1097/CCM.0000000000003342

86. Prescott HC, Cope TM, Gesten FC, et al. Reporting of Sepsis Cases for Performance Measurement Versus for Reimbursement in New York State. *Crit Care Med*. 2018;46(5):666-673. doi:10.1097/CCM.0000000000003005

87. Rhee C, Jentzsch MS, Kadri SS, et al. Variation in Identifying Sepsis and Organ Dysfunction Using Administrative Versus Electronic Clinical Data and Impact on Hospital Outcome Comparisons. *Crit Care Med*. 2019;47(4):493-500. doi:10.1097/CCM.0000000000003554

88. Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit Care Med*. 2013;41(5):1167-1174. doi:10.1097/CCM.0b013e31827c09f8

89. Wilhelms SB, Huss FR, Granath G, Sjöberg F. Assessment of incidence of severe sepsis in Sweden using different ways of abstracting International Classification of Diseases codes: difficulties with methods and interpretation of results. *Crit Care Med*. 2010;38(6):1442-1449. doi:10.1097/CCM.0b013e3181de4406

90. Rhee C, Murphy M V., Li L, Platt R, Klompas M, Centers for Disease Control and

Prevention Epicenters Program. Comparison of trends in sepsis incidence and coding using administrative claims versus objective clinical data. *Clin Infect Dis*. 2015;60(1):88-95. doi:10.1093/cid/ciu750

91. Walkey AJ, Lagu T, Lindenauer PK. Trends in sepsis and infection sources in the United States. A population-based study. *Ann Am Thorac Soc*. 2015;12(2):216-220. doi:10.1513/AnnalsATS.201411-498BC

92. Fleischmann-Struzek C, Mikolajetz A, Schwarzkopf D, et al. Challenges in assessing the burden of sepsis and understanding the inequalities of sepsis outcomes between National Health Systems: secular trends in sepsis and infection incidence and mortality in Germany. *Intensive Care Med*. 2018;44(11):1826-1835. doi:10.1007/s00134-018-5377-4

93. Jafarzadeh SR, Thomas BS, Marschall J, Fraser VJ, Gill J, Warren DK. Quantifying the improvement in sepsis diagnosis, documentation, and coding: the marginal causal effect of year of hospitalization on sepsis diagnosis. *Ann Epidemiol*. 2016;26(1):66-70. doi:10.1016/j.annepidem.2015.10.008

94. Rhee C, Murphy M V., Li L, Platt R, Klompas M, Centers for Disease Control and Prevention Epicenters Program. Improving documentation and coding for acute organ dysfunction biases estimates of changing sepsis severity and burden: a retrospective study. *Crit Care*. 2015;19(1):338. doi:10.1186/s13054-015-1048-9

95. Kadri SS, Rhee C, Strich JR, et al. Estimating Ten-Year Trends in Septic Shock Incidence and Mortality in United States Academic Medical Centers Using Clinical Data. *Chest*. 2017;151(2):278-285. doi:10.1016/j.chest.2016.07.010

96. Stevenson EK, Rubenstein AR, Radin GT, Wiener RS, Walkey AJ. Two Decades of Mortality Trends among Patients with Severe Sepsis: A Comparative Meta-analysis. *Crit Care Med*. 2014;42(3):625-631. doi:10.1097/CCM.0000000000000026

97. Wang HE, Addis DR, Donnelly JP, et al. Discharge diagnoses versus medical record review in the identification of community-acquired sepsis. *Crit Care*. 2015;19(1):42. doi:10.1186/s13054-015-0771-6

98. Henriksen DP, Laursen CB, Jensen TG, Hallas J, Pedersen C, Lassen AT. Incidence rate of community-acquired sepsis among hospitalized acute medical patients-a population-based survey. *Crit Care Med*. 2015;43(1):13-21. doi:10.1097/CCM.0000000000000611

99. Rhee C, Brown SR, Jones TM, et al. Variability in determining sepsis time zero and bundle compliance rates for the centers for medicare and medicaid services SEP-1 measure. *Infect Control Hosp Epidemiol*. 2018;39(8):994-996. doi:10.1017/ice.2018.134

100. Rhee C, Kadri SS, Danner RL, et al. Diagnosing sepsis is subjective and highly variable: a survey of intensivists using case vignettes. *Crit Care*. 2016;20(1):89. doi:10.1186/s13054-016-1266-9

101. Centers for Disease Control and Prevention. Hospital Toolkit for Adult Sepsis Surveillance. https://www.cdc.gov/sepsis/clinicaltools/index.html. Published 2018. Accessed July 10, 2019.

102. Rhee C, Dantes R, Epstein L, et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *JAMA*. 2017;318(13):1241-1249.

doi:10.1001/jama.2017.13836

103. Page B, Klompas M, Chan C, et al. Surveillance for Healthcare-Associated Infections: Hospital-Onset Adult Sepsis Events Versus Current Reportable Conditions. *Clin Infect Dis*. March 2021. doi:10.1093/cid/ciab217

104. Damiani E, Donati A, Serafini G, et al. Effect of performance improvement programs on compliance with sepsis bundles and mortality: a systematic review and meta-analysis of observational studies. *PLoS One*. 2015;10(5):e0125827. doi:10.1371/journal.pone.0125827

105. Evans L, Rhodes A, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med*. 2021;47(11):1181-1247. doi:10.1007/s00134-021-06506-y

106. Black MD, Schorr C, Levy MM. Knowledge translation and the multifaceted intervention in the intensive care unit. *Crit Care Med*. 2012;40(4):1324-1328. doi:10.1097/CCM.0b013e3182431673

107. Ferrer R, Artigas A, Levy MM, et al. Improvement in process of care and outcome after a multicenter severe sepsis educational program in Spain. *JAMA*. 2008;299(19):2294-2303. doi:10.1001/jama.299.19.2294

108. Rosenqvist M, Fagerstrand E, Lanbeck P, Melander O, Åkesson P. Sepsis Alert - a triage model that reduces time to antibiotics and length of hospital stay. *Infect Dis (London, England)*. 2017;4235(July):1-7. doi:10.1080/23744235.2017.1293840

109. Minton J, Clayton J, Sandoe J, Mc Gann H, Wilcox M. Improving early management of bloodstream infection: a quality improvement project. *BMJ*. 2008;336(7641):440-443. doi:10.1136/bmj.39454.634502.80

110. Place A. *Updated Report of a Working Party National Early Warning Score (NEWS) 2 Standardising the Assessment of Acute-Illness Severity in the NHS National Early Warning Score (NEWS) 2 Standardising the Assessment of Acute-Illness Severity in the NHS The Royal Col*.; 2017.

111. FitzGerald G, Jelinek GA, Scott D, Gerdtz MF. Emergency department triage revisited. *Emerg Med J*. 2010;27(2):86-92. doi:10.1136/emj.2009.077081

112. Pérez N, Nissen L, Nielsen RF, Petersen P, Biering K. The predictive validity of RETTS-HEV as an acuity triage tool in the emergency department of a Danish Regional Hospital. *Eur J Emerg Med*. 2016;23(1):33-37. doi:10.1097/MEJ.0000000000000173

113. Subbe CP, Kruger M, Rutherford P, Gemmel L. Validation of a modified Early Warning Score in medical admissions. *QJM An Int J Med*. 2001;94(10):521-526. doi:10.1093/qjmed/94.10.521

114. Alberto L, Marshall AP, Walker R, Aitken LM. Screening for sepsis in general hospitalized patients: a systematic review. *J Hosp Infect*. 2017;96(4):305-315. doi:https://doi.org/10.1016/j.jhin.2017.05.005

115. Harrison AM, Gajic O, Pickering BW, Herasevich V. Development and Implementation of Sepsis Alert Systems. *Clin Chest Med*. 2016;37(2):219-229. doi:10.1016/j.ccm.2016.01.004

116. Bhattacharjee P, Edelson DP, Churpek MM. Identifying Patients With Sepsis on the Hospital Wards. *Chest*. 2017;151(4):898-907. doi:10.1016/j.chest.2016.06.020

117. Surviving Sepsis Campaign. https://www.sccm.org/SurvivingSepsisCampaign/Resources/Protocols-and-Checklists.

118. Zhang Z, Chen L, Xu P, et al. Effectiveness of automated alerting system compared to usual care for the management of sepsis. *NPJ Digit Med*. 2022;5(1):101. doi:10.1038/s41746-022-00650-5

119. Rothman M, Levy M, Dellinger RP, et al. Sepsis as 2 problems: Identifying sepsis at admission and predicting onset in the hospital using an electronic medical record-based acuity score. *J Crit Care*. 2017;38:237-244. doi:10.1016/j.jcrc.2016.11.037

120. Kheterpal S, Singh K, Topol EJ. Digitising the prediction and management of sepsis. *Lancet (London, England)*. 2022;399(10334):1459. doi:10.1016/S0140-6736(22)00658-4

121. Shah ND, Steyerberg EW, Kent DM. Big Data and Predictive Analytics: Recalibrating Expectations. *JAMA*. 2018;320(1):27-28. doi:10.1001/jama.2018.5602

122. Naylor CD. On the Prospects for a (Deep) Learning Health Care System. *JAMA*. 2018;320(11):1099-1100. doi:10.1001/jama.2018.11103

123. Marafino BJ, Park M, Davies JM, et al. Validation of Prediction Models for Critical Care Outcomes Using Natural Language Processing of Electronic Health Record Data. *JAMA Netw open*. 2018;1(8):e185097. doi:10.1001/jamanetworkopen.2018.5097

124. Peiffer-Smadja N, Rawson TM, Ahmad R, et al. Machine learning for clinical decision support in infectious diseases: a narrative review of current applications. *Clin Microbiol Infect*. 2020;26(5):584-595. doi:10.1016/j.cmi.2019.09.009

125. Chen P-HC, Liu Y, Peng L. How to develop machine learning models for healthcare. *Nat Mater*. 2019;18(5):410-414. doi:10.1038/s41563-019-0345-0

126. Riley RD, Ensor J, Snell KIE, et al. Calculating the sample size required for developing a clinical prediction model. *BMJ*. 2020;368:m441. doi:10.1136/bmj.m441

127. Brandt BN, Gartner AB, Moncure M, et al. Identifying severe sepsis via electronic surveillance. *Am J Med Qual*. 2015;30(6):559-565. doi:10.1177/1062860614541291

128. Umscheid CA, Betesh J, VanZandbergen C, et al. Development, Implementation and Impact of an Automated Early Warning and Response System for Sepsis. *J Hosp Med*. 2015;10(1):26-31. doi:10.1002/jhm.2259

129. Khurana HS, Groves RH, Simons MP, et al. Real-Time Automated Sampling of Electronic Medical Records Predicts Hospital Mortality. *Am J Med*. 2016;129(7):688-698.e2. doi:10.1016/j.amjmed.2016.02.037

130. McRee L, Thanavaro JL, Moore K, Goldsmith M, Pasvogel A. The impact of an electronic medical record surveillance program on outcomes for patients with sepsis. *Heart Lung*. 2014;43(6):546-549. doi:10.1016/j.hrtlng.2014.05.009

131. Herasevich V, Pieper MS, Pulido J, Gajic O. Enrollment into a time sensitive clinical study in the critical care setting: results from computerized septic shock sniffer implementation. *J Am Med Inform Assoc*. 2011;18(5):639-644. doi:10.1136/amiajnl-

2011-000228

132. Nelson JL, Smith BL, Jared JD, Younger JG. Prospective trial of real-time electronic surveillance to expedite early care of severe sepsis. *Ann Emerg Med*. 2011;57(5):500-504. doi:10.1016/j.annemergmed.2010.12.008

133. Downing NL, Rolnick J, Poole SF, et al. Electronic health record-based clinical decision support alert for severe sepsis: a randomised evaluation. *BMJ Qual Saf*. March 2019:bmjqs-2018-008765. doi:10.1136/bmjqs-2018-008765

134. Gatewood MO, Wemple M, Greco S, Kritek PA, Durvasula R. A quality improvement project to improve early sepsis care in the emergency department. *BMJ Qual Saf*. 2015;24(12):787-795. doi:10.1136/bmjqs-2014-003552

135. Arabi YM, Al-Dorzi HM, Alamry A, et al. The impact of a multifaceted intervention including sepsis electronic alert system and sepsis response team on the outcomes of patients with sepsis and septic shock. *Ann Intensive Care*. 2017;7(1):57. doi:10.1186/s13613-017-0280-7

136. Hooper MH, Weavind L, Wheeler AP, et al. Randomized trial of automated, electronic monitoring to facilitate early detection of sepsis in the intensive care unit*. *Crit Care Med*. 2012;40(7):2096-2101. doi:10.1097/CCM.0b013e318250a887

137. Semler MW, Weavind L, Hooper MH, et al. An Electronic Tool for the Evaluation and Treatment of Sepsis in the ICU: A Randomized Controlled Trial. *Crit Care Med*. 2015;43(8):1595-1602. doi:10.1097/CCM.0000000000001020

138. Gyang E, Shieh L, Forsey L, Maggio P. A nurse-driven screening tool for the early identification of sepsis in an intermediate care unit setting. *J Hosp Med*. 2015;10(2):97-103. doi:10.1002/jhm.2291

139. MacQueen IT, Dawes AJ, Hadnott T, et al. Use of a Hospital-Wide Screening Program for Early Detection of Sepsis in General Surgery Patients. *Am Surg*. 2015;81(10):1074-1079. http://www.ncbi.nlm.nih.gov/pubmed/26463311.

140. Singer M. The new sepsis consensus definitions (Sepsis-3): the good, the not-so-bad, and the actually-quite-pretty. *Intensive Care Med*. 2016;42(12):2027-2029. doi:10.1007/s00134-016-4600-4

141. Torsvik M, Gustad LT, Mehl A, et al. Early identification of sepsis in hospital inpatients by ward nurses increases 30-day survival. *Crit Care*. 2016;20(1):244. doi:10.1186/s13054-016-1423-1

142. Warttig S, Alderson P, Evans DJW, Lewis SR, Kourbeti IS, Smith AF. Automated monitoring compared to standard care for the early detection of sepsis in critically ill patients. *Cochrane Database Syst Rev*. 2018;(6). doi:10.1002/14651858.CD012404.pub2

143. Franchini S, Duca A. qSOFA should replace SIRS as the screening tool for sepsis. *Crit Care*. 2016;20(1):409. doi:10.1186/s13054-016-1562-4

144. Park HK, Kim WY, Kim MC, Jung W, Ko BS. Quick sequential organ failure assessment compared to systemic inflammatory response syndrome for predicting sepsis in emergency department. *J Crit Care*. 2017;42:12-17. doi:10.1016/j.jcrc.2017.06.020

145. Song J-U, Sin CK, Park HK, Shim SR, Lee J. Performance of the quick Sequential (sepsis-related) Organ Failure Assessment score as a prognostic tool in infected patients outside the intensive care unit: a systematic review and meta-analysis. *Crit Care*. 2018;22(1):28. doi:10.1186/s13054-018-1952-x

146. Jiang J, Yang J, Mei J, Jin Y, Lu Y. Head-to-head comparison of qSOFA and SIRS criteria in predicting the mortality of infected patients in the emergency department: a meta-analysis. *Scand J Trauma Resusc Emerg Med*. 2018;26(1):56. doi:10.1186/s13049-018-0527-9

147. Rudd KE, Seymour CW, Aluisio AR, et al. Association of the quick sequential (sepsis-related) organ failure assessment (qSOFA) score with excess hospital mortality in adults with suspected infection in low- and middle-income countries. *JAMA - J Am Med Assoc*. 2018;319(21):2202-2211. doi:10.1001/jama.2018.6229

148. Haydar S, Spanier M, Weems P, Wood S, Strout T. Comparison of QSOFA score and SIRS criteria as screening mechanisms for emergency department sepsis. *Am J Emerg Med*. 2017;35(11):1730-1733. doi:10.1016/j.ajem.2017.07.001

149. Williams JM, Greenslade JH, McKenzie J V, Chu K, Brown AFT, Lipman J. Systemic Inflammatory Response Syndrome, Quick Sequential Organ Function Assessment, and Organ Dysfunction: Insights From a Prospective Database of ED Patients With Infection. *Chest*. 2017;151(3):586-596. doi:10.1016/j.chest.2016.10.057

150. Askim Å, Moser F, Gustad LT, et al. Poor performance of quick-SOFA (qSOFA) score in predicting severe sepsis and mortality - a prospective study of patients admitted with infection to the emergency department. *Scand J Trauma Resusc Emerg Med*. 2017;25(1):56. doi:10.1186/s13049-017-0399-4

151. Anand V, Zhang Z, Kadri SS, Klompas M, Rhee C, CDC Prevention Epicenters Program. Epidemiology of qSOFA Criteria in Undifferentiated Patients and Association with Suspected Infection and Sepsis. *Chest*. April 2019. doi:10.1016/j.chest.2019.03.032

152. Liu Y-C, Luo Y-Y, Zhang X, et al. Quick Sequential Organ Failure Assessment as a prognostic factor for infected patients outside the intensive care unit: a systematic review and meta-analysis. *Intern Emerg Med*. 2019;(0123456789). doi:10.1007/s11739-019-02036-0

153. Moskowitz A, Patel P V., Grossestreuer A V., et al. Quick Sequential Organ Failure Assessment and Systemic Inflammatory Response Syndrome Criteria as Predictors of Critical Care Intervention Among Patients With Suspected Infection. *Crit Care Med*. 2017;45(11):1813-1819. doi:10.1097/CCM.0000000000002622

154. Churpek MM, Snyder A, Han X, et al. Quick sepsis-related organ failure assessment, systemic inflammatory response syndrome, and early warning scores for detecting clinical deterioration in infected patients outside theintensive care unit. *Am J Respir Crit Care Med*. 2017;195(7):906-911. doi:10.1164/rccm.201604-0854OC

155. Maitra S, Som A, Bhattacharjee S. Accuracy of quick Sequential Organ Failure Assessment (qSOFA) score and systemic inflammatory response syndrome (SIRS) criteria for predicting mortality in hospitalized patients with suspected infection: a meta-analysis of observational studies. *Clin Microbiol Infect*. 2018;24(11):1123-1129. doi:10.1016/j.cmi.2018.03.032

156. Faisal M, Richardson D, Scally AJ, et al. Computer-aided National Early Warning Score to predict the risk of sepsis following emergency medical admission to hospital: a model development and external validation study. *CMAJ*. 2019;191(14):E382-E389. doi:10.1503/cmaj.181418

157. Brink A, Alsma J, Verdonschot RJCG, et al. Predicting mortality in patients with suspected sepsis at the Emergency Department; A retrospective cohort study comparing qSOFA, SIRS and National Early Warning Score. *PLoS One*. 2019;14(1):e0211133. doi:10.1371/journal.pone.0211133

158. Usman OA, Usman AA, Ward MA. Comparison of SIRS, qSOFA, and NEWS for the early identification of sepsis in the Emergency Department. *Am J Emerg Med*. November 2018. doi:10.1016/j.ajem.2018.10.058

159. Yan MY, Gustad LT, Nytrø Ø. Sepsis prediction, early detection, and identification using clinical text for machine learning: a systematic review. *J Am Med Inform Assoc*. 2022;29(3):559-575. doi:10.1093/jamia/ocab236

160. Fleuren LM, Klausch TLT, Zwager CL, et al. Machine learning for the prediction of sepsis: a systematic review and meta-analysis of diagnostic test accuracy. *Intensive Care Med*. January 2020. doi:10.1007/s00134-019-05872-y

161. Moor M, Rieck B, Horn M, Jutzeler CR, Borgwardt K. Early Prediction of Sepsis in the ICU Using Machine Learning: A Systematic Review. *Front Med*. 2021;8:607952. doi:10.3389/fmed.2021.607952

162. Kausch SL, Moorman JR, Lake DE, Keim-Malpass J. Physiological machine learning models for prediction of sepsis in hospitalized adults: An integrative review. *Intensive Crit care Nurs*. 2021;65:103035. doi:10.1016/j.iccn.2021.103035

163. Islam MM, Nasrin T, Walther BA, Wu C-C, Yang H-C, Li Y-C. Prediction of sepsis patients using machine learning approach: A meta-analysis. *Comput Methods Programs Biomed*. 2019;170:1-9. doi:10.1016/j.cmpb.2018.12.027

164. Johnson AEW, Pollard TJ, Shen L, et al. MIMIC-III, a freely accessible critical care database. *Sci data*. 2016;3:160035. doi:10.1038/sdata.2016.35

165. Deng H-F, Sun M-W, Wang Y, et al. Evaluating machine learning models for sepsis prediction: A systematic review of methodologies. *iScience*. 2022;25(1):103651. doi:10.1016/j.isci.2021.103651

166. Thiel SW, Rosini JM, Shannon W, Doherty JA, Micek ST, Kollef MH. Early prediction of septic shock in hospitalized patients. *J Hosp Med*. 2010;5(1):19-25. doi:10.1002/jhm.530

167. Delahanty RJ, Alvarez J, Flynn LM, Sherwin RL, Jones SS. Development and Evaluation of a Machine Learning Model for the Early Identification of Patients at Risk for Sepsis. *Ann Emerg Med*. 2019. doi:10.1016/j.annemergmed.2018.11.036

168. Nemati S, Holder A, Razmi F, Stanley MD, Clifford GD, Buchman TG. An Interpretable Machine Learning Model for Accurate Prediction of Sepsis in the ICU. *Crit Care Med*. 2017:1. doi:10.1097/CCM.0000000000002936

169. Mao Q, Jay M, Hoffman JL, et al. Multicentre validation of a sepsis prediction algorithm using only vital sign data in the emergency department, general ward and ICU. *BMJ Open*. 2018;8(1):e017833. doi:10.1136/bmjopen-2017-017833

170. Desautels T, Calvert J, Hoffman J, et al. Prediction of Sepsis in the Intensive Care Unit With Minimal Electronic Health Record Data: A Machine Learning Approach. *JMIR Med informatics*. 2016;4(3):e28. doi:10.2196/medinform.5909

171. Calvert J, Saber N, Hoffman J, Das R. Machine-Learning-Based Laboratory Developed Test for the Diagnosis of Sepsis in High-Risk Patients. *Diagnostics (Basel, Switzerland)*. 2019;9(1):20. doi:10.3390/diagnostics9010020

172. Giannini HM, Ginestra JC, Chivers C, et al. A Machine Learning Algorithm to Predict Severe Sepsis and Septic Shock: Development, Implementation, and Impact on Clinical Practice. *Crit Care Med*. 2019;47(11):1485-1492. doi:10.1097/CCM.0000000000003891

173. Henry KE, Hager DN, Pronovost PJ, Saria S. A targeted real-time early warning score (TREWScore) for septic shock. *Sci Transl Med*. 2015;7(299):299ra122. doi:10.1126/scitranslmed.aab3719

174. Tarabichi Y, Cheng A, Bar-Shain D, et al. Improving Timeliness of Antibiotic Administration Using a Provider and Pharmacist Facing Sepsis Early Warning System in the Emergency Department Setting: A Randomized Controlled Quality Improvement Initiative. *Crit Care Med*. 2022;50(3):418-427. doi:10.1097/CCM.0000000000005267

175. Nachimuthu SK, Haug PJ. Early detection of sepsis in the emergency department using Dynamic Bayesian Networks. *AMIA . Annu Symp proceedings AMIA Symp*. 2012;2012:653-662. http://www.ncbi.nlm.nih.gov/pubmed/23304338%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3540576.

176. Gultepe E, Green JP, Nguyen H, Adams J, Albertson T, Tagkopoulos I. From vital signs to clinical outcomes for patients with sepsis: a machine learning basis for a clinical decision support system. *J Am Med Informatics Assoc*. 2014;21(2):315-325. doi:10.1136/amiajnl-2013-001815

177. Burdick H, Pino E, Gabel-Comeau D, et al. Effect of a sepsis prediction algorithm on patient mortality, length of stay and readmission: a prospective multicentre clinical outcomes evaluation of real-world patient data from US hospitals. *BMJ Heal care informatics*. 2020;27(1). doi:10.1136/bmjhci-2019-100109

178. McCoy A, Das R. Reducing patient mortality, length of stay and readmissions through machine learning-based sepsis prediction in the emergency department, intensive care unit and hospital floor units. *BMJ open Qual*. 2017;6(2):e000158. doi:10.1136/bmjoq-2017-000158

179. Shimabukuro DW, Barton CW, Feldman MD, Mataraso SJ, Das R. Effect of a machine learning-based severe sepsis prediction algorithm on patient survival and hospital length of stay: a randomised clinical trial. *BMJ open Respir Res*. 2017;4(1):e000234. doi:10.1136/bmjresp-2017-000234

180. Wong A, Otles E, Donnelly JP, et al. External Validation of a Widely Implemented Proprietary Sepsis Prediction Model in Hospitalized Patients. *JAMA Intern Med*. 2021;181(8):1065-1070. doi:10.1001/jamainternmed.2021.2626

181. Bates DW, Sands K, Miller E, et al. Predicting bacteremia in patients with sepsis syndrome. Academic Medical Center Consortium Sepsis Project Working Group. *J*

Infect Dis. 1997;176(6):1538-1551. doi:10.1086/514153

182. Jaimes F, Arango C, Ruiz G, et al. Predicting bacteremia at the bedside. *Clin Infect Dis*. 2004;38(3):357-362. doi:10.1086/380967

183. Apostolopoulou E, Raftopoulos V, Terzis K, Elefsiniotis I. Infection Probability Score, APACHE II and KARNOFSKY scoring systems as predictors of bloodstream infection onset in hematology-oncology patients. *BMC Infect Dis*. 2010;10(11-12):135. doi:10.1186/1471-2334-10-135

184. Vasudevan A, Mukhopadhyay A, Li J, Yuen EGY, Tambyah PA. A prediction tool for nosocomial multi-drug Resistant Gram-Negative Bacilli infections in critically ill patients - prospective observational study. *BMC Infect Dis*. 2014;14(1):615. doi:10.1186/s12879-014-0615-z

185. Komatsu T, Takahashi E, Mishima K, et al. A Simple Algorithm for Predicting Bacteremia Using Food Consumption and Shaking Chills: A Prospective Observational Study. *J Hosp Med*. 2017;12(7):510-515. doi:10.12788/jhm.2764

186. Coburn B, Morris AM, Tomlinson G, Detsky AS. Does this adult patient with suspected bacteremia require blood cultures? *JAMA*. 2012;308(5):502-511. doi:10.1001/jama.2012.8262

187. Fröding I, Valik JK, Bolinder L, Nauclér P, Giske CG. Prediction of bloodstream infection caused by extended-spectrum β-lactamase-producing Enterobacterales in patients with suspected community-onset sepsis. *Int J Antimicrob Agents*. 2019;53(6):820-829. doi:10.1016/j.ijantimicag.2019.02.008

188. Rottier WC, van Werkhoven CH, Bamberg YRP, et al. Development of diagnostic prediction tools for bacteraemia caused by third-generation cephalosporin-resistant enterobacteria in suspected bacterial infections: a nested case-control study. *Clin Microbiol Infect*. 2018;24(12):1315-1321. doi:10.1016/j.cmi.2018.03.023

189. Paul M, Andreassen S, Nielsen AD, et al. Prediction of Bacteremia Using TREAT, a Computerized Decision-Support System. *Clin Infect Dis*. 2006;42(9):1274-1282. doi:10.1086/503034

190. Paul M, Nielsen AD, Goldberg E, et al. Prediction of specific pathogens in patients with sepsis: Evaluation of TREAT, a computerized decision support system. *J Antimicrob Chemother*. 2007;59(6):1204-1207. doi:10.1093/jac/dkm107

191. Ward L, Paul M, Andreassen S. Automatic learning of mortality in a CPN model of the systemic inflammatory response syndrome. *Math Biosci*. 2017;284:12-20. doi:10.1016/j.mbs.2016.11.004

192. Paul M, Andreassen S, Tacconelli E, et al. Improving empirical antibiotic treatment using TREAT, a computerized decision support system: Cluster randomized trial. *J Antimicrob Chemother*. 2006;58(6):1238-1245. doi:10.1093/jac/dkl372

193. Park HJ, Jung DY, Ji W, Choi C-M. Detection of Bacteremia in Surgical In-Patients Using Recurrent Neural Network Based on Time Series Records: Development and Validation Study. *J Med Internet Res*. 2020;22(8):e19512. doi:10.2196/19512

194. Pai K-C, Wang M-S, Chen Y-F, et al. An Artificial Intelligence Approach to Bloodstream Infections Prediction. *J Clin Med*. 2021;10(13). doi:10.3390/jcm10132901

195. Roimi M, Neuberger A, Shrot A, Paul M, Geffen Y, Bar-Lavie Y. Early diagnosis of bloodstream infections in the intensive care unit using machine-learning algorithms. *Intensive Care Med*. 2020;46(3):454-462. doi:10.1007/s00134-019-05876-8

196. Boerman AW, Schinkel M, Meijerink L, et al. Using machine learning to predict blood culture outcomes in the emergency department: a single-centre, retrospective, observational study. *BMJ Open*. 2022;12(1):e053332. doi:10.1136/bmjopen-2021-053332

197. Boerman AW, Schinkel M, Meijerink L, et al. Using machine learning to predict blood culture outcomes in the emergency department: a single-centre, retrospective, observational study. *BMJ Open*. 2022;12:53332. doi:10.1136/bmjopen-2021-053332

198. Paterson DL, Ko WC, Von Gottberg A, et al. International prospective study of Klebsiella pneumoniae bacteremia: implications of extended-spectrum β-lactamase production in nosocomial infections. *Ann Intern Med*. 2004;140(January 1996):26-32.

199. Battle SE, Augustine MR, Watson CM, et al. Derivation of a quick Pitt bacteremia score to predict mortality in patients with Gram-negative bloodstream infection. *Infection*. 2019;0(0):0. doi:10.1007/s15010-019-01277-7

200. Sawyer AM, Deal EN, Labelle AJ, et al. Implementation of a real-time computerized sepsis alert in nonintensive care unit patients. *Crit Care Med*. 2011;39(3):469-473. doi:10.1097/CCM.0b013e318205df85

201. Adams R, Henry KE, Sridharan A, et al. Prospective, multi-site study of patient outcomes after implementation of the TREWS machine learning-based early warning system for sepsis. *Nat Med*. 2022;28(7):1455-1460. doi:10.1038/s41591-022-01894-0

202. IDSA Sepsis Task Force. Infectious Diseases Society of America (IDSA) POSITION STATEMENT: Why IDSA Did Not Endorse the Surviving Sepsis Campaign Guidelines. *Clin Infect Dis*. 2018;66(10):1631-1635. doi:10.1093/cid/cix997

203. Andersson M, Brink M, Cronqvist J, et al. Vårdprogram Sepsis och septisk chock-tidig identifiering och initial handläggning. 2022.

204. Levy MM, Dellinger RP, Townsend SR, et al. The Surviving Sepsis Campaign: results of an international guideline-based performance improvement program targeting severe sepsis. *Intensive Care Med*. 2010;36(2):222-231. doi:10.1007/s00134-009-1738-3

205. Levy MM, Rhodes A, Phillips GS, et al. Surviving Sepsis Campaign: association between performance metrics and outcomes in a 7.5-year study. *Intensive Care Med*. 2014;40(11):1623-1633. doi:10.1007/s00134-014-3496-0

206. Leisman DE, Doerfler ME, Ward MF, et al. Survival Benefit and Cost Savings From Compliance With a Simplified 3-Hour Sepsis Bundle in a Series of Prospective, Multisite, Observational Cohorts. *Crit Care Med*. 2017;45(3):395-406. doi:10.1097/CCM.0000000000002184

207. Levy MM, Evans LE, Rhodes A. The Surviving Sepsis Campaign Bundle: 2018 Update. *Crit Care Med*. 2018;46(6):997-1000. doi:10.1097/CCM.0000000000003119

208. Barbash IJ, Davis B, Kahn JM. National Performance on the Medicare SEP-1 Sepsis Quality Measure. *Crit Care Med*. 2019;47(8):1026-1032. doi:10.1097/CCM.0000000000003613

209. Rhee C, Gohil S, Klompas M. Regulatory mandates for sepsis care--reasons for caution. *N Engl J Med*. 2014;370(18):1673-1676. doi:10.1056/NEJMp1400276

210. Rhee C, Yu T, Wang R, et al. Association Between Implementation of the Severe Sepsis and Septic Shock Early Management Bundle Performance Measure and Outcomes in Patients With Suspected Sepsis in US Hospitals. *JAMA Netw open*. 2021;4(12):e2138596. doi:10.1001/jamanetworkopen.2021.38596

211. Rosenqvist M, Fagerstrand E, Lanbeck P, Melander O, Åkesson P. Sepsis Alert - a triage model that reduces time to antibiotics and length of hospital stay. *Infect Dis (London, England)*. 2017;49(7):507-513. doi:10.1080/23744235.2017.1293840

212. Rosenqvist M, Bengtsson-Toni M, Tham J, Lanbeck P, Melander O, Åkesson P. Improved Outcomes After Regional Implementation of Sepsis Alert: A Novel Triage Model. *Crit Care Med*. 2020;48(4):484-490. doi:10.1097/CCM.0000000000004179

213. Viale P, Tedeschi S, Scudeller L, et al. Infectious diseases team for the early management of severe sepsis and septic shock in the emergency department. *Clin Infect Dis*. June 2017. doi:10.1093/cid/cix548

214. Schmitt S, McQuillen DP, Nahass R, et al. Infectious diseases specialty intervention is associated with decreased mortality and lower healthcare costs. *Clin Infect Dis*. 2014;58(1):22-28. doi:10.1093/cid/cit610

215. Bai a. D, Showler a., Burry L, et al. Impact of Infectious Disease Consultation on Quality of Care, Mortality, and Length of Stay in Staphylococcus aureus Bacteremia: Results From a Large Multicenter Cohort Study. *Clin Infect Dis*. 2015;60:1451-1461. doi:10.1093/cid/civ120

216. Paulsen J, Solligård E, Damås JK, DeWan A, Åsvold BO, Bracken MB. The Impact of Infectious Disease Specialist Consultation for Staphylococcus aureus Bloodstream Infections: A Systematic Review. *Open forum Infect Dis*. 2016;3(2):ofw048. doi:10.1093/ofid/ofw048

217. Kariv G, Paul M, Shani V, Muchtar E, Leibovici L. Benchmarking inappropriate empirical antibiotic treatment. *Clin Microbiol Infect*. 2013;19(7):629-633. doi:10.1111/j.1469-0691.2012.03965.x

218. Marquet K, Liesenborgs A, Bergs J, Vleugels A, Claes N. Incidence and outcome of inappropriate in-hospital empiric antibiotics for severe infection: a systematic review and meta-analysis. *Crit Care*. 2015;19(1):63. doi:10.1186/s13054-015-0795-y

219. Waagsbø B, Stuve N, Afset JE, et al. High levels of discordant antimicrobial therapy in hospital-acquired bloodstream infections is associated with increased mortality in an intensive care, low antimicrobial resistance setting. *Infect Dis (London, England)*. 2022;54(10):738-747. doi:10.1080/23744235.2022.2083672

220. Kadri SS, Lai YL, Warner S, et al. Inappropriate empirical antibiotic therapy for bloodstream infections based on discordant in-vitro susceptibilities: a retrospective cohort analysis of prevalence, predictors, and mortality risk in US hospitals. *Lancet Infect Dis*. 2021;21(2):241-251. doi:10.1016/S1473-3099(20)30477-1

221. Rhee C, Chiotos K, Cosgrove SE, et al. Infectious Diseases Society of America Position Paper: Recommended Revisions to the National Severe Sepsis and Septic Shock Early Management Bundle (SEP-1) Sepsis Quality Measure. *Clin Infect Dis*. 2021;72(4):541-552. doi:10.1093/cid/ciaa059

222. Singer M. Antibiotics for Sepsis: Does Each Hour Really Count, or Is It Incestuous Amplification? *Am J Respir Crit Care Med*. 2017;196(7):800-802. doi:10.1164/rccm.201703-0621ED

223. Nauclér P, Huttner A, van Werkhoven CH, et al. Impact of time to antibiotic therapy on clinical outcome in patients with bacterial infections in the emergency department: implications for antimicrobial stewardship. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2021;27(2):175-181. doi:10.1016/j.cmi.2020.02.032

224. Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob Agents Chemother*. 2010;54(11):4851-4863. doi:10.1128/AAC.00627-10

225. Ferrer R, Martin-Loeches I, Phillips G, et al. Empiric Antibiotic Treatment Reduces Mortality in Severe Sepsis and Septic Shock From the First Hour. *Crit Care Med*. 2014;42(8):1749-1755. doi:10.1097/CCM.0000000000000330

226. Liu VX, Fielding-Singh V, Greene JD, et al. The Timing of Early Antibiotics and Hospital Mortality in Sepsis. *Am J Respir Crit Care Med*. 2017;196(7):856-863. doi:10.1164/rccm.201609-1848OC

227. Seymour CW, Gesten F, Prescott HC, et al. Time to Treatment and Mortality during Mandated Emergency Care for Sepsis. *N Engl J Med*. 2017;376(23):2235-2244. doi:10.1056/NEJMoa1703058

228. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med*. 2006;34(6):1589-1596. doi:10.1097/01.CCM.0000217961.75225.E9

229. Kumar A, Ellis P, Arabi Y, et al. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. *Chest*. 2009;136(5):1237-1248. doi:10.1378/chest.09-0087

230. Savage RD, Fowler RA, Rishu AH, et al. The Effect of Inadequate Initial Empiric Antimicrobial Treatment on Mortality in Critically Ill Patients with Bloodstream Infections: A Multi-Centre Retrospective Cohort Study. *PLoS One*. 2016;11(5):e0154944. doi:10.1371/journal.pone.0154944

231. Sterling SA, Miller WR, Pryor J, Puskarich MA, Jones AE. The Impact of Timing of Antibiotics on Outcomes in Severe Sepsis and Septic Shock: A Systematic Review and Meta-Analysis. *Crit Care Med*. 2015;43(9):1907-1915. doi:10.1097/CCM.0000000000001142

232. Kumar A. Systematic Bias in Meta-Analyses of Time to Antimicrobial in Sepsis Studies. *Crit Care Med*. 2016;44(4):e234-5. doi:10.1097/CCM.0000000000001512

233. Lambregts MMC, Wijnakker R, Bernards AT, Visser LG, Cessie S le, Boer MGJ de. Mortality after Delay of Adequate Empiric Antimicrobial Treatment of Bloodstream Infection. *J Clin Med*. 2020;9(5). doi:10.3390/jcm9051378

234. Fitzpatrick JM, Biswas JS, Edgeworth JD, et al. Gram-negative bacteraemia; a multi-centre prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. *Clin Microbiol Infect*. 2016;22(3):244-251. doi:10.1016/j.cmi.2015.10.034

235. Alam N, Oskam E, Stassen PM, et al. Prehospital antibiotics in the ambulance for sepsis: a multicentre, open label, randomised trial. *Lancet Respir Med*. 2018;6(1):40-50. doi:10.1016/S2213-2600(17)30469-1

236. Puskarich MA, Trzeciak S, Shapiro NI, et al. Association between timing of antibiotic administration and mortality from septic shock in patients treated with a quantitative resuscitation protocol. *Crit Care Med*. 2011;39(9):2066-2071. doi:10.1097/CCM.0b013e31821e87ab

237. Hranjec T, Rosenberger LH, Swenson B, et al. Aggressive versus conservative initiation of antimicrobial treatment in critically ill surgical patients with suspected intensive-care-unit-acquired infection: a quasi-experimental, before and after observational cohort study. *Lancet Infect Dis*. 2012;12(10):774-780. doi:10.1016/S1473-3099(12)70151-2

238. Brunkhorst FM, Oppert M, Marx G, et al. Effect of empirical treatment with moxifloxacin and meropenem vs meropenem on sepsis-related organ dysfunction in patients with severe sepsis: a randomized trial. *JAMA*. 2012;307(22):2390-2399. doi:10.1001/jama.2012.5833

239. Sjövall F, Perner A, Hylander Møller M. Empirical mono- versus combination antibiotic therapy in adult intensive care patients with severe sepsis - A systematic review with meta-analysis and trial sequential analysis. *J Infect*. 2017;74(4):331-344. doi:10.1016/j.jinf.2016.11.013

240. Tabah A, Cotta MO, Garnacho-Montero J, et al. A Systematic Review of the Definitions, Determinants, and Clinical Outcomes of Antimicrobial De-escalation in the Intensive Care Unit. *Clin Infect Dis*. 2016;62(8):1009-1017. doi:10.1093/cid/civ1199

241. Tabah A, Bassetti M, Kollef MH, et al. Antimicrobial de-escalation in critically ill patients: a position statement from a task force of the European Society of Intensive Care Medicine (ESICM) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Critically Ill Patient. *Intensive Care Med*. 2020;46(2):245-265. doi:10.1007/s00134-019-05866-w

242. Thwaites GE, Scarborough M, Szubert A, et al. Adjunctive rifampicin for Staphylococcus aureus bacteraemia (ARREST): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet (London, England)*. 2018;391(10121):668-678. doi:10.1016/S0140-6736(17)32456-X

243. Paul M, Daikos GL, Durante-Mangoni E, et al. Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem-resistant Gram-negative bacteria: an open-label, randomised controlled trial. *Lancet Infect Dis*. 2018;18(4):391-400. doi:10.1016/S1473-3099(18)30099-9

244. Timsit J-F, Azoulay E, Schwebel C, et al. Empirical Micafungin Treatment and Survival Without Invasive Fungal Infection in Adults With ICU-Acquired Sepsis, Candida Colonization, and Multiple Organ Failure: The EMPIRICUS Randomized Clinical Trial. *JAMA*. 2016;316(15):1555-1564. doi:10.1001/jama.2016.14655

245. Reitz KM, Kennedy J, Li SR, et al. Association Between Time to Source Control in Sepsis and 90-Day Mortality. *JAMA Surg*. July 2022. doi:10.1001/jamasurg.2022.2761

246. López-Cortés LE, Del Toro MD, Gálvez-Acebal J, et al. Impact of an evidence-based

bundle intervention in the quality-of-care management and outcome of staphylococcus aureus bacteremia. *Clin Infect Dis*. 2013;57(9):1225-1233. doi:10.1093/cid/cit499

247. Martínez ML, Ferrer R, Torrents E, et al. Impact of Source Control in Patients With Severe Sepsis and Septic Shock. *Crit Care Med*. 2017;45(1):11-19. doi:10.1097/CCM.0000000000002011

248. Peake SL, Delaney A, Bailey M, et al. Goal-directed resuscitation for patients with early septic shock. *N Engl J Med*. 2014;371(16):1496-1506. doi:10.1056/NEJMoa1404380

249. Mouncey PR, Osborn TM, Power GS, et al. Trial of early, goal-directed resuscitation for septic shock. *N Engl J Med*. 2015;372(14):1301-1311. doi:10.1056/NEJMoa1500896

250. Rochwerg B, Alhazzani W, Sindi A, et al. Fluid resuscitation in sepsis: a systematic review and network meta-analysis. *Ann Intern Med*. 2014;161(5):347-355. doi:10.7326/M14-0178

251. Meyhoff TS, Hjortrup PB, Wetterslev J, et al. Restriction of Intravenous Fluid in ICU Patients with Septic Shock. *N Engl J Med*. 2022;386(26):2459-2470. doi:10.1056/NEJMoa2202707

252. Avni T, Lador A, Lev S, Leibovici L, Paul M, Grossman A. Vasopressors for the Treatment of Septic Shock: Systematic Review and Meta-Analysis. *PLoS One*. 2015;10(8):e0129305. doi:10.1371/journal.pone.0129305

253. Hernández G, Teboul J-L, Bakker J. Norepinephrine in septic shock. *Intensive Care Med*. 2019;45(5):687-689. doi:10.1007/s00134-018-5499-8

254. Rygård SL, Butler E, Granholm A, et al. Low-dose corticosteroids for adult patients with septic shock: a systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med*. 2018;44(7):1003-1016. doi:10.1007/s00134-018-5197-6

255. Gordon AC, Mason AJ, Thirunavukkarasu N, et al. Effect of Early Vasopressin vs Norepinephrine on Kidney Failure in Patients With Septic Shock: The VANISH Randomized Clinical Trial. *JAMA*. 2016;316(5):509-518. doi:10.1001/jama.2016.10485

256. Venkatesh B, Finfer S, Cohen J, et al. Adjunctive Glucocorticoid Therapy in Patients with Septic Shock. *N Engl J Med*. 2018;378(9):797-808. doi:10.1056/NEJMoa1705835

257. Annane D, Renault A, Brun-Buisson C, et al. Hydrocortisone plus Fludrocortisone for Adults with Septic Shock. *N Engl J Med*. 2018;378(9):809-818. doi:10.1056/NEJMoa1705716

258. Holst LB, Haase N, Wetterslev J, et al. Lower versus higher hemoglobin threshold for transfusion in septic shock. *N Engl J Med*. 2014;371(15):1381-1391. doi:10.1056/NEJMoa1406617

259. Hirano Y, Miyoshi Y, Kondo Y, Okamoto K, Tanaka H. Liberal versus restrictive red blood cell transfusion strategy in sepsis or septic shock: a systematic review and meta-analysis of randomized trials. *Crit Care*. 2019;23(1):262. doi:10.1186/s13054-019-2543-1

260. Frat J-P, Thille AW, Mercat A, et al. High-flow oxygen through nasal cannula in acute hypoxemic respiratory failure. *N Engl J Med*. 2015;372(23):2185-2196. doi:10.1056/NEJMoa1503326

261. Ni Y-N, Luo J, Yu H, Liu D, Liang B-M, Liang Z-A. The effect of high-flow nasal cannula in reducing the mortality and the rate of endotracheal intubation when used before mechanical ventilation compared with conventional oxygen therapy and noninvasive positive pressure ventilation. A systematic review a. *Am J Emerg Med*. 2018;36(2):226-233. doi:10.1016/j.ajem.2017.07.083

262. Chu DK, Kim LH-Y, Young PJ, et al. Mortality and morbidity in acutely ill adults treated with liberal versus conservative oxygen therapy (IOTA): a systematic review and meta-analysis. *Lancet (London, England)*. 2018;391(10131):1693-1705. doi:10.1016/S0140-6736(18)30479-3

263. Mackle D, Bellomo R, Bailey M, et al. Conservative Oxygen Therapy during Mechanical Ventilation in the ICU. *N Engl J Med*. 2020;382(11):989-998. doi:10.1056/NEJMoa1903297

264. Tonelli M, Manns B, Feller-Kopman D. Acute renal failure in the intensive care unit: a systematic review of the impact of dialytic modality on mortality and renal recovery. *Am J Kidney Dis*. 2002;40(5):875-885. doi:10.1053/ajkd.2002.36318

265. Zha J, Li C, Cheng G, Huang L, Bai Z, Fang C. The efficacy of renal replacement therapy strategies for septic-acute kidney injury: A PRISMA-compliant network meta-analysis. *Medicine (Baltimore)*. 2019;98(16):e15257. doi:10.1097/MD.0000000000015257

266. Bagshaw SM, Wald R, Adhikari NKJ, et al. Timing of Initiation of Renal-Replacement Therapy in Acute Kidney Injury. *N Engl J Med*. 2020;383(3):240-251. doi:10.1056/NEJMoa2000741

267. Barbar SD, Clere-Jehl R, Bourredjem A, et al. Timing of Renal-Replacement Therapy in Patients with Acute Kidney Injury and Sepsis. *N Engl J Med*. 2018;379(15):1431-1442. doi:10.1056/NEJMoa1803213

268. Diekema DJ, Hsueh P-R, Mendes RE, et al. The Microbiology of Bloodstream Infection: 20-Year Trends from the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother*. April 2019:AAC.00355-19. doi:10.1128/AAC.00355-19

269. Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. *Antimicrob Agents Chemother*. 2008. doi:10.1128/AAC.01169-07

270. Mauldin PD, Salgado CD, Hansen IS, Durup DT, Bosso JA. Attributable Hospital Cost and Length of Stay Associated with Health Care-Associated Infections Caused by Antibiotic-Resistant Gram-Negative Bacteria. *Antimicrob Agents Chemother*. 2010;54(1):109 LP - 115. doi:10.1128/AAC.01041-09

271. Parkins MD, Gregson DB, Pitout JDD, Ross T, Laupland KB. Population-based study of the epidemiology and the risk factors for Pseudomonas aeruginosa bloodstream infection. *Infection*. 2010;38(1):25-32. doi:10.1007/s15010-009-9145-9

272. Al-Hasan MN, Wilson JW, Lahr BD, Eckel-Passow JE, Baddour LM. Incidence of Pseudomonas aeruginosa Bacteremia: A Population-Based Study. *Am J Med*.

2008;121(8):702-708. doi:10.1016/j.amjmed.2008.03.029

273. Iversen BG, Brantsæter AB, Aavitsland P. Nationwide study of invasive Pseudomonas aeruginosa infection in Norway: Importance of underlying disease. *J Infect*. 2008;57(2):139-146. doi:10.1016/j.jinf.2008.05.010

274. Schechner V, Nobre V, Kaye KS, et al. Gram-Negative Bacteremia upon Hospital Admission: When Should Pseudomonas aeruginosa\n Be Suspected? *Clin Infect Dis*. 2009;48(5):580-586. doi:10.1086/596709

275. Werth BJ, Carreno JJ, Reveles KR. Shifting trends in the incidence of Pseudomonas aeruginosa septicemia in hospitalized adults in the United States from 1996-2010. *Am J Infect Control*. 2015;43(5):465-468. doi:10.1016/j.ajic.2015.01.028

276. Kang C-I, Kim S-H, Kim H-B, et al. Pseudomonas aeruginosa bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. *Clin Infect Dis*. 2003;37(6):745-751. doi:10.1086/377200

277. Wenzel RP, Tallent SM, Bischoff T, Edmond MB, Wisplinghoff H, Seifert H. Nosocomial Bloodstream Infections in US Hospitals: Analysis of 24,179 Cases from a Prospective Nationwide Surveillance Study. *Clin Infect Dis*. 2004;39(3):309-317. doi:10.1086/421946

278. Al-Hasan MN, Eckel-Passow JE, Baddour LM. Impact of healthcare-associated acquisition on community-onset Gram-negative bloodstream infection: A population-based study. *Eur J Clin Microbiol Infect Dis*. 2012;31(6):1163-1171. doi:10.1007/s10096-011-1424-6

279. Magiorakos A-P, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268-281. doi:10.1111/j.1469-0691.2011.03570.x

280. Raman G, Avendano EE, Chan J, Merchant S, Puzniak L. Risk factors for hospitalized patients with resistant or multidrug-resistant Pseudomonas aeruginosa infections: a systematic review and meta-analysis. *Antimicrob Resist Infect Control*. 2018;7:79. doi:10.1186/s13756-018-0370-9

281. Gómez-Zorrilla S, Camoez M, Tubau F, et al. Antibiotic pressure is a major risk factor for rectal colonization by multidrug-resistant pseudomonas aeruginosa in critically ill patients. *Antimicrob Agents Chemother*. 2014;58(10):5863-5870. doi:10.1128/AAC03419-14

282. Buhl M, Peter S, Willmann M. Prevalence and risk factors associated with colonization and infection of extensively drug-resistant *Pseudomonas aeruginosa* : a systematic review. *Expert Rev Anti Infect Ther*. 2015;13(9):1159-1170. doi:10.1586/14787210.2015.1064310

283. Peña C, Gómez-Zorrilla S, Suarez C, et al. Extensively drug-resistant Pseudomonas aeruginosa: risk of bloodstream infection in hospitalized patients. *Eur J Clin Microbiol Infect Dis*. 2012;31(10):2791-2797. doi:10.1007/s10096-012-1629-3

284. Daneman N, Elligsen M, Walker SAN, Simora A. Duration of hospital admission and the need for empirical antipseudomonal therapy. *J Clin Microbiol*. 2012;50(8):2695-2701. doi:10.1128/JCM.00758-12

285. Valenza G, Tuschak C, Nickel S, Krupa E, Lehner-Reindl V, Höller C. Prevalence, antimicrobial susceptibility, and genetic diversity of *Pseudomonas aeruginosa* as intestinal colonizer in the community. *Infect Dis (Auckl)*. 2015;47(9):654-657. doi:10.3109/23744235.2015.1031171

286. Estepa V, Rojo-Bezares B, Torres C, S??enz Y. Faecal carriage of Pseudomonas aeruginosa in healthy humans: Antimicrobial susceptibility and global genetic lineages. *FEMS Microbiol Ecol*. 2014;89(1):15-19. doi:10.1111/1574-6941.12301

287. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. *Drugs*. 2007;67(3):351-368. doi:10.2165/00003495-200767030-00003

288. Korte AKM, Vos JM. Ecthyma Gangrenosum. *N Engl J Med*. 2017;377(23):e32. doi:10.1056/NEJMicm1702302

289. Cheong HS, Kang C-I, Wi YM, et al. Clinical significance and predictors of community-onset Pseudomonas aeruginosa bacteremia. *Am J Med*. 2008;121(8):709-714. doi:10.1016/j.amjmed.2008.03.034

290. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. Pseudomonas aeruginosa bloodstream infection: importance of appropriate initial antimicrobial treatment. *Antimicrob Agents Chemother*. 2005;49(4):1306-1311. doi:10.1128/AAC.49.4.1306-1311.2005

291. Hung Y-P, Lee C-C, Ko W-C. Effects of Inappropriate Administration of Empirical Antibiotics on Mortality in Adults With Bacteraemia: Systematic Review and Meta-Analysis. *Front Med*. 2022;9:869822. doi:10.3389/fmed.2022.869822

292. Bassetti M, Russo C, Vena A, Giacobbe DR. New antibiotics for the treatment of nonfermenting Gram-negative bacteria. *Curr Opin Infect Dis*. 2021;34(6):701-709. doi:10.1097/QCO.0000000000000757

293. Horcajada JP, Montero M, Oliver A, et al. Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa Infections. *Clin Microbiol Rev*. 2019;32(4):e00031-19. doi:10.1128/CMR.00031-19

294. Zhanel GG, Lawrence CK, Adam H, et al. Imipenem-Relebactam and Meropenem-Vaborbactam: Two Novel Carbapenem-β-Lactamase Inhibitor Combinations. *Drugs*. 2018;78(1):65-98. doi:10.1007/s40265-017-0851-9

295. Bassetti M, Graziano E, Berruti M, Giacobbe DR. The role of fosfomycin for multidrug-resistant gram-negative infections. *Curr Opin Infect Dis*. 2019;32(6):617-625. doi:10.1097/QCO.0000000000000597

296. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D CC. Infectious Diseases Society of America Antimicrobial-Resistant Treatment Guidance: Gram-Negative Bacterial Infections. Infectious Diseases Society of America. Available at https://www.idsociety.org/practice-guideline/amr-guidance/. Accessed 15 Sep 2022.

297. Ito R, Mustapha MM, Tomich AD, et al. Widespread Fosfomycin Resistance in Gram-Negative Bacteria Attributable to the Chromosomal fosA Gene. *MBio*. 2017;8(4). doi:10.1128/mBio.00749-17

298. Burgess DS. Use of pharmacokinetics and pharmacodynamics to optimize

antimicrobial treatment of Pseudomonas aeruginosa infections. *Clin Infect Dis*. 2005;40 Suppl 2(Supplement_2):S99-104. doi:10.1086/426189

299. Fabre V, Amoah J, Cosgrove SE, Tamma PD. Antibiotic Therapy for Pseudomonas aeruginosa Bloodstream Infections: How Long is Long Enough? *Clin Infect Dis*. 2019;(Xx Xxxx):1-4. doi:10.1093/cid/ciz223

300. Yahav D, Franceschini E, Koppel F, et al. Seven Versus 14 Days of Antibiotic Therapy for Uncomplicated Gram-negative Bacteremia: A Noninferiority Randomized Controlled Trial. *Clin Infect Dis*. 2018;(Xx Xxxx):1-8. doi:10.1093/cid/ciy1054

301. Paul M, Leibovici L. Editorial commentary: combination therapy for Pseudomonas aeruginosa bacteremia: where do we stand? *Clin Infect Dis*. 2013;57(2):217-220. doi:10.1093/cid/cit220

302. Tan SH, Teng CB, Ng TM, Lye DCB. Antibiotic therapy and clinical outcomes of Pseudomonas aeruginosa (PA) bacteraemia. *Ann Acad Med Singapore*. 2014;43(11):526-534. http://www.ncbi.nlm.nih.gov/pubmed/25523856.

303. Paulsson M, Granrot A, Ahl J, et al. Antimicrobial combination treatment including ciprofloxacin decreased the mortality rate of Pseudomonas aeruginosa bacteraemia: a retrospective cohort study. *Eur J Clin Microbiol Infect Dis*. 2017. doi:10.1007/s10096-017-2907-x

304. Kwee F, Walker SAN, Elligsen M, Palmay L, Simor A, Daneman N. Outcomes in Documented Pseudomonas aeruginosa Bacteremia Treated with Intermittent IV Infusion of Ceftazidime, Meropenem, or Piperacillin-Tazobactam: A Retrospective Study. *Can J Hosp Pharm*. 2015;68(5):386-394. doi:10.4212/cjhp.v68i5.1485

305. Babich T, Naucler P, Valik JK, et al. Ceftazidime, Carbapenems, or Piperacillin-tazobactam as Single Definitive Therapy for Pseudomonas aeruginosa Bloodstream Infection: A Multisite Retrospective Study. *Clin Infect Dis*. 2020;70(11):2270-2280. doi:10.1093/cid/ciz668

306. Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. *Clin Microbiol Rev*. 2012;25(3):450-470. doi:10.1128/CMR.05041-11

307. Vardakas KZ, Tansarli GS, Bliziotis IA, Falagas ME. β-Lactam plus aminoglycoside or fluoroquinolone combination versus β-lactam monotherapy for Pseudomonas aeruginosa infections: A meta-analysis. *Int J Antimicrob Agents*. 2013;41(4):301-310. doi:10.1016/j.ijantimicag.2012.12.006

308. Mulet X, Cabot G, Ocampo-Sosa AA, et al. Biological Markers of Pseudomonas aeruginosa Epidemic High-Risk Clones. *Antimicrob Agents Chemother*. 2013;57(11):5527-5535. doi:10.1128/AAC.01481-13

309. Bliziotis IA, Petrosillo N, Michalopoulos A, Samonis G, Falagas ME. Impact of definitive therapy with beta-lactam monotherapy or combination with an aminoglycoside or a quinolone for Pseudomonas aeruginosa bacteremia. *PLoS One*. 2011;6(10):e26470. doi:10.1371/journal.pone.0026470

310. Tschudin-Sutter S, Fosse N, Frei R, Widmer AF. Combination therapy for treatment of Pseudomonas aeruginosa bloodstream infections. *PLoS One*. 2018;13(9):e0203295. doi:10.1371/journal.pone.0203295

311. Hilf M, Yu VL, Sharp J, Zuravleff JJ, Korvick JA, Muder RR. Antibiotic therapy for Pseudomonas aeruginosa bacteremia: outcome correlations in a prospective study of 200 patients. *Am J Med*. 1989;87(5):540-546. doi:10.1016/S0002-9343(89)80611-4

312. Bliziotis IA, Samonis G, Vardakas KZ, Chrysanthopoulou S, Falagas ME. Effect of Aminoglycoside and β-Lactam Combination Therapy versus β-Lactam Monotherapy on the Emergence of Antimicrobial Resistance: A Meta-analysis of Randomized, Controlled Trials. *Clin Infect Dis*. 2005;41(2):149-158. http://dx.doi.org/10.1086/430912.

313. Karaiskos I, Antoniadou A, Giamarellou H. Combination therapy for extensively-drug resistant gram-negative bacteria. *Expert Rev Anti Infect Ther*. 2017;15(12):1123-1140. doi:10.1080/14787210.2017.1410434

314. Reig S, Le Gouellec A, Bleves S. What Is New in the Anti-Pseudomonas aeruginosa Clinical Development Pipeline Since the 2017 WHO Alert? *Front Cell Infect Microbiol*. 2022;12:909731. doi:10.3389/fcimb.2022.909731

315. Hauser AR, Mecsas J, Moir DT. Beyond Antibiotics: New Therapeutic Approaches for Bacterial Infections. Weinstein RA, ed. *Clin Infect Dis*. 2016;63(1):89-95. doi:10.1093/cid/ciw200

316. Azeredo da Silveira S, Perez A. Improving the fate of severely infected patients: the promise of anti-toxin treatments and superiority trials. *Expert Rev Anti Infect Ther*. 2017;15(11):973-975. doi:10.1080/14787210.2017.1400908

317. Shao X, Xie Y, Zhang Y, et al. Novel therapeutic strategies for treating Pseudomonas aeruginosa infection. *Expert Opin Drug Discov*. 2020;15(12):1403-1423. doi:10.1080/17460441.2020.1803274

318. Jault P, Leclerc T, Jennes S, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect Dis*. 2019;19(1):35-45. doi:10.1016/S1473-3099(18)30482-1

319. François B, Luyt C-E, Stover CK, Brubaker JO, Chastre J, Jafri HS. New Strategies Targeting Virulence Factors of Staphylococcus aureus and Pseudomonas aeruginosa. *Semin Respir Crit Care Med*. 2017;38(3):346-358. doi:10.1055/s-0037-1602715

320. Bondareva NE, Soloveva A V, Sheremet AB, et al. Preventative treatment with Fluorothiazinon suppressed Acinetobacter baumannii-associated septicemia in mice. *J Antibiot (Tokyo)*. 2022;75(3):155-163. doi:10.1038/s41429-022-00504-y

321. Johansen HK, Gøtzsche PC. Vaccines for preventing infection with Pseudomonas aeruginosa in cystic fibrosis. *Cochrane database Syst Rev*. 2015;(8):CD001399. doi:10.1002/14651858.CD001399.pub4

322. Priebe GP, Goldberg JB. Vaccines for Pseudomonas aeruginosa: a long and winding road. *Expert Rev Vaccines*. 2014;13(4):507-519. doi:10.1586/14760584.2014.890053

323. Hart RJ, Morici LA. Vaccination to Prevent Pseudomonas aeruginosa Bloodstream Infections. *Front Microbiol*. 2022;13:870104. doi:10.3389/fmicb.2022.870104

324. Paterson DL. The epidemiological profile of infections with multidrug-resistant Pseudomonas aeruginosa and Acinetobacter species. *ClinInfectDis*. 2006;43 Suppl 2(1537-6591 (Electronic)):S43-S48. doi:10.1086/504476

325. Klockgether J, Cramer N, Wiehlmann L, Davenport CF, Tümmler B. Pseudomonas aeruginosa Genomic Structure and Diversity. *Front Microbiol*. 2011;2:150. doi:10.3389/fmicb.2011.00150

326. Kung VL, Ozer EA, Hauser AR. The accessory genome of Pseudomonas aeruginosa. *Microbiol Mol Biol Rev*. 2010;74(4):621-641. doi:10.1128/MMBR.00027-10

327. Berger C, Rückert C, Blom J, Rabaey K, Kalinowski J, Rosenbaum MA. Estimation of pathogenic potential of an environmental Pseudomonas aeruginosa isolate using comparative genomics. *Sci Rep*. 2021;11(1):1370. doi:10.1038/s41598-020-80592-8

328. Poulsen BE, Yang R, Clatworthy AE, et al. Defining the core essential genome of Pseudomonas aeruginosa. *Proc Natl Acad Sci U S A*. 2019;116(20):10072-10080. doi:10.1073/pnas.1900570116

329. Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG. Development of a Multilocus Sequence Typing Scheme for the Opportunistic Pathogen Pseudomonas aeruginosa Development of a Multilocus Sequence Typing Scheme for the Opportunistic Pathogen Pseudomonas aeruginosa. *J Clin Microbiol*. 2004;42(12):5644-5649. doi:10.1128/JCM.42.12.5644

330. Wiehlmann L, Wagner G, Cramer N, et al. Population structure of Pseudomonas aeruginosa. *Proc Natl Acad Sci U S A*. 2007;104(19):8101-8106. doi:10.1073/pnas.0609213104

331. Maatallah M, Cheriaa J, Backhrouf A, et al. Population structure of Pseudomonas aeruginosa from five Mediterranean countries: evidence for frequent recombination and epidemic occurrence of CC235. *PLoS One*. 2011;6(10):e25617. doi:10.1371/journal.pone.0025617

332. Ozer EA, Nnah E, Didelot X, Whitaker RJ, Hauser AR. The Population Structure of Pseudomonas aeruginosa Is Characterized by Genetic Isolation of exoU+ and exoS+ Lineages. *Genome Biol Evol*. 2019;11(1):1780-1796. doi:10.1093/gbe/evz119

333. Oliver A, Mulet X, López-Causapé C, Juan C. The increasing threat of Pseudomonas aeruginosa high-risk clones. *Drug Resist Updat*. 2015;21-22:41-59. doi:10.1016/j.drup.2015.08.002

334. Mulet X, Cabot G, Ocampo-Sosa AA, et al. Biological markers of Pseudomonas aeruginosa epidemic high-risk clones. *Antimicrob Agents Chemother*. 2013;57(11):5527-5535. doi:10.1128/AAC.01481-13

335. Gómez-Zorrilla S, Juan C, Cabot G, et al. Impact of multidrug resistance on the pathogenicity of Pseudomonas aeruginosa: In vitro and in vivo studies. *Int J Antimicrob Agents*. 2016;47(5):368-374. doi:10.1016/j.ijantimicag.2016.02.010

336. Sánchez-Diener I, Zamorano L, López-Causapé C, et al. Interplay among Resistance Profiles, High-Risk Clones, and Virulence in the Caenorhabditis elegans Pseudomonas aeruginosa Infection Model. *Antimicrob Agents Chemother*. 2017;61(12). doi:10.1128/AAC.01586-17

337. Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? *Clin Infect Dis*. 2002;34(5):634-640. doi:10.1086/338782

338. Poole K. Pseudomonas aeruginosa: resistance to the max. *Front Microbiol*.

2011;2(15):65. doi:10.3389/fmicb.2011.00065

339. Pragasam AK, Raghanivedha M, Anandan S, Veeraraghavan B. Characterization of Pseudomonas aeruginosa with discrepant carbapenem susceptibility profile. *Ann Clin Microbiol Antimicrob*. 2016;15:12. doi:10.1186/s12941-016-0127-3

340. Pestrak MJ, Chaney SB, Eggleston HC, et al. Pseudomonas aeruginosa rugose small-colony variants evade host clearance, are hyper-inflammatory, and persist in multiple host environments. *PLoS Pathog*. 2018;14(2):e1006842. doi:10.1371/journal.ppat.1006842

341. Fenner L, Richet H, Raoult D, Papazian L, Martin C, La Scola B. Are clinical isolates of Pseudomonas aeruginosa more virulent than hospital environmental isolates in amebal co-culture test? *Crit Care Med*. 2006;34(3):823-828. doi:10.1097/01.CCM.0000201878.51343.F1

342. Ledizet M, Murray TS, Puttagunta S, Slade MD, Quagliarello VJ, Kazmierczak BI. The Ability of Virulence Factor Expression by Pseudomonas aeruginosa to Predict Clinical Disease in Hospitalized Patients. *PLoS One*. 2012;7(11). doi:10.1371/journal.pone.0049578

343. Qin S, Xiao W, Zhou C, et al. Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct Target Ther*. 2022;7(1):199. doi:10.1038/s41392-022-01056-1

344. Hauser AR. The type III secretion system of Pseudomonas aeruginosa: infection by injection. *Nat Rev Microbiol*. 2009;7(9):654-665. doi:10.1038/nrmicro2199

345. Skindersoe ME, Alhede M, Phipps R, et al. Effects of antibiotics on quorum sensing in Pseudomonas aeruginosa. *Antimicrob Agents Chemother*. 2008;52(10):3648-3663. doi:10.1128/AAC.01230-07

346. Hentzer M, Wu H, Andersen JB, et al. Attenuation of Pseudomonas aeruginosa virulence by quorum sensing inhibitors. *EMBO J*. 2003;22(15):3803-3815. doi:10.1093/emboj/cdg366

347. Battle SE, Rello J, Hauser AR. Genomic Islands of Pseudomonas aeruginosa. *FEMS Microbiol Lett*. 2009;290(1):70-78. doi:10.1111/j.1574-6968.2008.01406.x

348. Garey KW, Vo QP, Larocco MT, Gentry LO, Tam VH. Prevalence of Type III Secretion Protein Exoenzymes and Antimicrobial Susceptibility Patterns from Bloodstream Isolates of Patients with *Pseudomonas aeruginosa* Bacteremia. *J Chemother*. 2008;20(6):714-720. doi:10.1179/joc.2008.20.6.714

349. Hauser AR, Cobb E, Bodí M, et al. Type III protein secretion is associated with poor clinical outcomes in patients with ventilator-associated pneumonia caused by Pseudomonas aeruginosa. *Crit Care Med*. 2002;30(3). http://journals.lww.com/ccmjournal/Fulltext/2002/03000/Type_III_protein_secretion_is_associated_with_poor.5.aspx.

350. El-Solh AA, Hattemer A, Hauser AR, Alhajhusain A, Vora H. Clinical outcomes of type III Pseudomonas aeruginosa bacteremia. *Crit Care Med*. 2012;40(4):1157-1163. doi:10.1097/CCM.0b013e3182377906

351. Luo R-G, Miao X-Y, Luo L-L, Mao B, Yu F-Y, Xu J-F. Presence of pldA and exoU in

mucoid Pseudomonas aeruginosa is associated with high risk of exacerbations in non–cystic fibrosis bronchiectasis patients. *Clin Microbiol Infect*. 2019;25(5):601-606. doi:10.1016/j.cmi.2018.07.008

352. Peña C, Cabot G, Gómez-Zorrilla S, et al. Influence of virulence genotype and resistance profile in the mortality of pseudomonas aeruginosa bloodstream infections. *Clin Infect Dis*. 2015;60(4):539-548. doi:10.1093/cid/ciu866

353. Sánchez-Diener I, Zamorano L, Peña C, et al. Weighting the impact of virulence on the outcome of Pseudomonas aeruginosa bloodstream infections. *Clin Microbiol Infect*. 2020;26(3):351-357. doi:10.1016/j.cmi.2019.06.034

354. Panayidou S, Georgiades K, Christofi T, Tamana S, Promponas VJ, Apidianakis Y. Pseudomonas aeruginosa core metabolism exerts a widespread growth-independent control on virulence. *Sci Rep*. 2020;10(1):9505. doi:10.1038/s41598-020-66194-4

355. Pincus NB, Ozer EA, Allen JP, et al. A Genome-Based Model to Predict the Virulence of Pseudomonas aeruginosa Isolates. *MBio*. 2020;11(4). doi:10.1128/mBio.01527-20

356. Chen L, Yang J, Yu J, et al. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res*. 2005;33(Database issue):D325-8. doi:10.1093/nar/gki008

357. Stover CK, Pham XQ, Erwin AL, et al. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. *Nature*. 2000;406(6799):959-964. doi:10.1038/35023079

358. Pelegrin AC, Palmieri M, Mirande C, et al. Pseudomonas aeruginosa: a clinical and genomics update. *FEMS Microbiol Rev*. 2021;45(6). doi:10.1093/femsre/fuab026

359. Forde BM, Bergh H, Cuddihy T, et al. Clinical implementation of routine whole-genome sequencing for hospital infection control of multi-drug resistant pathogens. *Clin Infect Dis*. September 2022. doi:10.1093/cid/ciac726

360. Winsor GL, Griffiths EJ, Lo R, Dhillon BK, Shay JA, Brinkman FSL. Enhanced annotations and features for comparing thousands of Pseudomonas genomes in the Pseudomonas genome database. *Nucleic Acids Res*. 2016;44(D1):D646-53. doi:10.1093/nar/gkv1227

361. Deurenberg RH, Bathoorn E, Chlebowicz MA, et al. Reprint of "Application of next generation sequencing in clinical microbiology and infection prevention". *J Biotechnol*. 2017;250(February 2017):2-10. doi:10.1016/j.jbiotec.2017.03.035

362. Pérez-Losada M, Arenas M, Castro-Nallar E. Microbial sequence typing in the genomic era. *Infect Genet Evol*. 2018;63(September):346-359. doi:10.1016/j.meegid.2017.09.022

363. Babich T, Naucler P, Valik JK, et al. Combination versus monotherapy as definitive treatment for Pseudomonas aeruginosa bacteraemia: a multicentre retrospective observational cohort study. *J Antimicrob Chemother*. 2021;76(8):2172-2181. doi:10.1093/jac/dkab134

364. Babich T, Naucler P, Valik JK, et al. Risk factors for mortality among patients with Pseudomonas aeruginosa bacteraemia: a retrospective multicentre study. *Int J Antimicrob Agents*. 2020;55(2):105847. doi:10.1016/j.ijantimicag.2019.11.004

365. Dalianis H, Henriksson A, Kvist M, Velupillai S, Weegar R. HEALTH BANK - A

Workbench for Data Science Applications in Healthcare. In: *Proceedings of the CAiSE-2015 Industry Track Co-Located with 27th Conference on Advanced Information Systems Engineering (CAiSE 2015)*. ; 2015.

366. Linder A, Arnold R, Boyd JH, et al. Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department. *Crit Care Med*. 2015;43(11):2378-2386. doi:10.1097/CCM.0000000000001265

367. Kahn F, Tverring J, Mellhammar L, et al. Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease Severity at the Emergency Department. *Shock*. 2019;52(6):e135-e145. doi:10.1097/SHK.0000000000001332

368. Calandra T, Cohen J, International Sepsis Forum Definition of Infection in the ICU Consensus Conference. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit Care Med*. 2005;33(7):1538-1548. doi:10.1097/01.ccm.0000168253.91200.83

369. Klein Klouwenberg PMC, Ong DSY, Bos LDJ, et al. Interobserver agreement of Centers for Disease Control and Prevention criteria for classifying infections in critically ill patients. *Crit Care Med*. 2013;41(10):2373-2378. doi:10.1097/CCM.0b013e3182923712

370. Quan H, Li B, Couris CM, et al. Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. *Am J Epidemiol*. 2011;173(6):676-682. doi:10.1093/aje/kwq433

371. Sayers S, Li L, Ong E, et al. Victors: a web-based knowledge base of virulence factors in human and animal pathogens. *Nucleic Acids Res*. 2019;47(D1):D693-D700. doi:10.1093/nar/gky999

372. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44(3):837-845.

373. Andreassen S, Leibovici L, Paul M, et al. A Probabilistic Network for Fusion of Data and Knowledge in Clinical Microbiology. In: Husmeier D, Dybowski R, Roberts S, eds. *Probabilistic Modeling in Bioinformatics and Medical Informatics*. London: Springer London; 2005:451-472. doi:10.1007/1-84628-119-9_15

374. Zuur AF, Ieno EN, Smith GM, eds. Principal coordinate analysis and non-metric multidimensional scaling BT - Analysing Ecological Data. In: New York, NY: Springer New York; 2007:259-264. doi:10.1007/978-0-387-45972-1_15

375. Biau G, Scornet E. A random forest guided tour. *TEST*. 2016;25(2):197-227. doi:10.1007/s11749-016-0481-7

376. Schonlau M, Zou RY. The random forest algorithm for statistical learning. *Stata J*. 2020;20(1):3-29. doi:10.1177/1536867X20909688

377. Kursa MB, Rudnicki WR. Feature Selection with the Boruta Package. *J Stat Softw*. 2010;36(11 SE-Articles):1-13. doi:10.18637/jss.v036.i11

378. Aakre C, Franco PM, Ferreyra M, Kitson J, Li M, Herasevich V. Prospective validation of a near real-time EHR-integrated automated SOFA score calculator. *Int J Med Inform*. 2017;103(October 2016):1-6. doi:10.1016/j.ijmedinf.2017.04.001

379. Shappell CN, Rhee C. Leveraging electronic health record data to improve sepsis surveillance. *BMJ Qual Saf*. 2020;29(9):706-710. doi:10.1136/bmjqs-2020-010847

380. Rhee C, Jones TM, Hamad Y, et al. Prevalence, Underlying Causes, and Preventability of Sepsis-Associated Mortality in US Acute Care Hospitals. *JAMA Netw Open*. 2019;2(2):e187571. doi:10.1001/jamanetworkopen.2018.7571

381. van Mourik MSM, van Rooden SM, Abbas M, et al. PRAISE: providing a roadmap for automated infection surveillance in Europe. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2021;27 Suppl 1:S3-S19. doi:10.1016/j.cmi.2021.02.028

382. Statens Serum Institut, Denmark. https://miba.ssi.dk/overvaagningssystemer/haiba.

383. Gubbels S. The development of a national surveillance system for hospital-acquired infections in Denmark the Hospital Acquired Infections Database - HAIBA. 2016.

384. Verberk JDM, Aghdassi SJS, Abbas M, et al. Automated surveillance systems for healthcare-associated infections: results from a European survey and experiences from real-life utilization. *J Hosp Infect*. 2022;122:35-43. doi:10.1016/j.jhin.2021.12.021

385. van der Werff SD, Thiman E, Tanushi H, et al. The accuracy of fully automated algorithms for surveillance of healthcare-associated urinary tract infections in hospitalized patients. *J Hosp Infect*. 2021;110:139-147. doi:https://doi.org/10.1016/j.jhin.2021.01.023

386. Condell O, Gubbels S, Nielsen J, et al. Automated surveillance system for hospital-acquired urinary tract infections in Denmark. *J Hosp Infect*. 2016;93(3):290-296. doi:10.1016/j.jhin.2016.04.001

387. Feiner JR, Severinghaus JW, Bickler PE. Dark skin decreases the accuracy of pulse oximeters at low oxygen saturation: the effects of oximeter probe type and gender. *Anesth Analg*. 2007;105(6 Suppl):S18-23, tables of contents. doi:10.1213/01.ane.0000285988.35174.d9

388. Riviello ED, Kiviri W, Twagirumugabe T, et al. Hospital Incidence and Outcomes of the Acute Respiratory Distress Syndrome Using the Kigali Modification of the Berlin Definition. *Am J Respir Crit Care Med*. 2016;193(1):52-59. doi:10.1164/rccm.201503-0584OC

389. Pandharipande PP, Shintani AK, Hagerman HE, et al. Derivation and validation of Spo2/Fio2 ratio to impute for Pao2/Fio2 ratio in the respiratory component of the Sequential Organ Failure Assessment score. *Crit Care Med*. 2009;37(4):1317-1321. doi:10.1097/CCM.0b013e31819cefa9

390. Rice TW, Wheeler AP, Bernard GR, Hayden DL, Schoenfeld DA, Ware LB. Comparison of the SpO2/FIO2 ratio and the PaO2/FIO2 ratio in patients with acute lung injury or ARDS. *Chest*. 2007;132(2):410-417. doi:10.1378/chest.07-0617

391. Andreassen S, Jensen F V, Olesen KG. Medical expert systems based on causal probabilistic networks. *Int J Biomed Comput*. 1991;28(1-2):1-30. doi:10.1016/0020-7101(91)90023-8

392. Schulam P, Saria S. Reliable decision support using counterfactual models. *Adv Neural Inf Process Syst*. 2017;30.

393. Leibovici L, Paul M, Nielsen AD, Tacconelli E, Andreassen S. The TREAT project: decision support and prediction using causal probabilistic networks. *Int J Antimicrob Agents*. 2007;30(SUPPL. 1):93-102. doi:10.1016/j.ijantimicag.2006.11.027

394. Schinkel M, Boerman AW, Bennis FC, et al. Diagnostic stewardship for blood cultures in the emergency department: A multicenter validation and prospective evaluation of a machine learning prediction tool. *EBioMedicine*. 2022;82:104176. doi:10.1016/j.ebiom.2022.104176

395. Van Gennip M, Christensen LD, Alhede M, et al. Inactivation of the rhlA gene in Pseudomonas aeruginosa prevents rhamnolipid production, disabling the protection against polymorphonuclear leukocytes. *APMIS*. 2009;117(7):537-546. doi:10.1111/j.1600-0463.2009.02466.x

396. Moser C, Jensen PØ, Thomsen K, et al. Immune Responses to Pseudomonas aeruginosa Biofilm Infections. *Front Immunol*. 2021;12:625597. doi:10.3389/fimmu.2021.625597

397. Balloy V, Verma A, Kuravi S, Si-Tahar M, Chignard M, Ramphal R. The role of flagellin versus motility in acute lung disease caused by Pseudomonas aeruginosa. *J Infect Dis*. 2007;196(2):289-296. doi:10.1086/518610

398. Subedi D, Vijay AK, Kohli GS, Rice SA, Willcox M. Comparative genomics of clinical strains of Pseudomonas aeruginosa strains isolated from different geographic sites. *Sci Rep*. 2018;8(1):15668. doi:10.1038/s41598-018-34020-7

399. van Rooden SM, Aspevall O, Carrara E, et al. Governance aspects of large-scale implementation of automated surveillance of healthcare-associated infections. *Clin Microbiol Infect*. 2021;27 Suppl 1:S20-S28. doi:10.1016/j.cmi.2021.02.026

400. Bachmann NL, Rockett RJ, Timms VJ, Sintchenko V. Advances in Clinical Sample Preparation for Identification and Characterization of Bacterial Pathogens Using Metagenomics. *Front public Heal*. 2018;6:363. doi:10.3389/fpubh.2018.00363