LUND UNIVERSITY

# Infections Related to the Use of Medical Devices and Changes in the Oropharyngeal Flora

Thorarinsdottir, Hulda

2020

*Document Version:*
Publisher's PDF, also known as Version of record

Link to publication

*Citation for published version (APA):*
Thorarinsdottir, H. (2020). *Infections Related to the Use of Medical Devices and Changes in the Oropharyngeal Flora*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Lund University, Faculty of Medicine.

*Total number of authors:*
1

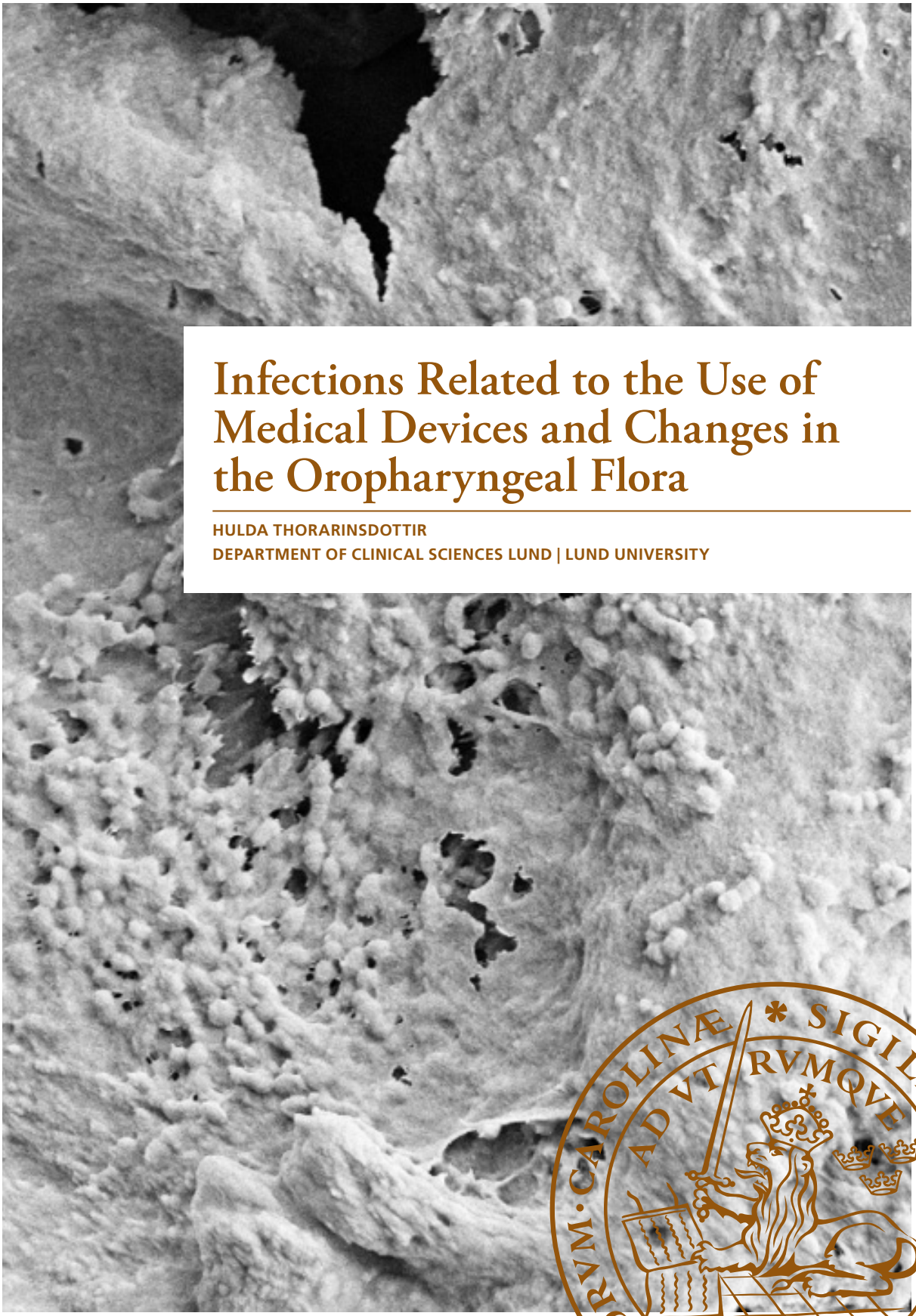
**General rights**
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

**Take down policy**
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00



# Infections Related to the Use of Medical Devices and Changes in the Oropharyngeal Flora

HULDA THORARINSDOTTIR
DEPARTMENT OF CLINICAL SCIENCES LUND | LUND UNIVERSITY

# Infections Related to the Use of Medical Devices and Changes in the Oropharyngeal Flora

Hulda Thorarinsdottir

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Belfragesalen, BMC, Klinikgatan 32
Friday, the 12th of June, 2020, at 1 p.m.

*Faculty opponent*
Peter Frykholm

*Supervisor*
Docent Thomas Kander

*Co-supervisors*
Bengt Klarin & Ulf Schött

| Organization<br>LUND UNIVERSITY | Document name<br>Doctoral Dissertation |
|---|---|
| | Date of issue<br>June 12th 2020 |
| Author Hulda Thorarinsdottir | Sponsoring organization |

Title and subtitle: Infections Related to the Use of Medical Devices and Changes in Oropharyngeal Flora


**Background:**
Humans exist in mutualistic balance with a large range of microbiota. Illness and hospitalization can disturb this balance and contribute to hospital-acquired infections (HAIs), which occur most often in critically ill patients. The use of medical devices such as central venous catheters (CVCs) and endotracheal tubes (ETTs) is essential in the care of critically ill patients. At the same time, they increase the risk of HAI by forcing or disrupting the normal barriers in the human body. All such devices eventually become colonized with microbes (usually normal flora), that form biofilms on the surface of the foreign material and subsequently lead to infection. The three types of devices related to the majority of HAIs in the intensive care unit are ETTs, urinary catheters, and CVCs.

**Aim:** The present research was conducted to study: (i) changes in oropharyngeal microbial flora during hospitalization; (ii) compare biofilm formation on widely used ETTs with different surface properties and to explore factors potentially predictive of biofilm formation; (iii) the incidence of catheter-related infections and the impact of implementing a simple hygiene insertion bundle; (iiii) compare the blood compatibility of widely used CVCs.

**Paper I:** In a clinical observational study, oropharyngeal cultures were collected from 487 individuals: 77 controls, 193 ward patients, and 217 critically ill patients. The results indicated that occurrence of an abnormal oropharyngeal flora is an early and frequent event in hospitalized patients, particularly the critically ill. Also, colonization with gut flora in the oropharynx was common in critically ill patients. Treatment with proton pump inhibitors was associated with colonization of gut flora in the oropharynx. The result of paper I reinforces the hypothesis that proton pump inhibitor use increases the risk of pneumonia by changing the oral flora, harboring gut bacteria which then may be micro aspirated into the lungs.

**Paper II:** In a clinical observational study, biofilm formation on three widely used ETTs was compared in critically ill patients. Biofilm formation on the tubes was found to be an early and frequent event, and high-grade biofilm formation on the ETTs was associated with development of VAP. Compared to uncoated polyvinyl chloride (PVC) ETTs, silicone-coated and noble-metal-coated PVC ETTs were independently associated with reduced high-grade biofilm formation. Methods aimed at the continuous monitoring of biofilm formation are warranted. Routines for biofilm removal need further study.

**Paper III:** This retrospective study compared the incidence of catheter-related infections and catheter-related bloodstream infections during a 2-year period starting 1 year before and ending 1 year after the implementation of a simple hygiene insertion bundle. A total of 1,722 catheter insertions were included. The incidence of catheter-related infections and catheter-related bloodstream infections in this Scandinavian cohort was low. Thus, it seems that the implementation of a simple hygiene insertion bundle was effective in reducing catheter-related infections. The use of multiple-lumen catheters was associated with increased risk of catheter-related infections.

**Paper IV:** In an experimental study, the blood compatibility of three coated and three uncoated CVC materials was evaluated in a modified Chandler loop model imitating the flow of blood in a vein. When in contact with blood, all the tested catheters had some impact on blood cells, contact coagulation, the complement system, or inflammatory markers, although the effects varied significantly. A polyurethane catheter coated with chlorohexidine and silver sulfadiazine showed the most unfavorable blood compatibility profile. A silicone dialysis catheter exhibited the greatest variation in the blood compatibility tests. Poor blood compatibility could cause inflammation and facilitate the development of catheter-related thrombosis in patients receiving these central venous catheters, but clinical significance has to be studied further.



Key words: critical illness, biofilm, intratracheal intubation, ventilator-associated pneumonia, central venous catheters, catheter-related infections, proton pump inhibitors, oropharynx, microbiota, foreign-body reaction, hemolysis, inflammation, thrombosis.

Classification system and/or index terms (if any)

| Supplementary bibliographical information | | Language English |
|---|---|---|
| ISSN and key title 1652-8220 | | ISBN 978-91-7619-942-8 |
| Recipient's notes | Number of pages 96 | Price |
| | Security classification | |



I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.


Signature Hulda Thorarinsdottir Date 2020-04-30

# Infections Related to the Use of Medical Devices and Changes in the Oropharyngeal Flora

Hulda Thorarinsdottir

Copyright Hulda Thorarinsdottir pp. 1–96
Paper 1 © Wiley
Paper 2 © by the Authors (Manuscript unpublished)
Paper 3 © Wiley
Paper 4 © by the Authors (Manuscript unpublished)

Faculty of Medicine
Department of Clinical Sciences Lund
Section of Anesthesiology and Intensive Care

ISSN 1652-8220
ISBN 978-91-7619-942-8
Lund University, Faculty of Medicine Doctoral Dissertation Series 2020:80

Printed in Sweden by Media-Tryck, Lund University
Lund 2020

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se
MADE IN SWEDEN

*To my family*

# Table of Contents

# List of publications

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Proton pump inhibitor medication is associated with colonization of gut flora in the oropharynx. Tranberg A[1], Thorarinsdottir HR[1], Holmberg A, Schött U, Klarin B. *Acta Anaesthesiol Scand*. 2018;62(6):791–800.

[1]These authors contributed equally.

II. Biofilm formation on three different endotracheal tubes: a prospective clinical trial. Thorarinsdottir HR, Kander T, Holmberg A, Petronis S, Klarin B. Manuscript submitted to *Crit Care*.

III. Catheter-related infections: A Scandinavian observational study on the impact of a simple hygiene insertion bundle. Thorarinsdottir HR, Rockholt M, Klarin B, Broman M, Fraenkel CJ, Kander T. *Acta Anaesthesiol Scand*. 2020;64(2):224–231.

IV. Blood compatibility of widely used central venous catheters. Thorarinsdottir HR, Johansson D, Nilsson B, Kander T, Klarin B, Sanchez J. Manuscript submitted to *J Thromb Haemost*.

All papers are reprinted with permission of the copyright owners.

# Abbreviations

| | |
|---|---|
| BMI | body mass index |
| CVC | central venous catheter |
| CI | confidence interval |
| CRI | catheter-related infection |
| CRBSI | catheter-related bloodstream infection |
| CHC | central hemodialysis catheter |
| ETT | endotracheal tube |
| HAI | healthcare-associated infection |
| HAP | healthcare-associated pneumonia |
| ICU | intensive care unit |
| IMI | Innovation against Infection (in Swedish: *Innovation mot infektion*) |
| MP | microparticle |
| NbMC | noble-metal-coated |
| OR | odds ratio |
| PPI | proton pump inhibitor |
| PVC | polyvinyl chloride |
| RISE | Research Institutes of Sweden |
| SC | silicone-coated |
| SIRS | systemic inflammatory response syndrome |
| VAP | ventilator-associated pneumonia |

# Background

Humans exist in a mutualistic balance with a large range of microbiota in which different communities of microbes inhabit different parts of the body[1–4]. Only in recent years has it become clearer that a balanced symbiosis with the microbiota plays a key role in the physiology of the human body, including nutrition, drug metabolism, vitamin synthesis, and protection against infection, as well as the susceptibility and response to disease[3,5]. This balance is often disturbed during illness and hospitalization, where overgrowth of pathogenic bacteria (dysbiosis) or translocation of normal flora to other sites can contribute to healthcare-associated infections (HAIs)[6]. Many hospital-related factors, such as surgery, immobilization, fasting, drugs, and medical devices, can foster dysbiosis or bacterial translocation and increase the risk of HAIs[7–9].

## Healthcare-associated infections

*Definitions and epidemiology*

An HAI, also known as a nosocomial or hospital-acquired infection, is an infection that is acquired in a hospital or other healthcare facility. HAIs constitute a heavy burden on modern healthcare, because they entail prolonged hospital stays and increased patient morbidity and mortality, and contribute to rises in both microbial antibiotic resistance and healthcare costs[10–13].

Large point-prevalence surveys in Europe have estimated that 5.7–6.5% of patients in acute care hospitals have at least one HAI and that about 3.8–4.5 million HAI episodes occur in acute care hospitals each year [10,12,14]. The World Health Organization has estimated that HAIs cause 16 million extra hospital days, lead to 37,000 hospital deaths, and contribute to an additional 110,000 deaths annually in Europe[15]. Notably, data from Swedish hospitals were very limited in these surveys.

In the United States, the prevalence of HAIs has been estimated to be 3.2–4.0%, representing 1.7 million affected patients and contributing to 99,000 deaths each year[15,16]. Although the indicated prevalence is lower than reported in Europe, it is difficult to compare studies conducted in different parts of the world due to variation in the methodology applied. In the United States, the incidence of HAI has been used as a marker for healthcare quality for years. The National Healthcare Safety

Network (NHSN) of the Centers for Disease Control and Prevention (CDC) tracks state and national prevalence and progress regarding the prevention of HAIs[17]. Such prevention efforts have gained national attention, which may contribute to lower prevalence, but at the same time US hospitals are being fined for cases of HAIs, and this may lead to underreporting. In developing countries, data on HAIs are lacking, although a pooled prevalence of 10–15% has been estimated[15,18].

In Sweden, the prevalence of HAIs has been measured nationally since 2008 in point-prevalence surveys conducted at approximately 60 acute care hospitals by municipal and regional authorities (SKR, *Sveriges Kommuner och Regioner*). A report from the SKR covering the years 2013–2018 showed that the prevalence of HAIs was significantly reduced from 5.2% to 4.4% after national implementation of preventive strategies[19]. Despite that finding, about 57,000 patients suffer HAIs each year, and HAIs contribute to 1,300 deaths annually in Sweden. HAIs leading to death are more common among older patients, and severe HAI episodes are more common among men[19]. The HAIs seen most frequently in Swedish hospitals are urinary tract infections (27%) and surgical site infections (22%), but sepsis (11%) and pneumonia (14%) are the HAIs most often leading to death[11,19]. Not only do HAIs involve suffering for patients, but they also entail substantial costs for the healthcare system, because they have been shown to double the length of hospital stay from 6.2 to 16.3 days[20]. It has been estimated that approximately 6% of healthcare costs and resources can be attributed to treatment of HAIs in Sweden. A large proportion of HAIs (35–55%) are assumed to be preventable[21], and, in the context of Swedish healthcare, this means that 400–650 deaths could be prevented and 1.5 to 2.2 billion SEK could be saved yearly. A report presented by the OECD in 2017 demonstrated that, as has been shown for many other diseases, the cost of preventing an HAI are much lower than treating a perceived infection[22].

*HAI in the intensive care unit*

The prevalence of HAIs is highest among intensive care unit (ICU) patients, and large European studies have shown that 19.2% to 20.6% of ICU patients have at least one HAI[10,12,23] compared to 5.2% on average for all other hospitalized patients[12]. The most common HAIs in the ICU are pneumonia (46.9%), urinary tract infections (17.8%), and bloodstream infections (12%)[23]. The microorganisms most often involved are *Escherichia coli* (16.1%), *Staphylococcus aureus* (11.6%), *Klebsiella* spp. (10.4%), *Enterococcus* spp. (9.7%), *Pseudomonas aeruginosa* (8.0%), *Clostridium difficile* (7.3%), coagulase-negative staphylococci (7.1%), *Candida* spp. (5.2%), *Enterobacter* spp. (4.4%), *Proteus* spp. (3.8%), and *Acinetobacter* (3.6%)[10,12]. Antimicrobial resistance is common (31.6%) in HAIs[10], and it is important to recognize how these infections contribute to widespread use of antibiotics and microbial resistance to antibiotics. Preventing HAIs, and thereby slowing the emergence of antimicrobial resistance, is an important public health

issue[24], again highlighting the importance of applying preventive strategies rather than treating established conditions.

*Medical devices contribute to HAIs*

The use of medical devices such as central venous catheters (CVCs) and endotracheal tubes (ETTs) is essential in the care of critically ill patients. However, at the same time they increase the risk of HAI[12,25] by forcing or disrupting the normal barriers in the human body. All such medical devices become colonized with microbes over time, most often with normal flora that forms biofilms on the surface of the foreign material and subsequently lead to infection. It is not unexpected that HAIs are common in ICU patients, considering the several invasive devices required to practice modern intensive care[25,26]. The three types of devices related to the majority of HAIs in the ICU are ETTs, urinary catheters, and CVCs[12].

# Biofilm formation on devices

Biofilms are structured communities of sessile microbes that are enclosed in a self-produced polymeric matrix that is attached to a surface[27,28]. This matrix consists primarily of polysaccharide material, although it can also contain mineral crystals, proteins, or blood components, depending on the environment in which the biofilm has developed[29,30]. Biofilms can arise on a wide variety of biological or non-biological surfaces, including living tissues and indwelling medical devices[31]. Once a biofilm has formed on a device, it is difficult to eradicate[27,32] and often leads to preterm and unwanted device removal[33]. Biofilms are involved in several different types of infections and indeed have been estimated to be associated with 65–80% of all human infections[34].

Biofilm formation is a complex process that is dependent on multiple factors, such as surface characteristics of the device, presence of a conditioning film, physical and chemical properties of the liquid in contact with the surface, and microbial properties[35]. Biofilm formation is often described as a three-step process involving attachment, maturation, and dispersal (Figure 1). In the first step, planktonic (free floating) microbes attach themselves to a surface, and this attachment is initially reversible and is achieved through weak forces (e.g., van der Walls and electrostatic forces). Thereafter, the microbes become more firmly and irreversibly attached via adhesins (anchors) present on the bacterial surface or on their pili and fimbriae[36] (Figure 1). Microcolonies formed by aggregated microbes simultaneously multiply and produce the extracellular polymeric matrix that glues the microbial community to the surface and forms a cover (the “house”). The exact composition of the matrix differs between microbial communities, and microbes differ regarding their diligence in producing a biofilm[37]. Biofilm formation can be initiated by a pioneer

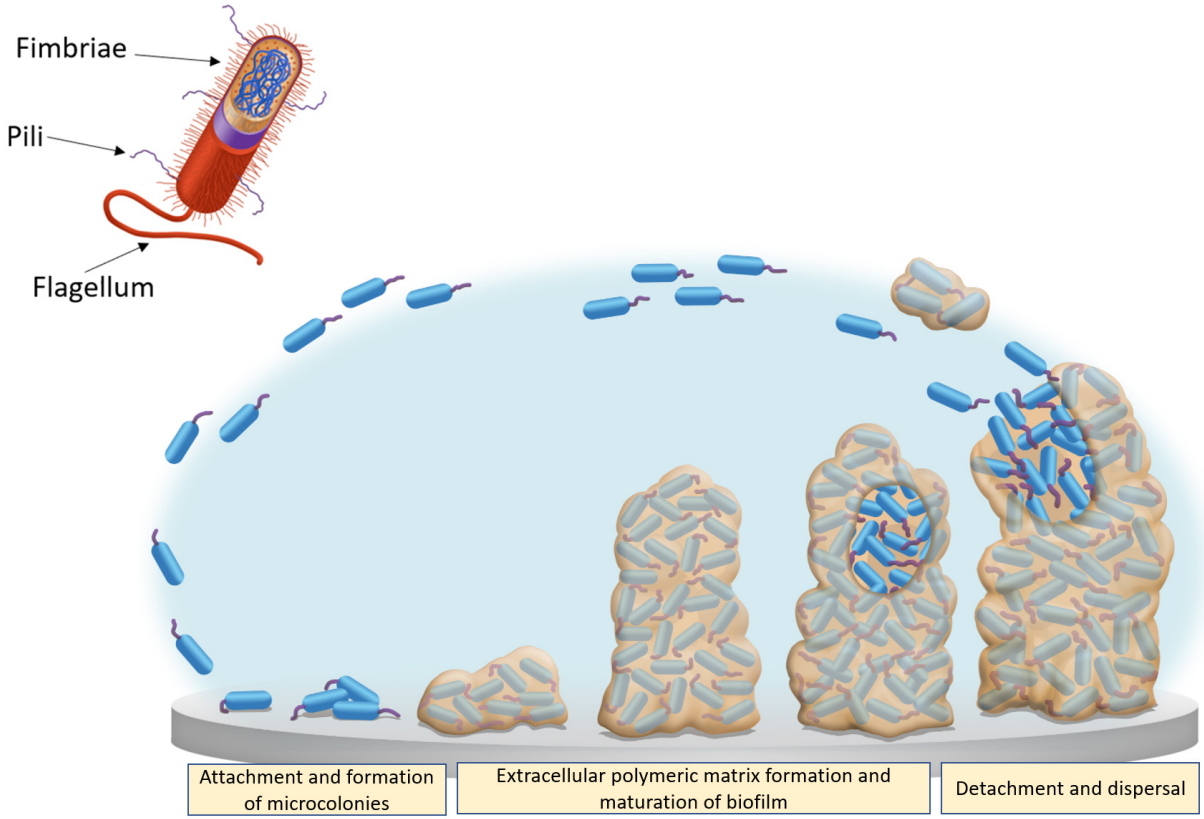



**Figure 1. Biofilm formation**
The figure shows different stages of biofilm formation on a surface: reversible attachment to a surface, irreversible attachment and formation of microcolonies, exopolysaccharide formation, maturation of biofilm, and finally, detachment and dispersal. The illustration of an enlarged bacterium shows the various types of cell surface appendages: pili, fimbriae, and a flagellum. Figure created by Lisbet Thorarinsdottir, printed with permission.

microbe and then inhabited by other species taking advantage of the shelter provided by the film[38].

With continued microbial growth, the biofilm becomes more mature, containing a high density of microbes[39] that often form pillar- and mushroom-shaped masses on the surface[40] (Figure 1). Fluid is retained in the hydrophilic polysaccharides in the matrix, and nutrients and waste products are efficiently transported in channels in the biofilm. Numerus microenvironments co-exist in the biofilm, with different microbes in different metabolic and reproductive states, depending on variation in oxygen concentrations, pH level, and nutrient availability throughout the film[41]. The microbes are in close proximity to each other within the biofilm, which creates a suitable environment for the development of antimicrobial resistance through processes such as plasmid exchange[42]. In addition, the biofilm formation makes the microbes more resistant to antibiotics and disinfectants through different mechanisms[43–45]. Sessile (immobile) microbes in biofilms also differ from their planktonic counterparts with respect to the genes they express[32,46], and hence also differ in their expression of surface molecules, nutrient utilization, and virulence factors[47]. A common occurrence in biofilms is cell-to-cell communication by quorum sensing, in which diffusible chemical signals modulate gene expression and microbial behavior in response to environmental changes in the biofilm[48].

The final stage of biofilm development includes detachment and dispersal of highly infective biofilm fragments and planktonic microbes to the surroundings, which spread the infection to new sites. Dispersal of mature biofilm can occur in a passive manner due to sheer forces or via an active process where the microbes in the biofilm produce enzymes that lead to dispersal[37,49]. Alterations of the environment inside the biofilm, such as a reduction in oxygen pressure or nutrient availability, are among the factors that can trigger biofilm dispersal[37].

# CVC-related infections

*Definitions and epidemiology*

According to the clinical guidelines of the Swedish Society of Anesthesiology and Intensive Care Medicine[50], a catheter-related infection (CRI) is present if the catheter-tip culture is positive and the patient has at least two of the four systemic inflammatory response syndrome (SIRS) criteria (i.e., fever > 38 or < 36°C, heart rate > 90 beats/minute, respiratory rate > 20 breaths/minute, or white blood cell count > 12,000/μL or <4000/μL) upon CVC removal with no likely explanation other than the catheter. Catheter-related blood stream infection (CRBSI) is defined as a bloodstream infection upon catheter removal with the same microorganism isolated on both the catheter tip and in peripheral blood in a patient fulfilling at least two of the four SIRS criteria with no likely explanation other than the catheter.

The use of CVCs is essential in the care of hospitalized patients, both in wards and in the ICU. It has been estimated that about 8% of hospitalized patients require a CVC during their hospital stay[51]. European point prevalence surveys have shown that 11% of HAIs are bloodstream infections, and 33% are related to indwelling CVCs[12]. CRIs and CRBSIs are among the most common life-threatening complications of CVC use, and they significantly increase mortality, length of stay, and hospital costs[52,53]. A previous study in Scandinavia estimated the incidence of CRBSI to be 0.6/1,000 catheter days, which is a relatively low incidence compared to other European countries with incidences varying between 1.2/1,000 and 11.4/1,000 catheter days in different patient populations[52,54–56]. Still, few investigations of cohorts in Scandinavia have addressed this issue[54,57].

*Pathogenesis of CVC infections*

As soon as the CVC is inserted, a microbial entry point from the skin into the vessel is created. This enables microorganisms to migrate along the catheter via the extraluminal or intraluminal route and form a biofilm on the catheter surface[58]. Disruption of the skin barrier, microbial colonization and biofilm formation can lead to soft tissue infection at the insertion site. A blood stream infection can also develop if microbes spread from a colonized catheter surface to the bloodstream, or if biofilm fragments (packed with microbes) are detached from the surface of a catheter that is in contact with blood[51]. CVCs can be colonized through hematogenous seeding of microbes from another source or by a contaminated infusion occurring via the intraluminal route, although these routes of infection are not as common. The source of microorganisms is often found in the patient's own commensal skin flora or as contamination from caregivers handling the CVC. Most infections are caused by coagulase-negative staphylococci, *Staphylococcus aureus*, enterococci, and *Candida* spp. Also, gram-negative strains such as *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa* have been increasingly reported as a cause of CVC-related infections[59,60].

*CVC materials in contact with blood and microbes*

When a CVC is inserted into the bloodstream, the surface of the device almost momentarily becomes covered with a layer of plasma proteins, which changes the surface characteristics of the material[60–62]. Subsequent activation of the host's defenses can induce inflammation and thrombus formation depending on the composition and the activation mechanisms of the proteins absorbed on the surface[63](Figure 2). Initially, inflammatory mediators are generated when blood comes in contact with the biomaterial surface. Later, inflammation can be spread in plasma by soluble activation products, activated leukocytes, and platelets[64,65]. The consequences of biomaterial-induced inflammation have been described extensively

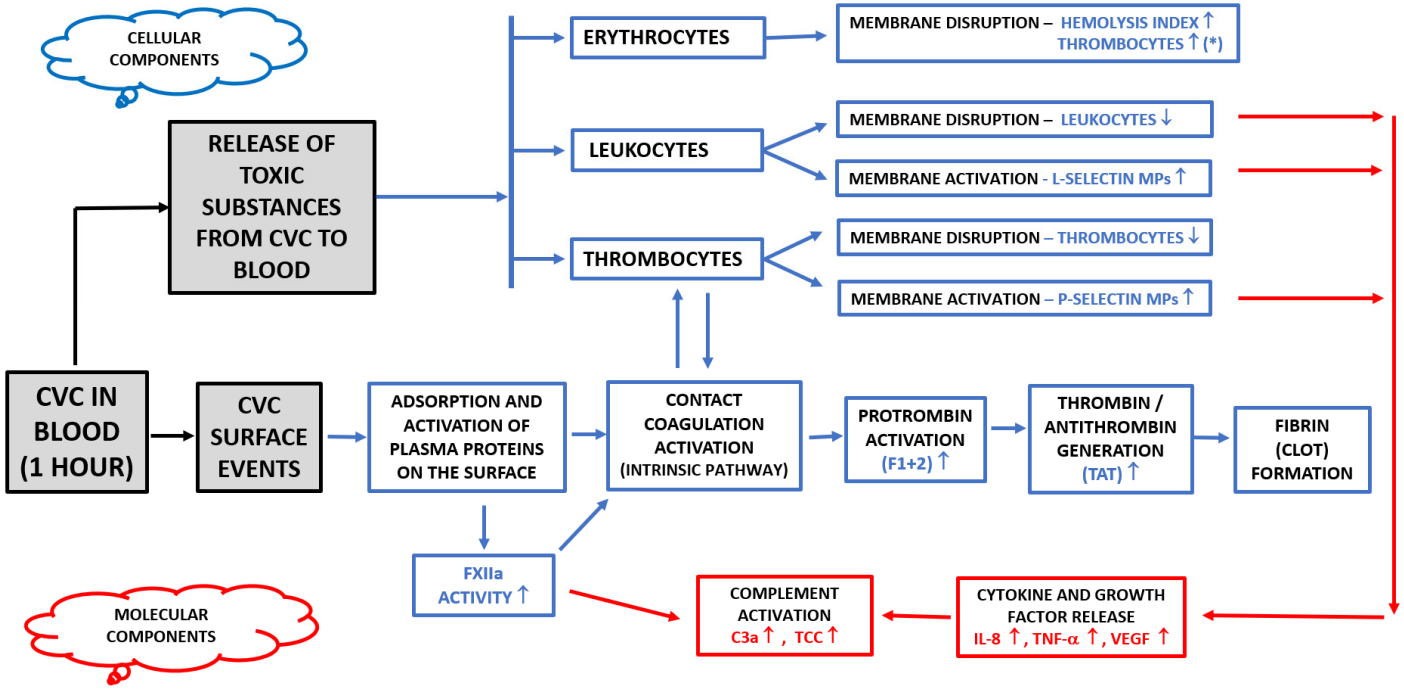



**Figure 2. Activation of the body's defense systems when CVC comes into contact with blood.**
When a foreign material such as a CVC is inserted into the bloodstream, various defense systems aimed at eliminating the foreign material are activated in the blood, including blood cells, contact coagulation, the complement system, and inflammation. *False increase in thrombocytes when fragments of erythrocytes and leukocytes membrane interfere with analysis of much smaller thrombocytes.

in patients who have end-stage renal disease and are receiving hemodialysis[64]. Although patients with a CVC are in much less extensive contact with foreign material compared to dialysis patients, in many cases the effects of different catheter materials in contact with blood are unknown.

Catheter-related thrombosis is an often underdiagnosed and potentially serious complication of CVC use[66,67]. There is accumulating evidence showing that CVC-associated thrombosis and infections are interrelated and cannot be regarded as separate entities[68,69]. The conditioning protein film that forms rapidly on a device in contact with blood provides an ideal platform for adherence of microorganisms, because many of the adsorbed proteins are known ligands for bacteria and can facilitate the development of biofilm formation[70]. Crosstalk between bacteria on the CVC surface and the plasma contact system can also result in further activation of coagulation and thus induce additional thrombus formation[71,72]. This association between infection and thrombus formation has led to emphasis on preventing catheter-related thrombus as an additional mechanism for reducing CRBSIs and vice versa.

*Device modifications to battle CRBSI*

As the surface material of a catheter plays an important role in the pathogenesis of infection and thrombus formation, modifications of the CVC itself in the form of different impregnations or coatings have been used in efforts to reduce CRBSIs and catheter-related thrombosis. CVCs coated with chlorohexidine and silver sulfadiazine (CHSS) or impregnated with minocycline plus rifampicin have been shown to lead to significantly reduced microbial colonization rates and a decrease in CRBSIs[73–76]. However, the overall benefits of those devices in reducing clinical sepsis and mortality remain uncertain[77]. Studies evaluating such CVCs regarding the risk of thrombosis and inflammation are scarce but would be relevant, considering that those devices release antimicrobial substances that are potentially toxic. Other CVCs coated with substances such as silver, heparin, and benzalkonium have also been evaluated in trials. A large meta-analysis showed that silver-impregnated CVCs were effective in reducing CRBSIs[77]. By comparison, benzalkonium-impregnated CVCs have not been studied as extensively but have been found to be effective in reducing bacterial colonization of the catheter[77].

# Ventilator-associated pneumonia

*Definitions and epidemiology*

Hospital-acquired pneumonia (HAP) is defined as an infection that arises in the pulmonary parenchyma 48 hours after hospital admission. Ventilator-associated

pneumonia (VAP) is a subset of HAP that occurs in intubated and mechanically ventilated patients > 48 hours after endotracheal intubation[78]. Data from European studies show that 1.3% of all hospitalized patients in acute care hospitals suffer from HAP[11,79]. The prevalence of HAP is highest among patients in the ICU (8.1%), and with intubation and mechanical ventilation, the risk of VAP increases more than tenfold (15%)[79]. Data on the prevalence of VAP vary substantially (from 5% to 67%) between studies and countries depending on study design and the definition of VAP[80]. The criteria for the diagnosis of VAP have been challenged in recent years, because the specificity of clinical and radiographic features can be low in critically ill patients, which makes it difficult to distinguish VAP from other conditions, such as ventilator-associated tracheobronchitis[81]. Despite that, it cannot be ignored that VAP is a heavy burden on modern healthcare and the most common HAI in the ICU[82].

VAP is often divided into early-onset (arising after < 5 days) and late-onset (occurring after ≥ 5days) disease, because both mortality and causative microbes differ between the two groups. Late-onset VAP has been associated with higher mortality than early-onset VAP[83]. The etiology of VAP varies considerably with patient populations, local ecology in the community and hospital unit, and the patient's prior length of hospital and ICU stay, days of mechanical ventilation, and exposure to antibiotics[78,84,85]. Early-onset VAP is often caused by pathogens that are common in community-acquired pneumonia. In contrast, late-onset VAP is frequently caused by gram-negative bacteria or multiresistant pathogens[78,85]. However, it should be mentioned that multiresistant pathogens are increasingly reported in early-onset VAP, possibly due to their worldwide emergence[86]. VAP increases the length of both mechanical ventilation and hospital stay by about 7–10 days, and the excess healthcare costs per VAP episode have been estimated to be approximately 40,000 USD (about 400,000 SEK)[87]. Estimations of the attributable mortality of VAP vary considerably between studies due to differences in the definitions used and the cohorts investigated[88]. A meta-analysis of 58 randomized studies estimated the attributable mortality to be 10% (range 3–17%). Another meta-analysis identified factors predictive of mortality in VAP as being malignancy, inappropriate initial antimicrobial treatment, acute respiratory distress syndrome, shock, and sepsis[89].

*Pathogenesis of VAP*

VAP arises when microbes invade the lower respiratory tract, where they overwhelm the host defense and establish an infection. The major route of microbial invasion is believed to be a process that requires colonization or overgrowth of the upper gastrointestinal tract with potentially causative pathogens[90–92], followed by micro-aspiration of contaminated oropharyngeal secretions that overcome lower respiratory tract defense mechanisms, resulting in an infection[90,93]. Other less common mechanisms are inhalation of pathogens in contaminated aerosols or

hematogenous spread from other sites such as infected intravascular catheters or via translocation from the gastrointestinal tract[88].

The ETT plays a central role in the pathogenesis of VAP[94]. This is not surprising, because endotracheal intubation involves opening the body's natural barrier (the vocal cords), giving pathogens access to and changing the microbiota of the lower respiratory tract[2]. In recent years, studies have indicated that biofilm formation on the surfaces of ETTs is an important link in the pathogenesis of VAP[95–101]. The concentration of microbes is high in the oropharynx[5], and soon after intubation, these microbes colonize the ETT and form a biofilm[99]. In the biofilm the microbes are sheltered from the host defense. As the biofilm matures, fragments of it are dispersed into the lungs and challenge the host defense[37]. Fragments containing high concentrations of microbes can also be released from the biofilm into the lungs as the result of manipulations such as suctioning with a catheter or due to sheer forces of airflow during mechanical ventilation[102]. Furthermore, if the patient develops VAP, and antimicrobial treatment is initiated, the ETT biofilm can act as a reservoir for sheltered pathogens that are believed to contribute to VAP relapses[99].

*Preventive measures*

The risk of VAP is determined by both patient-related factors and intervention-related factors (e.g., intubation and mechanical ventilation). Patients related factors are often not modifiable and include aspects such as old age, male gender, immunosuppression, and pre-existing comorbidity[103,104]. Several intervention-related factors have been studied with the aim of reducing VAP. Placing the patient in a semi-recumbent position, oral hygiene with chlorhexidine or probiotics, subglottic secretion drainage, continuous control of tracheal cuff pressure, and ETT device modifications are all measures that have shown effect on reducing VAP in randomized trials or meta analysis[80,105–108]. Notably, the preventive measures most consistently associated with reduced mortality in the ICU are those focused on avoiding intubation and speeding up extubation[109]. The risk of VAP increases with increased duration of mechanical ventilation, although studies have shown that the risk per day peaks at 1.5–3.3% between days 5 and 10 and subsequently decreases to 0.5–1.3% per day, after day 15[110,111]. Bundles of preventive measures have been developed, because no single measure is sufficient to eliminate VAP[112,113]. The concept of treatment bundles has gained widespread use and has been used with good results to battle other types of HAI[114].

*Device modifications to prevent VAP*

As biofilm formation and fragments of it entering the lower respiratory tract is considered a major source for VAP [97,99], a number of different ETT surfaces or materials that have an action against microbial adhesion or viability have been developed. Different biocide coatings (e.g. silver, chlorhexidine, sulfadiazine and gendine) have been tested in this context, although only silver-coated ETTs have

been subjected to multiple clinical trials that have shown some beneficial effects[115]. However, there are some impediments to widespread use, including concerns over antibiotic resistance and the relatively high costs. Today, several different ETT materials are commercially available and they differ markedly in price. Two materials that are used extensively for this purpose are polyvinyl chloride (PVC) and silicone-coated (SC) PVC. To the best of our knowledge, these two materials have not been compared regarding biofilm formation in a clinical setting. Another ETT coated with a thin layer of a noble metal alloy (NbMC) containing silver, gold, and palladium (Bactiguard® AB, Sweden), has been on the market since 2013, and the manufacturer claims that this coating does not release any silver ions into the environment. Urinary catheters with this coating have been successful in reducing urinary tract infections[116], but the effectiveness of the coating in preventing biofilm formation on ETTs has not been evaluated in intensive care settings. Clearly, studies comparing widely used ETT materials are warranted.

# Aims of the thesis

The research underlying this thesis was conducted to study the impact of different materials and coatings in medical devices on the development of device-related infections. Further, to investigate changes in oropharyngeal microbial flora during hospitalization and to survey the incidence of CRI. The specific aims of the four studies presented in Papers I–IV were as follows:

I. To determine whether there is an imbalance in the oropharyngeal flora early after hospital or ICU admittance and whether the flora differs between ICU, ward, and control subjects, and also to explore what characterizes patients with changes in the oropharyngeal flora.

II. To compare biofilm formation on three widely used ETTs with different surface properties and to explore factors potentially predictive of biofilm formation.

III. To investigate the CRI/CRBSI incidence and the association between potential risk factors, including the introduction of a simple hygiene insertion bundle and occurrence of CRIs at a large university hospital in Sweden.

IV. To evaluate the blood compatibility of six different catheter materials widely used in critically ill patients.

# Materials and methods

This chapter summarizes the methods outlined in Papers I–IV. Details of the techniques applied in the four studies are presented in the respective papers.

## Innovation against infection

Innovation against Infection (designated IMI, an abbreviation for the name in Swedish: *Innovation mot infection*) was a national project funded by the Swedish Innovation Agency VINNOVA (https://www.vinnova.se-/p/innovation-mot-infektion2/). The goal of this project was to increase the collaboration between hospitals, universities and industries and to analyze and develop new concepts for battling HAI. The studies presented in Papers II–IV were projects performed within the IMI collaboration.

## Ethics

All study protocols were approved by the ethics committees in the regions of Sweden where the study subjects were included: protocols for the studies in Papers I–III by the Regional Ethical Review Board in Lund, and the protocol for the investigation described in Paper IV by the Regional Ethical Review Board in Stockholm.

# Study design

**Table 1. Overview of study design.**
ICU: intensive care unit, CVC: central venous catheter, CHC; central hemodialysis catheter.

| Paper | I | II | III | IV |
|---|---|---|---|---|
| **Design** | Clinical observational study | Clinical observational study | Retrospective cohort study | Experimental study |
| **Study settings (all in Sweden)** | Skåne University Hospital in Lund and two tertiary hospitals in Region Skåne | Skåne University Hospital in Lund | Skåne University Hospital in Lund | Danderyd Hospital in Danderyd |
| **Informed consent** | Yes | Yes | No (waived) | Yes |
| **Study population** | Three study groups respectively comprising ICU patients, ward patients, and controls (n = 487) | ICU patients (n = 106) | Patients receiving a CVC or a CHC (n = 1,722) | Healthy volunteer blood donors (n = 10) |

# Selection of the devices studied in Papers II and IV

Information about the most commonly used CVCs and ETTs in Sweden were obtained from the procurement (se. *upphandling*) documentation compiled by three of the largest regions in Sweden (Region Västra Götaland, Region Skåne, and Region Stockholm). We assumed that these three regions would be representative, because they represent approximately 50% of the population of the country. Healthcare personnel at three university hospitals in the above regions (Sahlgrenska University Hospital in Gothenburg, Skane University Hospitalin Lund, and Karolinska Institute in Stockholm) were interviewed to ensure that the information given in the procurement documentation about the use of the CVCs and ETTs to be tested was consistent with clinical praxis.

Polyvinyl chloride (PVC) was the standard material in ETTs used in all three regions, although the devices came from different manufacturers (Kimberly-Clark, Irving, TX, USA; Medtronic, Dublin, Ireland; Teleflex, Wayne, PA, USA). Silicone-coated (SC) PVC ETTs (Smith's Medical, Minneapolis, MN, USA) were also used but to a lesser extent. Polyurethane (PU) was the standard material for CVCs in all regions, but again originated from different manufacturers (Merit Medical, South Jordan, UT, USA; Argon, Frisco, TX, USA; Teleflex, Wayne, PA, USA; Becton Dickinson, Franklin Lakes, NJ, USA). Exact composition of the materials in ETTs and CVCs can differ between manufacturers and is also a matter of trade secrets and hence was not accessible for use in the present research. According to our analysis, neither impregnated nor coated ETTs or CVCs were routinely used. Also, to the best of our knowledge, coated ETTs and CVCs are not

widely used in any region in Sweden. This differs markedly compared to the United States, where impregnated/coated devices are common. The IMI collaboration did estimate that about 65% of the CVCs and 45% of the urinary catheters used in the United States are impregnated or coated devices. The number of coated ETTs used in the United States could not be estimated in the IMI collaboration, but use of silver coated ETTs is described in studies from the United States [115]. However, are probably not as widely used as coated CVCs or urinary catheters.

After analyzing the information obtained from the procurement documentation and through the interviews, we chose to evaluate three different ETT materials in study II and six different venous access materials in study IV (Table 2). A central hemodialysis catheter (CHC) made of silicone was included in study IV because silicone is a commonly occurring material in CHCs and also to be able to compare silicone in two different environments in the body: in the blood in study IV, and in the respiratory tract in study II.

**Table 2. Material description**
Materials used in study II and IV. BIP, Bactiguard Infection Protection; ETT: endotracheal tube; CVC, central venous catheter; CHC, central hemodialysis catheter; Pd, Palladium; Au, gold; Ag, silver.

| Device | Type | Brand | Abbreviations used in Papers II & IV | Material(s) |
|---|---|---|---|---|
| CHC | Uncoated | Hemo-Cath ST®, MedComp | Si-1 | Silicone |
| CVC | Uncoated | Careflow®, Merit Medical | PU-1 | Polyurethane |
| CVC | Uncoated | Arrow with blue tip®, Teleflex | PU-2 | Polyurethane |
| CVC | Coated | ARROWg+ ard Blue® with blue tip, Teleflex | PU-2+CHSS | Polyurethane coated with chlorohexidine and silver sulfadiazine |
| CVC | Coated | Hydrocath Assure™, Argon Medical | PU-3+BZC | Polyurethane coated with a hydrophilic matrix, impregnated with Benzalkonium chloride |
| CVC | Coated | BIP CVC®, Bactiguard | PU4+NbMC | Polyurethane coated with a thin layer of noble metals (Pd, Au, and Ag) |
| ETT | Uncoated | Mallinckrodt™, Medtronic | PVC ETT | Polyvinyl chloride |
| ETT | Coated | Portex™, Smith's Medical | SC ETT | Silicone-coated polyvinyl chloride |
| ETT | Coated | BIP ETT®, Bactiguard | NbMC ETT | Polyvinyl chloride coated with a thin layer of noble metals (Pd, Au, and Ag) |

# Microbiological procedures

Samples for microbial cultures were collected from patients in study I and II. All cultures were processed at the Department of Clinical Microbiology, Skåne University Hospital, Lund, Sweden, in the same manner, using standardized extended microbiological procedures. Oropharyngeal swabs were collected from the oropharynx behind the posterior tonsillar pillar from all patients in study I and II. In study II endotracheal aspirate was cultured from ICU patients and the tip of the ETT was cultured after extubation.

All samples (oropharyngeal swabs, endotracheal aspirates, and ETT tips) were inoculated on three different selective agar plates, one differentiating agar plate, and one non-selective agar plate as shown in Figure 3. Plates were inspected for growth after 16 and 40 hours of aerobic, anaerobic or $CO_2$ incubation at 35–37 °C. Bacterial species were identified by matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF) mass spectrometry (MALDI Biotyper Microbial Identification system, Bruker, Boston, MA, USA). Differentiation of *Candida* spp. was based on colony appearance on CHROM Candida agar (CHROMagar, Hägersten, Sweden) after 48 hours of incubation at 35 °C.

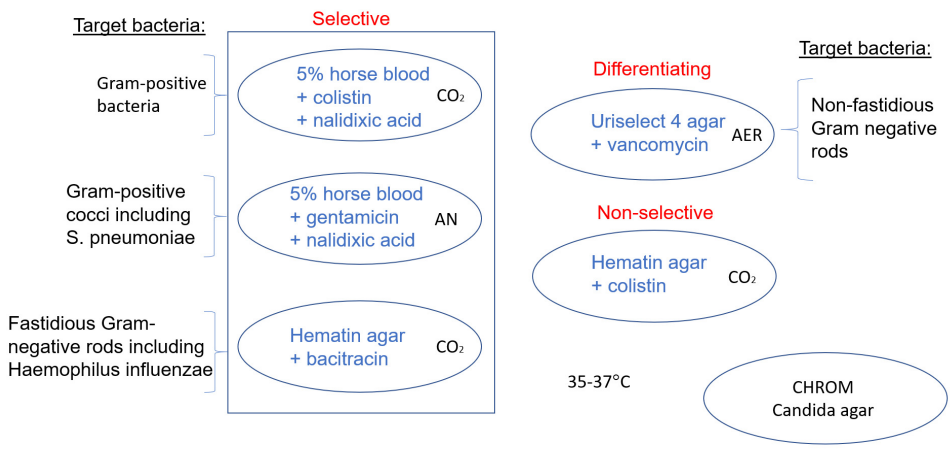



**Figure 3.** The six different agar plates used for microbial cultures of all samples (oropharyngeal swabs, endotracheal aspirates, and endotracheal tube tips) in study I and II. AN, anerobic incubation; AER, aerobic incubation.

In study III, we analyzed CVC tip cultures and blood cultures retrospectively for the years 2011–2012. The culture routine was as follows: the CVCs were removed after site treatment with 0.5% chlorhexidine in 70% alcohol; thereafter, the distal end of the CVC was submerged into a culture tube, and the distal 5 cm was cut off; the CVC tip was sonicated in 10 mL of broth, and 0.1 mL of the broth was quantitatively

cultured on blood agar plates. Growth of $> 10^2$ CFU/catheter was considered significant colonization. The BACT/ALERT system (BioMérieux) was used for blood cultures. All bottles were incubated until microbial growth was detected or for a maximum of 5 days.

# Study I:

*Patients and inclusion*

This clinical observational study included patients aged ≥ 18 years in three groups: (1) critically ill patients admitted to the hospital's ICU (mixed surgical and medical ICU) and requiring mechanical ventilation for at least 24 hours; (2) patients admitted to acute medical or surgical wards and not requiring intensive care; (3) control subjects in the community who had not been hospitalized or treated with antibiotics during the previous 2 months (Figure 4).

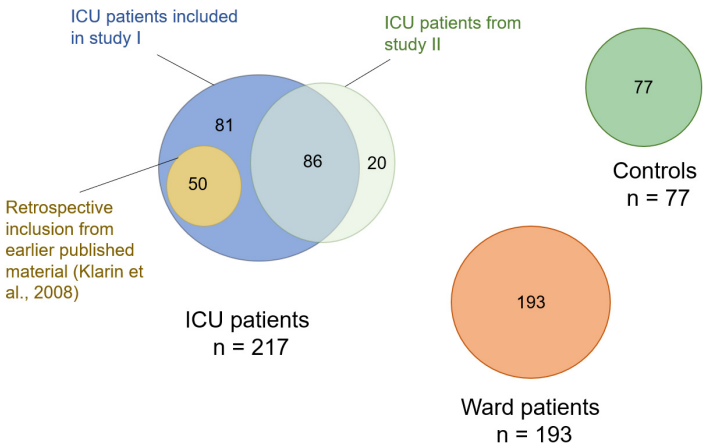



**Figure 4.** Patients included in study I.

A total of 217 ICU patients were included, 167 of them prospectively from May 2010 to February 2016, and 50 retrospectively from previously published material[117]. The same inclusion criteria were applied to all ICU patients. The 193 ward patients were prospectively included from January 2014 to December 2016. The 77 control subjects were prospectively included from March 2014 to April 2016; 30 of them were members of Skåne's Fire Department (*Raddningstjänsten Syd*, Lund, Sweden), and 47 were members of the Senior Citizens Organization (*Pensionarernas Riksorganisation*, Lund, Sweden).

*Patient data*

Patient data were recorded in a standardized form. Variables (methods and patient data) are listed in Paper I. Oropharyngeal swabs were collected from all patients within 24 hours of admission to the hospital (ward patients) or ICU (ICU patients). For the controls, oropharyngeal swabs were collected at the respective inclusion sites. Apart from the collection of oropharyngeal swabs, hospitalized patients received standard hospital care based on their diagnoses and clinical decisions of the responsible physicians.

*Definitions*

- *Normal oral flora*: specimens with growth of at least two species of bacteria usually found in the oral cavity.
- *Abnormal oral flora*: specimens with species not normally found in the oral cavity (pathogens or gut flora) or overgrowth of normal oral flora.
- *Gut flora*: Specimens with species not normally found in the oral cavity and originating in the gut.

# Study II

*Patients and inclusion*

This prospective observational study, included patients aged ≥18 years who were admitted to a tertiary general ICU and were expected to require invasive mechanical ventilation for at least 24 hours. Patients were allowed to participate only once and were included during six separate time periods from February 2014 to April 2017. Depending on the period, patients were intubated on clinical indications with one of the three different types of ETTs tested in study II (Table 2). Each type of ETT was used in two of the six periods. The use of different ETTs according to study period rather than by randomization was done for logistic reasons. Patients in study periods one and four received an uncoated PVC ETT (Oral/Nasal Endotracheal Tube, Mallinckrodt™, Medtronic, Dublin, Ireland), which is standard in our hospital; patients in periods two and six received an SC ETT (Siliconized PVC, Oral/Nasal Soft Seal® Cuffed Tracheal Tube, Portex™, Smith's Medical, Minneapolis, MN, USA); and patients in periods three and five received an NbMC PVC ETT, coated with a thin noble metal alloy coating consisting of gold, silver, and palladium (Bactiguard Infection Protection Endotracheal Tube, Bactiguard®, Tullinge, Sweden).

*Microbiological procedures*

For all patients in study II, samples for surveillance cultures (i.e., oropharyngeal swabs and endotracheal aspirates) were collected on days 1, 2, 3, 5, 7, 14, and 21

and thereafter once a week. On the day of extubation, cultures were collected if not previously scheduled. All oropharyngeal swabs and endotracheal aspirates were processed as described above.

*Patient data*

Data on patients' characteristics were recorded in a standardized form. Variables are listed in paper II (method, patient data). Information on the occurrence of VAP, together with data on microorganisms isolated in surveillance cultures and ETT biofilms were collected for all patients.

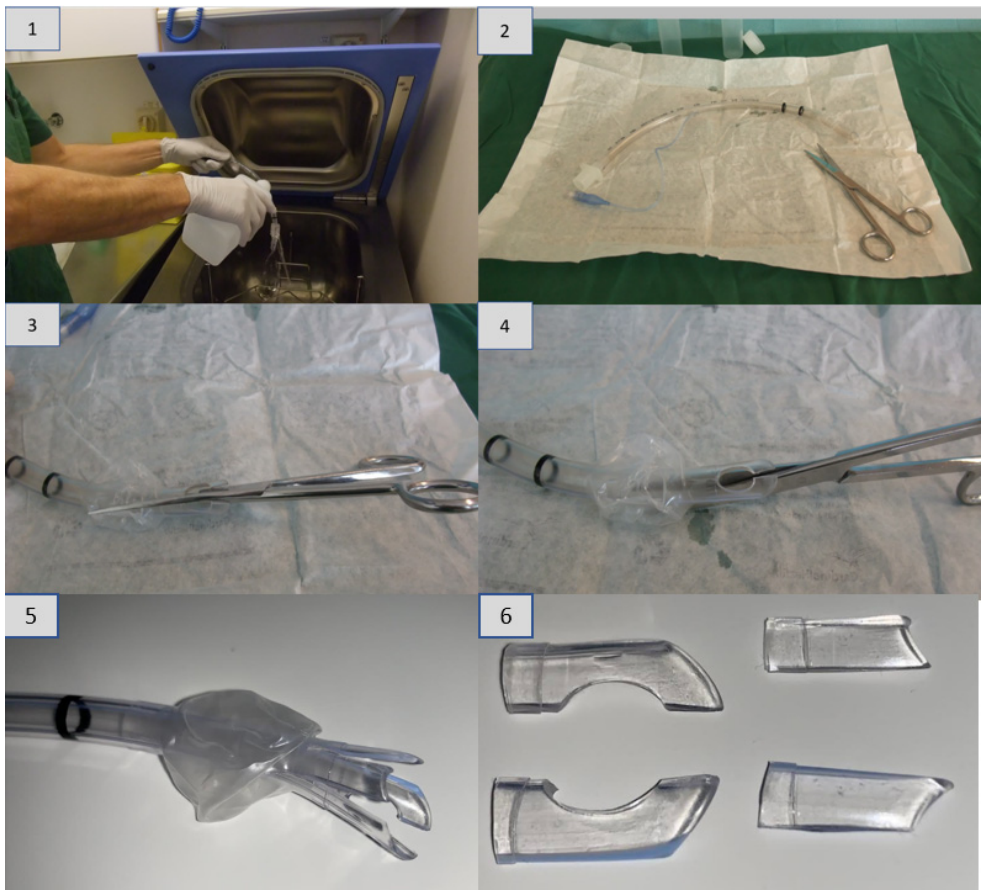



**Figure 5.**
Processing of endotracheal tubes (ETTs): (1) each ETT was rinsed with sterile saline, on the inside and outside, to eliminate excess mucus; (2–6) photographs showing how ETTs were cut for scanning electron microscopy (two pieces) and microbial culture (two pieces). Photos by Bengt Klarin and Hulda Thorarinsdottir.

*Processing of the ETT*

Patients were extubated at the discretion of the treating physician or in the case of a patient's death. After extubation, the ETTs were collected, avoiding contamination from other than oropharyngeal flora, and rinsed (inside and outside) with 1 L of sterile saline to eliminate excess mucus (Figure 5). Thereafter, the distal tip of the ETT was divided into four pieces for both scanning electron microscopy (SEM) (2 pieces) and microbial cultures (2 pieces). Finally, the ETT tip was cut in a cross-sectional manner 1.5 cm above the distal tip (Figure 5).

Pieces of an ETT for microbial cultures were sonicated to dislodge biofilm microbes. The solution was then homogenized by vortex mixing and subsequently cultured using the same procedures as described above. For SEM, ETT pieces were fixed in a solution of formaldehyde and dehydrated with crescent ethanol concentrations, air dried overnight, and then sent to Research Institutes of Sweden (RISE) in Borås for grading of the biofilm.

Before the start of study II, a pilot study with five ETTs, comparing two different methods for processing of the ETTs (scraping vs. sonication) indicated that the method outlined above was optimal for removing the biofilm from the ETT and for dislodging the biofilms' microbes before culturing. The fixation protocol for SEM was also evaluated in this pilot-study.

*Scanning electron microscopy and grading of the biofilm*

The inner and outer surfaces of the ETTs were examined by SEM (Zeiss Supra 40VP, Carl Zeiss Microscopy GmbH, Jena, Germany) at RISE. Grading of the biofilm was performed by a researcher at RISE who was blinded to all patient information including the type of ETT being analyzed. A summary of the grading system is shown in Table 3. The final grade of the biofilm (score 0 to 9) was calculated by adding together the scores for coverage, density, and thickness levels.

**Table 3. Scoring System used to grade biofilm formation in study II**
The biofilm grade was calculated by adding together the scores for biofilm coverage, density, and thickness. Mag: magnification.

| **Biofilm coverage**<br>Determined at<br>100x to 1,000x mag | **Biofilm density**<br>Determined at<br>10,000x to 30,000x mag | **Biofilm thickness scale**<br>Determined at<br>10,000x to 50,000x mag | **Biofilm grade**<br>(coverage + density<br>+ thickness) |
|---|---|---|---|
| 0 no biofilm<br>1 scarce (< 10% coverage)<br>2 clusters (10% to 70% coverage)<br>3 confluent ( > 70% coverage) | 0 no biofilm<br>1 low/very porous<br>2 intermediate<br>3 high/compact | 0 no biofilm<br>1 thin biofilm (0.1 to 1.0 mm)<br>2 medium biofilm (1.1 to 7 mm)<br>3 thick biofilm ( > 7 mm) | 0 no biofilm<br>1 - 3 low grade<br>4 - 6 medium grade<br>7 - 9 high grade |

*Definitions*

- *Negative culture (normal flora)*: surveillance cultures and ETT tip cultures with growth of at least two species of bacteria usually found in the oral cavity.
- *Positive culture (abnormal flora)*: surveillance cultures and ETT tip cultures with growth of species not normally found in the oral cavity, such as pathogens, gut flora, or overgrowth of normal oral flora.
- *High grade biofilm formation* was defined as a score of ≥ 7 in the above described scoring system.
- *VAP* was defined as: 1) new or progressive lobar infiltrate > 48 hours after intubation; 2) two or more of the minor criteria: fever, leukocytosis/leukopenia, and/or purulent respiratory secretions; and 3) microbiologically confirmed in endotracheal aspirate.
- *Colonization with common VAP pathogens* included cultures with *Enterococcus faecium*, *Enterococcus faecalis, Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumani, Pseudomonas aeruginosa, Streptococcus pneumoniae, Haemophilus influenzae*, and other Enterobacteriaceae
- *VAP relapse* was defined as 1) occurrence at least 72 hours after first VAP episode, 2) positive endotracheal aspirate with previously isolated strain, 3) new infiltrate or progression of previous infiltrate on chest x-ray, 4) two of the following: fever, leukocytosis/leukopenia, and/or purulent respiratory secretions, and 5) no evidence of extrapulmonary source of infection.

# Study III:

*Inclusion of cases*

All CVCs or central hemodialysis catheters (CHCs) inserted at the Department of Intensive and Perioperative Care at Skåne University Hospital, Lund, from January 2011 to December 2012, were included retrospectively. The catheters included were inserted at the centralized CVC clinic, the ICU, or in the operating theatre. We included all patients > 8 years old, because that was the age limit for admission to our department. Peripherally inserted CVCs and ports were excluded, because they were inserted by a different technique and with other hygiene precautions.

*Implementation of a hygiene insertion bundle*

In January 2012, a standardized hygiene bundle for catheter insertion was introduced at our institution. Before January 2012, the hygiene precautions utilized

at the time of CVC insertion were performed at the discretion of the inserting anesthesiologist. An English version of the description of the new hygiene insertion bundle is presented in in the appendix (Appendix S1) of Paper III.

*Data collection and culture routines*

All documented cannulation procedures (for CVCs and CHCs) during 2011 and 2012 were registered. Microbiological data were thereafter extracted from the accredited microbiology laboratory at the hospital using an automated script and then merged with insertion data into a database. According to guidelines at our hospital, the catheter tip, together with a simultaneous peripheral blood culture should be sent for culture only when CRI is suspected. Routines for CVC removal and culture methods are described in Paper III (method, cultures).

*Definitions*

- *CVC colonization* was defined as a positive tip culture regardless of clinical symptoms.
- *Catheter-related infection (CRI)* was present, if the catheter-tip culture was positive and the patient fulfilled at least two of four systemic inflammatory response syndrome (SIRS) criteria (fever > 38 or < 36 °C, heart rate > 90 beats/minute, respiratory rate > 20 breaths/minute, or white blood cell count >12,000/μL or < 4,000/μL) upon CVC removal with no likely explanation other than the catheter.
- *Catheter-related blood stream infection (CRBSI)* was defined as a bloodstream infection upon CVC removal with the same microorganism isolated on both the catheter tip and in the blood (within 48 hours prior to the removal of the CVC) in a patient fulfilling at least two of the four SIRS criteria with no likely explanation other than the catheter.

# Study IV:

Five CVCs and one central hemodialysis catheter (CHC) were selected for this experimental study and are described in Table 2. The three uncoated materials (two CVCs made of polyurethane [PU] and one CHC made of silicone) were chosen because they are widely used CVC materials in Europe, including Sweden. The three CVCs made of PU with anti-infective coatings were selected for comparison because they are widely used in the United States. All catheters were triple lumen and 7 Fr except for the CHC, which was double lumen and 13 Fr.

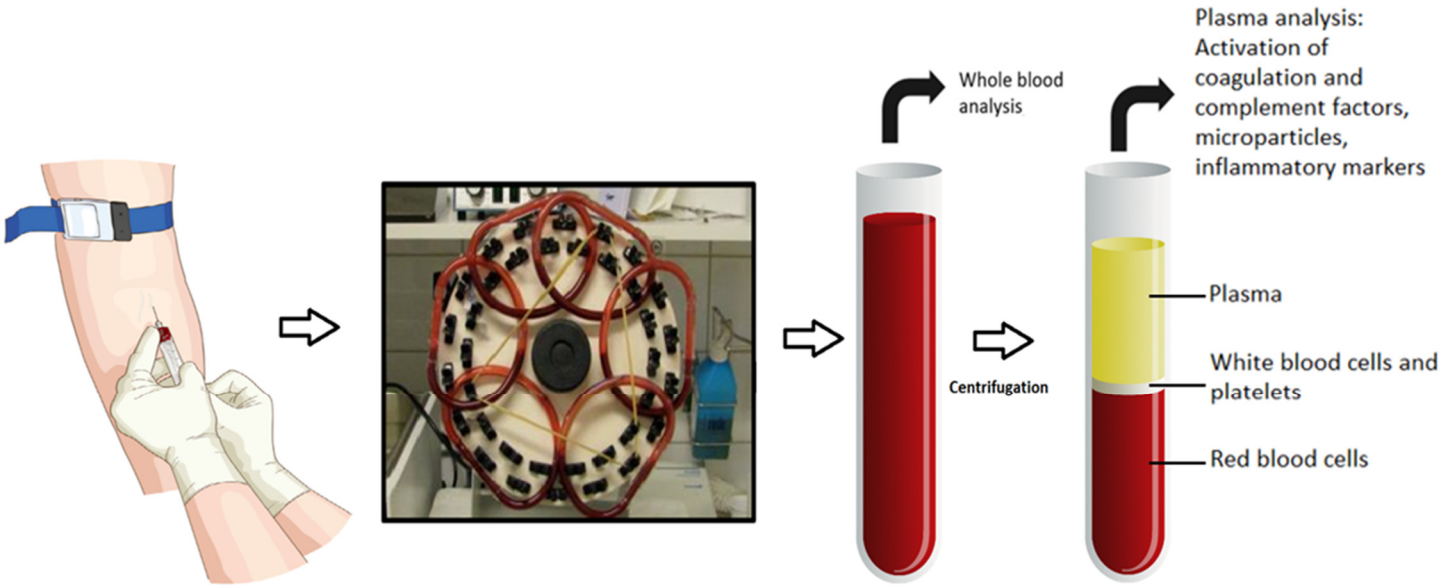



**Figure 6. The Chandler loop model**
The model imitates the flow of blood in a vein. Reprinted with permission. Picture on the left by Nick Grib, in the middle photo by Javier Sanchez, on the right, picture created by Lisbet Gudny Thorarinsdottir).

### The Chandler loop model

The blood compatibility of the six catheter materials was tested in a modified Chandler loop model (Figure 6) at Danderyd Hospital, Danderyd, Sweden. The Chandler loop model is a rotating, blood-filled tubing system, designed to imitate the flow of blood in a vein, and this model has also been used in previous laboratory studies[118–120]. The tubes in the Chandler loop are made of PVC (Medtronic, Minneapolis, USA) and have an internal thromboresistant heparin-based coating (Carmeda BioActive Surface, Vasby, Sweden). The tubing rotates at 10 rpm for 60 minutes in a water bath at 37 °C to keep a steady temperature.

Ten healthy volunteers who had not taken any medication 15 days prior to blood donation signed informed consent before entering the study. A 35-mL sample of blood was collected from each blood donor, and a 4.5 mL aliquot of the sample was poured into each of the loops in the Chandler system. A 1.4-$cm^2$ piece of each CVC material was put into each of the loops. One loop contained no CVC material and served as a "control loop". The blood circulated in the loops for 1 hour. Thereafter, the materials were removed from the blood, and either EDTA or citrate was added to stop any ongoing activation of blood components. Finally, the blood samples were centrifuged, and the plasma was stored at –70 °C.

*Blood compatibility assays*

Blood compatibility was evaluated using parameters related to the activation of coagulation, the complement system, and inflammation. The parameters were chosen according to ISO standard 10993-4: Biological evaluation of medical devices, Part 4: Selection of tests for interaction with blood. All blood compatibility tests were analyzed at the Clinical Chemistry Laboratory at Danderyd Hospital, Danderyd, Sweden. The blood compatibility assays are described in detail in Paper IV (methods, blood compatibility assays).

## Statistical analysis

All analyses were performed using SPSS 24 (SPSS Inc., Chicago, IL, USA).

*Power calculations*

Power calculations were performed for the primary endpoint in study I, II and IV and indicated a power of 0.80 with the selected sample sizes described above. Study III was a retrospective registry study and all patients available during the study period were included. Given the low incidence of outcomes (CRI and CRBSI), only

a limited number of potential risk factors could be evaluated in the multivariable regression model.

*Study I - III*

Results were expressed as median (range) for continuous variables and number (percentage) for categorical variables. Groupwise comparisons were conducted using Fisher's exact test for categorical variables and the Mann-Whitney U-test for continuous variables. Logistic regression was applied to analyze associations between dependent and independent variables. Univariable logistic regression analyses were performed to evaluate possible predictors that could be associated with the outcome. Thereafter, multivariable logistic regression analyses were carried out to determine independent factors associated with the outcome. If cases (e.g., of VAP or CRBSI) were too few, univariable regression or descriptive statistics was applied. Outcome measures in study I and III were binary (yes/no), whereas in study II the grade of biofilm formation (grade $< 7$ and $\geq 7$ in the scoring system described above) was dichotomized based on data from a previous study[98]. The Hosmer-Lemeshow test was used to test goodness of fit. A P value of $< 0.05$ was considered significant, and all statistical tests were two tailed.

*Study IV*

The results of blood compatibility tests were expressed as median (range). Non-parametric tests for dependent samples (Friedman test and Wilcoxon's signed rank sum test) were used for comparison of blood compatibility variables, because each patient served as his own control. The calculation of sample size for the primary endpoint (hemolysis) was based on a previous study[120] and a significance level of $< 0.0083$ for the Wilcoxon's signed rank sum test. Given a power of 0.80 and an alpha level of 0.0083, a sample size of 9 was needed. A $P < 0.0083$ was considered significant in group-wise comparison (Bonferroni correction) for the primary endpoint and $P < 0.05$ for the secondary endpoints. All statistical tests were two-tailed.

# Results

## Study I

In this clinical observational study, we included a total of 487 individuals: 77 control subjects, 193 ward patients, and 217 ICU patients. As expected, the groups differed significantly regarding baseline characteristics (Table 1, Paper I).

Oropharyngeal cultures were obtained for all 487 of the subjects, and the results for the three study groups are shown in Figure 7 and Table 4. Abnormal oropharyngeal flora was significantly more common among ICU and ward patients as compared to controls (62.2% vs. 1.3% [P < 0.001] and 10.4% vs. 1.3% [P = 0.010], respectively). Abnormal oropharyngeal flora was also more frequent in ICU patients than in ward patients (62.2% vs. 10.4%, P < 0.001). Colonization of gut flora in the oropharynx was significantly more common among ICU patients compared to ward patients or controls (26.3% vs. 4.7% [P < 0.001] and 26.3% vs. 1.3% [P < 0.001], respectively). The occurrence of gut flora in the ward patients was not significantly different from that seen in controls (4.7% vs. 1.3%, P = 0.29).

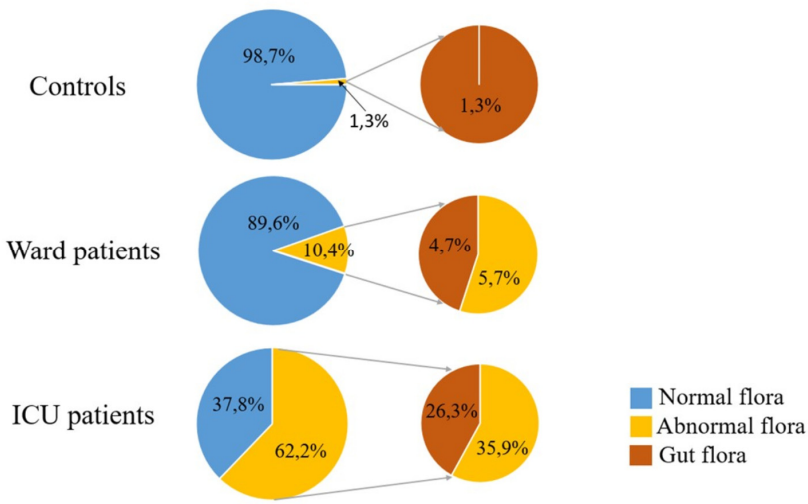



**Figure 7.**
Results of the oropharyngeal cultures obtained from the three study groups.

**Table 4. Microbial isolation from oropharyngeal cultures in the three study groups**
The percentage of positive cultures is related to the total number of positive cultures for all bacterial species in each group. ESBL, extended spectrum beta-lactamase-producing Enterobacteriaceae.

| Organism | Controls *n* (%) | Ward patients *n* (%) | ICU patients *n* (%) | All together *n* (%) |
|---|---|---|---|---|
| Respiratory tract pathogens | | | | |
| *Staphylococcus aureus* | | 4 (17.4) | 26 (14.0) | 30 (14.3) |
| *Haemophilus influenzae* | | 6 (26.1) | 6 (3.2) | 12 (5.7) |
| *Streptococcus* group B | | | 4 (2.2) | 4 (1.9) |
| *Moraxella catarrhalis* | | | 3 (1.6) | 3 (1.4) |
| Beta-haemolytic *streptococcus* group A | | | 2 (1.1) | 2 (1.0) |
| Beta-haemolytic *streptococcus* group G | | 1 (4.3) | 1 (0.5) | 2 (1.0) |
| *Streptococcus pneumoniae* | | | 1 (0.5) | 1 (0.5) |
| *Staphylococcus epidermidis* | | | 1 (0.5) | 1 (0.5) |
| Gut flora | | | | |
| *Escherichia coli* | | 2 (8.7) | 12 (6.5) | 14 (6.7) |
| *Enterobacter* species | | 1 (4.3) | 11 (5.9) | 12 (5.7) |
| *Enterococcus faecium* | | | 11 (5.9) | 11 (5.2) |
| *Enterococcus faecalis* | | 1 (4.3) | 10 (5.4) | 11 (5.2) |
| *Klebsiella* species | | 1 (4.3) | 9 (4.8) | 10 (4.8) |
| *Pseudomonas aeruginosa* | 1 (100) | 1 (4.3) | 7 (3.8) | 9 (4.3) |
| *Stenotrophomonas maltofilia* | | | 8 (4.3) | 8 (3.8) |
| *Citrobacter* species | | 2 (8.7) | 2 (1.1) | 4 (1.9) |
| *Serratia marcenes* | | | 3 (1.6) | 3 (1.4) |
| *Proteus* species | | 2 (8.7) | 1 (0.5) | 3 (1.4) |
| *Morganella morganii* | | | 1 (0.5) | 1 (0.5) |
| *ESBL* | | | 1 (0.5) | 1 (0.5) |
| Yeast | | | | |
| *Candida* species | | 2 (8.7) | 63 (33.9) | 65 (31.0) |
| Other origin | | 3 (1.5) | 3 (1.4) | |
| Total number of pathogens cultured overall | 1 (100) | 23 (100) | 186 (100) | 210 (100) |

We performed univariable and stepwise multivariable analysis to evaluate the determinants of abnormal oropharyngeal and gut flora in the oropharynx (Table 5, a and b). This showed that no factors were significantly associated with abnormal oropharyngeal flora among the ward and ICU patients. Restricting the analyses to growth of gut flora in the oropharynx, proton pump inhibitor (PPI) treatment emerged as the strongest independent predictor in ward and ICU patients in the multivariable analysis (OR 4.82 and 2.27, respectively). In the ICU group, higher BMI was also independently associated with the presence of gut flora in the oropharynx (OR 1.08). In contrast, diabetes was associated with lower occurrence of gut flora in the oropharynx (OR 0.34) in the ICU group. The length of hospital stay (number of days) prior to ICU admission was significantly associated with the presence of gut flora in the oropharynx (OR 1.05). Antibiotic therapy ≥ 24 hours before inclusion was not associated with changes in flora in either the ward or the ICU patients. An analysis of risk factors in the control group was not possible due to the low occurrence of abnormal flora and gut flora (n = 1, 1.3%; Table 4).

**Table 5 Analyzing potential predictors of abnormal flora in the oropharynx in (a) ward patients (b) ICU patients**
The table presents results of the univariable and multivariable logistic regression analyses. For the multivariable analysis, blanks (–) represent factors that were not significantly associated with changes in the oropharyngeal flora after stepwise regression. PPI, proton pump inhibitor; BMI, body mass index; APACHE II, Acute Physiology and Chronic Health Evaluation II; OR, odds ratio; CI, confidence interval.

| | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| Factor | OR | 95% CI | *P* value | OR | 95% CI | *P* value |
| (a) | | | | | | |
| Abnormal oropharyngeal flora | | | | | | |
| Age | 1.029 | 0.991–1.070 | 0.140 | – | – | – |
| Male gender | 0.867 | 0.342–2.197 | 0.763 | – | – | – |
| Current or ex-smoker | 1.551 | 0.599–4.018 | 0.366 | – | – | – |
| Alcohol consumption > 2x per week | 0.000 | 0.000 | 0.999 | – | – | – |
| PPI use ≥ 24 h before culture sampling | 1.551 | 0.599–4.018 | 0.366 | – | – | – |
| Antibiotic use ≥ 24 h before culture sampling | 1.859 | 0.488–7.076 | 0.363 | – | – | – |
| BMI | 0.989 | 0.912–1.072 | 0.780 | – | – | – |
| Diabetes | 0.215 | 0.028–1.664 | 0.141 | – | – | – |
| Gut flora in the oropharynx | | | | | | |
| Age | 1.039 | 0.979–1.102 | 0.211 | – | – | – |
| Male gender | 0.854 | 0.222–3.281 | 0.818 | – | – | – |
| Current or ex-smoker | 1.829 | 0.473–7.066 | 0.382 | – | – | – |
| Alcohol consumption > 2x per week | 0.000 | 0.000 | 0.999 | – | – | – |
| PPI use ≥ 24 h before culture sampling | 4.815 | 1.162–19.96 | 0.030 | 4.815 | 1.162–19.96 | 0.030 |
| Antibiotic use ≥ 24 h before culture sampling | 3.000 | 0.574–15.67 | 0.193 | – | – | – |
| BMI | 0.939 | 0.828–1.066 | 0.331 | – | – | – |
| Diabetes | 0.551 | 0.067–4.557 | 0.581 | – | – | – |
| (b) | | | | | | |
| Abnormal oropharyngeal flora | | | | | | |
| Age | 1.006 | 0.987–1.025 | 0.543 | – | – | – |
| Male gender | 1.483 | 0.847–2.596 | 0.168 | – | – | – |
| Current or ex-smoker | 0.694 | 0.384–1.255 | 0.227 | – | – | – |
| Alcohol consumption > 2x per week | 1.154 | 0.466–2.856 | 0.757 | – | – | – |
| PPI use ≥ 24 h before culture sampling | 1.585 | 0.872–2.881 | 0.131 | – | – | – |
| Antibiotic use ≥ 24 h before culture sampling | 1.107 | 0.632–1.937 | 0.723 | – | – | – |
| BMI | 1.009 | 0.964–1.055 | 0.712 | – | – | – |
| Diabetes | 0.797 | 0.412–1.542 | 0.500 | – | – | – |
| APACHE II | 1.013 | 0.975–1.054 | 0.505 | – | – | – |
| Days in the hospital before ICU admission | 1.024 | 0.991–1.059 | 0.150 | – | – | – |
| Gut flora in the oropharynx | | | | | | |
| Age | 1.028 | 1.004–1.053 | 0.022 | – | – | – |
| Male gender | 0.912 | 0.495–1.680 | 0.767 | – | – | – |
| Current or ex-smoker | 0.981 | 0.514–1.873 | 0.953 | – | – | – |
| Alcohol consumption > 2x per week | 1.55 | 0.617–3.891 | 0.351 | – | – | – |
| PPI use ≥ 24 h before culture sampling | 1.767 | 0.948–3.295 | 0.073 | 2.272 | 1.022–5.054 | 0.044 |
| Antibiotic use ≥ 24 h before culture sampling | 1.226 | 0.657–2.287 | 0.522 | – | – | – |
| BMI | 1.047 | 0.998–1.098 | 0.062 | 1.083 | 1.021–1.148 | 0.008 |
| Diabetes | 0.625 | 0.287–1.363 | 0.238 | 0.338 | 0.121–0.943 | 0.038 |
| APACHE II | 1.038 | 0.993–1.084 | 0.098 | – | – | – |
| Days in the hospital before ICU admission | 1.047 | 1.015–1.079 | 0.004 | 1.052 | 1.015–1.090 | 0.005 |

# Study II

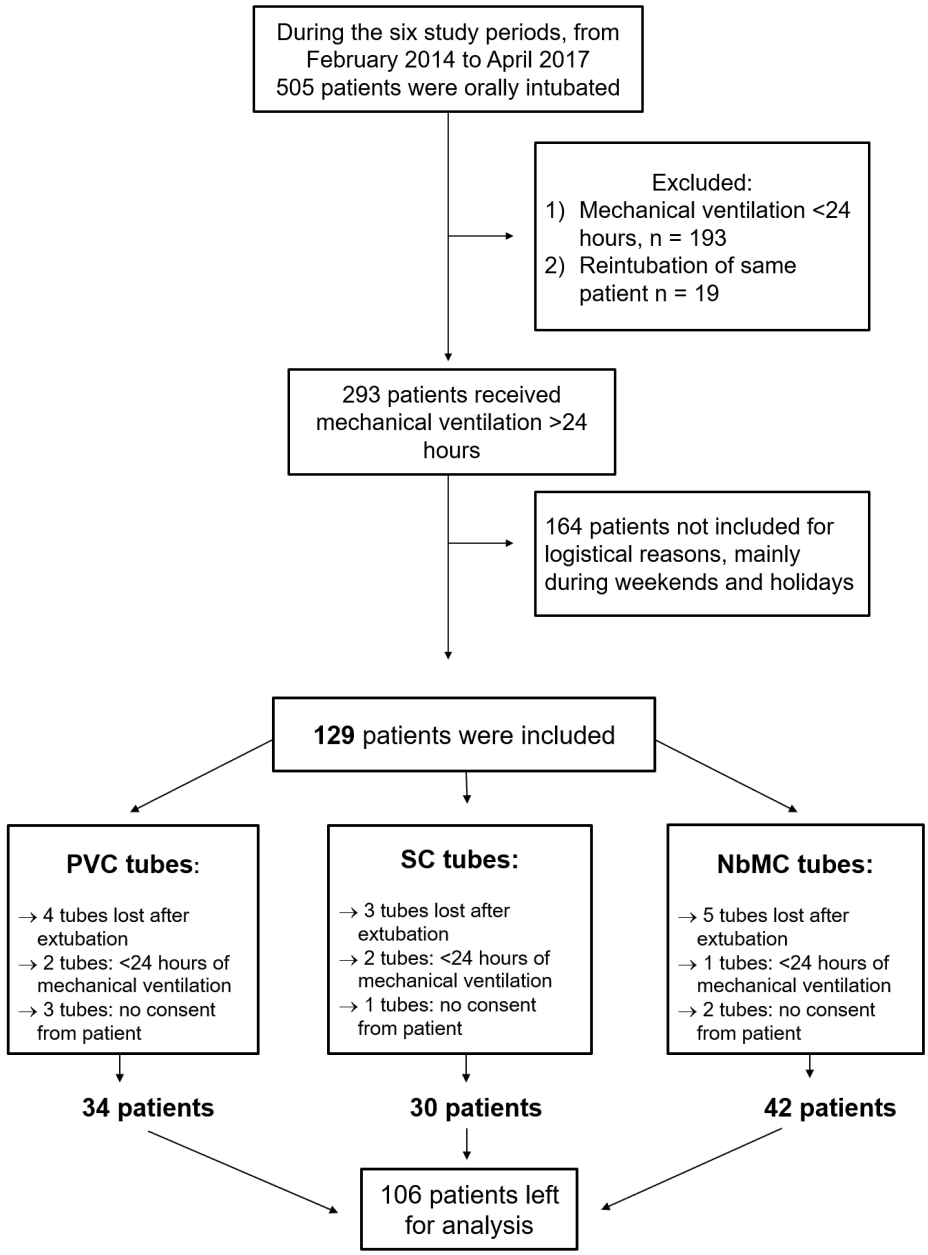



**Figure 8. Flow chart showing inclusion of patients in study II**
"No consent" refers to cases in which the patient did not give his/her consent to participate after the ICU stay. Endotracheal tube materials: PVC, polyvinyl chloride; SC, silicone-coated; NbMC, noble-metal-coated.

During the six study periods 505 patients were orally intubated. Among the 293 patients receiving mechanical ventilation for >24 hours, 129 patients were included in our study (Fig. 1). One-hundred and sixty-four patients were not included for logistical reasons and during weekends and holidays. Twenty-three of the included patients (n=129) were excluded because the ETT was lost after extubation, mechanical ventilation lasted < 24 hours and/or informed consent was not obtained from the patient or a relative. The remaining 106 patients were included in the final analysis as follows: 34 were intubated with an uncoated PVC tube; 30 were intubated with an SC PVC tube; and 42 were intubated with the NbMC PVC tube (Figure 1). Patient characteristics and main diagnosis on ICU admission were similar in the three study groups (Table 2 in Paper II).

*Biofilm formation*

After patient extubation, biofilm was present on the majority of ETTs (97%, n = 103), but the grade of biofilm formation varied from low, porous (Figure 9a) to confluent, abundant (Figure 9b) biofilm matrices. SEM often revealed colonies of different microorganisms embedded in the biofilm matrix (Figure 9c).

Multivariable logistic regression analysis performed to evaluate possible predictors of high-grade biofilm formation (score ≥ 7 ) on the ETTs showed that the SC and the NbMC PVC ETTs were independently associated with reduced high-grade biofilm formation compared to the uncoated PVC tubes (OR 0.18 [95% CI 0.06–0.59; p = 0.005] and OR 0.34 [95% CI 0.13–0.93; p = 0.036], respectively). There was no significant difference between SC and NbMC ETTs (OR 0.54 [95% CI 0.17–1.65]; p = 0.278). Age, sex, days with invasive ventilation, and colonization with common VAP pathogens in surveillance cultures did not predict higher biofilm formation in univariable or multivariable analyses (Table 6).

*Surveillance cultures*

As reported in Paper I, a majority of the patients developed abnormal oropharyngeal flora (82%, n = 87) and became colonized in the lower airways during invasive ventilation (endotracheal aspirate, 79%, n = 84). The surveillance cultures turned positive for several different microbes during mechanical ventilation (Table 4, Paper II). Colonization with common VAP pathogens was present in oropharyngeal cultures from 33% (n = 35) of the patients and in endotracheal cultures from 27% (n = 29).

*Microbial isolation from endotracheal tubes*

At extubation, 99% (n = 105) of the ETTs were cultured and 47% (n = 49) turned positive. No significant difference in positive ETT cultures was observed between the groups (uncoated PVC, 41% [n = 14], SC PVC 45% [n = 13]; NbMC PVC 52% [n = 22]; p = 0.61). Microbial isolations from the different ETTs are presented in Table 5 in Paper II. For the ETT tips that turned positive, the microbe was found in

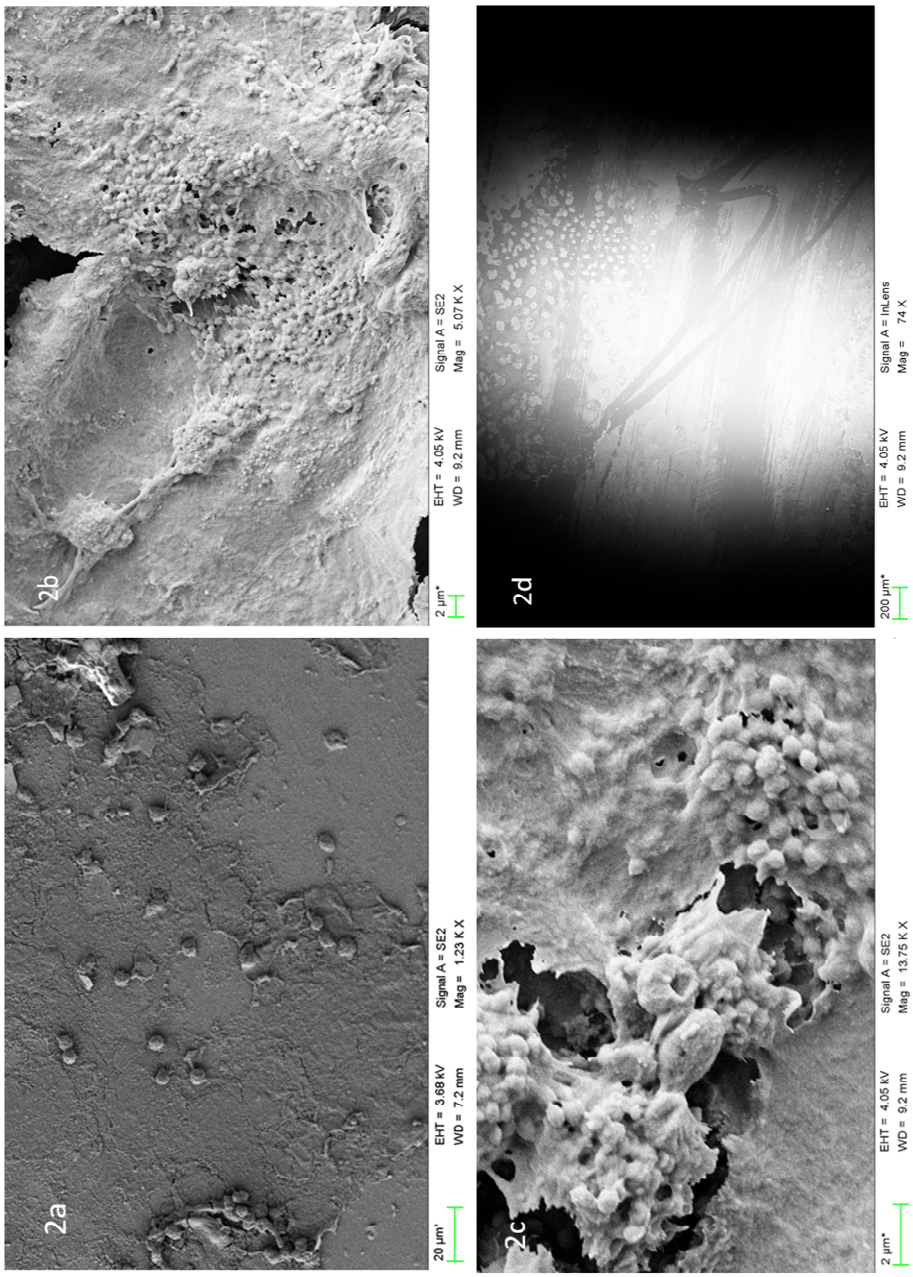



**Figure 9.**
Scanning electron microscopy showing (a) typical low grade (score < 4) biofilm and (b) typical high grade (score ≥ 7) biofilm formation on the surface of an endotracheal tube (low magnification); (c) colonies of microorganisms embedded in biofilm matrix (high magnification); (d) scrape marks on the ETT surface, probably after a suction catheter.

**Table 6. Possible predictors of high-grade (score ≥ 7) biofilm formation on the endotracheal tube**

Data are presented as median (range) or number (percentage). OR, odds ratio; CI, confidence interval, ETT, endotracheal tube; PVC, polyvinyl chloride; SC, silicone coated; NbMC, noble metal coated; VAP, ventilator-associated pneumonia; Ref, reference; NA, not applicable. "Colonized" refers to patients colonized with common VAP pathogens in surveillance cultures (oropharyngeal or endotracheal cultures).

| Factor | No n = 65 | Yes n = 41 | Univariable Analysis | | | Multivariable Analysis | | |
|---|---|---|---|---|---|---|---|---|
| | | | OR | 95% CI | *p* - value | OR | 95% CI | *p* - value |
| **ETT type** | | | | | | | | |
| **PVC (reference)** | 15 (23) | 19 (46) | NA | NA | NA | NA | NA | NA |
| **SC** | 23 (35) | 7 (17) | 0.24 | 0.08 - 0.71 | 0.010 | 0.18 | 0.06 - 0.59 | 0.005 |
| **NbMC** | 27 (42) | 15 (37) | 0.44 | 0.17 - 1.11 | 0.081 | 0.34 | 0.13 - 0.93 | 0.036 |
| **Age** | 69 (58 - 76) | 66 (53 - 75) | 0.98 | 0.95 - 1.00 | 0.088 | 0.98 | 0.95 - 1.02 | 0.388 |
| **Sex, male** | 34 (52) | 26 (63) | 1.58 | 0.71 - 3.52 | 0.262 | 1.78 | 0.72 - 4.39 | 0.211 |
| **Days with invasive ventilation** | 3.1 (2.0 - 5.8) | 3.8 (2.0 - 9.2) | 1.06 | 0.98 - 1.14 | 0.157 | 1.06 | 0.97 - 1.15 | 0.219 |
| **Colonized** | 33 (51) | 18 (44) | 0.76 | 0.35 - 1.67 | 0.491 | 0.59 | 0.25 - 1.43 | 0.245 |

oropharyngeal cultures in 86% (n = 42) of the patients and in endotracheal cultures in 88% (n = 43). The microbes found on the ETTs at points of extubation could be detected in the first oropharyngeal culture in 60% (n = 29) of the patients and in the first endotracheal cultures in 58% (n = 28).

*VAP*

Twelve patients developed 15 episodes of VAP during their stay in the ICU, and three of those episodes were VAP relapses. The most common pathogens involved in VAP were *Enterococcus faecium*, *E. faecalis, Staphylococcus aureus, Klebsiella* spp., and *Acinetobacter* spp. High-grade biofilm formation (score ≥ 7) on the ETTs, days of invasive ventilation, and age were significantly associated with the development of VAP (OR, 4.17; 95% CI 1.14–15.3; p = 0.031 and OR 1.11; 95% CI 1.01–1.22; p = 0.026 and OR 0.96; 95% CI 0.92–0.99; p = 0.046, respectively). ETT material, sex, and colonization with common VAP pathogens in surveillance cultures were not associated with development of VAP (Table 7).

Microbial persistence in surveillance cultures could be evaluated in seven of 12 patients who developed VAP, and it occurred in five patients (71%) after appropriate antibiotic treatment for VAP. At extubation, the microbes previously causing VAP could be found in the ETT biofilm in 56% of the cases (5 out of 9 patients) despite appropriate antibiotic therapy. The microbes most often involved in microbial persistence were *Klebsiella* spp., *Candida parapilosis*, *Enterococcus faecium*, and *E. faecalis*.

**Table 7. Analyzing possible predictors of ventilator associated pneumonia (n = 83)**
"Colonized" refers to patients colonized with common VAP pathogens in surveillance cultures (oropharyngeal or endotracheal cultures). OR, odds ratio; CI, confidence interval, ETT, endotracheal tube; PVC, polyvinyl chloride; SC, silicone coated; NbMC, noble metal coated; Ref, reference; NA, not applicable.

| | Univariable analysis | | |
|---|---|---|---|
| **Factor** | **OR** | **95% CI** | **p-value** |
| ETT type | | | |
| PVC (reference) | NA | NA | NA |
| SC | 0.66 | 0.14–3.16 | 0.606 |
| NbMC | 0.54 | 0.13–2.26 | 0.400 |
| Age | 0.96 | 0.92–0.99 | 0.046 |
| Sex, male | 0.68 | 0.19–2.48 | 0.563 |
| Days with invasive ventilation | 1.11 | 1.01–1.22 | 0.026 |
| High-grade biofilm formation on ETT | 4.17 | 1.14–15.3 | 0.031 |
| Colonized | 1.52 | 0.44–5.26 | 0.505 |

# Study III

During the two years studied, a total of 1,722 catheter insertions (94% CVCs and 6% CHCs) in 1,428 patients (52% male, median age 66 years) were registered (Table 1 in Paper III). The patient selection is shown in Figure 10.

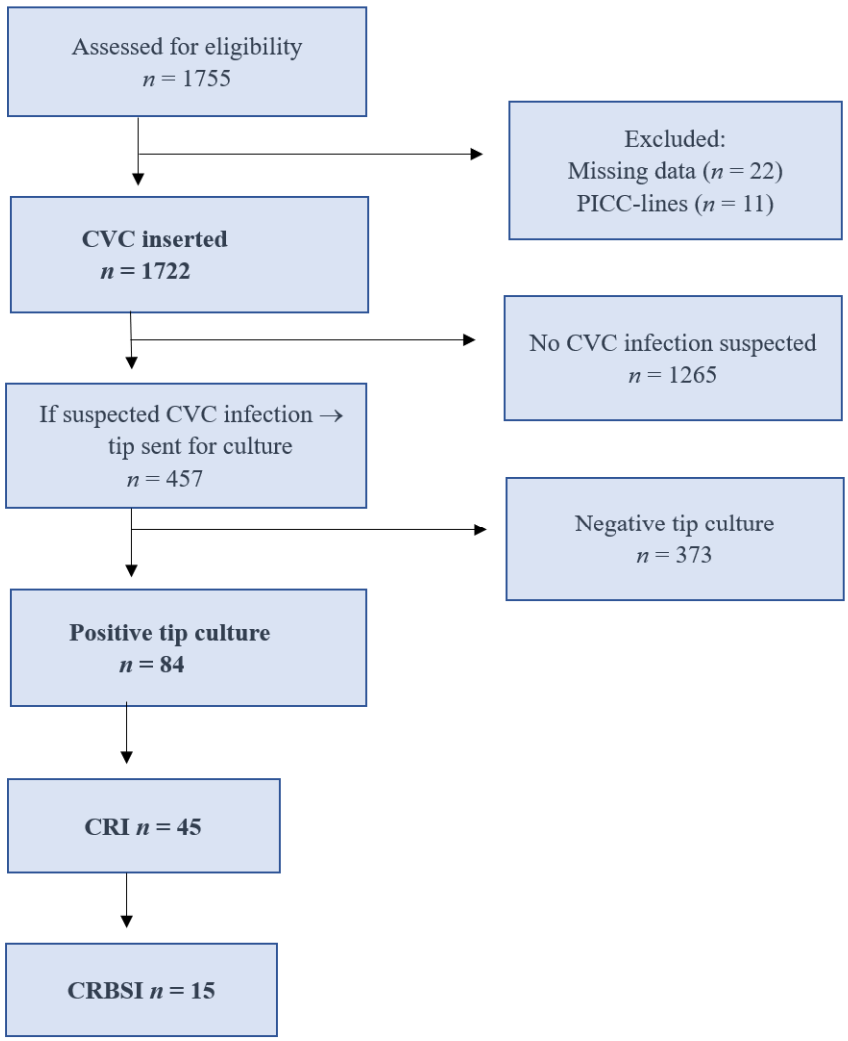



**Figure 10. Flowchart showing the selection of CRI and CRBSI cases.**
Abbreviations: CRBSI, catheter-related blood stream infection; CRI, catheter-related infection; PICC, peripherally inserted central catheter. CRI was present if the catheter-tip culture was positive and the patient fulfilled at least two of four systemic inflammatory response syndrome (SIRS) criteria with no likely explanation other than the catheter. CRBSI was defined as having the same microorganism isolated on both the catheter tip and in the peripheral blood culture and at least two of four SIRS criteria with no likely explanation other than the catheter.

Catheters were most often single lumen (62%), and the most-preferred insertion site was the jugular vein (76%). The median catheter duration time was 9 days (range 1–144 days). The baseline characteristics of catheters inserted before the implementation of a hygiene insertion bundle were similar to the characteristics of those inserted after the implementation (Table 1 in Paper III). One fourth (457) of the catheter tips were sent for culture at removal, and 18% (n = 84) of those cultures were positive (Figure 10). Sixty-nine percent of the patients were on antibiotics when the CVC tip was sent for culture. CRI was present in 45 of the cases (2.6% of the 1,722 catheters), with an incidence of 1.86/1,000 catheter days. When the tip was sent for culture, a simultaneous blood culture was taken in 124 cases and was positive in 37 of those cases. In 16 cases, the same microbe was identified on the catheter tip and in the blood culture, and 15 of those cases (0.9% of the 1,722 catheters) met the CRBSI criteria, yielding an incidence of 0.62/1,000 catheter days.

After implementation of the hygiene insertion bundle, the rates of catheter-tip colonization, CRI, and CRBSI decreased from 4.5/1,000 to 2.6/1,000, from 2.7/1,000 to 1.1/1,000, and from 0.8/1,000 to 0.5/1,000, respectively. Number needed to treat was 40 for catheter-tip colonization, 48 for CRI, and 215 for CRBSI. This means that 23 cases of catheter-tip colonization, 19 cases of CRI, and four cases of CRBSI were avoided at our department the year after the hygiene insertion bundle was implemented.

Microbial isolation from CVC tips and patients with CRI and CRBSI is shown in Table 2 in Paper III. The pattern of microbes was similar in patients with catheter-tip colonization and CRI, although the frequency of *Staphylococcus aureus* (19% of positive catheter tip cultures) and *Candida* spp. (12%) was higher in CRI cases. *S. aureus* (33.2% of CRBSI cases) and *Candida* spp. (26.6% of CRBSI cases) were the most common pathogens in CRBSI cases.

We used a multivariable logistic regression model to analyze the determinants of catheter-tip colonization and CRI (Tables 8 and 9). The implementation of a simple hygiene insertion bundle was the independent factor most strongly associated with significantly lower catheter tip colonization (95% CI of OR 0.34–0.98, P = 0.042) and CRI (95% CI of OR 0.23–0.92, P = 0.029) when compared with the year before implementation. An increase in the number of catheter lumens was also significantly associated with increasing catheter-tip colonization and CRI. Catheters placed in the subclavian vein were associated with lower catheter-tip colonization compared to those placed in the jugular vein (95% CI of OR 0.24–0.95, P = 0.036). A multivariable regression model was not applicable because there were few cases of CRBSI (n = 15). A description of patients with CRBSI is presented in Table 9.

**Table 8. Catheter-tip colonization**

Data are presented as median (range) or number (percentage). "Bundle" refers to the hygiene bundle introduced in January 2012. Abbreviations: OR, odds ratio; CHC, central hemodialysis catheter; CI, confidence interval; CVC, central venous catheter.

| Independent variable | No | Yes | Multivariable analysis | | |
|---|---|---|---|---|---|
| | n = 373 | n = 84 | OR | 95% CI | *P*-value |
| Age | 60 (16-93) | 62 (18-92) | 1.01 | 0.99-1.03 | .092 |
| Male gender | 188 (50) | 51 (61) | 1.50 | 0.88-2.54 | .136 |
| Days with catheter | 14 (1-144) | 13 (1-140) | 1.01 | 0.99-1.02 | .607 |
| Site of insertion | | | | | |
| Jugular vein | 213 (59) | 63 (79) | – | – | – |
| Subclavian vein | 140 (39) | 16 (20) | 0.47 | 0.24-0.95 | .036 |
| Femoral vein | 9 (3) | 1 (1) | 0.28 | 0.03-2.27 | .232 |
| Number of catheter lumens | – | – | 1.61 | 1.18-2.19 | .002 |
| CHC vs CVC | 40 (11) | 16 (20) | 1.93 | 0.94-3.96 | .073 |
| After bundle | 191 (51) | 34 (41) | 0.58 | 0.34-0.98 | .042 |

**Table 9. CRI and CRBSI**

Data are presented as median (range) or number (percentage). "Bundle" refers to the hygiene bundle introduced in January 2012. Abbreviations: CHC, central hemodialysis catheter; CRBSI, catheter-related bloodstream infection; CRI, catheter-related infection; CI, confidence interval; CVC, central venous catheter; OR, odds ratio.

| | CRI | | | | | CRBSI | |
|---|---|---|---|---|---|---|---|
| | No | Yes | Multivariable analysis | | | No | Yes |
| Independent variable | n = 412 | n = 45 | OR | 95% CI | *P*-value | n = 109 | n = 15 |
| Age | 60 (16-93) | 59 (18-83) | 1.01 | 0.99-1.03 | .375 | 59 (16-92) | 60 (18-83) |
| Male gender | 210 (51) | 28 (62) | 1.56 | 0.79-3.10 | .188 | 64 (59) | 11 (73) |
| Days with catheter | 13 (1-144) | 14 (4-99) | 1.01 | 0.99-1.03 | .524 | 11 (1-113) | 18 (6-41) |
| Site of insertion | | | | | | | |
| Jugular vein | 243 (89) | 30 (11) | – | – | – | 71 (65) | 8 (53) |
| Subclavian vein | 145 (93) | 11 (7) | 0.82 | 0.35-1.90 | .641 | 32 (29) | 5 (33) |
| Femoral vein | 9 (90) | 1 (10) | 0.70 | 0.08-5.93 | .747 | 3 (3) | 1 (7) |
| Number of catheter lumens | – | – | 1.63 | 1.11-2.39 | .013 | – | – |
| CHC vs CVC | 49 (89) | 6 (11) | 1.42 | 0.53-3.79 | .495 | 11 (10) | 1 (7) |
| After bundle | 208 (51) | 15 (33) | 0.46 | 0.23-0.92 | .029 | 59 (54.1) | 6 (40) |

# Study IV

Table 10a and 10b show the results of the blood compatibility tests for the six catheter materials and the control loop after circulating in the Chandler loop system. Detailed description of the blood compatibility results and P values from multiple comparisons are presented in Paper IV.

***The Blood cell system***

*Hemolysis*

All catheters except PU-2+CHSS showed "non-hemolytic" properties. In blood exposed to the PU-2+CHSS material, significant hemolysis occurred with a median hemolytic index of 29%. To determine whether the hemolysis was due to complement activation, the PU-2+CHSS material was exposed to eculizumab (50ug/mL), which is a C5 inhibitor that prevents formation of the membrane attack complex and complement-mediated lysis. However, the hemolysis rate was still 27% to 36% after introducing the inhibitor, indicating that the hemolysis was not due to complement activation but rather to a toxic effect of the PU-2+CHSS coating.

*Leukocytes*

*Remaining leukocytes*

Membrane disruption of leukocytes leads to a decrease in their count in blood analysis. Only plasma exposed to PU-2+CHSS showed significantly lower number of remaining leukocytes than noted in the control loop. Also, PU-2+CHSS resulted in lower remaining leukocytes compared to all the other materials except Si-1.

*L-selectin microparticles (MPs)*

The activation of leukocyte membranes leads to the release of the cell adhesion protein L-selectin MPs (CD62L) from the surface of platelets. The release of MPs was significantly higher (282/mL) in plasma exposed to the PU-3+BZC material than in the control loop ($P = 0.043$). When comparing the different materials, PU-3+BZC induced significantly higher L-selectin levels than PU-2 and PU-4+NbMC.

*Platelets and coagulation*

*Remaining platelets*

Both the disruption of platelet membranes and the aggregation of platelets leads to a decrease in remaining platelet counts after circulation in the Chandler system. The uncoated catheters (Si-1, PU-1, and PU-2) all showed significantly lower levels of remaining platelets than the control loop. When comparing the tested materials,

**Table 10a. Blood compatibility tests for the six catheter materials and the control loop after circulation in the Chandler loop system**

Results are shown as median (range). Colors: light orange, significantly different from control loop; orange, significantly different from control loop and at least one other CVC material; red, significantly different from control loop and all other CVC materials. *This catheter showed a higher platelet count after exposure to the tested material than in fresh blood (110%) because fragments of disrupted erythrocytes and leukocytes interfered with the platelet measurements, resulting in a false high platelet count.

| | **The blood cell system** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Erythrocytes** | **Leukocytes** | | **Platelets and coagulation** | | | | |
| | **Hemolysis index (%)** (membrane disruption) | **Remaining leukocytes (%)** (membrane disruption and activation) | **L-Selectin (mp/mL)** (leukocyte activation) | **Remaining platelets (%)** (aggregation, membrane disruption) | **P-Selectin (mp/mL)** (thrombocyte activation) | **FXIIa Activity (Abs 450 nm)** (surface activation) | **Prothrombin fragment F1+2 (pmol/mL)** | **TAT (mg/L)** (coagulation activation, TAT is pre-stage for clot) |
| **n** | 10 | 10 | 5 | 10 | 8 | 6 | 6 | 10 |
| **Control loop** | **0.75** (0.40–0.98) | **97** (93–102) | **37** (4–87) | **94** (88–101) | **31** (11–141) | n.a. | **300** (158–358) | **48** (19–74) |
| **Uncoated catheters** | | | | | | | | |
| **Si-1** | **0.62** (0.21–1.21) | **97** (90–100) | **37** (12–108) | **72** (44–82) | **309** (191–1365) | **0.24** (0.17–0.32) | **1078** (556–2410) | **218** (118–558) |
| **PU-1** | **0.90** (0.47–1.47) | **99** (95–104) | **17** (13–38) | **81** (72–88) | **178** (24–673) | **0.25** (0.15–0.29) | **617** (154–1563) | **370** (300–479) |
| **PU-2** | **0.93** (0.45–1.28) | **100** (97–105) | **25** (4–36) | **79** (51–87) | **228** (122–545) | **0.07** (0.04–0.08 | **719** (272–1576) | **132** (105–170) |
| **Coated catheters** | | | | | | | | |
| **PU-2+CHSS** | **29.0** (21.3–47.6) | **93** (90–100) | **65** (18–134) | **110*** (73–130) | **337** (42–752) | **0.07** (0.06–0.11) | **514** (180–983) | **250** (186–333) |
| **PU-3+BZC-H** | **0.90** (0.43–1.27) | **97** (93–104) | **282** (1–374) | **94** (77–104) | **75** (14–238) | **0.04** (0.02–0.10) | **660** (16 –1112) | **183** (113–238) |
| **PU-4+NbMC** | **0.91** (0.51–1.41) | **98** (92–107) | **25** (11– 135) | **86** (67–99) | **162** (20–679) | **0.06** (0.04–0.08) | **666** (250–865) | **185** (146–259) |

**Table 10b. Blood compatibility tests for the six catheter materials and the control loop after circulation in the Chandler loop system**

Results are shown as median (range). Colors: light orange, significantly different from control loop; orange, significantly different from control loop and at least one other CVC material; red, significantly different from control loop and all other CVC materials.

| | The innate immune system | | | | |
|---|---|---|---|---|---|
| | Complement activation by FXII or leukocytes | | Acute immune reaction: cytokine and growth factors released from leukocytes/platelets | | |
| | C3a (ng/mL) (anaphyla-toxin) | sC5b-9 (ng/mL) (membrane lysis) | IL-8 (pg/mL) (chemotaxis, phagocytosis) | TNF-α (pg/mL) (acute phase) | VEGF (pg/mL) (angio-genesis) |
| n | 10 | 10 | 10 | 10 | 8 |
| Control loop | 854 (228–984) | 282 (168–420) | 2.09 (1.1–7.4) | 1.54 (0.88–3.64) | 36 (8–70) |
| Uncoated catheters | | | | | |
| Si-1 | 873 (665–1926) | 626 (419–1576) | 5.53 (2.3–20.7) | 1.59 (1.17–4.25) | 71 (27–144) |
| PU-1 | 853 (652.1–1009) | 626 (370–904) | 3.64 (2.0–7.2) | 1.34 (1.09–4.35) | 42 (16–62) |
| PU-2 | 837 (562–978) | 637 (323–816) | 4.20 (2.5–7.5) | 1.34 (1.07–4.03) | 44 (16–75) |
| Coated catheters | | | | | |
| PU-2+CHSS | 1528 (809–1876) | 1218 (466–1859) | 12.50 (3.5–26.8) | 6.64 (3.40–17.7) | 47 (42–87) |
| PU-3+BZC-H | 920 (710–1132) | 621 (378–896) | 2.41 (1.5–5.1) | 1.25 (0.77–7.17) | 34 (12–70) |
| PU-4+NbMC | 834 (752–1341) | 672 (423–832) | 3.74 (2.0–8.6) | 1.60 (0.81–3.33) | 44 (15–63) |

Si-1 resulted in significantly lower levels of remaining platelets than all the other materials except PU-2. The CHSS catheter led to higher platelets counts (110%), because fragments of disrupted erythrocytes and leukocytes interfered with the platelet measurements, resulting in a falsely high count.

*P- selectin MPs*

Activation of platelet membranes leads to release of the cell adhesion glycoprotein, P-selectin MPs (CD62P) from the surface of the platelets. The P-selectin levels were significantly higher in plasma exposed to all the materials except PU3+BZC in comparison with the control loop. When comparing the materials, PU-2 + CHSS and Si-1 showed significantly higher levels of P-selectin MPs than all the other materials except PU-2.

*FXIIa activity*

Factor XII (FXII) is the first of the plasma proteins to be activated by contact with a foreign material present in the blood, resulting in formation of FXIIa. Measurement of FXIIa was not applicable in the control loop, because FXII is activated by the surface of a catheter and there was no catheter in the control loop. The FXIIa activity was significantly higher in plasma exposed to the Si-1 (0.24) and PU-1 (0.25) surfaces compared to all other materials.

*Prothrombin fragment F1+2*

Prothrombin fragment F1+2 is a peptide released from prothrombin during its activation. The Si-1, PU-2, and PU4+NbMC materials all resulted in significantly higher levels of F1+2 than noted in the control loop. We found no significant difference in generation of F1+2 between the six materials ($P = 0.071$).

*Thrombin-anti-thrombin (TAT) complex*

TAT is an indicator of activation of the coagulation cascade and is generated when thrombin neutralizes anti-thrombin. The PU-1 catheter led to the highest TAT level of 370 µg/L, which was significantly higher than compared to all the other materials except Si-1. PU-2+CHSS resulted in a TAT level of 251 µg/L, which was significantly higher than the levels noted for PU-2, PU-3+BZC, and PU-4+NbMC.

***Molecular markers of the innate immune system***

*Activation of the complement system*

Activation of the complement system via the cellular system (leukocytes and platelets) or via protein absorption on the material surface was assessed by analyzing the increase in inflammatory anaphylatoxin C3a and soluble terminal complement complex sC5b-9, a marker of cell membrane lysis, after exposure to the six catheter materials and the control loop.

*C3a anaphylatoxin*

The PU-2+CHSS and Si-1 materials generated significantly higher levels of C3a compared to the control loop. The median value for Si-1 was low (873 ng/mL) but varied markedly (range 665–1,926 ng/mL), resulting in statistical significance in paired comparisons. The PU-2+CHSS catheter generated the highest C3a levels (1,528 ng/mL), which were significantly higher than compared to the control loop and all the materials except Si-1.

*sC5b-9 marker*

The results of the analysis of sC5b-9 showed a trend similar to that observed in the C3a analysis, which was expected. As for C3a, the PU-2+CHSS catheter generated the highest median level of sC5b-9 of 1218 ng/mL, which was significantly higher than noted for all the other materials except Si-1. In contrast to C3a, all catheters generated significantly higher levels of sC5b-9 than the control loop.

*Acute inflammatory reaction: IL-8, TNF-α, and VEGF*

To assess the acute inflammatory reaction caused by leukocyte and thrombocyte activation or membrane disruption, we analyzed IL-8, TNF-α, and VEGF released from leukocytes or injured endothelial cells into plasma upon exposure to the six different catheter materials and the control loop.

*IL-8*

The Si-1, PU-2, PU2+CHSS, and PU4+NbMC materials generated significantly higher IL-8 levels compared to the control loop. Also, PU-2+CHSS generated significantly higher IL-8 than all the other materials except Si-1.

*TNF-α*

The PU-2+CHSS catheter generated significantly higher levels of TNF-α than the control loop and all the other materials.

*VEGF*

The Si-1 material, PU-2, and PU-2+CHSS catheters generated significantly higher VEGF levels compared to the control loop. The Si-1 generated the highest VEGF levels, which were significantly higher than the levels induced by all the other materials except PU-2+CHSS.

# Discussion

## Changes in oropharyngeal flora during hospitalization

In the study presented in Paper I, changes in the oropharyngeal flora were found to be an early and frequent event in hospitalized patients, particularly in the critically ill. Furthermore, early oropharyngeal colonization by gut flora was common in critically ill ICU patients. The use of PPIs was independently associated with presence of microbial gut flora in the oropharynx in both ward and critically ill ICU patients.

The observation of a change in the oropharyngeal flora in both ward and ICU patients was in accordance with earlier studies showing that abnormal oropharyngeal flora is common in hospitalized patients[90,91], especially in the critically ill[90,121]. Previous investigations have demonstrated that early after hospital or ICU admittance, patients are often colonized with pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae.* A shift towards enteric gram-negative bacteria is often observed with increasing length of stay, which often entails worsening of an illness and a greater number of procedures and treatments performed[90–92,122]. Being colonized with gram-negative bacilli in the oropharynx increases the risk of developing VAP[92].

When analyzing factors that might be associated with abnormal oropharyngeal flora with multivariable regression analysis, we could not identify any independent factors associated with abnormal oropharyngeal flora, although such associations have been found by other researchers[123]. The definition of abnormal oropharyngeal flora includes a wide range of pathogens/microbes of various origins (e.g., candida, gut bacteria, contamination from caregivers, or an overgrowth of the patient's own flora) with varying pathogenesis, and hence it is likely that the risk factors associated with the appearance of these microorganisms also differ. The heterogeneity in the oral flora may explain why we found no factors that were significantly associated with abnormal flora in study I.

When applying multivariable logistic regression to analyze possible predictors of gut flora in the oropharyngeal tract (Table 4a and b in Paper I), PPI use emerged as an independent risk factor in both ward and ICU patients. Several studies have shown that treatment with PPIs increases the risk of pneumonia in people in the community and patients in both hospital wards and in ICUs[124–127]. However, the

prevalence of pneumonia is much lower in the community compared to hospitalized patients, and this is a reminder that the development of pneumonia is multifactorial and a question of balance between host defense and microbes. So, in a person in the community taking PPI the risk of pneumonia is low, but with sickness and hospitalization, more risk-factors are added together, resulting in a substantial risk of gut flora in the oropharynx and development of pneumonia. Recognizing this difference will aid in taking the appropriate measures, such as the following: considering the risks and benefits of the PPI treatment early during hospitalization, obtaining oropharyngeal cultures, taking patient positioning into account, providing physiotherapy, applying oral decontamination, and starting treatment with probiotics at hospital admission.

Many ICU studies performed in recent years have focused on choosing the 'right' antacid medication, that is, finding a treatment that minimizes the risk of gastrointestinal bleeding while not increasing the risk of VAP[128,129]. The majority of such investigations have indicated that PPIs are more effective than histamin-2 receptor blockers in reducing the risk of GI bleeding[125,130,131], but at the same time it seems that stress ulcer prophylaxis increases the risk of pneumonia[125,129,132]. Results are conflicting, with some randomized trials not showing a clear benefit of PPI use[133], especially in certain ICU groups such as sepsis patients[132] and patients receiving enteral nutrition[134]. This has made clinicians question the widespread recommendations to use PPI treatment in ICU patients e.g., as suggested in the Surviving Sepsis Campaign[135]. In a newly published large meta-analysis by Wang et al. [124], PPI reduced the risk of clinically significant GI bleeding in patients at high risk for bleeding while in low risk patients, the effect was considered minimal. PPIs again increased the risk of pneumonia in all groups. The results reported in Paper I reinforce the hypothesis that PPI use increases the risk of pneumonia by changing the oral flora to harbor gut bacteria that in turn can subsequently be micro-aspirated into the lungs. Oropharyngeal colonization with enteric gram-negative flora is a known risk factor for VAP[92]. The benefit of PPI treatment probably depends on the patient's risk of GI bleeding. Risk-benefit evaluations should be performed before treatment with PPI is initiated, considering the evidence that PPIs increase the risk of pneumonia. Also, re-evaluation of PPI treatment should be performed, because the patient's risk of GI bleeding might change during the ICU stay (e.g., upon start of enteral nutrition or after stabilization of shock and coagulopathy).

In addition to PPI use, our analysis showed that body mass index (BMI), diabetes, and days of hospitalization were significantly associated with either increased or decreased presence of gut flora in the ICU patients but not in ward patients. Higher BMI was associated with increased prevalence of gut flora in the oropharynx in ICU patients. A probable explanation for these observations is a higher incidence of gastro-esophageal reflux disease (GERD) and lower sphincter tonus in obese patients (BMI > 30 $kg/m^2$) due to a distorted pressure gradient across the gastro-esophageal junction[136,137], which provides gut bacteria access to the oropharynx.

Still, there are some conflicting results. A study by Frandah et al.[123] showed that an increase in BMI significantly reduced the prevalence of abnormal flora in the oropharyngeal tract, although this applied to all abnormal flora and not specifically gut flora. The results outlined in Paper I demonstrated that elevated BMI is not associated with rising prevalence of abnormal flora, only with increasing gut flora, which supports the explanation suggested above. Furthermore, in the ICU group, diabetes was associated with reduced frequency of gut flora in the oropharynx. Diabetes is known to be associated with an increased risk of developing oral candidiasis, with several mechanisms potentially contributing to that effect, such as changes in oral pH and salivary production and neural dysfunction[138]. It is possible that those changes in the environment in the oropharynx in patients with diabetes or an increased incidence of oral candida infection influences the ability of the gut flora to thrive in the oral cavity.

We also found that increased length of hospital stay prior to ICU admission was associated with gut flora colonization, but not with abnormal flora. These patients were hospitalized for a few days, receiving medical treatment and/or undergoing surgery, frequently having some sort of complication/aggravation that led to a transfer to the ICU. Johanson et al.[90] concluded that the severity of illness in the patients they investigated was the factor that was best correlated with the emergence of a Gram-negative oropharyngeal flora. This finding can probably explain the association with length of stay noted in study I.

Paper I did not indicate that antibiotic use prior to hospitalization and/or ICU admission was associated with a higher incidence of abnormal oropharyngeal flora in the evaluated population. This observation agrees with the study published by Johansson in 1969[90] and a more recent report published by Frandah et al.[123] in 2013, which showed that antibiotic therapy was not associated with a higher incidence of abnormal oropharyngeal flora. A likely explanation for this is that, compared to the flora in the intestines, the oropharyngeal flora is affected more slowly and to a lesser extent by both digested and intravenous antibiotics[139–141].

# Biofilm formation on different endotracheal tubes

As described in Paper II, we found that biofilm formation on ETTs was an early and frequent event in intubated patients. Furthermore, we noted that high-grade biofilm formation on ETTs was associated with the development of VAP. Among the studied ETT materials, both the SC ETT and the NbMC ETT were associated with reduced high-grade biofilm formation as compared to the standard uncoated PVC tube. The microbes detected in the ETT biofilm were frequently found in surveillance cultures obtained at intubation. Microbial persistence despite

appropriate antibiotic treatment was common in ICU patients with VAP, and the causative microbes were often found in the ETT biofilm at extubation.

The results reported in Paper II confirm earlier studies showing that biofilm formation on the ETT surface is an early and frequent event in intubated patients[95–99]. In that context, Paper II adds important knowledge illustrating that formation of a high-grade biofilm on the ETT surface (not just the presence of biofilm at any stage) is associated with development of VAP. Given that 60% of the first oropharyngeal cultures and 58% of the first endotracheal cultures contained microbes that were found in the ETT biofilm, this study also demonstrates a microbial link between early surveillance cultures and microbial content of biofilms. In addition, we found that microbial persistence was common, despite appropriate antibiotic treatment, when an ETT was left in place after an episode of VAP. These observations also indicate that the choice of ETT material can influence the grade of biofilm formation on the ETT surface. Sottile et al.[96] were among the first to use SEM to describe biofilm formation on ETTs, but only in recent years has the role of biofilm formation in the pathogenesis of VAP become clearer. There is increasing evidence[99], including the present results, suggesting that the ETT biofilm is a significant and persistent source of infection that should be taken into account when treating critically ill patients.

The determinants of high-grade biofilm formation on ETTs in critically ill patients are not clear and are probably multifactorial. Our study (Paper II) showed that high-grade biofilm formation on ETTs was associated with the development of VAP, and this has also been observed in only one prior investigation that included 32 patients[98]. Thus, study II confirms this finding but in a much larger population (n = 106). Unfortunately, there is no gold standard for biofilm grading when using SEM, which makes it difficult to compare the results of different studies. The majority of investigations that have applied SEM to analyze biofilms have only used the coverage or the existence of a biofilm to grade biofilm formation[99,142–144]. The disadvantage of such an approach is that two biofilms exhibiting the same coverage can differ substantially in thickness and density, and most likely also with regard to their degree of maturity and how prone they are to dispersal[37]. This may be one reason why the authors of some earlier studies have not found an association between biofilm coverage/existence and VAP development[99,145].

Although biofilm formation is a rapid process, we were unable to predict biofilm grade solely by determining the duration of invasive ventilation. This is also seen in earlier studies[95,98,99], indicating that the grade of biofilm formation is dependent on additional factors. It is not clear what causes high-grade biofilm to develop rapidly on the ETT surface in one patient, whereas it happens much more slowly or not at all in another. Similarly, not all intubated patients ultimately develop VAP[98]. Being able to monitor or predict the grade of biofilm formation is of clinical importance, considering that the findings presented in Paper II imply that high-grade biofilm formation (not just biofilm formation at any stage) is associated with the

development of VAP. Methods for continuous monitoring of biofilm formation on ETTs are described in laboratory models utilizing optical fiber sensors incorporated in the lumen of the ETT[146]. This may prove to be an interesting tool for clinical use but needs clinical evaluation.

Despite the knowledge that VAP is the most common hospital acquired infection in the critically ill, and that ETT biofilms act as a significant and persistent source of infection in the intubated patient, routines for biofilm removal including ETT exchange are not well studied, and in many cases, not considered safe. As biofilms are hard to eradicate once they have formed, it would be interesting to study if changing the ETT in selected VAP patients could reduce microbial persistence and the risk of VAP relapse. The risk-to-benefit ratio must be evaluated for each patient, but the benefits of an ETT change may be greater than the risks in some cases. It has been pointed out that reintubation is associated with VAP, although some of the data underlying that conclusion have not been obtained in evaluations of ETT exchange, but rather in extubation trials[147]. Nevertheless, well-controlled studies in this field are warranted before any recommendations can be made. Early performance of a tracheostomy has been considered to be a measure that can reduce VAP. However, a systematic review found no such reduction[148], possibly because the tracheostomy tube is also a foreign material that is prone to biofilm formation in the same manner as the conventional ETT. With the emergence of multiresistant bacteria and fewer choices for antimicrobial treatment of VAP, ETT routines may change[149]. Methods aimed at the continuous monitoring of biofilm formation[146] are needed, because few predictive factors are known at present. Biofilm removal without ETT removal by use of tools such as the mucus shaver or photodynamic inactivation are promising but must be further evaluated[150,151].

Considering the life cycle of a biofilm, it is likely that high-grade biofilm formation (grade $\geq 7$ in study II) reflects a more mature biofilm containing several pillar- and mushroom-shaped masses that are susceptible to breakage caused by manipulation such as suction with a catheter (as shown in Figure 9d) or due to turbulent airflow. Detachment and dispersal are natural developments in a mature biofilm that lead to the spread of highly contagious biofilm fragments into the lower airways[40]. On the other hand, a low-grade biofilm is thinner and more firmly anchored to the surface when host- and tissue-specific adhesins on pili and fimbria are attached[36].

We noted that colonization with common VAP pathogens in surveillance cultures was not associated with high-grade biofilm formation or VAP in study II. This finding was somewhat unexpected, considering that colonization with pathogenic bacteria is assumed to precede the development of VAP[90,152]. Previous research has shown that there is a microbial link between oropharyngeal, tracheal, and biofilm cultures [99], but, to the best of my knowledge, the correlation between positive cultures and biofilm grade has not yet been elucidated. Vandecandelaere et al. [153] used both culture and culture-independent methods and found no significant difference in biofilm flora between patients who developed VAP and those who did

not. Furthermore, those authors observed no difference in biofilm flora between patients with longer (> 5 days) and shorter (< 5 days) intubation periods. It has been shown that culture-dependent methods (as in study II) detect only a small proportion of the microorganisms present in ETT biofilms. Biofilms are multi-microbial, and culture-independent methods have revealed that they contain approximately 70% oral flora[153]. It has been suggested that many microbes in the oral flora initiate biofilm formation that may facilitate colonization of more pathogenic bacteria, although those floral microbes may not be responsible for development of VAP per se [43]. One such interaction has been observed between *Candida albicans* and *Pseudomonas aeruginosa*[154], in which an antifungal treatment was found to be associated with a reduced risk of *P. aeruginosa* VAP [155]. The impact of each microbe or combination of microbes is not clear and must be elucidated in larger trials.

Study II is the first study of critically ill patients to compare biofilm formation on widely used ETT materials. We found that, compared to the standard uncoated PVC ETT, the SC ETT and the NbMC ETT were associated with reduced high-grade biofilm formation. Although silicone is applied extensively in healthcare, there are no previous evaluations of use of SC ETTs in the critical care setting. Silicone is also used for industrial purposes: in fluids (free chains) for producing cosmetics; in gels (crosslinked chains) for fabricating soft tissue implants; in resins (heavily crosslinked chains) for producing optically clear silicone; and in elastomers (crosslinked and reinforced chains) for manufacture of medical catheters including ETTs and various kinds of prostheses (e.g., breast, hand, foot, ear, nose, eye, and voice prostheses). It should be mentioned that there are a number of subtypes of silicone elastomers, and the exact composition of the particular subtype used in the present SC ETTs is the manufacturer's trade secret and hence was not available to us. Silicone elastomers have gained widespread use mainly due to their durability, flexibility, and biocompatibility when in contact with skin or human tissue[156]. On the other hand, some studies indicate that silicone elastomers have reduced blood compatibility[157,158], and assessments in rabbit models have shown that, compared to CVCs made of PVC, such catheters made of silicone increase the risk of infection[159]. These differences imply that there may not be only one successful surface or material in particular that fits all environments of the body.

Bacterial adhesion and biofilm formation on different catheter materials have been studied in vitro. Although results are conflicting, some authors have reported significantly reduced bacterial adhesion and biofilm formation on silicone surfaces compared to PVC surfaces[160,161]. Also, research has demonstrated that the properties of PVC and silicone catheters from different manufacturers vary, indicating differences in the composition of the materials[162]. Hence, our findings in study II may not apply to all silicone or PVC ETTs. It has also been observed that biofilm formation is facilitated on surfaces with greater roughness[163]. Even though evaluation of surface topography was not one of the objectives of study II, we

examined the surfaces of unused ETTs by SEM and found no subjective signs of differences between the tube materials.

The NbMC ETTs showed reduced high-grade biofilm formation compared to uncoated PVC tubes, but although both those tube types were made of PVC, they were not from the same manufacturer. As for silicone, the composition of PVC can differ between manufacturers. Thus far, NbMC ETTs have not been evaluated in the intensive care settings, with the exception of a short-term intubation trial during elective surgery[164]. During study II no adverse reactions were registered that could be related to the NbMC ETT coating. According to some reports, the noble metal coating has been shown to reduce catheter-related urinary tract infections with long-term use[165–167]. However, it should be noted that the NbMC urinary catheter is made of silicone or latex, not PVC like the NbMC ETT we tested. PVC ETTs have been shown to be prone to bacterial adhesion in vitro[168], which indicates that silicone coated ETTs with NbMC may be more efficient in preventing microbial adhesion and biofilm formation. This possibility must be further assessed before any conclusions can be drawn.

The association between PPI use and colonization with gut flora in the oropharynx was observed in study I, but, unfortunately the association between PPI use and development of VAP could not be evaluated in study II because the majority of patients (99/106) received PPI treatment after intubation. The association between use of PPIs, colonization with more pathogenic bacteria in the oropharynx, and development of VAP has been described in previous reports[123,128,169]. The same applied to the impact of antibiotics on biofilm formation and VAP in our investigation, because 103 of the 106 patients received antibiotics. Previous studies considering the effect of systemic antibiotics on bacterial persistence in the respiratory tract have shown that the persistence of colonization after antibiotic treatment differs significantly among pathogens[170]. Thus, it is likely that the effectiveness of antibiotics in reducing the bacterial load on ETTs varies depending on the microbes present. Moreover, it is well established that a biofilm that has formed on the surface of a medical device is highly resistant to antibiotics[43,171]. The impact of PPI and/or antibiotic therapy on biofilm formation and VAP has to be further studied in other types of trials.

# Catheter-related infections

Paper III shows low incidence of both CRI (1.86/1,000 catheter days) and CRBSI (0.62/1,000 catheter days) in this Scandinavian observational study. Furthermore, the introduction of a simple hygiene insertion bundle was associated with decreased incidence of catheter-tip colonization and CRI, and large-bore catheters with

multiple lumens were associated with increased incidence of catheter-tip colonization and CRI.

The data in Paper III also demonstrate that the rate of CRI decreased from 2.7/1,000 to 1.1/1,000 catheter days after the implementation of a simple hygiene insertion bundle. This hygiene bundle was based on recommendations from the Swedish Society of Anesthesiology and Intensive Care Medicine, and introduction of the bundle entailed low cost and required no new staff or expensive equipment[50]. Prior to implementation of the hygiene insertion bundle, hygiene precautions taken at CVC insertion had been performed at the discretion of the inserting anesthesiologist. The new hygiene bundle included standardization of the insertion routines. A key factor in the success of the bundle may have been that that the staff member who was to assist in CVC insertion after implementation of the intervention was educated about the bundle and made responsible for compliance with its use.

The hygiene bundle used in the classic study by Pronovost et al.[114] consisted of hand washing, full-barrier precautions during the insertion, cleaning the skin with chlorhexidine, avoiding the femoral site if possible, and removing unnecessary catheters. The hygiene insertion bundle implemented during study III was similar (see Appendix S1 in Paper III) but also included washing the insertion site twice with 0.5% chlorhexidine in 70% alcohol, placing a clean sheet under the patient before insertion, and giving the assistant responsibility for compliance with the use of the bundle. Pronovost et al.[114] gave no description of the insertion routines that were applicable prior to the study intervention. It should be noted that our study was performed a few years after the investigation by Pronovost et al., and that the department we assessed had already embraced the advantages of good insertion hygiene. Accordingly, it is reasonable to assume that even though previously established hygiene insertion routines were acceptable, the additional simple hygiene precautions described in Paper III may further reduce CRIs.

In the last 20 years, incidence of both CRI and CRBSI has decreased globally, but the burden of CRI is still substantial; increasing costs, hospital stays and possibly also mortality[13,52,172]. The efficiency of hygiene bundles in reducing CRBSI has been validated in various trials[114,172–174]. In a European study conducted by Van der Kooi et al.[173], the CRBSI incidence was reduced from 2.4/1,000 to 0.9/1,000 catheter days. In the investigation reported by Pronovost et al.[114] (performed in Michigan in the United States), the incidence of CRBSI was reduced from 7.7/1,000 to 1.4/1,000 catheter days. In the United Kingdom, Longmate et al.[174] found a reduction in CRBSI from 3.4/1,000 to 0/1,000 catheter days after implementing hygiene bundles. Notably, in an international perspective, the incidence of and reduction in CRBSI vary markedly between centers and cannot always be compared due to differences in the patient populations evaluated and the definitions of CVC infection applied (CRI, CRBSI, or central line-associated infection [CLABSI])[52,55,56,175,176]. Scandinavian data on both CRI/CRBSI incidence and the efficiency of bundles are scarce, although Paper III confirms the low incidence of CRI and CRBSI noted in

two other Scandinavian single-center studies performed at another regional hospital in Sweden[54,57].

The microorganisms identified in our study (Paper III) are similar to those previously reported[60,177]. Notably, there were no methicillin-resistant S. *aureus* or vancomycin-resistant enterococci isolates, which reflects the low prevalence of these bacteria in Sweden. By comparison, in other parts of Europe, the emergence of multiresistant bacteria in hospital-associated infections is substantial, and in some European countries multiresistant bacteria constitute up to 30% of CRBSI pathogens[12,149,178]. We found that the number of lumens was independently associated with CRI and catheter colonization. Each added CVC lumen increased the odds of CRI approximately 63%. To some extent, this difference might be explained by an increased area of catheter surface to which microbes could adhere and a larger number of ports where microbes could be injected. The results in study III are consistent with some prior studies[177,179,180], although the literature is to some extent conflicting[181]. It should be noted that no large study, adjusting for comorbidities and the degree of illness, has confirmed these findings and that the multivariable regression model used in this study did not adjust for the degree of illness or comorbidities. Studies of more homogeneous groups of patients have indicated benefits of choosing single-lumen over multiple-lumen catheters in various patient populations, including critically ill and cancer patients[177,179,180]. Nevertheless, in Paper III, we concluded that even though the evidence is scarce, multiple-lumen catheters should be used restrictively, and the need for extra lumens should be carefully evaluated before insertion.

It has been demonstrated that increased time with a CVC is associated with CRI[176]. This was not the case in our investigation (Paper III), which may be explained by the adherence to routines, including the active removal of unnecessary catheters. This was demonstrated in a Scandinavian study conducted by Hammarskjöld et al.[54], in which only a weak association (OR 1.002) with time was observed and was explained by strong adherence to the use of bundles, including active and early removal of catheters that were no longer necessary. Still, these findings must be confirmed in larger trials, evaluating similar patient populations and hygiene routines.

In Paper III, multivariable logistic regression analysis showed that catheters placed in the subclavian vein were associated with lower risk of colonization but not CRI. The preferred choice of the subclavian vein has recently been evaluated in a large trial comparing the jugular, femoral, and subclavian sites, and it was found that the subclavian site conferred a stronger benefit than the alternatives[56]. At our institution, the subclavian vein was the primary choice for more seriously ill and immunocompromised patients (e.g., those receiving chemotherapy), which may explain why the subclavian route was not associated with lower risk of CRI than the standard internal jugular route.

# Blood compatibility of widely used central venous catheters

In the experimental study outlined in Paper IV, we assessed differences in the blood compatibility of six commonly used venous access devices (five CVCs and one CHC) in a modified Chandler loop model, imitating the flow of blood in a vein. All the catheters activated coagulation, the complement system, and inflammation to some extent, but there were significant differences between the devices. The polyurethane catheter coated with chlorohexidine and silver sulfadiazine exhibited reduced blood compatibility compared to the other catheters we evaluated. The silicone catheter showed the greatest variation in blood compatibility test results. Poor blood compatibility could cause inflammation and facilitate the development of catheter-related thrombosis in patients receiving these central venous catheters, but clinical significance has to be studied further.

*Blood coagulation and inflammation in the presence of foreign materials*

Entry of a foreign CVC material into the bloodstream triggers hemostatic processes (Figure 2). Surface-related events depend on the structure and/or chemistry of the surface in question, and they initially involve adsorption and the activation of plasma proteins. FXII is the first protein to be activated in the contact activation system. The resulting FXIIa initiates contact coagulation activation, generation of thrombin (measured as TAT level), and fibrin clot formation. Complement is also activated by other mechanisms, such as the adsorption of immunoglobulins, Ficolin-2, and C3 (leading to increased C3a and sC5b-9). FXIIa can also trigger complement activation via C1s.

If a material inserted into the bloodstream releases any substances, leached either from the polymer itself or from an active substance in a coating, these substances can trigger coagulation and inflammatory processes directly via toxic actions and/or through the activation of blood cells. Also, substances released from a catheter coating may cause cell membrane disruption, leading to hemolysis and/or decreased counts of all types of blood cells (erythrocytes, leukocytes, and thrombocytes). Even if the substances that are released do not disrupt the cell membrane, they may induce the cell membranes to release MPs. L-selectin (CD62L) MPs are released from activated leukocytes and play an important role in interactions between leukocytes and endothelial cells. P-selectin MPs (CD62P) are released from activated platelets (thrombocytes), and P-selectin has an essential function in the initial recruitment of leukocytes to the site of injury during inflammation. VEGF is also released from activated platelets. Thus, the release of L- and P-selectin MPs and VEGF indicates that the inflammatory defense mechanism of the human body has been activated. Blood cell membrane disruption and activation may also influence the acute

immune system, leading to release of proinflammatory cytokines such as IL-8 and TNF-α, as well as inflammatory responses induced by complement activation.

*Differences between the tested materials*

The study reported in Paper IV showed that, compared to the control loop, all the materials triggered either the blood cell system or the molecular innate immune system when tested in the Chandler model (illustrated in Table 10a and b and Figure 2).

Among the uncoated catheter materials, silicone (Si-1) showed the lowest blood compatibility and also the greatest variation in such compatibility between test runs. Silicone seemed to trigger contact coagulation, as indicated by increased levels of FXIIa, prothrombin fragment F1+2, and TAT, leading to platelet activation and aggregation with the release of P-selectin MPs and VEGF. Activation of both the complement system and inflammation was also demonstrated by increased levels of C3a, sC5b-9, and IL-8. The activation of contact coagulation and thrombocytes by silicone materials has been observed in previous studies in which modifications of the silicone surface were found to result in improved blood compatibility[157,182]. A potential mechanism for this effect is that the negative charge of the silicone surface activated not only FXII, but also thrombocytes, via the intrinsic contact coagulation pathway[183,184]. The pronounced variation in surface properties between test runs might have been due to different amounts of silicone oil slowly exuding to the surface of the material during storage, thereby renewing its negative charges. This problem has been observed in studies of silicone breast prostheses[185]. Such exudation of silicone oil is also potentially toxic to thrombocytes, representing the same type of negative effect[182]. In animal studies, compared to polyurethane catheters, silicone catheters have been found to show increased susceptibility to staphylococcal infections and a stronger local inflammatory response[70,159]. These effects have been demonstrated to be secondary to increased complement activation via the alternative pathway, thus leading to reduced opsonizing ability and increased risk of infection[158]. Our results corroborate those findings but also further describe the different mechanisms (see Table 10, a and b) that are activated when silicone comes in contact with blood.

The two polyurethanes we tested (PU-1 and PU-2) differed significantly in blood compatibility, indicating variation in the surface composition of polyurethanes from different manufacturers. PU-2 exhibited good blood compatibility with only a slight increase in P-selectin MPs, F1+2, TAT, and complement activation compared to the control loop (see Table 10a and 10b). PU-1, on the other hand, induced contact coagulation (increased FXIIa and TAT), which can theoretically increase the risk of catheter-related thrombosis[186,187]. Differences in the composition of the two polyurethane catheters may include the presence of sulphate groups, which would give the surface a negative charge and thereby trigger FXII and activation of contact coagulation (i.e. increased TAT is seen)[188].

The surface of a catheter coated with an anti-infective substance can impair the blood compatibility of the catheter. In our laboratory investigation (Paper IV), this effect was pronounced for the chlorhexidine and silver sulfadiazine coated polyurethane catheter (PU-2+CHSS), which exhibited the most unfavorable blood compatibility by inducing substantial hemolysis, platelet activation, TAT, F1+2 generation, complement activation, and release of proinflammatory cytokines. The effectiveness of the PU-2+CHSS catheter in reducing CRBSI has been extensively investigated[189–191], and international guidelines recommend the use of this catheter to reduce CRBSIs in critically ill patients[192]. As far as we know, the blood compatibility of the PU-2-CHSS catheter has not been described by other investigators.

The results in Paper IV suggest that the PU-2+CHSS catheter may increase the risk of catheter-related thrombosis and hemolysis, but this must be confirmed in clinical trials. As far as I know, thrombotic complications associated with the use of the PU-2+CHSS catheters have not been observed in clinical studies. Still, it should be mentioned that the majority of thrombotic events (5–18%) remain undiagnosed even though they are relatively common[66,67]. Hemolysis secondary to the use of a PU-2+CHSS catheter has apparently not been described in clinical settings, but such an effect may be masked by patients' critical conditions in which PU-2+CHSS catheters are often indicated[193].

An in vitro investigation found that the PU-2+CHSS catheter induced hemolysis when in contact with human blood[120], and animal experiments have also shown that chlorhexidine diacetate in a range of concentrations is damaging to rabbit erythrocytes[194]. The results in Paper IV, indicate that this hemolysis is due to a toxic effect of the coating rather than primary complement activation, because the hemolysis occurs after complement inhibition with eculizumab or calcium. The strong complement activation seen with PU-2+CHSS catheters might be explained by massive leakage of heme into plasma, which is known to activate the complement system[195]. The release of proinflammatory cytokines has been associated with an increased risk of venous thrombosis, but the contribution of each cytokine remains to be elucidated[196,197]. Both silver sulfadiazine and chlorhexidine are known allergens[198,199], which is consistent with the activation of pro-inflammatory markers seen in our study. Staphylococcal species, the most common catheter-infecting organisms, show receptor-mediated binding with fibronectin, fibrin, and other components of the fibrin sheath[63,200]. Hence, it is possible that impaired blood compatibility and limited duration of the coating[201] (< 15 days in laboratory studies) can partly explain why it appears that antimicrobial CVCs do not clearly reduce clinically diagnosed sepsis or mortality[189].

Chlorhexidine is a commonly used synthetic antiseptic and disinfectant that was introduced in the 1950s, and it affects both gram-negative and gram-positive bacteria, and also Candida albicans and some viruses[202]. The use of chlorhexidine in healthcare settings has increased substantially in recent years, and the results of

large studies have indicated that this might lower the rates of healthcare-associated infections in different settings[203,204]. Case reports have described allergic reactions associated with use of chlorhexidine-impregnated CVCs in several countries[205–208]. It appears that the prevalence of allergic reactions is greater when chlorhexidine comes in contact with mucosal membranes or blood as compared to the skin, especially in persons of Japanese descent[199,203,204,209], although more recent case reports describe allergic reactions in other ethnic groups as well[205]. Few investigations have focused on the systemic effects of chlorhexidine, but there are some case reports describing chlorhexidine as highly toxic and causing acute respiratory distress syndrome and shock[209,210]. The use of chlorhexidine in oral care of critically ill patients has been questioned, because trials have shown increased mortality with its use, possibly due to micro-aspiration and lung injury[211].

The second coated CVC we tested, PU-3+BZC-H, was coated with both a cationic surfactant benzalkonium chloride and a hydrophilic hydromer, the latter of which is intended to improve the blood compatibility of the surface. All blood compatibility parameters except L-selectin were found at low levels. It is possible that the release of L-selectin from leukocytes can be triggered by a toxic effect of benzalkonium chloride, as has been suggested by other researchers, and this could potentially have a negative impact on the patients' immune defense[212].

The CVC coated with a noble metal alloy, PU-4+NbMC, induced a slight increase in P-selectin MPs, F1+2, TAT, and complement activation compared to the control loop. The durability of this coating has been evaluated in a small clinical investigation, which showed good durability for up to 16 days[213]. The blood compatibility of the noble metal coating has also been evaluated in a previous laboratory study[120]. Although the reporting authors described fewer blood compatibility parameters and values differing from those noted in Paper IV (a TAT level of ~300 µg/L compared to ~200 µg/L in study IV). Those investigators also described similar behavior in contact with blood as observed in study IV.

# Limitations

*Logistic regression analysis*

Univariable and multivariable logistic regression analyses were used in the calculations presented in Papers I–III. Due to low incidence, only univariable or descriptive statistics were applied for certain outcomes (i.e., VAP and CRBSI). When the outcomes abnormal oropharyngeal flora, gut flora (Paper I), high grade biofilm formation (Paper II), catheter colonization and CRI (Paper III) were subjected to multivariable logistic regression analysis, it was not possible to include an unlimited number of factors in the model due limitations in the number of outcomes. Hence, there are factors that have been observed as significant in other

studies but were not evaluated in our studies. For example, considering Paper I, other investigators have reported that renal failure reduces the frequency of abnormal flora[123]. In Paper II, the impact of each microbe or combination of microbes is not clear and could not be evaluated due to the lack of statistical power. Regarding Paper III, other investigators have observed that parenteral nutrition and trauma are associated with CRI[176,180]. Similarly, we did not adjust for all patient comorbidities such as cancer and immunosuppression in Papers I–III. Finally, although there are significant association between factors and outcomes in Papers I–III, we cannot claim causality between the two due to study design and lack of randomization.

*Microbial procedures*

Cultures results were evaluated in Papers I–III, and there are several factors related to sampling and analysis of cultures that are difficult to control. Considering the oropharyngeal cultures, it was not possible to assess recent tooth brushing or water consumption occurring before sampling. Likewise, it was not possible to have an overview of individual culture technique, delay in storage before culture, and many other steps. Furthermore, microbiological procedures applied can differ between institutions, making external validation and comparison with other studies difficult.

*Paper I*

Given that some of the data on the critically ill patients were collected up to 12 years ago, there is a risk that the definition of normal oropharyngeal flora has changed since then. However, according to the microbiology department at our hospital, the definition of a normal oropharyngeal flora applied in southern Sweden has not been changed during the indicated period. In the same way, the occurrence of multi-resistant bacteria remained low, reflecting the slower emergence of antibiotic resistance in Sweden compared to other parts of Europe[214].

*Paper II*

Our ambition was to include all patients that were expected to receive mechanical ventilation for >24 hours. For logistical reasons and during weekends and holidays, a large number of patients were not included (n = 164). The authors have speculated if this could have led to any kind of selection bias. As patient characteristics are similar among the three groups, and the same method of inclusion was applied to all six inclusion periods, we find that unlikely. The six study periods were also equally spread through the year, containing some holiday periods and summer months. Second, one would expect to see the same significant association between ETT material and VAP as is seen between ETT material and high-grade biofilm formation. Not all patients with high grade biofilm formation on the ETT surface are though expected to develop VAP, the odds are only increased according to the analyses. One probable explanation is lack of power as a much larger sample size is

needed to observe a difference in VAP than in grade of biofilm formation. In the same way, the impact of each microbe or combination of microbes is not clear and could not be evaluated due to the lack of statistical power. Finally, microbial persistence could not be assessed in many cases due to the death of a patient or because a patient underwent a tracheostomy a few days after the VAP episode.

*Paper III*

Although guidelines for taking peripheral blood cultures when CRI was suspected did apply, they were not always followed, resulting in a drop-out of cases (in 333 cases (73%)), and possible underestimation of CRBSI incidence. Correspondingly, it has previously been described that critically ill patients can fulfill the SIRS criteria and still not have CRI, because the SIRS criteria are not designed or specific for CRI. This may result in overestimation of CRI incidence and make it difficult to differentiate between colonization and CRI. The implemented hygiene insertion bundle was widely distributed in the department, and the CVC insertion assistants noticed that the staff members were highly aware of the new hygiene insertion bundle. Nevertheless, individual compliance with guidelines could not be evaluated. Furthermore, the findings depend on additional important factors remaining unchanged between the year before and the year after the implementation of the bundle. Although we investigated this issue carefully and found that no changes had been made in other important factor in either the insertion routine or the maintenance of CVCs, such potential time-dependent bias should be considered. This study evaluated a hygiene insertion bundle implemented in January 2012, and preserved low incidence of CRI/CRBSI to date cannot be guaranteed.

*Paper IV*

The reported comparison was based on an experimental study. Clinical significance of the results remains to be explored and may also vary for different patient groups depending on their diseases and comorbidities. The investigation was conducted with limited samples from one or two different product lots, and possible variability between lots has not been evaluated.

# Conclusions

*Changes in oropharyngeal flora with hospitalization (Paper I)*

- Abnormal oropharyngeal flora is an early and frequent event in hospitalized patients, particularly in the critically ill. Colonization with gut flora in the oropharynx is common in critically ill ICU patients.
- Proton pump inhibitor medication is associated with colonization of gut flora in the oropharynx.

*Biofilm formation on different endotracheal tubes (Paper II)*

- Biofilm formation on ETTs is an early and frequent event in critically ill patients. High-grade biofilm formation on ETTs is associated with the development of VAP. Compared to the uncoated PVC ETTs, the silicone-coated and noble-metal coated PVC ETTs are associated with reduced high-grade biofilm formation.
- Methods aimed at the continuous monitoring of biofilm formation are warranted. Routines for biofilm removal need further study.

*Catheter-related infections in a cohort in Scandinavia and the impact of a simple hygiene insertion bundle (Paper III)*

- The incidence of CRI and CRBSI in this cohort in Scandinavia was low. The implementation of a simple hygiene insertion bundle seems to be an effective intervention for reducing CRI. The use of multiple-lumen catheters is associated with increased risk of catheter-related infections.

*Blood compatibility of widely used central venous catheters (Paper IV)*

- A polyurethane catheter coated with chlorohexidine and silver sulfadiazine showed the most unfavorable blood compatibility profile. A silicone dialysis catheter elicited the greatest variation in blood compatibility test results between test runs.
- Poor blood compatibility may cause inflammation and facilitate the development of catheter-related thrombosis in patients receiving these catheters, although clinical significance has to be studied further.

# Future perspectives

*Changes in oropharyngeal flora during hospitalization*

Changes in the normal human flora resulting in loss of health-promoting microbes and overgrowth of pathogenic bacteria are common with disease and hospitalization. These changes may contribute to hospital-acquired infections, sepsis and multiple organ failure in the critically ill[6]. With the emergence of multi-resistant microbes and the knowledge how dysbiosis can affect the body's normal physiology, methods for monitoring and re-establishing balance in the normal flora are needed that do not include antibiotics or antiseptics. Probiotics and fecal microbiota transplantation are promising alternatives in that context.

*Biofilm formation on different ETTs*

The predictors of high-grade biofilm formation on ETTs in critically ill patients are not clear and are most likely multifactorial. Predicting the grade of biofilm formation is of clinical importance, as biofilm grade seems to be associated with the development of VAP. Also, tools to monitor biofilm formation on the ETT surface[146] are warranted as biofilm grade cannot be predicted solely by duration of invasive ventilation. Biofilm removal without ETT removal by use of tools such as the mucus shaver are promising but must be further investigated[150,151]. Furthermore, routines for changing the ETT after an episode of VAP may be indicated in selected patients, but need evaluation.

*Catheter-related infections in a cohort in Scandinavia and the impact of a simple hygiene insertion bundle*

Standardization of hygiene insertion protocols seems to be an effective measure to prevent CRI. There is a need for standardization of care bundles after CVC insertion, and for developing large-scale monitoring systems that can give feedback and guide further work in the battle against CRI/CRBSI.

*Blood compatibility of widely used CVCs*

Few studies have focused on how widely used CVC materials behave when in contact with blood. Given that experimental settings have limitations, further clinical investigations are needed, because it is possible that reduced blood compatibility can induce inflammation and thrombus formation, and also facilitate the development of CRI.

# Populärvetenskaplig sammanfattning

Människan lever i symbios med biljontals bakterier som finns inuti och utanpå vår kropp. Under det senaste årtiondet har stora framsteg gjorts inom detta forskningsområde. Det har visat sig att en balanserad symbios med bakteriefloran är viktig för många av kroppens olika funktioner, såsom matsmältning, immunförsvar samt såväl fysisk som mental hälsa. I samband med sjukhusvård är det stor risk att denna normala balans i bakteriefloran rubbas. Katetrar, dränage, antibiotika, operation, sängläge och fasta är faktorer som bidrar till att balansen i bakteriefloran ändras. Dessa förändringar ökar risken för vårdrelaterade infektioner.

*Vårdrelaterad infektion*

> ”En vårdrelaterad infektion är en infektion som uppkommer hos en person under slutenvård eller till följd av åtgärd i form av diagnostik, behandling eller omvårdnad inom övrig vård och omsorg” (SKR, *Sveriges Kommuner och Regioner*).

Bland intensivvårdspatienter är förekomsten av vårdrelaterade infektioner högst och vissa studier har visat att en av fem patienter drabbas. Intensivvårdsutrustning såsom central ven kateter och endotrakealtub är oundgängliga redskap för att bedriva rationell vård för kritiskt sjuka patienter. Central venkateter är en plastslang som förs in i stort centralt blodkärl via halsen, under nyckelbenet eller i ljumsken och används som ingång till blodbanan hos en stor del kritiskt sjuka patienter. Endotrakealtub är ett rör, vanligtvis av plast, som förs ner i luftstrupen (processen nämns intubering) för att försäkra att patienter kan andas alternativt få hjälp med andningen via respirator. Både central venkateter och endotrakealtub forcerar eller bryter kroppens naturliga barriärer. Detta ger bakteriefloran möjlighet att förflytta sig till delar av kroppen där de vanligtvis inte hör hemma vilket ökar risken för vårdrelaterad infektion.

Mikrober (oftast patientens egna flora) som fastnar på centrala venkatetrar eller endotrakealtuber kan bilda biofilmer i vilken de blir mycket svåråtkomliga för antibiotika och kroppens immunförsvar. Biofilm kan liknas vid en form av vävnad där bakterier ligger inbäddade i en egenproducerad matrix som skyddar mot angrepp. Inuti biofilmen kan mikrober kommunicera och näringsämnen kan flyttas via kanaler till olika delar av biofilmen. Biofilmen bildas i några steg, och när den blir mogen kan bitar som innehåller hög koncentration av mikrober lossna från ytan

och orsaka spridning av infektionen. Man tror att biofilmsbildning på endotrakealtuber bildar en reservoar där sjukdomsframkallande bakterier kan komma undan kroppens försvarsmekanismer och sedan bidra till upprepade lunginflammationer trots adekvat antibiotikabehandling.

När en central venkateter kommer i kontakt med blod i bildas det snabbt en proteinpäls på kateterns yta beroende på ytans egenskaper. Ett främmande föremål i blodbanan kan bidra till både inflammation och blodproppsbildning beroende på vilka proteiner som adsorberats till ytan och vilka mekanismer i blodet som aktiveras. Proteinpäls- och blodproppsbildning på kateterns yta underlättar för bakterier att få fäste och bilda biofilm på kateterns yta. Bakterier på kateterns yta kan späda på ytterligare blodlevring och blodproppsbildning. Man tror därför att biofilmsbildning och blodproppsbildning är sammankopplade fenomen som potentierar varandra.

Biofilmsbildning på centrala venkatetrar eller endotrakealtuber utgör en stor fara för patienten, och försämrar patienternas överlevnad.

Målsättningen med denna avhandling var att undersöka endotrakealtuber och centrala venkatetrar tillverkade i olika material och med olika ytbeläggningar. I projektet undersöktes förändringar i svalgfloran hos sjukhusvårdade och kritiskt sjuka patienter och jämfördes med individer ute i samhället. Graden av biofilmsbildning på tre olika endotrakealtuber efter avlägsnade undersöktes hos intensivvårdspatienter. Vidare kartlades infektioner relaterade till centrala venkatetrar i en stor Skandinavisk kohort. Slutligen undersöktes sex olika centrala venkatetrar, tillverkade i olika material och med olika ytbeläggningar, i en experimentell modell med avseende på hur blod reagerade i kontakt med katetrarna.

Innovation Mot Infektion (IMI) var ett projekt stött av innovationsfonden VINNOVA med, Research Institutes of Sweden (RICE) som samordnare mellan sjukvård, industri och universitetsinstitutioner. Arbete II, III och IV i detta forskningsprojekt var delprojekt inom IMI.

*Delstudie I* är en klinisk observationsstudie där det togs svalgodlingar på patienter inom första dygnet från ankomst till sjukhus eller intensivvårdsavdelning samt på jämförbara individer ute i samhället. Resultaten visade att förändringar i svalgfloran var vanliga och tidigt förekommande bland sjukhusvårdade patienter samt att fynd av tarmbakterier i svalget hos kritiskt sjuka intensivvårdspatienter var förhöjd. Vi såg även en ökad förekomst av tarmbakterier i svalget bland sjukhusvårdade patienter som medicinerat med protonpumpshämmare (läkemedel som minskar produktion av magsyra) innan ankomst till sjukhus eller intensivvårdsavdelning. Att ha tarmbakterier i svalget är från tidigare studier en känd riskfaktor för att utveckla sjukhusförvärvad lunginflammation. Resultaten i Delstudie I stöder hypotesen att behandling med protonpumpshämmare inom intensivvården ökar förekomsten av tarmbakterier i svalget och medför ökad risk för sjukhusförvärvad lunginflammation.

*Delstudie II* är en klinisk observationsstudie på intensivvårdspatienter, där graden av biofilmsbildning på tre olika endotrakealtuber efter avslutad ventilator-behandling undersöktes. Det bildades biofilm på nästan alla endotrakealtuber (97%) men graden (hur mogen den var) av biofilmsbildning varierade. Patienter som hade höggradig (mogen) biofilm på endotrakealtubens yta fick oftare ventilator associerad lunginflammation (en undergrupp av sjukhusförvärvad lunginflammation). Intressant nog så var tiden patienten haft slangen i luftstrupen (vart intuberad) inte kopplad till vilken grad av biofilm som bildades. Om endotrakealtuben var ytbelagd med silikon eller med ett tunt lager av ädelmetaller var förekomsten av höggradig biofilm på ytan mindre vanligt jämfört med vanliga endotrakealtuber av polyvinylklorid (en typ av plast) som för närvarande är standard inom intensivvården. Metoder för att monitorerna biofilmsbildning på endotrakealtubens yta skulle behöva studeras då vår studie talar för att höggradig biofilm är kopplad till ökad förekomst av ventilator associerad pneumoni. Det skulle även vara intressant att undersöka om det finns tillräcklig vinst i att byta endotrakealtuben hos valda patienter som fått ventilator associerad lunginflammation. Detta med avsikt att bli av med biofilmen.

*Delstudie III* är en retrospektiv studie där förekomsten av infektioner relaterade till centrala venkatetrar undersöktes året före och året efter införandet av checklista för bibehållen sterilitet vid inläggning av centrala venkatetrar. Sammanfattningsvis var förekomsten av kateterrelaterade infektioner låg i denna Skandinaviska kohort och om den centrala venkatetetern var inlagd året efter införandet av hygien-checklistan var risken för kateterrelaterad infektion lägre. Analysen av data visade också att centrala venkatetrar med flera lumen (inre kanaler) gav större risk för kateterrelaterad infektion.

*Delstudie IV* är en experimentell studie där reaktionen i blod (blodkompatibilitet) för sex olika centrala venkatetrar, tillverkade i olika material och med olika ytbeläggningar undersöktes. Alla sex materialen aktiverade blodlevring, inflammation eller komplement systemet (en rad av olika plasmaproteiner) i någon grad men i varierande omfattning. En central venkateter ytbelagd med klorhexidin och silver sulfadiazin gav den kraftigaste reaktionen i blodet. Denna kateter används frekvent i USA. Om katetermaterialet orsakar en kraftig reaktion i kontakt med blod (dålig blodkompatibilitet) kan det leda till blodpropp i anslutning till katetern. Dessa fynd behöver dock styrkas i kliniska studier.

# Acknowledgements and grants

This thesis required interest and support from many people. I would like to express my great appreciation to all of you, with special gratitude to the following:

**Thomas Kander**, my supervisor, for your excellent guidance, insightfulness, determination, and sincere interest in research. Thank you for always believing in me and for your constant encouragement and time.

**Bengt Klarin**, my former head supervisor and during second half, my co-supervisor for teaching me the hard work of performing clinical trials! Thank you for your patience and for taking me with you on this journey of unconventional and interesting research ideas. You have opened up a new field (microbiota) that inspires to future studies.

**Ulf Schött**, professor and my co-supervisor, for your sincere kindness and support, which have been invaluable! Thank you also for sharing all your funny stories and optimism on PhD-rainy days.

**Anna Holmberg** my college and friend, for sharing your great knowledge on biofilm formation and infections. Your support and help since the very start has been something special.

**Anna Tranberg**, for the hard work and collaboration leading to Paper I. Thank you for your friendship over the years and for introducing me to all the enjoyable non-research things in life.

**Mika Rockholt**, for your unlimited enthusiasm and determination to reach the goal set in Paper III.

**Helene Jacobson**, my dear statistician, for all your patience and help, and not least the small talk while analyzing data.

**Anne Adolfsson** and **Susann Schrey**, my great research nurses—without you, I wouldn't have any data! It has been a pleasure getting to know you both, thank you for all your help and kindness.

The **ICU staff**, for always doing your best no matter what!

To all co-workers at the **Department of Clinical Microbiology** for their help processing microbial cultures

**Javier Sanchez**, scientist and blood compatibility specialist at Bactiguard—thank you for all the laboratory work resulting in Paper IV and for leading me through the jungle of blood compatibility.

**Dorota Johansson**, former Clinical Research Director at Bactiguard, for professional collaboration throughout all my years of research. Your ability to structure long-distance meetings and maintain discipline in the workflow is admiring.

**Stefan Grass**, CMO at Bactiguard, for your interest in research and support in finalizing Paper IV.

**Erika Södergren**, Microbiology Laboratory Manager at Bactiguard, for your kind help in the IMI collaboration.

**Bo Nilsson**, professor at Uppsala University, for sharing your great knowledge in the field of thromboinflammation.

**Sarunas Petronis**, researcher at RISE (Research Institutes of Sweden), for your hard work analyzing all SEM images and your wise comments on Paper II.

**Patricia Ödman** for her good humor and excellent work correcting the English language in both my thesis and papers II and IV (let´s hope there's not a spelling error in this sentence!).

**Mikael Bodelsson**, professor at our department, for giving me the opportunity to teach at the university and for all your support throughout these years. We are so lucky to have you as our professor!

**Johan Bonnevier**, ICU director, for your honesty, enthusiasm, coaching, and support. You are one of a kind.

**Anders Rehn, Carolina Samuelsson, Marie Martinson** and **Bengt Roth**, past and present heads of the Department of Anesthesia and Intensive Care, for making research an integral part of the department.

To the many **friends** and **colleagues** who have supported me: you are the best!

My roommates, **Emilie Krite Svanberg**, **Ingrid Berkestedt**, **Lisa Boström**, **Malin Rundgren**, and **Viveka Björck**, for the laughs, the talks, the support and the friendship.

To **Attila** for your kindness, sincere advice and statistical help.

To **Maria Lengquist** for being the smart, kind and organized person you are.

To my friends **Berglind** and **Birna**, for your honest advice and ability to put things in the right context.

**Hallgerdur** and **Gudny**, for many, many years of invaluable friendship.

To **Helga**, my friend and babysitter, for being an everyday hero, always ready to help with the unexpected tasks in daily life.

My sister **Elin Helga**, for being the wise and calm middle sister always willing to help. My brother **Gisli Hrafn**, for solving technical urgencies and for being the kind and funny person he is. To my sister **Lisbet Gudny**, for her help with figures and for always taking such good care of my girls.

To my parents, **Bryndis** and **Thorarinn**, for their unconditional love and for encouraging me to always perform at my best.

To my kind father in-law, **Omar V**, and the great in-law family!

To my dearest daughters, **Bryndis**, **Hulda**, and **Lisbet Helga**, for always making me happy and giving life an important purpose.

To my life companion, **Omar Gunnar**, for your endless love and understanding.

*Financial support:*

I gratefully acknowledge funding of this PhD research provided by grants from Regional research support, Region Skåne and Vinnova, the Swedish governmental innovation agency.

# References


1. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the Human Intestinal Microbial Flora. Science. 2005;308(5728):1635-1638.
2. Segal LN, Blaser MJ. A brave new world: the lung microbiota in an era of change. Ann Am Thorac Soc. 2014;11 Suppl 1:S21-7.
3. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489:220-230.
4. Huttenhower C, Gevers D, Knight R, et al. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486:207-214.
5. Adlerberth, I, Wold A. Människans Normalflora. Lund, Studentlitteratur; 2017.
6. Wischmeyer PE, McDonald D, Knight R. Role of the microbiome, probiotics, and "dysbiosis therapy" in critical illness. Curr Opin Crit Care. 2016;22:347-353.
7. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal Microbiome in recurrent Clostridium difficile-associated diarrhea. J Infect Dis. 2008;197:435-438.
8. Young PY, Khadaroo RG. Surgical site infections. Surg Clin North Am. 2014;94:1245-1264.
9. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol. 2015;64:323-334.
10. 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.
11. 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.
12. Zarb P, Coignard B, Griskeviciene J, et al. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro Surveill 2012;17(46).
13. 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 system. JAMA. 2013;173:2039-2046.

14. T, Plachouras D, Cassini A, Suetens C. Burden of healthcare-associated infections in European acute care hospitals. Wiener Medizinische Wochenschrift. 2019;169(Suppl 1):3-5.
15. World Health Organization. Report on the Burden of Endemic Health Care-Associated Infections Worldwide, a Systematic Review.; 2011. www.who.int/patientsafety/en/.
16. Magill SS, O'Leary E, Janelle SJ, et al. Changes in prevalence of health care-associated infections in U.S. Hospitals. N Engl J Med. 2018;379:1732-1744.
17. The National Healthcare Safety Network of the Centre's for Disease Control and Prevention. 2018 National and State Healthcare-Associated Infections Progress Report.; 2018. https://www.cdc.gov/hai/data/portal/progress-report.html.
18. Allegranzi B, Nejad SB, Combescure C, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011;377:228-241.
19. Sveriges Kommuner och Regioner. Vårdrelaterade Infektioner, En Kunskapssammanställning Baserad På Markörbaserad Journalgranskning 2013-2018.; 2019. https://webbutik.skr.se/sv/artiklar/vardrelaterade-infektioner-2.html.
20. Sveriges Kommuner och Regioner. Vårdrelaterade Infektioner, Kostnader Och Konsekvenser. Kortversion Av SKR Rapporten.; 2019. https://webbutik.skr.se/sv/artiklar/vardrelaterade-infektioner-3.html.
21. Schreiber PW, Sax H, Wolfensberger A, Clack L, Kuster SP. The preventable proportion of healthcare-associated infections 2005-2016: Systematic review and meta-analysis. Infect Control Hosp Epidemiol. 2018;39:1277-1295.
22. Slawomirski L, Auraaen A, Klazinga N. THE ECONOMICS OF PATIENT SAFETY Strengthening a Value-Based Approach to Reducing Patient Harm at National Level.OECD report; March 2017.
23. Vincent JL, Bihari DJ, Suter PM, et al. The Prevalence of Nosocomial Infection in Intensive Care Units in Europe: Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. JAMA. 1995;274:639-644.
24. Gröndal H. The Emergence of Antimicrobial Resistance as a Public Matter of Concern: A Swedish History of a "Transformative Event." Sci Context. 2018;31:477-500.
25. Safdar N, Crnich CJ, Maki DG. Nosocomial infections in the intensive care unit associated with invasive medical devices. Curr Infect Dis Rep. 2001;3:487-495.
26. Vincent JL. Nosocomial infections in adult intensive-care units. Lancet. 2003;361:2068-2077.
27. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284:1318-1322.
28. Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as Complex Differentiated Communities. Annu Rev Microbiol. 2002;56:187-209.
29. Donlan RM. Biofilms: Microbial Life on Surfaces. Emerg Infect Dis. 2002;8:881-90.
30. Flemming HC, Neu TR, Wozniak DJ. The EPS matrix: the "house of biofilm cells." J Bacteriol. 2007;189:7945-7947.

31. Flemming HC. Biofouling in water systems - Cases, causes and countermeasures. Appl Microbiol Biotechnol. 2002;59:629-640.
32. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15:167-193.
33. Lindsay D, von Holy A. Bacterial biofilms within the clinical setting: what healthcare professionals should know. J Hosp Infect. 2006;64:313-325.
34. Coenye T, Nelis HJ. In vitro and in vivo model systems to study microbial biofilm formation. J Microbiol Methods. 2010;83:89-105.
35. Donlan RM. Biofilm elimination on intravascular catheters: important considerations for the infectious disease practitioner. Clin Infect Dis. 2011;52:1038-1045.
36. Rosan B, Lamont RJ. Dental plaque formation. Microbes Infect. 2000;2:1599-1607.
37. Kaplan JB. Biofilm Dispersal: Mechanisms, Clinical Implications, and Potential Therapeutic Uses. J Dent Res. 2010;89:205-218.
38. Marsh PD. Dental plaque as a microbial biofilm. In: Caries Research. Vol 38. ; 2004:204-211.
39. Vandecandelaere I, Matthijs N, Nelis HJ, Depuydt P, Coenye T. The presence of antibiotic-resistant nosocomial pathogens in endotracheal tube biofilms and corresponding surveillance cultures. Pathog Dis. 2013;69(2):142-148.
40. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2:95-108.
41. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8:623-633.
42. Wang BY, Chi B, Kuramitsu HK. Genetic exchange between Treponema denticola and Streptococcus gordonii in biofilms. Oral Microbiol Immunol. 2002;17:108-112.
43. Vandecandelaere I, Coenye T. Microbial composition and antibiotic resistance of biofilms recovered from endotracheal tubes of mechanically ventilated patients. Adv Exp Med Biol. 2015;830:137-155.
44. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358:135-138.
45. Holmberg A, Rasmussen M. Mature biofilms of Enterococcus faecalis and Enterococcus faecium are highly resistant to antibiotics. Diagn Microbiol Infect Dis. 2016;84:19-21.
46. Prigent-Combaret C, Lejeune P. Monitoring gene expression in biofilms. Methods Enzymol. 1999;310:56-79.
47. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: Development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med. 2013;3.
48. Cámara M, Williams P, Hardman A. Controlling infection by tuning in and turning down the volume of bacterial small-talk. Lancet Infect Dis. 2002;2:667-676.
49. Kaplan JB, Meyenhofer MF, Fine DH. Biofilm growth and detachment of Actinobacillus actinomycetemcomitans. J Bacteriol. 2003;185:1399-1404.

50. Frykholm P, Pikwer A, Hammarskjöld F, et al. Clinical guidelines on central venous catheterisation. Swedish Society of Anaesthesiology and Intensive Care Medicine. Acta Anaesthesiol Scand. 2014;58:508-524.
51. Yousif A, Jamal MA, Raad I. Biofilm-based central line-associated bloodstream infections. Adv Exp Med Biol. 2015;830:157-179.
52. Tacconelli E, Smith G, Hieke K, Lafuma A, Bastide P. Epidemiology, medical outcomes and costs of catheter-related bloodstream infections in intensive care units of four European countries: literature- and registry-based estimates. J Hosp Infect. 2009;72:97-103.
53. Rosenthal VD, Al-Abdely HM, El-Kholy AA, et al. International Nosocomial Infection Control Consortium report, data summary of 50 countries for 2010-2015: Device-associated module. Am J Infect Control. 2016;44:1495-1504.
54. Hammarskjöld F, Berg S, Hanberger H, Taxbro K, Malmvall B-E. Sustained low incidence of central venous catheter-related infections over six years in a Swedish hospital with an active central venous catheter team. Am J Infect Control. 2014;42:122-128.
55. Tarpatzi A, Avlamis A, Papaparaskevas J, et al. Incidence and risk factors for central vascular catheter-related bloodstream infections in a tertiary care hospital. New Microbiol. 2012;35:429-437.
56. Parienti J-J, Mongardon N, Mégarbane B, et al. Intravascular Complications of Central Venous Catheterization by Insertion Site. N Engl J Med. 2015;373:1220-1229.
57. Hammarskjöld F, Wallén G, Malmvall BE. Central venous catheter infections at a county hospital in Sweden: a prospective analysis of colonization, incidence of infection and risk factors. Acta Anaesthesiol Scand. 2006;50:451-460.
58. Mermel LA. What is the predominant source of intravascular catheter infections? Clin Infect Dis An Off Publ Infect Dis Soc Am. 2011;52:211-212.
59. Safdar N, Maki DG. The pathogenesis of catheter-related bloodstream infection with noncuffed short-term central venous catheters. Intensive Care Med. 2004;30:62-67.
60. The Joint Commision. Preventing Central Line-Associated Bloodstream Infections: A Global Challenge, a Global Perspective.Oak Brook, IL; Joint Commision Resources May 2012. http://www.PreventingCLABSIs.pdf.
61. Borow M, Crowley JG. Prevention of thrombosis of central venous catheters. J Cardiovasc Surg. 1986;27:571-574.
62. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;49:1-45.
63. Horbett TA. Fibrinogen adsorption to biomaterials. J Biomed Mater Res A. 2018;106:2777-2788.
64. Ekdahl KN, Soveri I, Hilborn J, Fellström B, Nilsson B. Cardiovascular disease in haemodialysis: role of the intravascular innate immune system. Nat Rev Nephrol. 2017;13:285-296.

65. Nilsson B, Nilsson Ekdahl K, Mollnes TE, Lambris JD. The role of complement in biomaterial-induced inflammation. Mol Immunol. 2007;44:82-94.
66. Kamphuisen PW, Lee AYY. Catheter-related thrombosis: lifeline or a pain in the neck? Hematol Am Soc Hematol Educ Progr. 2012;2012:638-644.
67. Verso M, Agnelli G. Venous thromboembolism associated with long-term use of central venous catheters in cancer patients. J Clin Oncol. 2003;21:3665-3675.
68. Boersma RS, Jie K-SG, Verbon A, van Pampus ECM, Schouten HC. Thrombotic and infectious complications of central venous catheters in patients with hematological malignancies. Ann Oncol. 2008;19:433-442.
69. Raad II, Luna M, Khalil SA, Costerton JW, Lam C, Bodey GP. The relationship between the thrombotic and infectious complications of central venous catheters. JAMA. 1994;271:1014-1016.
70. Mehall JR, Saltzman DA, Jackson RJ, Smith SD. Fibrin sheath enhances central venous catheter infection. Crit Care Med. 2002;30:908-912.
71. Nickel KF, Renné T. Crosstalk of the plasma contact system with bacteria. Thromb Res. 2012;130:S78-S83.
72. Oehmcke-Hecht S, Köhler J. Interaction of the Human Contact System with Pathogens-An Update. Front Immunol. 2018;9:312.
73. Lai NM, Chaiyakunapruk N, Lai NA, O'Riordan E, Pau WSC, Saint S. Catheter impregnation, coating or bonding for reducing central venous catheter-related infections in adults. Cochrane Database Syst Rev. 2016;3.
74. Wang H, Tong H, Liu H, et al. Effectiveness of antimicrobial-coated central venous catheters for preventing catheter-related blood-stream infections with the implementation of bundles: a systematic review and network meta-analysis. Ann Intensive Care. 2018;8:71.
75. Hanna H, Benjamin R, Chatzinikolaou I, et al. Long-term silicone central venous catheters impregnated with minocycline and rifampin decrease rates of catheter-related bloodstream infection in cancer patients: a prospective randomized clinical trial. J Clin Oncol. 2004;22:3163-3171.
76. Darouiche RO, Raad II, Heard SO, et al. A comparison of two antimicrobial-impregnated central venous catheters. Catheter Study Group. N Engl J Med. 1999;340:1-8.
77. Chong HY, Lai NM, Apisarnthanarak A, Chaiyakunapruk N. Comparative Efficacy of Antimicrobial Central Venous Catheters in Reducing Catheter-Related Bloodstream Infections in Adults: Abridged Cochrane Systematic Review and Network Meta-Analysis. Clin Infect Dis. 2017;64:S131-S140.
78. Torres A, Niederman MS, Chastre J, et al. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). Eur Respir J. 2017;50:1700582.

79. Walter J, Haller S, Quinten C, et al. Healthcare-associated pneumonia in acute care hospitals in European union/European economic area countries: An analysis of data from a point prevalence survey, 2011 to 2012. Eurosurveillance. 2018;23.
80. Barbier F, Andremont A, Wolff M, Bouadma L. Hospital-acquired pneumonia and ventilator-associated pneumonia: recent advances in epidemiology and management. Curr Opin Pulm Med. 2013;19:216-228.
81. Grgurich PE, Hudcova J, Lei Y, Sarwar A, Craven DE. Diagnosis of ventilator-associated pneumonia: controversies and working toward a gold standard. Curr Opin Infect Dis. 2013;26:140-150.
82. Vincent J-L, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA. 2009;302:2323-2329.
83. Vallés J, Pobo A, García-Esquirol O, Mariscal D, Real J, Fernández R. Excess ICU mortality attributable to ventilator-associated pneumonia: the role of early vs late onset. Intensive Care Med. 2007;33:1363-1368.
84. Park DR. The microbiology of ventilator-associated pneumonia. Respiratory Care.2005;50:742-763.
85. Hyllienmark P, Martling C-R, Struwe J, Petersson J. Pathogens in the lower respiratory tract of intensive care unit patients: impact of duration of hospital care and mechanical ventilation. Scand J Infect Dis. 2012;44:444-452.
86. Martin-Loeches I, Deja M, Koulenti D, et al. Potentially resistant microorganisms in intubated patients with hospital-acquired pneumonia: the interaction of ecology, shock and risk factors. Intensive Care Med. 2013;39:672-681.
87. Kollef MH, Hamilton CW, Ernst FR. Economic Impact of Ventilator-Associated Pneumonia in a Large Matched Cohort. Infect Control Hosp Epidemiol. 2012;33:250-256.
88. Timsit JF, Esaied W, Neuville M, Bouadma L, Mourvllier B. Update on ventilator-associated pneumonia. F1000Research. 2017;6:2061.
89. Siempos II, Vardakas KZ, Kyriakopoulos CE, Ntaidou TK, Falagas ME. Predictors of mortality in adult patients with ventilator-associated pneumonia: A meta-analysis. Shock. 2010;33:590-601.
90. Johanson WG, Pierce AK, Sanford JP. Changing pharyngeal bacterial flora of hospitalized patients. Emergence of gram-negative bacilli. N Engl J Med. 1969;281:1137-1140.
91. Ewig S, Torres A, El-Ebiary M, et al. Bacterial colonization patterns in mechanically ventilated patients with traumatic and medical head injury: Incidence, risk factors, and association with ventilator-associated pneumonia. Am J Respir Crit Care Med. 1999;159:188-198.
92. George DL, Falk PS, Wunderink RG, et al. Epidemiology of ventilator-acquired pneumonia based on protected bronchoscopic sampling. Am J Respir Crit Care Med. 1998;158:1839-1847.
93. A'Court C, Garrard CS. Nosocomial pneumonia in the intensive care unit: mechanisms and significance. Thorax. 1992;47:465-473.

94. Girou E, Schortgen F, Delclaux C, et al. Association of noninvasive ventilation with nosocomial infections and survival in critically ill patients. J Am Med Assoc. 2000;284:2361-2367.
95. Inglis TJ, Millar MR, Jones JG, Robinson DA. Tracheal tube biofilm as a source of bacterial colonization of the lung. J Clin Microbiol. 1989;27:2014-2018.
96. Sottile FD, Marrie TJ, Prough DS, et al. Nosocomial pulmonary infection: possible etiologic significance of bacterial adhesion to endotracheal tubes. Crit Care Med. 1986;14:265-270.
97. Adair CG, Gorman SP, Feron BM, et al. Implications of endotracheal tube biofilm for ventilator-associated pneumonia. Intensive Care Med. 1999;25:1072-1076.
98. Wilson A, Gray D, Karakiozis J, Thomas J. Advanced endotracheal tube biofilm stage, not duration of intubation, is related to pneumonia. J Trauma Acute Care Surg. 2012;72:916-923.
99. Gil-Perotin S, Ramirez P, Marti V, et al. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept. Crit Care. 2012;16:R93.
100. Sands KM, Wilson MJ, Lewis MAO, et al. Respiratory pathogen colonization of dental plaque, the lower airways, and endotracheal tube biofilms during mechanical ventilation. J Crit Care. 2017;37:30-37.
101. Feldman C, Kassel M, Cantrell J, et al. The Presence and Sequence of Endotracheal Tube Colonization in Patients Undergoing Mechanical Ventilation. Eur Respir J. 1999;13 :546-551.
102. Choi YC, Morgenroth E. Monitoring biofilm detachment under dynamic changes in shear stress using laser-based particle size analysis and mass fractionation. Water Sci Technol.2003;47:69-76.
103. Fitch ZW, Whitman GJR. Incidence, risk, and prevention of ventilator-associated pneumonia in adult cardiac surgical patients: A systematic review. J Card Surg. 2014;29:196-203.
104. Wu D, Wu C, Zhang S, Zhong Y. Risk factors of ventilator-associated pneumonia in critically III patients. Front Pharmacol. 2019;10:482.
105. Su M, Jia Y, Li Y, Zhou D, Jia J. Probiotics for the Prevention of Ventilator-Associated Pneumonia: A Meta-Analysis of Randomized Controlled Trials. Respir Care. 2020(Epub ahead of print).
106. Bo L, Li J, Tao T, et al. Probiotics for preventing ventilator-associated pneumonia. Cochrane Database Syst Rev. 2014.
107. Wang L, Li X, Yang Z, et al. Semi-recumbent position versus supine position for the prevention of ventilator-associated pneumonia in adults requiring mechanical ventilation. Cochrane Database Syst Rev. 2016.
108. Pozuelo-Carrascosa DP, Herráiz-Adillo Á, Alvarez-Bueno C, Añón JM, Martínez-Vizcaíno V, Cavero-Redondo I. Subglottic secretion drainage for preventing ventilator-associated pneumonia: An overview of systematic reviews and an updated meta-analysis. Eur Respir Rev. 2020;29:155.

109. Papazian L, Klompas M, Luyt C-E. Ventilator-associated pneumonia in adults: a narrative review. Intensive Care Med. 2020. (Epub ahead of print).
110. Cook DJ, Walter SD, Cook RJ, et al. Incidence of and risk factors for ventilator-associated pneumonia in critically ill patients. Ann Intern Med. 1998;129:433-440.
111. Bouadma L, Sonneville R, Garrouste-Orgeas M, et al. Ventilator-Associated Events: Prevalence, Outcome, and Relationship with Ventilator-Associated Pneumonia. Crit Care Med. 2015;43:1798-1806.
112. Rello J, Lode H, Cornaglia G, Masterton R. A European care bundle for prevention of ventilator-associated pneumonia. Intensive Care Med. 2010;36:773-780.
113. Okgün Alcan A, Demir Korkmaz F, Uyar M. Prevention of ventilator-associated pneumonia: Use of the care bundle approach. Am J Infect Control. 2016;44:173-176.
114. Pronovost P, Needham D, Berenholtz S, et al. An intervention to decrease catheter-related bloodstream infections in the ICU. N Engl J Med. 2006;355:2725-2732.
115. Tokmaji G, Vermeulen H, Müller MCA, Kwakman PHS, Schultz MJ, Zaat SAJ. Silver-coated endotracheal tubes for prevention of ventilator-associated pneumonia in critically ill patients. Cochrane Database Syst Rev. 2015;2015(8).
116. Karchmer TB, Giannetta ET, Muto CA, Strain BA, Farr BM. A randomized crossover study of silver-coated urinary catheters in hospitalized patients. Arch Intern Med. 2000;160:3294-3298.
117. Klarin B, Molin G, Jeppsson B, Larsson A. Use of the probiotic Lactobacillus plantarum 299 to reduce pathogenic bacteria in the oropharynx of intubated patients: a randomised controlled open pilot study. Crit Care. 2008;12:R136.
118. Gustafson EK, Elgue G, Hughes RD, et al. The instant blood-mediated inflammatory reaction characterized in hepatocyte transplantation. Transplantation. 2011;91:632-638.
119. Sinn S, Scheuermann T, Deichelbohrer S, Ziemer G, Wendel HP. A novel in vitro model for preclinical testing of the hemocompatibility of intravascular stents according to ISO 10993-4. J Mater Sci Mater Med. 2011;22:1521-1528.
120. Homann MV, Johansson D, Kan Wallen H, Sanchez J. Improved ex vivo blood compatibility of central venous catheter with noble metal alloy coating. J Biomed Mater Res B Appl Biomater. 2016;104:1359-1365.
121. George DL, Falk PS, Wunderink RG, et al. Epidemiology of ventilator-acquired pneumonia based on protected bronchoscopic sampling. Am J Respir Crit Care Med. 1998;158:1839-1847.
122. Sopena N, Heras E, Casas I, et al. Risk factors for hospital-acquired pneumonia outside the intensive care unit: A case-control study. Am J Infect Control. 2014;42:38-42.
123. Frandah W, Colmer-Hamood J, Amiri HM, Raj R, Nugent K. Oropharyngeal flora in patients admitted to the medical intensive care unit: clinical factors and acid suppressive therapy. J Med Microbiol. 2013;62:778-784.
124. Wang Y, Ye Z, Ge L, et al. Efficacy and safety of gastrointestinal bleeding prophylaxis in critically ill patients: Systematic review and network meta-analysis. BMJ. 2020;368:l6744.

125. Alhazzani W, Alshamsi F, Belley-Cote E, et al. Efficacy and safety of stress ulcer prophylaxis in critically ill patients: a network meta-analysis of randomized trials. Intensive Care Med. 2018;44:1-11.
126. Laheij RJ, Sturkenboom MC, Hassing RJ, Dieleman J, Stricker BH, Jansen JB. Risk of community-acquired pneumonia and use of gastric acid-suppressive drugs. Jama. 2004;292:1955-1960.
127. Herzig SJ, Howell MD, Ngo LH, Marcantonio ER. Acid-suppressive medication use and the risk for hospital-acquired pneumonia. Jama. 2009;301:2120-2128.
128. MacLaren R, Reynolds PM, Allen RR. Histamine-2 receptor antagonists vs proton pump inhibitors on gastrointestinal tract hemorrhage and infectious complications in the intensive care unit. JAMA Intern Med. 2014;174:564-574.
129. Bateman BT, Bykov K, Choudhry NK, et al. Type of stress ulcer prophylaxis and risk of nosocomial pneumonia in cardiac surgical patients: cohort study. BMJ. 2013;347:5416.
130. Alshamsi F. Belley-Cote E. et al. Efficacy and safety of proton pump inhibitors for stress ulcer prophylaxis in critically ill patients: A systematic review and meta-analysis of randomized trials. Crit Care. 2016;20:120.
131. Toews I, George AT, Peter J V., et al. Interventions for preventing upper gastrointestinal bleeding in people admitted to intensive care units. Cochrane Database Syst Rev. 2018;2018:6.
132. Sasabuchi Y, Matsui H, Lefor AK, Fushimi K, Yasunaga H. Risks and benefits of stress ulcer prophylaxis for patients with severe sepsis. Crit Care Med. 2016;44:464-469.
133. Krag M, Marker S, Perner A, et al. Pantoprazole in patients at risk for gastrointestinal bleeding in the ICU. N Engl J Med. 2018;379:2199-2208.
134. Huang H-B, Jiang W, Wang C-Y, Qin H-Y, Du B. Stress ulcer prophylaxis in intensive care unit patients receiving enteral nutrition: a systematic review and meta-analysis. Crit Care. 2018;22:20.
135. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43:304-377.
136. Pandolfino JE, El-Serag HB, Zhang Q, Shah N, Ghosh SK, Kahrilas PJ. Obesity: a challenge to esophagogastric junction integrity. Gastroenterology. 2006;130:639-649.
137. Friedenberg FK, Xanthopoulos M, Foster GD, Richter JE. The association between gastroesophageal reflux disease and obesity. Am J Gastroenterol. 2008;103:2111-2122.
138. Soysa NS, Samaranayake LP, Ellepola ANB. Diabetes mellitus as a contributory factor in oral candidosis. Diabet Med A J Br Diabet Assoc. 2006;23:455-459.
139. Edlund C, Bergan T, Josefsson K, Solberg R, Nord CE. Effect of norfloxacin on human oropharyngeal and colonic microflora and multiple-dose pharmacokinetics. Scand J Infect Dis. 1987;19:113-121.
140. Nord CE, Heimdahl A, Lundberg C, Marklund G. Impact of cefaclor on the normal human oropharyngeal and intestinal microflora. Scand J Infect Dis. 1987;19:681-685.

141. Zaura E, Brandt BW, de Mattos MJT, et al. Same Exposure but two radically different responses to antibiotics: Resilience of the salivary microbiome versus long-term microbial shifts in feces. MBio. 2015;6:01693-15.
142. Zur KB, Mandell DL, Gordon RE, Holzman I, Rothschild MA. Electron microscopic analysis of biofilm on endotracheal tubes removed from intubated neonates. Otolaryngol Head Neck Surg. 2004;130:407-414.
143. Bayazian G, Sayyahfar S, Safdarian M, Kalantari F. Is there any association between adenoid biofilm and upper airway infections in pediatric patients? Turk Pediatr Ars. 2018;53:71-77.
144. Chole RA, Faddis BT. Anatomical evidence of microbial biofilms in tonsillar tissues: A possible mechanism to explain chronicity. Arch Otolaryngol - Head Neck Surg. 2003;129:634-636.
145. Diaz-Blanco J, Clawson RC, Roberson SM, Sanders CB, Pramanik AK, Herbst JJ. Electron microscopic evaluation of bacterial adherence to polyvinyl chloride endotracheal tubes used in neonates. Crit Care Med. 1989;17:1335-1340.
146. Kurmoo Y, Hook AL, Harvey D, et al. Real time monitoring of biofilm formation on coated medical devices for the reduction and interception of bacterial infections. Biomater Sci. 2020. (Epub ahead of print)
147. Torres A, Gatell JM, Aznar E, et al. Re-intubation increases the risk of nosocomial pneumonia in patients needing mechanical ventilation. Am J Respir Crit Care Med. 1995;152:137-141.
148. Andriolo BN, Andriolo RB, Saconato H, Atallah ÁN, Valente O. Early versus late tracheostomy for critically ill patients. Cochrane Database Syst Rev. 2015.
149. Werner G, Coque TM, Hammerum AM, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Euro Surveill. 2008;13:47.
150. Berra L, Coppadoro A, Bittner EA, et al. A clinical assessment of the Mucus Shaver: A device to keep the endotracheal tube free from secretions. Crit Care Med. 2012;40:119-124.
151. Zangirolami AC, Inada NM, Bagnato VS, Blanco KC. Biofilm Destruction on Endotracheal Tubes by Photodynamic Inactivation. Infect Disord Drug Targets. 2018;18:218-223.
152. Estes RJ, Meduri GU. The pathogenesis of ventilator-associated pneumonia: I. Mechanisms of bacterial transcolonization and airway inoculation. Intensive Care Med. 1995;21:365-383.
153. Vandecandelaere I, Matthijs N, van Nieuwerburgh F, et al. Assessment of microbial diversity in biofilms recovered from endotracheal tubes using culture dependent and independent approaches. PLoS One. 2012;7:e38401.
154. Azoulay E, Timsit J-F, Tafflet M, et al. Candida colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. Chest. 2006;129:110-117.
155. Nseir S, Jozefowicz E, Cavestri B, et al. Impact of antifungal treatment on Candida-Pseudomonas interaction: A preliminary retrospective case-control study. Intensive Care Med. 2007;33:137-142.

156. Rahimi A, Mashak A. Review on rubbers in medicine: Natural, silicone and polyurethane rubbers. Plast Rubber Compos. 2013;42:223-230.
157. Melvin ME, Fissell WH, Roy S, Brown DL. Silicon induces minimal thromboinflammatory response during 28-day intravascular implant testing.ASAIO J. 2010;4:344-8.
158. Marosok R, Washburn R, Indorf A, Solomon D, Sherertz R. Contribution of vascular catheter material to the pathogenesis of infection: depletion of complement by silicone elastomer in vitro. J Biomed Mater Res. 1996;30:245-250.
159. Sherertz RJ, Carruth WA, Marosok RD, Espeland MA, Johnson RA, Solomon DD. Contribution of vascular catheter material to the pathogenesis of infection: the enhanced risk of silicone in vivo. J Biomed Mater Res. 1995;29:635-645.
160. ShR M, Mohammadi S. The evaluation of Candida albicans biofilms formation on silicone catheter, PVC and glass coated with titanium dioxide nanoparticles by XTT method and ATPase assay. Bratisl Lek List. 2012;113:12.
161. Lopez-Lopez G, Pascual A, Perea EJ. Effect of plastic catheter material on bacterial adherence and viability. J Med Microbiol. 1991;346:349-353.
162. Hawser SP, Douglas LJ. Biofilm formation by Candida species on the surface of catheter materials in vitro. Infect Immun. 1994;62:915-921.
163. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res. 2006;17:68-81.
164. Bjorling G, Johansson D, Bergstrom L, et al. Tolerability and performance of BIP endotracheal tubes with noble metal alloy coating--a randomized clinical evaluation study BIP studien om tubens säkerhet. BMC Anesthesiol. 2015;15:174-z.
165. Chung PHY, Wong CWY, Lai CKC, et al. A prospective interventional study to examine the effect of a silver alloy and hydrogel–Coated catheter on the incidence of catheter-associated urinary tract infection. Hong Kong Med J. 2017;23:239-245.
166. Magnusson B, Kai-Larsen Y, Granlund P, et al. Long-term use of noble metal alloy coated urinary catheters reduces recurrent CAUTI and decreases proinflammatory markers. Ther Adv Urol. 2018;11.
167. Brosnahan J, Jull A, Tracy C. Types of urethral catheters for management of short-term voiding problems in hospitalised adults. Cochrane Database Systematic Reviews. 2004.
168. Jones DS, Mcgovern JG, Woolfson AD, Gorrnan SP. Role of Physiological Conditions in the Oropharynx on the Adherence of Respiratory Bacterial Isolates to Endotracheal Tube Poly(Viny1 Chloride). Biomaterials. 1994;6;503-510.
169. Tranberg A, Thorarinsdottir HR, Holmberg A, Schött U, Klarin B. Proton pump inhibitor medication is associated with colonisation of gut flora in the oropharynx. Acta Anaesthesiol Scand. 2018;62:791-800.
170. Visscher S, Schurink CAM, Melsen WG, Lucas PJF, Bonten MJM. Effects of systemic antibiotic therapy on bacterial persistence in the respiratory tract of mechanically ventilated patients. Intensive Care Med. 2008;34:692-699.

171. Gorman S, Adair C, O'Neill F, Goldsmith C, Webb H. Influence of selective decontamination of the digestive tract on microbial biofilm formation on endotracheal tubes from artificially ventilated patients. Eur J Clin Microbiol Infect Dis. 1993;12:9-17.
172. Kim JS, Holtom P, Vigen C. Reduction of catheter-related bloodstream infections through the use of a central venous line bundle: epidemiologic and economic consequences. Am J Infect Control. 2011;39:640-646.
173. van der Kooi T, Sax H, Pittet D, et al. Prevention of hospital infections by intervention and training (PROHIBIT): results of a pan-European cluster-randomized multicentre study to reduce central venous catheter-related bloodstream infections. Intensive Care Med. 2018;44:48-60.
174. Longmate AG, Ellis KS, Boyle L, et al. Elimination of central-venous-catheter-related bloodstream infections from the intensive care unit. BMJ Qual Saf. 2011;20:174-180.
175. Worth LJ, Spelman T, Bull AL, Brett JA, Richards MJ. Central line-associated bloodstream infections in Australian intensive care units: Time-trends in infection rates, etiology, and antimicrobial resistance using a comprehensive Victorian surveillance program, 2009-2013. Am J Infect Control. 2015;43:848-852.
176. Timsit JF, L'Hériteau F, Lepape A, et al. A multicentre analysis of catheter-related infection based on a hierarchical model. Intensive Care Med. 2012;38:1662-1672.
177. Templeton A, Schlegel M, Fleisch F, et al. Multilumen central venous catheters increase risk for catheter-related bloodstream infection: prospective surveillance study. Infection. 2008;36:322-327.
178. Köck R, Becker K, Cookson B, et al. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill. 2010;15:19688.
179. Early TF, Gregory RT, Wheeler JR, Snyder SO, Gayle RG. Increased infection rate in double-lumen versus single-lumen Hickman catheters in cancer patients. South Med J. 1990;83:34-36.
180. Santacruz E, Mateo-Lobo R, Riveiro J, et al. Infectious complications in home parenteral nutrition: A long-term study with peripherally inserted central catheters, tunneled catheters, and ports. Nutrition. 2019;58:89-93.
181. Dezfulian C, Lavelle J, Nallamothu BK, Kaufman SR, Saint S. Rates of infection for single-lumen versus multilumen central venous catheters: a meta-analysis. Crit Care Med. 2003;31:2385-2390.
182. Li M, Neoh KG, Xu LQ, et al. Surface modification of silicone for biomedical applications requiring long-term antibacterial, antifouling, and hemocompatible properties. Langmuir ACS J surfaces colloids. 2012;28:16408-16422.
183. Steuer H, Krastev R, Lembert N. Metallic oxide nanoparticles stimulate blood coagulation independent of their surface charge. J Biomed Mater Res B Appl Biomater. 2014;102:897-902.
184. Arvidsson S, Askendal A, Tengvall P. Blood plasma contact activation on silicon, titanium and aluminium. Biomaterials. 2007;28(7):1346-1354.

185. Lykissa ED, Kala S V, Hurley JB, Lebovitz RM. Release of low molecular weight silicones and platinum from silicone breast implants. Anal Chem. 1997;69:4912-4916.
186. Brash JL, Horbett TA, Latour RA, Tengvall P. The blood compatibility challenge. Part 2: Protein adsorption phenomena governing blood reactivity. Acta Biomater. 2019;94:11-24.
187. Nurdin N, François P, Mugnier Y, et al. Haemocompatibility evaluation of DLC- and SiC-coated surfaces. Eur Cell Mater. 2003;5:17-28.
188. Gorbet M, Sperling C, Maitz MF, Siedlecki CA, Werner C, Sefton M V. The blood compatibility challenge. Part 3: Material associated activation of blood cascades and cells. Acta Biomater. 2019;94:25-32.
189. Chong HY, Lai NM, Apisarnthanarak A, Chaiyakunapruk N. Comparative Efficacy of Antimicrobial Central Venous Catheters in Reducing Catheter-Related Bloodstream Infections in Adults: Abridged Cochrane Systematic Review and Network Meta-Analysis. Clin Infect Dis.2017;64:S131-S140.
190. Casey AL, Mermel LA, Nightingale P, Elliott TSJ. Antimicrobial central venous catheters in adults: a systematic review and meta-analysis. Lancet Infect Dis. 2008;8:763-776.
191. Brun-Buisson C, Doyon F, Sollet J-P, Cochard J-F, Cohen Y, Nitenberg G. Prevention of intravascular catheter-related infection with newer chlorhexidine-silver sulfadiazine-coated catheters: a randomized controlled trial. Intensive Care Med. 2004;30:837-843.
192. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. Clin Infect Dis An Off Publ Infect Dis Soc Am. 2011;52:162.
193. Napolitano LM. Anemia and Red Blood Cell Transfusion: Advances in Critical Care. Crit Care Clin. 2017;33:345-364.
194. Ansel HC. Hemolysis of erythrocytes by antibacterial preservatives. IV. Hemolytic activity of chlorhexidine diacetate. J Pharm Sci. 1967;56:616-619.
195. Merle NS, Grunenwald A, Rajaratnam H, et al. Intravascular hemolysis activates complement via cell-free heme and heme-loaded microvesicles. JCI insight. 2018;3:12.
196. Gao Q, Zhang P, Wang W, et al. The correlation analysis of tumor necrosis factor-alpha-308G/A polymorphism and venous thromboembolism risk: A meta-analysis. Phlebology. 2016;31:625-631.
197. Matos MF, Lourenço DM, Orikaza CM, Bajerl JAH, Noguti MAE, Morelli VM. The role of IL-6, IL-8 and MCP-1 and their promoter polymorphisms IL-6 -174GC, IL-8 -251AT and MCP-1 -2518AG in the risk of venous thromboembolism: a case-control study. Thromb Res. 2011;128:216-220.
198. de la Hoz Caballer B, Fernandez-Rivas M, Lazaro JF, et al. Management of sulfadiazine allergy in patients with acquired immunodeficiency syndrome. J Allergy Clin Immunol. 1991;88:137-138.
199. Ohtoshi T, Yamauchi N, Tadokoro K, et al. IgE antibody-mediated shock reaction caused by topical application of chlorhexidine. Clin Allergy. 1986;16:155-161.

200. Herrmann M, Vaudaux PE, Pittet D, et al. Fibronectin, fibrinogen, and laminin act as mediators of adherence of clinical staphylococcal isolates to foreign material. J Infect Dis. 1988;158:693-701.
201. de Sousa JKT, Haddad JPA, de Oliveira AC, Vieira CD, dos Santos SG. In vitro activity of antimicrobial-impregnated catheters against biofilms formed by KPC-producing Klebsiella pneumoniae. J Appl Microbiol. 2019;127:1018-1027.
202. Karpiński TM, Szkaradkiewicz AK. Chlorhexidine-pharmaco-biological activity and application. Eur Rev Med Pharmacol Sci. 2015;19:1321-1326.
203. Huang SS, Septimus E, Kleinman K, et al. Chlorhexidine versus routine bathing to prevent multidrug-resistant organisms and all-cause bloodstream infections in general medical and surgical units (ABATE Infection trial): a cluster-randomised trial. Lancet (London, England). 2019;393:1205-1215.
204. Climo MW, Yokoe DS, Warren DK, et al. Effect of daily chlorhexidine bathing on hospital-acquired infection. N Engl J Med. 2013;368:533-542.
205. Toomey M. Preoperative chlorhexidine anaphylaxis in a patient scheduled for coronary artery bypass graft: a case report. AANA J. 2013;81:209-214.
206. Stephens R, Mythen M, Kallis P, Davies DW, Egner W, Rickards A. Two episodes of life-threatening anaphylaxis in the same patient to a chlorhexidine-sulphadiazine-coated central venous catheter. Br J Anaesth. 2001;87:306-308.
207. Weng M, Zhu M, Chen W, Miao C. Life-threatening anaphylactic shock due to chlorhexidine on the central venous catheter: a case series. Int J Clin Exp Med. 2014;7:5930-5936.
208. Terazawa E, Nagase K, Masue T, et al. Anaphylactic shock associated with a central venous catheter impregnated with chlorhexidine and silver sulfadiazine. Masui. 1998;47:556-561.
209. Ishigami S, Hase S, Nakashima H, et al. Intravenous chlorhexidine gluconate causing acute respiratory distress syndrome. J Toxicol Clin Toxicol. 2001;39:77-80.
210. Hirata K, Kurokawa A. Chlorhexidine gluconate ingestion resulting in fatal respiratory distress syndrome. Vet Hum Toxicol. 2002;44:89-91.
211. Price R, MacLennan G, Glen J, SuDDICU Collaboration. Selective digestive or oropharyngeal decontamination and topical oropharyngeal chlorhexidine for prevention of death in general intensive care: systematic review and network meta-analysis. BMJ. 2014;348:2197.
212. Berg ØH, Bakken AM, Steinsvåg SK, Farstad M. Benzalkonium chloride interferes with energy production, secretion and morphology in human blood platelets. Platelets. 1999;10(2-3):97-104.
213. Björling G, Johansson D, Bergström L, et al. Evaluation of central venous catheters coated with a noble metal alloy-A randomized clinical pilot study of coating durability, performance and tolerability. J Biomed Mater Res B Appl Biomater.2018;106:2337-2344.
214. Mölstad S, Löfmark S, Carlin K, et al. Bulletin of the World Health Organization Lessons learnt during 20 years of the Swedish strategic programme against antibiotic resistance. Bull World Health Organ. 2017;95:764-773.

Printed by Media-Tryck, Lund 2020 NORDIC SWAN ECOLABEL 3041 0903

# About the author

Hulda Thorarinsdottir was born in Reykjavik, Iceland in 1978. She is currently working as a specialist in anesthetics and intensive care at Skåne University Hospital in Lund. Her current research, and this thesis, studies the impact of different materials and coatings in medical devices on the development of device-related infections. Further, she investigates changes in oropharyngeal microbial flora during hospitalization.

LUND UNIVERSITY

FACULTY OF MEDICINE

Section of Anesthesiology and Intensive Care
Department of Clinical Sciences Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2020:80
ISBN 978-91-7619-942-8
ISSN 1652-8220