Pathogen colonisation, transmission and infection control measures in burn patients

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Abstract

Burn patients are susceptible to infections due to impaired natural defences when the epidermal barrier is damaged. Moreover, the trauma affects the patient's immune system through local and systemic inflammatory reactions. Adding to that, the need for intensive care treatment with multiple surgical interventions increases the risk of infection. The need for vascular, airway, etc. catheterisations further strains the burned patient. Microbes, especially those with extraordinary survival properties in the healthcare environment, are essential sources of infections in burn wounds.

This thesis aimed to investigate microbiological aspects in burn care, including bacterial colonisation, transmission and possible measures to combat this and reduce infection rates.

In paper I, we investigated Staphylococcus aureus-colonisation in burn patients. Thirty-nine (48.8%) patients were S. aureus positive, and 41 (51.3%) negative at admission. Whole-genome sequencing of the 53 (39 + 14) S. aureus positive showed that the patients carried unique isolates, most likely due to their flora at admission and not by cross-contamination.

In paper II, through a systematic investigation, we showed that higher awareness and education of contamination routes increased healthcare workers' compliance with hand hygiene and dress code. Intensified cleaning procedures of the environment and equipment could successfully be established, which significantly contributed to stopping the further spread of Acinetobacter baumannii in the burn unit.

In paper III, we observed that the decontamination-efficacy of Ultraviolet–C (UVC) of bacteria in fabrics in a clinical setting is not as effective as laundering and, thus, it’s not an alternative to UVC decontamination.

In paper IV, we established that received UVC dose varied with the device's location when decontaminating a wardroom. The validation of single-use UVC dose indicators showed their usefulness in ensuring adequate decontamination doses.

This thesis shows that the patient’s colonisation of S. aureus during the burn treatment is primarily due to the S. aureus carried at admission and not due to transmission. It also shows that a high level of knowledge about transmission routes, infection prevention, and cleaning protocols is essential to burn care. Decontamination using UVC light seems a plausible adjunct to routine cleaning.

Keywords: Whole-genome sequencing, Burn injury, Acinetobacter baumannii outbreak, infection control prevention, UVC decontamination

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To my family
List of Papers

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


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## Abbreviations

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<tr>
<td>AP-PCR</td>
<td>Arbitrarily primed polymerase chain reaction</td>
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<td>BICU</td>
<td>Burn intensive care unit</td>
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<td>CFU</td>
<td>Colony-forming unit</td>
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<tr>
<td>HAI</td>
<td>Healthcare-associated infections</td>
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<td>HEPA</td>
<td>High-efficiency particulate air</td>
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<tr>
<td>HCW</td>
<td>Healthcare worker</td>
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<td>HH</td>
<td>Hand hygiene</td>
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<td>ICU</td>
<td>Intensive care unit</td>
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<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
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<td>MSSA</td>
<td>Methicillin-sensitive <em>Staphylococcus aureus</em></td>
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<td>MLST</td>
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<td>PFGE</td>
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<td>PPE</td>
<td>Personal protection equipment</td>
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<td>UVC</td>
<td>Ultraviolet-C</td>
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<td>WGS</td>
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Introduction

The burn patient is often perceived to have significantly more resistant bacteria detected than any other patient category. This is especially true for burn patients treated in an intensive care unit (ICU) [1]. The burn trauma markedly compromises the patient's immune system by, e.g.:

- eliciting a massive (local and systemic) inflammatory reaction and open skin wounds with necrotic tissue [2].
- often need intensive care treatment.
- (repeated) surgical interventions and wound cleaning/dressing changes.
- (repeated) vascular, airway, oral, urinary, rectal, and other catheterisations [3, 4].

Thus, burn patients are particularly susceptible to infections. The use of antibiotics can be massive to treat occurring infections, not seldom from arising (multi-) resistant microbes. Recurring antibiotic courses due to immunosuppression select for multiresistant bacteria in the endogenous flora, which in turn gives rise to refractory infections and increases the risk of outbreaks with multiresistant bacteria. The most vulnerable patients are cared for in the burn intensive care unit (BICU), and these units constantly balance on the verge of (multiresistant) microbial outbreaks. The BICU thus cares for immunosuppressed patients susceptible to infections, often developing multiresistant microbes in a setting with a high risk of microbial transmission. This situation is further aggravated by the large number of personnel, visitors, consults, and contacts with contaminated surfaces (e.g., bed rails, medical equipment, and computers) in the ward rooms and sometimes also insufficient hygiene routines and behaviour [5]. Investigations by active surveillance have shown that compliance with hygiene routines among personnel often could be better [6]. Care-related activities and procedures, such as catheters, tubes, injections, and surgical procedures, all add to the risk of transmission [4, 7].

The risk for cross-transmissions of pathogens between patients is high in ICU and BICU. The risk factors in every aspect of the chain of infection (the chain of microorganisms, the source, the way/routes, the mode, the portal and the host) [8] are several. This thesis focused on three of the six components in the chain of infection:

- The host
- The source
- The routes of transmission
The host

Burn patients, their pathogens and risk of infection

In the BICU environment, burn patients are exposed to several sources of infection, e.g., other people (patients, healthcare workers (HCW) and relatives), equipment, and invasive catheters. These sources make them susceptible to healthcare-associated infections (HAI), e.g., central line-associated bloodstream infection, ventilator-associated pneumonia, and urinary tract infection.[5]. Directly after the burn trauma, the wounds are considered clean/sterile due to the heat inflicting the burn [9]. However, the wounds rapidly become colonised and the early colonisation includes primarily Gram-positive bacteria such as *Staphylococcus aureus* [4]. Over the following days, the Gram-negative bacteria come to dominate the colonisation, e.g., *Pseudomonas aeruginosa* and *Enterobacter* species (by approximately day 7) with subsequently more antibiotic resistance mechanisms due to selection from possible antibiotic treatment. Yeasts usually occur later in the process [4]. It has been shown that mortality from infections is higher in burn patients than in hospitalised patients in general, especially as a result of Gram-negative infections [10]. Furthermore, antibiotic prophylaxis has no, or limited effect and poses a risk of facilitating the development of resistant microbes [11, 12]. In the 1960s Zora Janžekovič discovered [13] that early removal of necrotic tissue and early complete wound coverage led to decreased infections of the wounds and increased survival of burn patients. The procedure with early tangential excision, necrosectomy, is now considered the "gold standard" in burn treatment (deep burns) to prevent infected wounds.

The source of infection

Endogenous and exogenous sources

The source of infection could be endogenous, i.e. the normal flora of the patient itself, or exogenous, depending on whether the patient brings the bacteria to the healthcare setting or acquires it in the hospital via HCW’s hands, medical equipment, or through the hospital environment. The sources of some of the most common bacteria in infections of the burned patient are described below.

*Staphylococcus aureus*

*Staphylococcus aureus* occurs in the normal bacterial flora in approximately 30% of the population in the community and colonises, preferably, the nasal cavity, mouth, axillae, and groins, without necessarily causing infections [14-16]. However, *S. aureus* may lead to life-threatening infections (e.g., sepsis or
endocarditis) in predisposed individuals. The person's immune defence is a significant factor in whether *S. aureus* will remain a harmless colonising microorganism or become an invading pathogen. Extensive burns with large areas of damaged skin and remaining necrosis are risk factors for life threatening infections. *S. aureus* infections are common in burns as they constitute approximately 50%–60% of the infections [10]. Previously published studies have shown that the mortality rate of burns due to infections and sepsis caused by *S. aureus* in some research sites has been as high as 51%-75% [17, 18].

A study by Boncompain et al. [19] marks the relevance of the nasal occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) in HCW. This study found that 30% of physicians and 22% of nurses had *S. aureus* as a colonising organism in their normal flora of the nasal cavity. They also observed that more nurses were carrying resistant *S. aureus* than physicians. They concluded that recommendations for screening and decolonisation strategies are needed to minimise the risk of infection. In one previous study in an ICU surgical setting, they investigated the number of *S. aureus* colonisation rates during hospitalisation. They found that 36.5% of the patients in the study were colonised by *S. aureus* on admission to the hospital. The primary site of *S. aureus* colonisation was the nasal cavity. This study concluded that colonisation with endogenous bacteria led to HAI (pneumonia and septicaemia) [20].

In certain parts of the world, high incidences of MRSA close to 80%, in BICU have been noted. The high incidence leads to an increased risk of morbidity and mortality and, thus, increased use of broad-spectrum antibiotics to combat these risks [21]. In Scandinavia, the incidence of MRSA is lower (2%, 2012) than most other European countries [22].

**Acinetobacter baumannii**

Few strains of multidrug-resistant Gram-negative *Acinetobacter baumannii* are detected in the environment outside the hospital, and obviously the bacterium has its ecological niche in healthcare settings [23]. However, from manure tanks and surface water in an agricultural environment in Eastern Ontario, Canada, *A. baumannii* was isolated from samples in a study investigating the prevalence of *Campylobacter* spp. The study team got secondary findings of *A. baumannii* strains with low antibiotic sensitivity [24]. In a review by Boucher et al. [25], the *Acinetobacter* species (>30 species) was described as early as 1911. In 1974, it was noted that community-acquired *A. baumannii* was mostly found during the summer months. Furthermore, community-acquired *A. baumannii* have been found in people infected with pneumonia in Australia and Asia, and the reason to this could have been the tropical environment [26, 27].

Multidrug-resistant *A. baumannii* clones are spread worldwide in healthcare settings and have been observed for several decades, causing outbreaks
in ICUs and BICUs [28-31]. The bacteria's ability to produce biofilm have made them adaptable to both moist and dry surfaces, especially on medical devices in healthcare environments. Thereby, *A. baumannii* both colonise and infect immunosuppressed multimorbid patients cared for in the ICU and BICU [29]. In November 2014, an outbreak of *A. baumannii* started in our burn centre. The outbreak came to involve a total of nine patients over a period of five months. The primary case was thought to be a patient admitted from outside our region. In addition to the numerous interventions and precautions mentioned in this thesis, we introduced an ultraviolet-C (UVC) device to adjunct infection control interventions to terminate the outbreak. Thus, in March 2015, a Tru-D™ SmartUVC decontamination [32] (process to eliminate pathogenic microorganism) device was added to the traditional manual cleaning of rooms and equipment.

The routes of transmission

In hospital settings, there are several routes of transmission for pathogens between the source and the susceptible host [8]:

- Contact transmission with two subgroups:
  - Direct transmission
  - Indirect transmission
- Airborne transmission
- Droplet transmission
- Water-borne transmission
- Blood-borne transmission

In this thesis, the focus is on describing transmission of pathogens by direct/indirect contacts and the interventions undertaken to break the chain of transmission.

Prevention and intervention

Cleaning and disinfection prevent indirect contact transmission of pathogens from the environment and is critical in reducing the risk of transmission of pathogens between patients and HCW [3]. Proper infection prevention education, training, and practice by HCW are also highly significant in patient care to prevent direct contact [9]. Training and practice must be repeated and constantly focused on to reduce the risk of microbial transmission. Knowing the different microbes in general, and local specific microbes in particular, in BICU patients, and how the microbes spread should be basic knowledge for HCW in a BICU [10, 12]. Furthermore, proper use of personal protection equipment (PPE), compliance with hand hygiene (HH) [3], dress code, and environmental cleaning are essentials for HCW [7, 33].
An Infection Prevention Competency model published by the American infection control society, APIC (Association for Professionals in Infection Control and Epidemiology) [34] can serve as a model for how to prevent contact transmission of pathogens in healthcare settings and this model will be applied here. The components of the model are (Figure 1):

- Cleaning, Disinfection, and Sterilisation
- Surveillance and Infection Rounding
- Outbreak Detection and Management
- Educational Responsibility
- Emerging Technologies
- Antimicrobial and Diagnostic Stewardship
- Laws and Regulations

Figure 1, with the patient placed in the centre, describes the patient's context in relation to the infection prevention interventions that must be undertaken to prevent contact transmission [35]. How and what we do as HCW should always be with the patient's best interests in mind. The nurse and assistant nurse are closest and committed to the patient 24/7. However, the infection prevention approach applies to all HCW, such as the other burn team members: doctors, physiotherapists, occupational therapists, dietitians, consultants, surgical staff, and cleaners.

Figure 1. The patient’s context in relation to infection prevention and control interventions.
The four manuscripts of this thesis and its relation to prevention and control interventions are described in Figure 2.

Figure 2. Description of this thesis' papers in relation to prevention and control interventions. Paper I) the patient’s nasal cavity as a source of infection. Paper II) transmission via the HCWs’ hands. Paper III) UVC-decontamination of textiles. Paper IV) UVC dose indicators in different places.

Numerous actions and precautions have been suggested and undertaken to prevent the transmission of microbes in healthcare [36]. As one effort to reduce the risk of transmission of pathogens, Uppsala University Hospital has, at the request of The Swedish Association of Local Authorities and Regions (SALAR) [33] since the year 2010, run a quality registry on compliance with HH, PPE, and dress code. The quality register is a point-prevalence measurement on personnel's compliance with the HH, PPE, and dress code regarding seven practice dimensions. The compliance with HH, PPE, and dress code is monthly monitored by direct observations, noted, and results are fed back to the personnel in staff meetings by the ward leadership. The head of the burn centre and the infection prevention and control department at the hospital is contacted for further investigations and actions when needed.
The dimensions observed are:

Hand hygiene

1. Hands and forearms are disinfected with alcohol before contact with a patient.

2. Hands and forearms are disinfected with alcohol immediately after contact with a patient.

Personal protective equipment

3. Gloves are used in contact with bodily fluids (e.g., wounds, blood, secretion, urine) and surface disinfectants. Protective gloves are discarded immediately after use. Gloves are to be changed between patients and different chores.

4. A disposable apron or protective gown is used in patient care, direct contact with the patient's bed, and handling dirty/contaminated items.

Dress code:

5. Scrubs have short sleeves and are changed daily and/or when they get wet or visibly contaminated.

6. Hands and forearms are free of rings, watches, bracelets, etc.

7. Hair is set or cut short.

UVC light
Besides infection prevention and control measures such as education, high awareness, cohorting, personal hygiene, other decontaminating adjunctive tools and devices such as e.g. specific detergents for manual cleaning, automated hydrogen peroxide vapour [33, 37], and UVC equipment have been described in the literature. UVC light for decontamination has been used in e.g. water, operating theatres, and research facilities [38]. UVC light induces changes in the cell structures of bacteria, viruses, fungi, and spores so their replicative abilities are decreased [38]. UVC light has previously been used, and shown effective, in multidrug-resistant strains, especially on hard surfaces [39-42]. UVC is increasingly being used as an adjunct to manual cleaning of
ward rooms [43]. The Tru-DTM SmartUVC (Lumalier Corporation, Mem-
phis, TN, USA) decontamination equipment was introduced in the United
States in 2010 and came to use in Sweden in 2014. According to the manufac-
turer's data, the device can disinfect an entire room in about 30 minutes using
UVC light [43]. The device is equipped with sensors that measure the amount
of UVC light reflected to the unit, thus determining when enough irradiation
has been reached [44]. By the light's reflections on walls and other surfaces in
the room, a place not directly (in the line of sight) exposed to the UVC source
can also be reached.
Aims

This thesis aim is to investigate the microbiological aspects in burn care, including bacterial colonisation, transmission, and possible measures to combat this to reduce infection rates. The specific aims were:

- to determine the frequency of *S. aureus* colonisation in 80 patients and to investigate the role of colonisation status during the first ten days of hospitalisation with regard to total length of stay at the burn centre, number of days before antibiotic treatment was started, and mortality.

- to describe the course of an outbreak and infection control measures taken to stop the spread of *A. baumannii*.

- to evaluate the efficacy of automated UVC decontamination on *Enterococcus faecium* in fabrics in a clinical setting.

- to investigate the UVC dose received in different areas in a BICU ward room after an automated UVC decontamination and to validate a disposable UVC-dose indicator with parallel radiometer readings.

Ethical approval

I. Ethical approval was obtained from the Regional Ethical Review Board Uppsala University (IRB No: 2013/359. Amendments were made. IRB No: 2021-03889).

II. The Swedish Ethical Review Authority approved a waiver of the requirement (IRB No: 2020-06285) for informed consent for this study.

III. + IV. Since these studies were conducted in an experimental clinical setting in a hospital room not involving human subjects, no ethical approval was necessary nor obtained.
Materials and Methods

Setting and periods
The studies in this thesis were conducted at Uppsala University Hospital's burn centre in Sweden. In paper I, the patients were recruited from 2014 to 2016 (29 months). Paper II; the A. baumannii outbreak lasted from November 2014 to the end of April 2015. Paper III was conducted in 2016 and paper IV in 2017.

Descriptive
In paper I, 80 acute burn patients were recruited from the year 2014 consecutively. The inclusion criteria were ages of 18 years or older and an expected length of stay of seven days. Patients with malignant disease, immunosuppressive disease, immunosuppressive drugs, and suspected or known risk of HIV infection or hepatitis were excluded. The patients were sampled with swabs from the nasal cavity, throat, and perineum (from day three supplemented with wound cultures). Clinical routine bacterial swabs/samples were also obtained from e.g. wounds, the bloodstream, trachea, and urine. The following patient data were noted: age, gender, date/time of injury, length of stay (LOS) in the hospital, mortality, type of burn (flame, scald, contact), height, weight, % total body surface area burned (%TBSA); on admission, medications and current diseases were noted. In all patients, the revised Baux score [45] (%TBSA + age + X*17 (X=1 if inhalation injury present, X=0 if no inhalation injury) was calculated.

In paper II, nine of 54 (17 %) patients did have resistant A. baumannii during the outbreak period (five months). The nine patients’ A. baumannii strains’ antibiotic susceptibility and microbes in the environment were retrospectively investigated.

Papers III and IV used the same experimental design with UVC light exposure to contaminated polycotton swatches and surface areas in the Burn Centre ward room, respectively. The Tru-D™ SmartUVC was used in both studies. The device was started outside the room using a tablet. When the decontamination circle had ended (set amount of irradiation reflected back to the device), the device deactivates, and completion was indicated on the tablet.
Bacterial sample

The bacterial samples in papers I and II were collected with cotton swabs by clinical routine and transported in charcoal medium (Copan diagnostics, Murrieta, CA, USA) to the Department of clinical microbiology, Uppsala University Hospital. Screening samples, additional clinical samples, and samples from the ward’s environment were cultured on specific agar plates.

Standard laboratory procedures and species identification, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) using Maldi Biotyper Microflex, Bruker (Bruker Corporation, Billerica, Ma, USA) was used.

Molecular analysis

Whole-genome sequences (WGS)

In paper I, the S. aureus isolates and bacterial genomic DNA were analysed with whole–genome sequencing (WGS) and sequence typing. DNA was prepared using a Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's recommendations for Gram-positive bacteria. The extracted DNA was controlled by gel electrophoresis and spectrophotometry for quality. DNA concentrations were measured using Quant-iT dsDNA B.R. assay and a Qubit instrument (Invitrogen, Waltham, Massachusetts, USA).

In papers I and II, after standardising the DNA extracts, the samples were transferred to the National Genomics Infrastructure (Stockholm, Sweden) for library preparation and WGS. Fragmented DNA in paper I was end-repaired, A-tailed, adapter-ligated, and amplified using Nextera DNA Library Prep kit (Illumina, San Diego, California, USA). Sequencing in both papers was performed on a NovaSeq SP-300 platform and NovaSeq 6000 SP platform (Stockholm, Sweden), generating 150 bp paired-end reads.

Multilocus sequence typing (MLST)

In paper I, multilocus sequence typing (MLST) profiles were assigned in Ridom SeqSphere+ [46] using the scheme for S. aureus available at https://pubmlst.org/organisms/staphylococcus-aureus. The Ridom SeqSphere+ software [46] was also used for core genome (cg) MLST analysis and to construct a minimum spanning tree (MST:s). The cgMLST scheme for S. aureus used consisted of 1861 targets. Isolates with a maximum of 24 allelic differences were designated as belonging to the same MST cluster.
Multi-locus sequence typing was performed in paper II and antimicrobial resistance genes were analysed according to the Pasteur and Oxford schemes [47, 48].

Arbitrarily primed polymerase chain reaction (AP-PCR) and Pulsed-field gel electrophoresis (PFGE)

In paper II, the carbapenem-resistant A. baumannii from the nine patients was first investigated with arbitrarily primed polymerase chain reaction (AP-PCR) at the Uppsala University Hospital's department of clinical microbiology. The pulsed-field gel electrophoresis (PFGE) analyses of the bacterial isolates were then analysed at the National Public Health Institute, Stockholm, Sweden.

Antibiotic susceptibility testing

After inoculating the culture in paper II, NordiCAST's (http://www.nordicast.org/) description for the antimicrobial susceptibility test was used on the isolates. Carbapenems such as meropenem and imipenem; trimethoprim/sulfamethoxazole; ciprofloxacin; tigecycline, the aminoglycosides gentamicin, tobramycin, amikacin; and colistin susceptibility were tested. Isolates with reduced susceptibility to one of the carbapenems were analysed for the presence of beta-lactamases in a Check-MDR CT103XL (Check-Points Health B.V., Wageningen, The Netherlands) according to the manufacturer's recommendations.

Infection prevention and control interventions

Several measures and interventions in paper II were investigated to identify the environmental source of infection (Figure 3). A separation in infection control measures before the outbreak and methods implemented during the outbreak could be seen. Stricter HH, PPE, dress code routines, stricter cleaning routines, disposal of broken materials, information, and teaching; weekly ward meetings concerning infection prevention and transmission of bacteria, especially A. baumannii; and education were performed in all wards involved in the outbreak. HH, PPE, and dress code were monitored, and the results were fed back to the staff which led to an increase in compliance. During the outbreak, numerous pieces of equipment and furniture were discarded due to rough or broken surfaces.
Figure 3. Timeline of the outbreak. A) Colours indicate hospitalisation time and location (ward). Arrow = first positive sample detected; yellow boxes – BICU; green boxes – Plastic surgery inpatient ward; orange boxes – Central ICU. B) Timeline of hand hygiene, personal protection equipment and dress code monthly measurements. C) Measurement timeline. Figure from paper II.
Experimental design

In paper III, ten polycottons (50/50 polyester/cotton) swatches, of which nine were contaminated with *Enterococcus faecium* (NCTC7171/CCUG33573) were used. The bacteria were obtained from the U.K. Health Security Agency and the Culture Collection University of Gothenburg (and were chosen for their ability to resist dehydration and being a common bacterium in humans), and 1 ml broth with $10^8$ colony-forming unit (CFU)/ml was used. One swatch was a negative (not contaminated) control, and two positive controls (contaminated) remained in the laboratory. Thus, seven swatches were transported to the burn centre, and five were distributed to five predefined locations in a ward room, two became control references outside the room. The UVC device was in the middle of the wardroom next to the bed, as it was done in the clinical setting, and the decontamination setting with a UVC dose of 22 000 µWs/cm² (sporicidal setting) emitted was used. The swatches were transported to the laboratory and put in Stomacher bags, where peptone water was added. The bags were processed for five minutes in the Stomacher 400 (Seward Medical, Worthing, UK) circulator, and the suspension subsequently vortexed for 10 minutes and quantitatively cultured. The experiment was repeated ten times.

In paper IV, disposable UVC-indicators (Intelleco Technologies AB, Gothenburg, Sweden) and an electronic (UVC) radiometer (Opsytec Dr. Gröbel GmbH, Ettingen, Germany) were used and positioned in ten different locations in an unoccupied manually cleaned burn centre ward room (five of the locations were the same as in paper III). The room was then decontaminated using the Tru-D™-UVC device positioned in the middle next to the bed, and the sporicidal setting was again chosen (22 000 µWs/cm²). Colour changes of the disposable indicators and the electronic radiometer readings were noted and compared for validation. The experiment was repeated ten times.

Statistical analyses

In Table 1, the statistical analyses are summarised. In paper I, the statistical analyses of the descriptive data were performed using basic R (version 4.1.1, 2021). Continuous data were expressed as median and range, and frequencies were calculated for categorical data. Logistic regression analysis was used for predictions of dependent and independent variables. Categorical data were analysed with odds ratio and Fisher’s exact test. Probabilities of $< 0.05$ were accepted as statistically significant.

The descriptive statistics of the nine patients’ in the outbreak, were presented in median and range in paper II; no other statistical analyses were performed.
The Wilcoxon signed-rank test was used in paper III to observe any statistically significant differences between median log_{10} reductions and surveillance samples.

Spearman's rank correlation coefficient was used to analyse the correlation between the variables “distance” and “dose of UVC” in paper IV. For calculations of differences in the median dose of UVC, the Mann-Whitney U-test (two-tailed) was used. Data were presented as box-and-whiskers and scatter plots. IBM SPSS Statistics for Windows (version 23, IBM Corp, Armonk, NY, USA) was used for the statistical analyses, and probabilities < 0.05 were accepted as statistically significant.

Table 1. *Statistical analyses*

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<td>Mann-Whitney U-test</td>
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Results

For more detailed results, see the manuscript and publications.

Paper I.

Eighty patients were included in the study (Figure 4). The median (range) age was 51 (19-93) years, more male patients were included 53/80 (66%), and the median BMI was 27kg/m² (15-47). The overall mortality among the study patients was 13/80 (16%). The median (range) stay at the burn centre was 13 days (1-94). The majority, 66/80 (82%), of the patients suffered from a flame injury, and 20/66 (30%) had a concomitant inhalation injury. On admission, *S. aureus* was found in the nasal cavity in 69% (n = 27/39) of the patients, followed by the throat in 54% (n = 21/39), and perineum in 28% (n = 11/39) (Figure 5).

![Figure 4. Schematic study inclusion flow chart. *16 dropouts due to death, early discharge, or sampling failures, remaining 64 patients at the end of the study period.*](image-url)
Figure 5. Venn diagram. Cohort 1. Percent, positive number of *S. aureus* isolates from 39 patients in the nasal cavity, throat, and perineum on admission.

The number of persistently colonised patients 25/80 (31%) was isolated from at least one sample from each period (1-5 days and 6-10 days). Twenty-one of 80 (26%) patients were intermittently positive over the three periods and 9/21 (43%) were positive on admission. In the group not colonised, the number of patients amounted to 18/80 (20%).

The number of samples for patients colonised on admission was significantly higher than for patients not colonised on admission (p < 0.05). Similarly, fewer samples were taken from patients who were never colonised compared to patients who were intermittently colonised or persistently colonised (p < 0.05).

There were no significant relations for *S. aureus* colonisation at the admission of type of colonisation with the patient's age, BMI or rBaux score. The patients that were colonised on admission tended to stay longer at the burn centre, particularly when admitted with more severe conditions corresponding to rBaux score >70 (p=0.05).

No statistical relation between the start of antibiotic therapy or colonisation status on admission or type of colonisation, was seen regarding antibiotic-free days from admission. Nevertheless, the lower the isolation frequency of *S. aureus* was in a patient, the shorter the antibiotic-free period from admission; this association was even stronger for more severely injured patients with rBaux score >70. No association between colonisation status and mortality was seen.

All patients carried unique isolates according to core genome multi-locus sequence analysis with a minimal allele difference of 91 alleles each. Of the patients with positive *S. aureus* isolates, 53 patients were available for further
analysis regarding the genetic relationship with WGS. The 258 isolates belonging to the 53 patients gave high–quality reads and *S. aureus* identity was confirmed with WGS. Sequences had a median (range) coverage of 181x (34 – 184) and covered a median (range) of 99.2% (97.0 – 99.7) of the cgMLST-scheme. A median (range) of 5 (1 - 18) WGS analysed isolates per patient were available.

**Paper II**

A patient's first positive sample of *A. baumannii* was noticed on Nov 4, 2014. Then eight more patients were observed during the following months. The *A. baumannii* isolates from the nine patients in paper II were resistant to several antibiotics, such as imipenem, meropenem, ciprofloxacin, and co-trimoxazol. The safety bed rail was the only place in the ward environment with the same band pattern in the PFGE analysis as the nine patients’ *A. baumannii* in the outbreak. The AP-PCR’s robust patterns for all isolates showed consistent patterns, and MLST showed that the isolates belonged to ST236 according to Oxford's MLST scheme and ST15 in Pasteur's MLST scheme. The *A. baumannii* outbreak was eventually contained at the end of April 2015, related to when the last infected patient was discharged. No further outbreak or cross-contaminations of *A. baumannii* was noted for five years following.

**Paper III**

All decontamination cycles had a median (range) time of 111 minutes (108 – 165) and the number of bacteria counted (CFUs) after decontamination showed a mean value of 1.37 log₁₀ units. The differences in the number between the samples and controls of CFU were statistically significant for all cycles. Under the bed, the most noticeable reduction of bacteria was seen (-1.97). The area with the minimum reduction in bacteria was in the wardrobe at the far end of the room (-0.57). A statistically significant decrease was, however, shown in all samples (Table 2).
Table 2. Reduction of CFUs of Enterococcus faecium after exposure to ultraviolet C (UVC) light compared with control samples serving as references for statistical analysis. From paper III.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Transported to burn centre</th>
<th>Exposed to UVC light (22/00 μW/cm² reflected dose)</th>
<th>Samples</th>
<th>Median (range) log₁₀CFU count</th>
<th>Log₁₀ reduction (compared with control sample(s))</th>
<th>P-value (Wilcoxon signed rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>No</td>
<td>No</td>
<td>10</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive control</td>
<td>No</td>
<td>No</td>
<td>20</td>
<td>8.12 (7.68–9.10)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control (refluence)</td>
<td>Yes</td>
<td>Yes</td>
<td>20</td>
<td>7.91 (7.50–8.01)</td>
<td>9</td>
<td>1.000</td>
</tr>
<tr>
<td>On bed</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>6.30 (5.59–7.36)</td>
<td>1.42</td>
<td>0.005</td>
</tr>
<tr>
<td>Under bed</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>5.95 (4.98–7.14)</td>
<td>1.97</td>
<td>0.005</td>
</tr>
<tr>
<td>Writing desk</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>5.99 (5.97–6.08)</td>
<td>1.93</td>
<td>0.005</td>
</tr>
<tr>
<td>Sink</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>6.91 (6.55–7.55)</td>
<td>1.01</td>
<td>0.005</td>
</tr>
<tr>
<td>Cupboard</td>
<td>Yes</td>
<td>Yes</td>
<td>10</td>
<td>7.35 (6.80–7.38)</td>
<td>0.57</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Paper IV

The decontamination cycles had a total median (range) time of 74 minutes (70 – 82). The median UVC dose delivered to different areas in the room was 266 ml/cm² and the range variation was between 15.9 ml/cm² and 1068 ml/cm² (Figure 6). In places where there was no shadowing and in direct line of sight, there were statistically significant higher UVC doses measured ($p = 0.019$). The disposable indicators adequately detected the UVC dose received compared with the readings of the radiometer used (Figure 7).

**Figure 6.** A) on the nurse’s desk, B) on the bed, C) under the bed, D) in the basin, E) in the wardrobe, F) on the ledge on the wall, G) in the drawer of the left ceiling mounted pendant, H) on the infusion pump, I) on the drawing surface of the right ceiling mounted pendant, and J) behind the desk chair. From paper II.
Figure 7. Representative picture samples of the disposable indicators' colour changes from different locations receiving different UVC doses. Positions A, B, C, F, and H - (pink); Positions G, I, and J (Orange); Positions D and E (Yellow). The letters indicate the positions of the indicators. Yellow – not significant, Orange – enough for MRSA decontamination, Pink – enough for Clostridium difficile decontamination. From paper II.
The main aim of this thesis was to investigate microbiological aspects in burn care, including bacterial transmission, and possible measures to combat this and reduce infection rates.

In paper I, we noted that 49% of the patients on admission were colonised by *S. aureus*, and that the *S. aureus* was part of the patient’s microbiological flora. Although 49% is a higher prevalence of *S. aureus* colonisation than 30% previously described in the general population [16, 49, 50], it is in line with other Swedish burn patient data [51].

Bacterial screening swabs were used to explore *S. aureus* colonisation in patients. The most accurate procedure, i.e. "gold standard," though, for bacterial culturing, is a tissue biopsy where CFU > 10^5 is regarded as infection and < 10^5 is regarded as colonisation [52, 53]. However, the most common clinical procedure is the ordinary swab from the anterior nares [54, 55]. Our results confirm that the nasal cavity is the best locale from which to obtain *S. aureus* (69%), as previously have been shown in other studies [56]. In the group of patients who will undergo surgery, it is essential to have good infection prevention. Several studies have shown that the strains found in the wounds are mainly the patient's own, by analogy with what was found in this study [57, 58]. Furthermore, several different kinds of studies, e.g., liver transplant and diabetic foot wound studies [59, 60], have also demonstrated a correlation between nasal carriage and wound/bloodstream infections with *S. aureus*.

The Gram-negative bacteria, *A. baumannii*, which is described in paper II, has successively become an increasing problem in intensive care and (especially) burn units [61] worldwide related to its increasing antibiotic resistance profile. High antibiotic pressure and the bacteria’s inherent properties in terms of the ability to form biofilms, its high tolerance to biocides and desiccation, and the ability to survive a long time on dry surfaces in the environment and its ability to adapt its genome quickly has made *A. baumannii* successful in developing antibiotic resistance [62]. Hence, *A. baumannii* has gained crucial benefits and made it possible for numerous clones to spread in hospital environments around the world [63]. In paper II, we showed several infection control measures performed to impede the spread of the bacteria in the 2014/2015 outbreak in our burn centre. During December 2014, compliance with HH, PPE, and dress code had decreased to 70%. Similar decreases in compliance have been shown in several other studies highlighting that higher compliance
with HH, PPE, and dress code is needed to keep patients safe from contracting bacteria from other patients [64, 65]. A study by Chang et al. showed that a high workload affected the HCW adherence to correct HH routines [66]. Manually monitoring of compliance with HH can be challenging due to observation bias, the Hawthorne’s effect and resource consuming. Automatic monitoring is in demand and discussed worldwide, especially in light of the COVID-19 pandemic when the problem was further actualised [67].

The burn centre’s plastic surgeons consulted with, and changed dressings in the burn patients treated in other wards than the burn centre (patients 4 and 7) along with one or two HCW from the burn centre. Hence, the HCW and plastic surgeons were likely to have transmitted the bacteria to these outsourced patients from the burn centre.

The knowledge of the outbreak and the infection control measures increased over time, with increased teaching opportunities and attention [68]. In a qualitative review by Chatfield et al. it was clear that adherence to routines among the HCW was connected to adequate training, management, and resource support [69].

Chatfield et al. showed that a “hidden” bacterial source could be found in broken items such as textiles in furniture, worn instruments, and medical equipment. However, only one sample with the outbreak strain was isolated from the hospital environment in our study; on a broken bed rail. It can be seen as an indication to that the bacteria were transmitted in the first place via the patient /HCW’s hands and that the cleaning was insufficient. On the other hand, at that time the protocol for samplings in the environment was inconsistent and without structure. Regular reviews, structured cleaning and environmental sampling protocols are necessary for all HCW to complete [70].

Clonal complex ST15 has been found in isolates from around the world, e.g., South America, Eastern Arabia, and Europe, and isolates producing OXA-23 were shared among these [71-74]. OXA-23-producing and OXA-23-non-producing ST-15 isolates are highly resistant to meropenem and imipenem, as has been described in other studies [73]. We noted that all findings of isolates in this study were susceptible to colistin and tobramycin.

Weinberg et al. concluded that there should be other complements than antibiotics to fight multiresistant pathogens. Specific, already existing, alternatives to antibiotics are, e.g., bacteriophages and vaccines/antimicrobial peptides [75]; however, most of the previous studies are in vitro studies and more clinical studies are needed for development in the future.

Infection prevention strategies, protocols, and checklists at the ward level when suspicion of an outbreak arises, are essential for effective combat and containment. During our work with paper I, where we investigated S. aureus colonisations in patients cared for at the burn centre (coinciding with the A. baumannii outbreak described in Paper II), we did not have any spread of S. aureus. A hypothesis is that the S. aureus isolates were less virulent, and the increased infection prevention focus, due to the A. baumannii outbreak also
prevented *S. aureus* transmission. However, this hypothesis has not been further explored.

UVC has been used in the food and water industry for a long time for decontamination and purifying of contagions. During a bacterial outbreak in a hospital, all routines must be reviewed and measures must be taken, which could include exploring non-hospital settings and scrutinising other supplementary methods to handle the outbreak. Very little data were available at the time of our outbreak concerning the use of UVC decontamination in a clinical setting. As we could not find any publications on UVC decontamination in a burn centre setting, we set up two studies to substantiate the science. As the UVC decontamination device was introduced in our burn centre in March 2015 in connection with the *A. baumannii* outbreak, we also wanted to investigate how well it lived up to the manufacturer's statement regarding decontamination of bacteria in different locations and with different textiles.

Paper III and IV had the same basic experimental set-up; however, there was a difference in decontamination time between the studies. The different lengths of time for the decontamination processes were probably related to the light tubes being changed on the device between the studies. However, the device measures the amount of UVC reflected to ensure that the setting (22 000 µWs/cm²) is reached before it turns off, thus compensating for the lower light tube effect with a longer cycle.

When compared to the laundering of textiles (60° C/140° F) that reduces *E. faecium* CFUs with 3-4 log₁₀ and if tumble drying or ironing is added, up to 9 log₁₀ CFU [76], paper III showed that UVC-decontamination of textiles was inferior in terms of reducing *E. faecium* CFU (however still statistically significant: log₁₀-reduction = 1.97, i.e., 99% eradication). The structure of the fabric makes it difficult for UVC to reach everywhere within the textile. Hence, the bacteria were shielded from the UVC light's bactericidal effect [77]. Thus, when it comes to textiles, UVC cannot replace laundering but may be seen as an adjunct or an alternative when laundering is not possible. Nevertheless, one may as well conclude that textiles that cannot be sufficiently decontaminated have no place in a hospital setting and should be replaced.

In paper IV, we examined the received dose of UVC in different areas in a BICU ward room after an automated UVC decontamination. Previous studies have shown that bacterial decontamination using UVC-based devices [40, 44, 78] is feasible, and there are many appropriate uses for this technology. We concluded that the received UVC dose varied related to shadowing objects. Thus, our findings, along with others [79, 80], suggest that one needs to be cautious in areas that are not in a direct line of sight with the light source. As we expected, the readings from the radiometer were significantly lower in such areas. For example, position G (shadowed) was within 100 cm from the light source but received a low UVC dose.

For disposable indicators further away, such as position H over 100 cm, but in a direct line from the light source, we could see that a full colour change
occurred from yellow (not significant) to the highest level (corresponding to Clostridium difficile eradication level). Since UVC decontamination technology is increasingly used in healthcare, it is crucial to access tools that offer quality control and assurance that the decontamination process has been adequate. Simple colour-changing, disposable indicators are cheap and easy to handle as they can be read immediately.

Methodological considerations
Sample
The patients in papers I and II were population-based. Sweden's national health care for severe burns is shared by two centres, Linköping and Uppsala. Both burn centres cover Sweden's (2016) 10 million population [81].

In paper I, there was a decrease in the number of samples after day six. This could, of course, have affected the outcome. Equivalent number of samples should have been ensured on all study days. Furthermore, infection and sepsis parameters should have been included in the protocol.

Paper II, a descriptive retrospective study, has several constraints due to the design. There was some lack of documentation, e.g., the patient's movement patterns to and from the different wards. Various documents interpreted in retrospect tend to include so-called recall bias when events are to be reported and interpreted after a long time has passed [82]. For example, when (non-research) data in documents are interpreted afterwards, the person who interprets them may misunderstand the text if it is missing information/not sufficiently clearly documented. The environmental sampling seemed not to follow a specific schedule but depended on who was performing the sampling at the time. The isolates of \textit{A. baumannii} are challenging to analyse with reference to their specific biology. However, a long time has passed since the outbreak, and analysis methods with progressive technical innovations and more genetically efficient analyses, such as WGS, have been developed. Hence, the study probably benefited from the write-up latency.

Paper III is defined by the fact that the number of samples, pathogens, and a blend of fabric, was limited due to the capacity of the laboratory and the ward's capability to handle the samples. Still, enough data was collected to demonstrate a reduction of bacteria in textiles.

Paper IV was also limited by the number of indicators available and the different angles/positions in the wardroom. By using more indicators and positions, it would have been possible to investigate angles, distances, and shadowing effects more thoroughly.

Generalisability and Clinical implementations
Regarding generalisability [83], ethnicity, and consideration of which ethnic group the patient belonged to, we did not examine ethnicity in paper I. Hence
it has been shown that certain ethnic groups have a higher incidence of \textit{S. aureus} [84, 85]. That could have been an interesting point of view to investigate. Furthermore, the sample demands a larger population. Men were overrepresented in our study population, as in other studies investigating the burn population [84]. Regarding age, we examined only adult patients. This limitation means that knowledge about the child population remains unknown.

Considering a Letter to the Editor, by Py et al. [86] regarding our descriptive article on \textit{A. baumannii}, they were highlighting the same infection prevention dilemmas in their burn centre, and it shows that more studies need to be compiled and evaluated for more knowledge of aggressive infection prevention when \textit{A. baumannii} is colonising the burn patient [85].

The COVID-19 pandemic 2020-2022 showed an increased interest in UVC and other decontamination methods in ICU wards/hospitals and general society. A UVC decontamination device is still used in our burn centre, and the cleaning protocols are continuously updated. A greater focus on infection prevention education for new employees has been established and HH, PPE, and dress code surveillance are continuously monitored. The close dialogue with the infection prevention and control and cleaning departments continues.

Future research

The findings in paper I could be a start in a future study to examine the relationships and differences in patients’ other bacterial flora and \textit{S. aureus} and see if we can explore any pattern. For example, are patients with \textit{S. aureus} on admission more likely to be affected by Gram-negative multi-resistant bacteria and various fungi? Is there a relationship between different \textit{S. aureus} strains and mortality in burn victims? A multicentre study would give more strength to the study.
The main conclusions in this thesis are:

- The \textit{S. aureus} colonisations in burn patients, during hospital treatment, arise mainly from the patients' endogenous flora brought to the hospital on admission and are probably strongly unrelated to transmission.

- That \textit{S. aureus} colonisation in burn patients contributes to longer hospitalisation at the BICU but in this study did not contribute to higher mortality.

- That high awareness, and increased compliance with, hand hygiene, personal protection equipment, dress code, cleaning protocols for the environment and equipment contributed to a concept of success for stopping the spread of ST 15 OXA-23 producing \textit{A. baumannii}.

- The efficacy of automated UVC decontamination on \textit{Enterococcus faecium} in fabrics in a clinical setting is inferior to laundering, and is not an alternative for decontamination.

- The UVC-dose received in different areas in a BICU ward room, after an automated UVC decontamination, varies significantly. Moreover, measures must be taken to ensure adequate UVC dose in all areas, e.g., using strategically placed indicators or UVC detectors.

- The disposable UVC-dose indicator we trialled correlated well with parallel radiometer readings and was thus validated.
Sammanfattning på svenska


I artikel I, vilken är en del av ett större projekt där bakterieförekomst hos brännskadepatienter kommer att undersökas, studerades specifikt brännskadade patienters kolonisation av *S. aureus* vid inläggning och vidare under vårtdagens tio första dagar. *S. aureus* är en bakterie vilken snabbt koloniserar brännskadepatienten och kan ge upphov till flertalet farliga vårdrelaterade infektioner såsom pneumoni, infartsrelaterad sepsis och kolonisation av hjärtklaffar. Det framkom i denna studie att de *S. aureus* bakterieisolat (isolat = en koloni av identiska mikroorganismer) med vilka patienterna var koloniserade med i samband med inläggningen var de samma de hade under vårdtiden. Av de 53/80 patienter som var koloniserade med *S. aureus* hade ingen patients *S. aureus* samma typ av isolat som en annan patients *S. aureus*. Således hade patienterna sannolikt inte blivit smittade av miljön eller personalen under de 10 första dagarna efter inläggning, utan av den *S. aureus* de hade med sig vid inläggningen. Vidare visade studien att *S. aureus* påverkade vårtdistlängden på brännskadecentret hos de större brännskadorna, dock sågs ingen påverkan på mortaliteten.

I det andra arbetet undersökte vi olika åtgärder, bl. a. följsamheten till basala hygien- och klädrutiner, vid ett utbrott av bakterien *A. baumannii* på vårt brännskadecentrum i Mellansverige. Slutsatsen i det arbetet var att den förbättrade följsamheten till basala hygien- och klädrutiner tillsammans med det utökat samarbetet med vårdfysiska avdelningen, utvecklandet av nya städprotokoll, omfattande städaåtgärder, undervisning och förhöjd medvetenhet hos hela personalstyrkan utgjorde ett lyckat koncept för att bemästra smittspridningen. Provtagningar i miljön och av patienter bidrog ytterligare.

Vi vet att städningen i ett vårdrum kan variera i noggrannhet, men genom att lägga till en UVC städrobot, där UVC-strålningen förstör DNA/RNA i bl.a. bakterier och därigenom skadar deras förplantningsförmåga, kan det komplettera städningen. Hur garanterar man dock tillräcklig dekontamination? I
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)