The effect of preoperative skin preparation on bacterial growth during cardiac surgery
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The effect of preoperative skin preparation on bacterial growth during cardiac surgery
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Abstract


Routine products are used and procedures are followed in order to prevent and minimize the bacterial contamination of the surgical wound, and thus reduce the risk of postoperative wound infections. The overall aim of this thesis was to investigate the effect of different preoperative skin preparation before cardiac surgery.

In study I, 10 healthy volunteers were compared in time to recolonization of the skin and bacterial growth with or without plastic adhesive drape. Bacterial samples were taken as paired samples on both side of the sternum. Plastic drape on disinfected skin seems to hasten recolonization compared with bare skin. In study II, 135 cardiac surgery patients were comparing plastic adhesive drape versus bare skin on the chest regarding intra-operative bacterial growth. Plastic adhesive drape did not reduce the bacterial recolonization or wound contamination, *P. acnes* colonizes males more often than females and *P. acnes* is not affected by disinfection with 0.5% chlorhexidine in ethanol. Study III, compared the leg harvesting site with or without microbial skin sealant in 135 CABG patients regarding intraoperative bacterial growth and postoperative wound infection. Almost no bacterial growth was found during surgery regardless of the use of microbial skin sealant and bare skin. A high incidence of postoperative wound infections (16.8%) in 2 month follow up was present and SSI was largely caused by *S. aureus*, i.e. other bacterial species than observed intraoperative. Study IV, a descriptive study using phenotypic and genotypic methods investigate susceptibility to chlorhexidine among *S. epidermidis* indicating that *S. epidermidis* isolates following preoperative skin disinfection are sensitive to chlorhexidine.

*Keywords*: OR, plastic adhesive drape, microbial skin sealent, chlorhexidine

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# ABBREVIATIONS AND DEFINITIONS

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<tr>
<td>AORN</td>
<td>Association of Perioperative Registered Nurses</td>
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<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CABG</td>
<td>Coronary artery bypass graft</td>
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<td>CDC</td>
<td>Centers for disease control and prevention</td>
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<td>CoNS</td>
<td>Coagulase-negative staphylococcus</td>
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<td>CFU</td>
<td>Colony-forming units</td>
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<tr>
<td>CPB</td>
<td>Cardiopulmonary bypass</td>
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<td>CVK</td>
<td>Central venous catheter</td>
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<td>ICU</td>
<td>Intensive care unit</td>
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<td>HAI</td>
<td>Healthcare-associated infections</td>
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<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<td>MDR</td>
<td>Multi-drug resistant</td>
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<td>OR</td>
<td>Operating room</td>
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<td>P. acnes</td>
<td>Propionibacterium acnes</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PJI</td>
<td>Prosthetic joint infection</td>
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<td>S. aureus</td>
<td>Staphylococcus aureus</td>
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<tr>
<td>SALAR</td>
<td>Swedish Association of Local Authorities and Regions</td>
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<td>SIS</td>
<td>Swedish Standards Institute</td>
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<td>SSI</td>
<td>Surgical site infection</td>
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<td>SWI</td>
<td>Sternal wound infection</td>
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<td>QAC</td>
<td>Quaternary ammonium compounds</td>
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CONCEPTS USED IN THIS THESIS

Colonization  The skin is colonized by the normal bacterial flora

Recolonization  Colonization on the skin after preoperative skin disinfection

Contamination  A contamination of bacteria in the wound

Surgical Wound Classification

Class I/ Clean: An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow nonpenetrating (blunt) trauma should be included in this category if they meet the criteria.

Class II/ Clean-contaminated: An operative wound in which the respiratory, alimentary, genital, or urinary tract are entered under controlled conditions and without unusual contamination. Operations involving the biliary tract, appendix, vagina and oropharynx are also included in this category, provided no evidence of infection or major break in technique is encountered.

Class III/Contaminated: Open, fresh accidental wounds, operations with major breaks in sterile technique (e.g. open cardiac massage), or gross spillage from gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included.

Class IV/Dirty-Infected: Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation.

Ref Mangram 1999
LIST OF PUBLICATIONS

This thesis is based on following papers, which will be referred to in the text by their Roman numerals:


III. Bacterial growth and wound infection following saphenous vein harvesting in cardiac surgery; a randomized controlled trial of the impact of microbial sealant. Falk-Brynhildsen K, Söderquist B, Friberg O, Nilsson U. Submitted


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INTRODUCTION

Surgical site infection (SSI) is a wound infection, which occurs after a surgical procedure. It causes serious healthcare problems, human suffering, increased morbidity and mortality, prolonged length of hospital stay\textsuperscript{1} increased risk for re-admission,\textsuperscript{2,3} and increased costs for society\textsuperscript{4-7} It is therefore an important duty for the healthcare system to work preventively to decrease SSI.\textsuperscript{1}

The greatest risk for a patient being contaminated by bacteria occurs from the time of incision to the time of wound closure.\textsuperscript{8,9} SSI after surgery are predominantly caused by pathogens that originating from the patient’s endogenous skin flora.\textsuperscript{8,10-15} However, there is also a risk of surgical site infections due to exogenous sources of contamination such as operating room (OR) environment, surgical team and hospital staff.\textsuperscript{16-21}

The responsibility of an OR nurse is to systematically plan and organize the work associated with a surgical procedure, such as infection control and aseptic principles, and to optimize the patient’s skin before surgery. The focus of this work is to minimize the bacteria present at and around the proposed incision site.\textsuperscript{22}

The questions for this research have emerged from the daily work as an OR-nurse in cardiac surgery. Routine products are used and procedures are followed in order to prevent and minimize bacterial contamination of the surgical wound, and thus reduce the risk of postoperative wound infections. The overall aim of this thesis is to investigate the effect of different approaches to preoperative skin preparation before cardiac surgery.

Knowledge has been lacking in this surgical area. It is hoped that the findings of this research can contribute to evidence-based guidelines, and thereby increase patient safety by decreasing the risk of SSI.
BACKGROUND

History
The history of microbiology and infection control is short. In the middle of the 19th century, Louis Pasteur and Robert Koch discovered, by experiment, that specific microorganisms developed certain diseases. Louis Pasteur introduced e.g. pasteurization and rabies vaccine 23 and discovered major pathogens as *streptococci* and *pneumococci*.24 Robert Koch introduced staining of bacteria and solid media and he also discovered cholera and tuberculosis bacteria. The methods they developed defined many bacterial diseases and microorganisms. *Staphylococci aureus* was discovered in 1880 by the surgeon Alexander Ogston. Ogston discovered it in pus from surgical abscesses. 25

During the same era, Joseph Lister learned about Pasteur’s principles and started to use the antiseptic solution carbolic acid to eliminate bacteria, thereby decreasing wound infection in patients with severe bone fractures. Lister also introduced disinfection of patients’ skin, hand disinfection of surgeons, wearing of gloves, sutures, and disinfection of instruments.24 These practices resulted in a decrease in surgical mortality after amputations from 46% to 15%.23 Lister also spraying of carbolic acid for air cleaning of airborne sources of infection. This procedure was without success.26

The methods used by Florence Nightingale during the Crimean war, pivotal for the development of infection control. Nightingale reduced mortality from 42% to 2% by improving several hygiene principles and routines. After the war she contributed to reforming health care in England and her nursing school became a model for other countries.27-29

In the early 1900’s, George Emerson Brewer introduced autoclaves to sterilize instruments. This led to a decreased infection rate for clean surgery from 39% to 3.2%. Later, special work clothes were introduced for use in the OR (known as scrubs), as well as changing of surgical gowns between patients.23

The discovery of antibiotics was one of the major medical breakthroughs of all time. Prior to 1950, before antibiotics were widely used, nearly 50% of all deaths were caused by infections.30 In 1969 Polk et al. reported a decreased infection rate after abdominal surgery due to preoperative antibiotic treatment.31 Cardiac surgery was one of the last medical procedures to implement preoperative antibiotic prophylactic routinely.24
The Skin

The skin is the largest organ of the human body and acts as a protective barrier both mechanically, chemically and against microorganisms. It consists of three main layers - the epidermis, dermis and hypodermis. The bacterial population on the skin helps us to inhibit establishment of harmful yeast and fungal infections, and produces bactericidal compounds which protect the skin against bacterial invasion. The normal flora is both a defense mechanism against infection and a source of potentially pathogenic organisms. The numbers of the flora vary on different parts of the body and can vary depending on the age and physiologic condition.

An average of five million bacteria lives permanently on each square centimeter of human skin. A person emits about $10^4$ skin particles per minute upon movement and nearly 10% of these skin particles are bacteria-carrying. The epidermis is composed mainly of dead skin cells that are constantly being shedded, and new epidermal cells are normally regenerated in 5-6 weeks.

Temporary bacteria on the skin are known as transient flora. The bacteria present on and in the skin are called resident flora. Transient colonization occurs through contact with other people or by touching a contaminated surface in the environment. These bacteria are temporarily present for short or extended periods of time. Transient skin flora is likely to include potential pathogens and antibiotic resistant bacterial strains. However, transient flora lives in the superficial layers of skin and is easier to eliminate through skin washing and disinfection. In contrast, the resident skin bacteria, which lives both on the skin surface and also in the deeper skin layers (20% buried in hair follicles and sweat glands), cannot be eliminated. The types of resident skin flora that dominate the skin are coagulase negative staphylococci (CoNS), Diphteroids, and Propionibacterium sp. All of which are present all over the skin. On the body, S. aureus is present in 20 to 30% of the population, and the anterior nares serve as a nest. Nasal S. aureus colonization is an important contributing factor in the development of infection.
Microbiology

Staphylococcus

The genus *Staphylococcus* consists of 40 species but not all cause human infections (Fig. 1). Eleven species are present on the skin, in the nose and the throat.

The bacteria are Gram-positive cocci and are about 0.5-1.0 μm in diameter. In microscopy they are present as clustered grapes, hence the name.

Staphylococcus species can be divided into two groups: the coagulase-positive group which can clot plasma; and the coagulase negative group which doesn’t have the clotting ability. *S. aureus* is coagulase-positive.

Two of the most common microorganisms causing infection after cardiothoracic surgery belong to the *Staphylococcaceae* family. These are *S. aureus* and CoNS, most often *S. epidermidis*. *S. aureus* is a highly virulent bacteria that can cause purulent wound infections, such as surgical site infections, endocarditis, and infections of foreign materials.

*S. epidermidis*, the most commonly isolated CoNS, are low-virulence pathogens which were previously considered as opportunistic bacteria. CoNS are on the skin overlaying the sternum in 80 to 90% of the population. During the last 20 years however this perspective has changed and *S. epidermidis* is now considered to have important virulence factors such as adhesion to surfaces and production of slime. After foreign material implantations, CoNS are the most common cause of wound infection because the bacteria produce a thick biofilm which makes them inaccessible to the immune system. Further, antibiotic treatment is difficult and lengthy.
**P. acnes**

The genus *P. acnes*, is a gram-positive facultative anaerobe (Fig. 2), and the bacterium consists of different strains, Type I and Type II.66,67 Recently, several species have been identified and classified into subtype groups 1A, 1B, II and III.68,69

*P. acnes* is commonly found on the skin flora, associated with hair follicles and sebaceous glands, and resides mostly on the face, back, shoulder and the chest. It has also been identified in the oral cavity, intestinal tract and conjunctiva.70 Men have a greater prevalence of *P. acnes* than women71,72 and *P. acnes* is well-known as a cause of acne vulgaris.70 *P. acnes* has also been reported to cause sternal wound infection (SWI) after cardiothoracic surgery5,73-78 neurosurgery79,80 shoulder surgery81 and surgeries with prosthetic body materials82 such as orthopedic implants.83 *P. acnes* has also been identified in prostate tissue and may have a role in prostate carcinogenesis.84

**Wound Healing**

Wounds can be classified as acute or chronic. Acute wounds usually include surgical operation wounds and trauma wounds. Within 48 hours, most wounds are sealed and bacteria can no longer penetrate into the wound.85 Risk of infection varies, not only depending on the type and amount of bacteria that reach the wound, but also on patient factors, anesthesia, surgery, and preoperative procedures.
Factors Which Affect Wound Healing and May Influence the Risk of SSI

**Surgery**

- Surgical classification \(^8\)
- Presence of foreign implantations \(^{33,46,86}\)
- Surgical /Suturing technique \(^{11,12,87-89}\)
- Hematoma \(^90\)
- Diathermy \(^{91,92}\)
- Dead space \(^{12,89}\)
- Poor Hemostasis \(^{8,12,93}\)
- Duration of wound drainage \(^{12}\)
- Duration of operation time \(^{8,11,12,94}\)
- Wound care, maintain sterile coverage \(^{8,12,89,92,95}\)

**Anesthesia**

- Prophylactic antibiotics and timing \(^{12,89,96-102}\)
- Body temperature, maintain normothermia intraoperatively (besides ECC) and postoperatively \(^{12,89,96,103}\)
- Blood transfusion \(^{8,12,96,104}\)
- Inspired oxygen \(^{89,92,105,106}\)
- Contamination/duration of central venous catheter (CVK) \(^{18,107-111}\)

**Patients Factors**

- Obesity \(^{8,12,14,94,112-117}\)
- Diabetic, tight blood glucos control postoperatively \(^{11,12,58,88,89,94,96,115-119}\)
- Smoking \(^{11,12,117,120,121}\)
- Poor nutrition \(^{122}\)
- Age \(^{8,11,117}\)
- *S. aureus* nares carriers \(^{11,12,54,123-125}\)
- Female gender \(^{88,113,115,126,127}\)
- Malnutrition 12
- Immunosuppressive therapy 12,89
- Ongoing systematic or local infections 89
- Long preoperative hospital care 9,12,128
- Infection registration 129-131

Factors that Reduce the Risk of Contamination during Clean Surgery
- Hand cleansing/disinfection 8,12,92,132-135
- Preoperative chlorhexidine showers 12,32,136,137
- Preoperative skin disinfection 11,12,138-140
- Removing hair with clipper or depilatory creams 12,89,96,141
- Adequate ventilation in the OR 12,26,142
- Tightly woven OR clothing 20,143
- Door-openings minimized 92,144
- Sterile sheet drapes used 12,145,146
- OR nurse and surgeon: sterile gown, doubled gloved with indicator gloves, wearing long surgical hoods, and facemasks 12,23,92,147
- OR team: facemasks and wearing surgical hoods 12,23
- As few persons as possible in the OR 8,10
- No staff with skin infection on their hands permitted in the OR 12,23,24

These above-mentioned factors influence the exposure to bacteria which may affect wound healing and increase the risk of SSI. To prevent infection, all members of the OR team need to have evidence based knowledge on how this can be accomplished. They also need knowledge of microorganisms such as virulence, antigen, toxin production, sensitivity to antibiotics, disinfectants, ability to survive outside the host and adaptability. 148
Incidence of Sternal Wound Infection and SSI after Vein Harvesting

About 6000 patients undergo cardiac surgery in eight different hospitals in Sweden every year. Approximately 500 cardiac surgery procedures are performed annually at Örebro University Hospital. The total incidence of complications from postoperative sternal wound or/and leg wound infection has been reported to range between 2% and 20% of patients who underwent a coronary artery bypass surgery (CABG). In SWI only, the total reported incidence varies from 0.5% to 9.7% of patients. Leg wound infection is a more common complication than SWI with the incidence rate after saphenous vein harvesting ranging between 1.6% and 17.7%.

One reason for the wide range in reported incidence is likely to be the inconsistent diagnostic criteria used.

Definitions of SSI

The most commonly used definition of SSI is the one published by the US Center for Disease Control and Prevention (CDC). Briefly, SSIs are divided in superficial and deep infections where a superficial SSI involves skin and subcutaneous tissues, and a deep SSI involves muscle, fascia and organ-space tissues. The infection has to occur within 30 days of the operation, or within a year in the case of prosthetic implant surgery. The diagnosis is determined by positive bacterial cultures, purulent drainage or by a physician’s clinical judgment. The classification can be criticized for not including infections presenting later than 30 days postoperatively, and for including a subjective criterion such as “definition of SSI by a physician” that overrides other criteria.

There are other classification systems such as the ASEPSIS-score which is a numerical score that is based on a number of specified signs and symptoms of impaired wound healing such as purulent discharge, antibiotic treatment, need for surgical revision etc. It thus provides an objective measure of the severity of a wound complication irrespective of the cause thus avoiding the more or less impossible dichotomous definition of SSI or no SSI.
OR Infection Control

The aim of strict hygiene routines in the OR is to prevent bacterial contamination of the wound and thereby reduce the risk of postoperative wound infection.

The main sources of infection and routes of transmission vary with the type of operation and the hygienic and aseptic measures taken. In clean surgery such as orthopedic surgery, cardiac surgery, and vascular surgery with implantations, both the patient and staff skin flora provide a risk of infection. Nonetheless, it’s the patient’s own endogenous skin flora which is the most common cause of SSI in these procedures. OR staff members cause exogenous bacterial contamination, and they are also the primary cause of airborne infection. Skin particles are the major source of airborne contamination in the OR, and their amount varies based on the number of people in the OR. These airborne bacteria contaminate the surgical wound either directly by falling into the wound or indirectly by contaminating surgical instruments, fluids, materials, or implants. The risk of contamination is also influenced by the amount of time the tissue is exposed.

An OR typically has a special ventilation system to reduce the number of bacteria. The number of bacteria is measured in colony-forming units (CFU/m³). The SIS OR 39:2012 (Swedish Standards Institute) is a new stricter standard aimed at reducing airborne infection through special ventilation and more tightly woven scrub suits. To maintain proper ventilation, air pressure, and air flow in the operating room, doors should be kept closed. Frequent door opening during surgery results in increased contamination of the air. A special working uniform, (Standard EN 13795:2011), must be used in the OR department. In cardiac surgery the surgical team wears masks to prevent droplets falling into and infecting the wound and to cover beards. OR staff hair should be covered with surgical hoods. Skin friction, such as that from clothing rubbing, increases the number of skin particles in the OR. Combining appropriate ventilation with the usage of special working uniforms decreases the level of bacteria to 5 cfu/m³.

Direct bacterial transmission from staff to patient may occur if staff members have a skin infection, atopic eczema or psoriasis. In this case the staff members in question should not be allowed in the OR due to the risk of S. aureus colonization. Bacteria from the hands of the surgical team can also contaminate the patient through holes in the gloves.
Patients Preoperative Skin Preparation

Disinfection
To prevent SSI, the preoperative skin preparation procedure begins with showers twice at three separate times, using 4% chlorhexidine soap prior to surgery. This happens i) before admission to the ward ii) the evening before surgery at the ward and iii) in the morning on the day of surgery. These procedures are set out in the guidelines for patients undergoing cardiac surgery, vascular surgery, and orthopaedic surgery in Sweden. However, there is no evidence that confirms the effectiveness of performing this number of full body showers before an operation in order to minimize the risk of SSI. The aim of the chlorhexidine showers is to remove transient flora and reduce the number of resident organisms from the skin to a minimal level, and thus prevent SSI. However, as published in a Cochrane Review and in a study of Chlebicki et al., no evidence was found that chlorhexidine showers led to fewer SSI, although reduced patient skin flora was shown.

Immediately before surgical incision, antiseptic skin disinfection is performed at the skin incision site. The following guidelines are recommended by several organizations, for example; Swedish National Board of Health and Welfare / SALAR CDC AORN Several antiseptic agents are available for preoperative preparation. Larson describes six types of antiseptics for local application: Iodine/iodophors, chlorhexidine gluconate, alcohol, hexachlorophene, parachlorometaxylenol, and triclosan.

The most suitable antiseptics for preparation are iodine/iodophors, and chlorhexidine. These bactericidal antiseptics are equally effective against gram positive and gram negative bacteria. Iodine/Iodophors are not used for skin disinfection in Sweden. One reason for this is that they may cause irritation and skin damage, as well as allergic or toxic reactions. The advantage of chlorhexidine usage is that it has a prolonged effect lasting for several days due to its bactericidal properties that destroy bacteria cell membranes as well as the cytoplasmic membrane. However, the efficacy of chlorhexidine may be reduced by the presence of biological material or a biofilm. Additionally, bactericidal efficacy is pH dependent. In a randomized study, Darouiche et al. show that chlorhexidine-alcohol reduces the risk of SSI by 41% compared with povidone-iodine. Resistance to chlorhexidine has been reported in gram negative bacteria, but despite prolonged use, no
significantly relevant resistance development has been shown. However, several genes identified encoding resistance mechanisms to quaternary ammonium compounds (QACs) including chlorhexidine, predominantly efflux pumps. Presence of these QAC genes has been reported both from *S. aureus* - including methicillin-resistant *Staphylococcus aureus* (MRSA) and various CoNS species.

In Sweden it is normal practice for 0.5% chlorhexidine in alcohol to be used for preoperative skin disinfection. Chlorhexidine with alcohol has a much faster effect than alcohol by itself. One important task performed by the OR nurse is to prepare and optimize the patient’s skin before surgery. Knowledge and skill in applying correct aseptic techniques of skin preparation is essential for providing a safe environment for the patient. Guidelines for preparation techniques may look different in different countries. There is no evidence for which method is the most effective. In Sweden, traditional application techniques have been used for more than 40 years on the basis of proven experience, lateral, and medial technique (Fig. 3).

The patient is washed thoroughly using forceps and a swab soaked in chlorhexidine in alcohol skin disinfection. The procedure is performed from the top of the incision down, first out to the right side of the site of incision by as large a margin as possible, and then with a new swab, out to the left side. Skin preparation then continues within the marked area and concludes with a swab being wiped over the incision site. Disinfection of the skin can also be applied transversely. Larson describes that skin disinfection should be performed from the operative site outward: a wide skin area should be cleansed thoroughly, and gently scrubbed thoroughly. The CDC Guideline for prevention of SSI proposes other practices: the patient’s skin is prepared by applying an antiseptic skin preparation in concentric circles, beginning in the area of the proposed incision made in larger and larger circles outwards and then the process begins again. The general principle in preparing the patient’s skin is to prepare the cleanest area first and then move to the less clean areas thereafter.
Draping

The purpose of sterile drapes is to maintain the surgical field as a sterile work surface, and thereby prevent the patient's skin bacteria contaminating the surgical wound. For this to be achieved, the patient's skin must be dry so that drape can be attached to the patient's skin. The adhesive side should be attached approximately 10 cm into the disinfected area, and it is essential to drape as close to the incision area as possible, without the risk of the drape being cut.146

Plastic Adhesive Drapes

The plastic adhesive drape used in this study is integrated into sterile thoracic sheets and consists of polyurea and polyacrylate adhesives. There are other transparent plastic adhesive drapes on the market that are single packed (Fig. 4) or are integrated into different draping sheets, as well as iodophor-impregnated antimicrobial adhesive drapes (Ioban 2).189,190

Plastic poly vinyl drapes were introduced into surgical care in the 1950s,191 and were applied after the skin had been prepared with a spray-on adhesive. The drapes were introduced both for practical reasons - cotton towels often failed despite the use of towel clips - and because it was hoped that the drape would constrain skin bacteria so they could not contaminate the surgical site, and thus reducing the risk of SSI192,193

Plastic adhesive drapes are routinely used today in areas such as cardiothoracic surgery despite a Cochrane analysis 194 revealing that their use does not reduce the number of postoperative wound infections. Theoretically, plastic drapes may increase recolonization of the skin by creating a moist “green-house” effect that enables rapid bacterial regrowth.195
**Microbial Skin Sealant**

Another product used for preventing SSI is Kimberly-Clark’s Integuseal microbial skin sealant. This is a sterile, film forming product. It is made up of monomer n-butyl-cyanoacrylate, plasticizers, stabilizers and colorant, packaged in a glass ampoule and delivered in a ready-to-use applicator.\(^{163}\)

The disadvantage of this product is that when the glass ampoule is broken it has a sharp smell during application, which many experience as unpleasant. One layer of this skin sealant only is applied gently to the surgical site after preoperative skin disinfection (Fig. 5).

Before incision, the skin sealant is meant to dry for 2 - 4 minutes. According to the manufacturer, it reduces skin bacteria contamination of the wound by immobilising bacteria remaining on the skin after conventional skin disinfection.\(^{163}\)

Theoretically, this product’s advantage over plastic adhesive drapes is that it penetrates deeper into the skin and also immobilizes resident bacteria that are more deeply located. The skin sealant is left on the skin after surgery and wears off gradually and stays on the skin for up to 5–7 days.\(^{163,196}\)

Microbial sealants have been reported to protect against bacterial contamination of surgical wounds at both the chest and the saphenous vein graft site,\(^{197}\) which might reduce the rate of SSIs.\(^{198}\) A reduced rate of SSI after vein harvesting was described of Iyer et al.\(^{199}\) However, Waldow et al. found that skin sealant did not reduce SWIs after open-heart surgery.\(^{200}\)
RATIONAL FOR THE THESIS

SSI after cardiac surgery is a serious postoperative complication that causes suffering to the patients and may contribute to morbidity and even death. Organisms once thought to be harmless of the skin can today cause SSI under certain circumstances and bacterial antibiotic resistance complicates therapy further.

The development of a surgical site infection is very complex, due to the various sources of infection and the interacting routes of transmission. In this context, the focus of my thesis is the preparation of the patient's skin before cardiac surgery.

In cardiac surgery, the patient's chest skin is often routinely prepared with plastic adhesive drape despite the fact that its usefulness in preventing infections has previously been called into question. However, the updated Cochrane review which assessed the evidence of the effectiveness of plastic adhesive drape in preventing SSI is old - it included studies from 1971 to 2002. Therefore conducting a randomized controlled trial of the effects of plastic adhesive drape is of great importance. Further, there is a lack of independent research on microbial skin sealant. Existing research has mostly been performed by the manufacturing company itself. There is also a need for more knowledge of the time it takes for recolonization after skin preparation during surgery. According to regular routines, 0.5% chlorhexidine in alcohol is used for preoperative skin disinfection. However, the efficacy of chlorhexidine may be reduced by the presence of biological material or biofilm, so decreased bacterial susceptibility to chlorhexidine also need to be investigated further.
AIMS OF THE THESIS

The overall aim of this thesis was to evaluate how different preoperative skin procedures in cardiac surgery patients, impact recolonization of the skin after disinfection and bacterial contamination in wound. The thesis also, investigates the existence of decreased bacterial susceptibility to chlorhexidine.

The specific aims are presented in the following four studies:

Study I: To compare time to recolonization of the skin and bacterial growth with and without plastic adhesive drape on the skin in healthy volunteers.

Study II: To compare the use of plastic adhesive drape versus bare skin regarding bacterial growth in wound and time to recolonization of the adjacent skin intraoperatively, in cardiac surgery patients.

Study III: To compare the use of microbial skin sealant versus bare skin regarding bacterial growth in the wound and time to recolonization of the adjacent skin intraoperatively, at the saphenous vein harvesting site in CABG surgery patients. A second aim was to examine the postoperative wound infection rate.

Study IV: To investigate whether there was decreased susceptibility to chlorhexidine among S. epidermidis isolates by using both phenotypic and genotypic methods.
Table 1 Overview of design and methods

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Participants</th>
<th>Data collection</th>
<th>Analysis</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Experimental</td>
<td>Healthy volunteers (n=10)</td>
<td>Bacterial samples from the skin of the chest</td>
<td>McNemar</td>
<td>Time to recolonization of the skin and bacterial growth with or without plastic adhesive drape</td>
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<tr>
<td></td>
<td>comparative</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>II</td>
<td>RCT</td>
<td>Cardiac surgery patients n=135</td>
<td>Bacterial samples from the skin of the chest and subcutaneous wound tissue</td>
<td>Chi-square test</td>
<td>Intraoperative recolonization of the skin and surgical wound contamination with or without plastic adhesive drape</td>
</tr>
<tr>
<td></td>
<td>Single-blinded</td>
<td></td>
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<td>Fishers’s exact test</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Student’s t-test</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Mann-Whitney U</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>RCT</td>
<td>CABG patients n= 135</td>
<td>Bacterial samples from the skin of the saphenous vein harvesting site and subcutaneous wound tissue</td>
<td>Chi-square test</td>
<td>Intraoperative recolonization of the skin and surgical wound contamination with or without cyanoacrylate based skin sealant</td>
</tr>
<tr>
<td></td>
<td>Single-blinded</td>
<td></td>
<td></td>
<td>Student’s t-test</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=125</td>
<td>Two months follow up after surgery</td>
<td></td>
<td>Postoperative wound infection rate</td>
</tr>
<tr>
<td>IV</td>
<td>Descriptive study</td>
<td>Patients with clinical infections (n=92) healthy individuals (n=51)</td>
<td>S. epidermidis isolates (n=143)</td>
<td>Mann-Whitney U</td>
<td>Decreased susceptibility to chlorhexidine among S. epidermidis</td>
</tr>
</tbody>
</table>
Setting
All data collection was performed at Örebro University Hospital. Studies I, II and III were conducted in the OR department of Cardiothoracic and Vascular Surgery. Study IV was carried out in the Laboratory Medicine and Clinical Microbiology department.

Participant

Study I
The study took place in October 2009. Ten healthy volunteers, 5 women and 5 men participated in the study which involved their undergoing a simulation preoperative preparation for cardiac surgery. All participants resided in Örebro, had no association with the healthcare system and were drawn from the author’s circle of friends. The mean age of the participants was 40 years (range 22-60 years) and all had a body mass index (BMI) <30 kg/m2. No participants had diabetes mellitus, allergies, or skin lesions, none were taking on-going medication, and none had suffered any recent infection or other disease during the three weeks prior to the start of the trial.

Studies II and III
The patients were informed about the study the day before surgery, either by the research nurse or by the author. For practical reasons, the nurse/author selected patients into the study consecutively.

Patients included in study II were scheduled for CABG, valve repair, or some other cardiac procedure during the period between May 2010 and May 2011. 107 of the 140 patients in study II were also enrolled into study III which was conducted in the period between May 2010 and Oct 2011. Patients were scheduled for CABG using the saphenous vein for at least two coronary artery bypass grafts, with or without other cardiac procedures (Table 2.) The exclusion criteria for studies II and III were; emergency operations, previous cardiac surgery, long-term treatment with corticosteroid or/and antibiotic treatment within 14 day prior to the operation, preoperative skin disease, active skin infection, and presence of preoperative intra-aortic balloon pump.
### Table 2 Comparison of patients’ characteristics and surgical factors

<table>
<thead>
<tr>
<th>Study</th>
<th>Plastic adhesive drape, n=68</th>
<th>Bare skin group, n=67</th>
<th>Skin sealant group, n=67</th>
<th>Bare skin group, n=68</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year mean</td>
<td>66.3</td>
<td>68.7</td>
<td>68.4</td>
<td>65.0</td>
</tr>
<tr>
<td>Gender Male/female</td>
<td>60 (88.2%)</td>
<td>49 (73.1%)</td>
<td>50 (74.6%)</td>
<td>60 (88.2%)</td>
</tr>
<tr>
<td>BMI mean</td>
<td>27.1</td>
<td>27.3</td>
<td>26.8</td>
<td>27.8</td>
</tr>
<tr>
<td>Diabetes mellitus, n</td>
<td>16</td>
<td>10</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Current smokers, n</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Duration of surgery (min), mean</td>
<td>233.0</td>
<td>228.0</td>
<td>231.7</td>
<td>233.0</td>
</tr>
<tr>
<td>Incision length, cm mean</td>
<td>21.2</td>
<td>20.9</td>
<td>40.8</td>
<td>38.1</td>
</tr>
</tbody>
</table>

In both studies II and III five different patients were excluded after inclusion, in some cases these were the same patients in both groups. Consequently, a total of 135 patients were included in the analysis. In the secondary outcome analysis in study III, ten patients were excluded, leaving a total of 125 patients that were included in the analysis. Fig. 6 and 7 show a flowchart of samples taken in studies II and III.
Fig. 6 Flowchart of the procedure, including timing and type of bacteria sampling from the chest
Fig. 7 Flowchart of the procedure, including timing and type of bacteria sampling from the leg. Participant flowchart, two-month follow-up
Study IV

Bacterial Isolates
143 clinical *S. epidermidis* isolates were included in study IV. The study cohort was assembled from several different sources:

- Isolates were analysed from 61 different patients during revision surgery for prosthetic joint infections (PJIs) with extraction or exchange. These *S. epidermidis* isolates were collected from patients treated from 1993 to 2008, who had infected hip (n=46), knee (n=13), elbow (n=1), or shoulder (n=1) joint prostheses.
- 31 *S. epidermidis* isolates from two cardiothoracic trials that had caused deep surgical site infections including mediastinitis and/or sternitis following cardiothoracic surgery.
- From study II, we used *S. epidermidis* isolates (n=27) cultured from the skin of the chest from 12 patients after routine pre-operative skin preparation using disinfection with 0.5% chlorhexidine solution in 70% ethanol. The patients were scheduled for cardiac surgery between May 2010 and May 2011 and the isolates were investigated at that time.
- The last group of samples was collected from 24 healthy individuals who hadn’t had any recent contact with healthcare system. These *S. epidermidis* isolates (n=24) were obtained from the nares (n=13) or the skin of the wrist (n=11) between 2000-2005.

Interventions
In study I all participants (five per session) were placed on separate operating beds in a single operating room. After skin preparation, the patients’ chests were draped with Klinidrape Cardiovascular set, removing the plastic adhesive drape before placing it. The left chest area was covered with eight 6×7 cm plastic adhesive drapes, while the skin on the right chest area was not covered with plastic adhesive drapes. Bacterial samples from the skin of the chest were taken on 8 occasions, starting from 30 minutes after being in the OR continuing until 360 minutes in the OR (Fig. 8). In addition, samples from the anterior nares were taken from all participants on the day of the trial, using rayon swabs.
Fig. 8 Flowchart of the procedure and bacterial samples from the chest
Ro=Rodac plate; Ra=Rayon swab; E=ESwab
In studies II and III, the information detailing the groups to which patients were assigned was concealed in a locked cupboard. Patient allocation to the different groups took place in the OR immediately before skin preparation. The responsible OR nurse was aware of which group the patient belonged to before preoperative skin disinfection was performed.

In study II, patients were randomly allocated to the adhesive drape group (chest covered with plastic adhesive drape; n=70) and bare skin group (no drape; n=70) and in study III, the patients were randomized into bare skin group; (n =70) or the group in which microbal skin sealant was applied on the patient’s leg at the saphenous vein harvest site.

An external statistician, who was not involved in the enrolment or assessment of the patients, produced the randomization sequence. The allocation sequence was computer-generated block randomized.

No group assignments were required for study IV.

Procedures in Study I, II and III

Preoperative

All study participants carried out skin cleansing, using 4% chlorhexidine soap, twice at three separate occasions: i) at home, before admission to the ward, usually the day before surgery; ii) in the hospital the day before surgery; and iii) on the morning of surgery. Hair was removed using electric clippers the night before surgery, but only if the hair was located at the incision site, on the chest or leg. The participants changed into clean hospital gowns and were then moved to a clean bed.

Once the participants arrived in the OR, they were given a disposable cap and placed on an OR bed. In study II and III after patient’s anesthesia induction, the OR nurse started to prepare the patients skin for surgery. Depending on the surgical procedure to be performed, the chest, abdomen or/and the legs were disinfected with 0.5% chlorhexidine solution in 70% ethanol. The disinfection solution was applied for at least 2 minutes on the surgical site which was then dried completely before the draping process began using the Cardiovascular set, with or without plastic adhesive drape on the chest (study II).

Intraoperative antibiotic prophylaxis - usually cloxacillin 2g - was administered 15 to 30 minutes before incision. This was repeated every 2 hours during surgery and every 8 hours after wound closure until the following day, for a maximum duration of 24 hours. All procedures were
Preoperative skin preparation performed in an operating room which had upward displacement ventilation and a temperature of 19°C.

In study I, the cardiac surgery procedure was simulated, and it replicated the hygienic preoperative preparations in study II and III.

**Surgical Procedure**

All cardiac surgery was performed using standard surgical techniques for cardiopulmonary bypass (CPB) surgery.

In study II, the incision was made with scalpel and the division of subcutaneous tissues was done predominantly using electrocautery. At wound closure, the fascial, subcutaneous and intracutaneous layers were closed separately using resorbable monofilament running sutures. Surgical technique with surrounding tissue was used, harvesting the saphenous vein (study III). The incisions began anterior to the medial malleolus which was resected based on the number of grafts needed (at least two). Electrocoagulation was used for hemostasis and the surgical wound was closed, either before or after the cardiopulmonary bypass, using resorbable monofilament sutures for the subcutaneous tissue and the intracutaneous layer. In cases where the wound was not sewn directly after vein harvesting was complete, the leg was wrapped intraoperatively with an elastic compression dressing. After surgery, a wound dressing was placed over the incision and the leg was wrapped with an elastic compression bandage.

**Bacterial Sampling and Datacollection**

In study I, the nursing team (n=5) consisted of: two OR nurses; one nurse of infection control with OR experience who handled all skin samples, and; two nurses anesthetist. The nursing team members all had previous experiences of clinical research. One of the OR nurse performed all bacterial samplings.

For study II and III the data were registered and monitored prospectively intraoperative by a research nurse, or the author of the thesis following a study specific schedule. The research nurse assisted with the trial, and samples were performed by the OR nurses working at the OR department (n=14) with assistance by the cardiac surgeons. Before the study, the staff involved in the sample procedure were informed about the procedure and educated in the sample technique.
Preoperative Sampling
In studies I, II, and III, skin samples were taken on three occasions before the interventions were carried out. In study I baseline samples were taken; i) 3 days before the trial (conducted by the author in her home) (Fig. 9); ii) the day of the trial after the pre-wash with 4% chlorhexidine soap; and iii) immediately after skin disinfection with 0.5% chlorhexidine in 70% ethanol. The samplings from the skin of the chest in were taken first with the Rodac plate, followed by the rayon swab, and lastly an ESwab. The results from study I showed that Rayon swabs were the most efficient method for obtaining bacterial samplings so this method was used in studies II and III. All rayon swabs were moistened in studies I, II, III. In study I the rayon swab was moistened with the transport medium, and in studies II and III the swabs were moistened with sodium chloride 0.9%, before rubbing was carried out. In studies II and III the first samples were taken preoperatively on arrival at the ward, and samplings then followed the same schedule as in study I. In studies II and III samplings were taken from the sternal area or the calf.

Sampling During Intervention
In study I, samples were taken in pairs from both the right side (bare skin area) and left side (skin covered with plastic adhesives) of the sternum. Each sample was taken from a separate, pre-defined area starting proximally. On the left side, each of the eight plastic adhesives was separately removed immediately before the sample was taken. All sampling was performed by the same OR nurse, and sterile gloves were used and changed between participants. The swabs were rubbed back and forth 12 times against the skin and Rodac plates were then pressed against the skin for 15 seconds. (Fig. 9)
The swabs were subcultured on blood agar medium (Columbia Blood Agar Base, Acumedia Neogen Corporation, Lansing, MI, USA) supplemented with 6% defibrinated horse blood (SVA, Uppsala, Sweden) at 36°C aerobically. The bacterial growth was examined after 1 day and 5 days.

Two sedimentation plates (diameter 14 cm) with Trypticase Soy Agar II 4% w/v (BBL, Sparks, MD, USA) were placed on a table in the OR next to the participants at the beginning of the study, remaining in place for six hours until the last sampling was performed.

In study II samples were collected intraoperatively both from the exposed subcutaneous wound tissue and the adjacent skin every hour during surgery, commencing one hour after surgery started. The last sample was collected immediately before skin closure (Fig. 10).

Each sample was taken from a different predefined area. In the adhesive drape group, a 30-mm piece (approximately) of the drape was removed from the skin using scissors, immediately before sampling to prevent contamination. Samples were taken using rayon swabs (COPAN Italia...
Subcutaneous wound tissue sampling

Sternal sprider

Skin sampling below the sternal sprider

Sternal incision

Sampling swab to be dipped in sodium chloride before sample is taken
Sampling should be carried out for 15 seconds
Grip the swab like a pen and spin the tip
Rub it against the skin
Use gentler pressure in the subcutaneous tissue
Samples to be stored in a fridge in the OR

Fig. 11 Protocol for bacteria sampling
In study III intraoperative cultures (n=8) were sampled at four points in time. All bacterial samples were taken both from the skin adjacent to the wound and from the subcutaneous wound tissue at these times: 1) directly after the incision was made (distal); 2) when the incision was completed (proximal); 3) proximally before skin suturing began; and 4) distally before suturing the skin (Fig.12). The samples were obtained from the skin, or from the subcutaneous tissues, following a standardized procedure by rubbing the swab back and forth on an area of 3 cm and spinning the tip for 15 seconds.

**Culture Conditions**

The swabs in studies II and III were aerobically and anaerobically subcultured in a microbiological standardized manner as follows:

- On haematin agar medium (Columbia Blood Agar Base, Acumedia Neogen Corporation, Lansing, MI, USA) 4.3% (w/v) supplemented with 6% (v/v) chocolatized defibrinated horse blood, blood agar medium (Columbia Blood Agar Base 4.3% (w/v) supplemented with 6% (v/v) defibrinated horse blood) at 36°C aerobically for 2 days.
- On FAA plates (LAB 90 Fastidious Anaerobe Agar) 4.6% (w/v) (LAB M, Lancashire, United Kingdom) supplemented with defibrinated horse blood, 5% (v/v) incubated under anaerobic conditions (10% H₂, 10% CO₂, 80% N₂) at 37°C incubated for 5 days.
Bacterial growth was determined semi-quantitatively after 1 and 2 days following aerobic incubation, and after 5 days for anaerobic growth. Culture diagnostics and species verification was performed in concordance with routine diagnostic procedures, such as DNAse, coagulase, and Pastorex Staph-plus (Bio-Rad, Marnes-la-Coquette, France) for the staphylococci, as well as by characteristic colony morphology.

Gram-positive polymorphic rods were diagnosed by microscopy, and positive catalase, as well as with indole tests for the *Propionibacterium acnes*. The final species verification was performed using API20A (bioMérieux, Marcy l'Étoile, France) in selected cases.

**Patients Follow-Up**

All patients in study III were contacted two months postoperatively to assess whether leg wound infection was present. Leg wound infection was defined as wound complication requiring antibiotic treatment, prescribed by a physician. The follow-up was carried out by phone by a nurse from the department of cardiovascular and thoracic surgery. In cases where the patient had been treated with antibiotics the microbiologic findings were recorded.

**Study IV**

**Determination of the MIC of Chlorhexidine Using the Agar Dilution Method**

Mueller Hinton (MH) agar plates (3.8% w/v BBL™ Mueller Hinton II Agar [BD corporation, Franklin Lakes, New Jersey, USA]) were supplemented with 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg/L chlorhexidine digluconate (Sigma-Aldrich, St Louis, USA). The isolates were subcultured in aerobic atmosphere at 37°C overnight on MH agar plates, without chlorhexidine digluconate. Approximately five to seven colonies were suspended in 2.5 ml of sterile saline solution (0.9% NaCl), and the density was adjusted to 0.5 McFarland using an Oxoid Turbidometer (Integrated Technologies Limited, Basingstoke, UK). An inoculum of 1Sl of the bacterial suspension was then inoculated onto the MH agar plates with serial dilutions of chlorhexidine digluconate using a Multipoint Elite dispenser (Mast Group LTD, Merseyside, UK). As growth controls, MH agar plates without chlorhexidine digluconate were inoculated before and after the MH agar plates with serial dilutions of chlorhexidine digluconate. Bacterial growth on the agar plates was assessed after 24 hours and 48 hours of incubation in an aerobic atmosphere at 37°C.
Isolation of DNA
DNA was isolated from bacterial colonies by using the Bullet BUGS’n BEADS kit on the NorDiag Bullet extraction instrument, in accordance with the instructions from the manufacturer (NorDiag ASA, Oslo, Norway). All DNA preparations were stored at 4°C prior to the PCR.

Real-time PCR
Amplification of the QAC resistance genes, qacA/B, smr, qacG, qacH and qacJ was performed in a LightCycler PCR system 1.2 and 1.0 (Roche Molecular Biochemicals, Mannheim, Germany) using SYBR Green I fluorescence melting curve analysis to detect the specific amplicon. The PCR mixture contained 0.5 SM of respectively primers (Scandinavian Gene Synthesis AB, Koping, Sweden), 3 mM MgCl2, 1×LightCycler FastStart DNA Master SYBR Green I (Roche Molecular Biochemicals). The PCR programs started with a pre-incubation at 95°C for 10 minutes, followed by 40 cycles of amplification and ended with melting curve analysis by rapid heating to 95°C, followed by 65°C for 15 seconds. The temperature was then slowly raised to 95°C at a rate of 0.1°C /second. In each PCR run, one positive control and one negative control (water instead of DNA template) were included.

Initial confirmation of the PCR product was determined by means of 2% agarose gel electrophoresis, and DNA sequencing used the ABI PRISM BigDye Terminator v 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems).

Antibiotic Susceptibility Testing
Antibiotic susceptibility testing was performed on Mueller–Hinton agar (3.8% w/v BBL™ Mueller Hinton II Agar, BD Diagnostics, Sparks, MD, USA) using the disk diffusion method (Oxoid, Cambridge, UK) and incubated in an aerobic atmosphere at 35°C for 16-20 hours. The break points applied for antibiotic susceptibility were in accordance with the Nordic Committee on Antimicrobial Susceptibility Testing (www.nordicast.org).
**STATISTICAL METHODS**

**Study I**
The bacterial cultures on the chest were categorized dichotomously as positive (growth) or negative (no growth) and were compared using the McNemar test for paired proportions. A p-value less than 0.05, two tailed, was considered statistically significant. Descriptive statistics are presented as minutes, numbers, and percentages. Data were analyzed with SPSS 15.0 (SPSS, Inc. Chicago, IL, USA).

**Study II**
Sample size calculation was based on assumptions to detect a difference of 20% between the patients (10% in the bare skin group vs. 30% in the adhesive drape group) for the primary endpoint of CoNS-positive bacterial cultures at 60 minutes, with a 5% significance level and a power of 80%, guided by study I. These assumptions suggested a sample size of 124 patients, and we obtained a sample size of 140 patients.

Semi-quantitative bacterial growth analysis was treated as follows: 0=no growth, 1=growth of <10 colonies, 2=growth in the primary streak on the agar plate), 3=growth in the secondary streak, and 4=growth in the tertiary streak.

Descriptive statistics are presented as numbers, percentages, means, and standard deviations. Student's t-test and the Mann-Whitney U-test were used to detect differences between groups in medical and demographic characteristics. Chi Square or Fisher’s exact test were used to dichotomously categorize bacterial growth as positive (growth) or negative (no growth), and to compare between groups and between gender. A two-tailed P value <0.05 was considered statistically significant. Data was analyzed using SPSS 17.0 (SPSS, Inc. Chicago, IL, USA).

**Study III**
Though data published on bacterial contamination of the vein harvest sites are rare the sample size of 140 patients was originated from study II. The sample size was considered as sufficient in the present study since wound infections, and thereby probable bacterial contamination, occur more frequently at the vein harvest site than at the sternal wound. A sample size based on the primary end point only, not secondary endpoints such as the infection rate was chosen. Therefore the same sample size (140 patients) was considered sufficient.
Descriptive statistics are presented as numbers, percentages, means, and standard deviations. Student’s T-test was used for comparisons of continuous variables and the Chi Square test was used for categorical variables.

The Chi Square test was also used to dichotomously categorize antibiotic treatment as positive (antibiotic treatment) or negative (no antibiotic treatment) and to categorize bacterial growth as positive (growth), and negative (no growth). A two-tailed P value of $<0.05$ was considered statistically significant. Data was analyzed using SPSS (20) (SPSS, Inc. Chicago, IL, USA).

**Study IV**
Fisher’s exact test was used to estimate differences in proportions and the Mann-Whitney $U$-test was used to detect differences between groups. A p-value of $<0.05$ was considered to be statistically significant.
ETHICAL CONSIDERATIONS

All studies were conducted following the ethical principles of medical research involving humans or animal subjects, outlined in the Helsinki Declaration, Ethical principles for medical research involving human subjects.204

All participants in studies I, II and III were given verbal and written information about the studies, and all gave their written informed consent before data collection. Per the Helsinki Declaration, participation was voluntary and no harm was affected on the participants. The participants were informed that they had the right to withdraw from participating in the study at any moment, without any explanation, and that it was possible to contact the author with concerns related to the study.

Studies I-III were approved by the Regional Ethical Review Board in Uppsala, Sweden (D.no. 2009/061 and 2010/046). Study II and III was registered in ClinicalTrials.gov (NCT0 1316588). Study IV including bacterial isolates from earlier studies. Bacterial isolates is not covered by the Swedish ethical law.

The patients in studies II and III were asked to participate in the studies the day before surgery. Before asking the patient about participation and on the patient’s own request, the research nurse or the author had a discussion and answered questions about the surgery that the patient raised. A number of patients were concerned that they would suffer a post-operative wound infection and were therefore positive to that the research being carried out. If the patient was in any doubt, they were encouraged to reflect further before making the decision and suggested that the decision would be carried out later.
RESULTS AND DISCUSSION

The results are presented in terms: Preoperative bacteria cultures; Plastic adhesive drape; recolonization and wound contamination; Microbial skin sealant recolonization and wound contamination; Correlation between presence of QAC genes and decreased susceptibility to chlorhexidine.

Preoperative Bacterial Cultures

In study I, prior to antiseptic preparation with 0.5% chlorhexidine in 70% alcohol, all skin samples showed bacterial growth of CoNS on the chest, but none showed \textit{S. aureus} growth. Three days before the trial, positive cultures were found with all three different sampling methods. After showering with 4% chlorhexidine soap, positive bacterial growth was found on all participants but only from two of the sampling methods used (Fig. 13).

![Fig. 13 Percentage of positive cultures by three different sampling methods](image-url)
In studies II and III the most common bacteria detected were CoNS and *P. acnes* (Table 3). No differences were found between the intervention groups and the control groups (studies II and III). No decreased bacterial colonization was noted after preoperative skin preparation using 4% chlorhexidine soap neither CoNS (studies I and II) nor *P. acnes* (study II). After skin disinfection with chlorhexidine with alcohol, CoNS colonization decreased (studies I and II) but *P. acnes* colonization did not (study II). In contrast to studies I and II, in study III there was a decrease in bacterial growth of CoNS and *P. acnes* after the skin preparations.

There were significant differences in *P. acnes* growth between the genders (Table 4). Note - semi-quantitative bacterial growth analysis was used). After using 4% chlorhexidine soap, the mean of *P. acnes* growth was 3.7 for men (indicating heavy growth) and 1.5 for women (range 0-4).

### Table 3 Proportions of bacteria growth on the chest (study II) and the leg (study III)

<table>
<thead>
<tr>
<th>SKIN SAMPLES</th>
<th><em>S. aureus</em> chest/leg</th>
<th>CoNS chest/leg</th>
<th><em>P. acnes</em> chest/leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>The ward (n=135)</td>
<td>6.7%/5.2%</td>
<td>94.8%/67.4%</td>
<td>74.1%/47.4%</td>
</tr>
<tr>
<td>After pre wash with 4% chlorhexidine soap (n=135)</td>
<td>1.5%/0%</td>
<td>86%/24.4%</td>
<td>72.6%/31.9%</td>
</tr>
<tr>
<td>After using 0.5% chlorhexidine solution in 70% ethanol (n=134)</td>
<td>0%/0%</td>
<td>9%/0.7%</td>
<td>53.7%/3.7%</td>
</tr>
</tbody>
</table>
Table 4 Proportions of bacteria growth on the chest between gender

<table>
<thead>
<tr>
<th>SKIN SAMPLES on the chest of the sternal area</th>
<th>CoNS</th>
<th>P. acnes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=109</td>
</tr>
<tr>
<td>The ward</td>
<td>88.5%</td>
<td>96.3%</td>
</tr>
<tr>
<td>After pre wash with 4% chlorhexidine soap</td>
<td>80.8%</td>
<td>87.2%</td>
</tr>
<tr>
<td>After using 0.5% chlorhexidine solution in 70% ethanol</td>
<td>11.5%</td>
<td>8.3%*</td>
</tr>
</tbody>
</table>

*p <0.001  
^=one missing, n= 108

Discussion

The results of the preoperative skin sampling were surprising. The chlorhexidine soap did not decrease the frequency of CoNS or *P. acnes* on the skin of the chest, (studies I and II). This is in contrast to the findings of several previous studies [12,167,205-208] which have shown decreased bacterial growth, and also a reduced number of microbes over time after the patient showered with chlorhexidine soap. [209] There were also gender differences concerning *P. acnes* growth in study II. Men have a higher bacterial burden than women, which is in line with other studies. [15,71] In contrast to the chest, bacterial growth decreased on the leg (study III) after the preoperative skin preparations. On the chest however, *P. acnes* growth was found in 53.7% of patients even after they used chlorhexidine solution in alcohol.

In clinical praxis and following national guidelines, patients that are about to undergo an elective cardiac surgery, routinely wash their bodies with chlorhexidine soap at three separate times before the operation. [23] The value of body disinfection in reducing the rate of SSI has been discussed for decades, and there is still no evidence for its efficacy. [32,167] However, one earlier study comparing chlorhexidine with regular bar soap did show a statistically significant reduction in SSI. [137] Since the patient’s skin is a major source of SSI, this is an extremely important issue to discuss. Why did not the number of positive bacterial cultures on the chest decrease after the chlorhexidine soap was used? Is it possible that there are more resident
bacteria, sebaceous glands, and moisture on the chest and therefore bacteria is more difficult to eliminate? Further research is needed in this area.

It is well-known that while skin preparation reduces the number of bacteria on the skin it does not eliminate it completely, and that 20% of skin flora is unaffected by skin disinfection. Nonetheless, these results still reflect a very high number of *P. acnes* present on the skin. The results from study II may put into question whether 0.5% chlorhexidine in alcohol is effective enough to eliminate *P. acnes* in skin preparation of the chest. A study by Darouiche et al demonstrated a 41% reduction in total SSI among patients undergoing clean surgery who used a 2% chlorhexidine in 70% alcohol solution compared to an aqueous povidone-iodine solution. Is it possible that different types of skin antiseptics are more effective for reducing the number of *P. acnes*?

Another explanation could be inadequate body cleaning. Do patients closely follow the instructions provided on how to carry out the body washing process? Do they manage to do it properly on their own? In Sweden patients usually only receive advance written instructions about the preoperative showering process to be carried out at home. It is important that the letter with the instructions clearly highlights the benefits of getting help from a friend or relative for the preoperative body wash as it can be difficult to do it properly alone. In studies I, II, and III no control of this was carried out. Interestingly, comparing the healthy participants in study I with the patient population in study II, there was less preoperative CoNS growth on the chest of the participants in study I. Study I showed <10 colonies (same as range 1 in the semi-quantitative bacterial growth analysis), while in the clinical study (study II) the mean growth was 2.1 (range 0-4). One hypothesis is that the hospitalized patients may have been more exposed to colonization by transmission from other patients or staff. The participants in study I had not had any recent contact with the healthcare system, so this may be one reasonable explanation.

The results from the preoperative disinfection studies provide important new knowledge, and hopefully will prompt a discussion and study of the clinic's practices regarding preoperative chlorhexidine showers. This would aim to ascertain the benefits of preoperative body showers in relation to costs, both in terms of chlorhexidine soap and postoperative wound infections.
Intraoperative Bacterial Growth and Time to Recolonization and Contamination to the Wound

Plastic Adhesive Drape: Recolonization and Wound Contamination
In study I all positive bacterial cultures contained CoNS, and three samples showed CoNS mixed with diphtheroid rods. No positive samples were found using Rodac plates during the trial. Samples taken with the Rayon swabs showed positive results at all time-points except at 120 minutes, and showed a higher proportion of positive results compared with the ESwabs (Fig. 14).

![Fig. 14 Time to recolonization from the skin versus plastic drape](image)

Recolonization was detected on the skin covered with the plastic adhesive drape after 30 minutes, whereas it was detected after 60 minutes on the skin without plastic adhesive drape. There was a consistent trend throughout the eight-sample time series: positive cultures were derived from a significantly (p<0.01) higher proportion of samples from the skin covered with plastic drape (31%, 25/80) than from skin without the drape (7.5%, 6/80), see Figure 5. The two sedimentation agar plates showed 17 and 23 colonies of CoNS respectively. A consistent trend throughout the eight-sample time series showed a more rapid recolonization of the skin covered with plastic drape. The result persuaded to design a clinical study on patients undergoing cardiac surgery to test and verify the observations, as well as investigate if there was any correlation between recolonization on the skin and in the surgical wound i.e. study II.
In study II patients with plastic adhesive drape had significantly more CoNS growth on the skin 44.6% (29/65) after 2 hours, compared with those with bare skin 23.8% (15/63), p=0.013. A significantly higher proportion of *P. acnes* was also noted: 63.1% (41/65) versus 44.4% (28/63), p=0.034. No differences were found between the groups at wound closure (Fig. 15.) In the subcutaneous wound, positive cultures were found only at the end of surgery, showing higher proportions of CoNS in the patients with plastic adhesive drape compared to those with bare skin, 14.7% (10/68) and 4.5% (3/67), respectively (Fig. 16).

Fig. 15 Proportions of *P. acnes* and CoNS on the skin
Fig. 16 Proportions of *P. acnes* and CoNS on the subcutaneous wound tissues

**Discussion**

There is concordance between the results of study I and study II. Plastic adhesive drapes did not reduce bacterial contamination of the wound, and there were significantly higher proportions of *P. acnes* and CoNS-positive skin cultures in the adhesive drape group at 120 minutes compared with the bare skin group. Different types of incision drapes have been manufactured during the last 60 years, but none have been shown to reduce postoperative wound infections. The recently updated Cochrane Review\textsuperscript{194} found that a significantly higher proportion of patients in the adhesive drape group developed a SSI when compared to those without drapes (risk ratio (RR) 1.23, 95% confidence interval (CI) 1.02 to 1.48, p=0.03). It was also found that Iodine-impregnated adhesive drapes had no effect on SSI (RR 1.03, 95% CI 0.06 to 1.66, p=0.89).

Studies I and II provide only one possible theory and explanation for why there is no reduction in infection rates. This theory is that the plastic adhesive drape retains increased moisture next to the skin, which may cause rapid bacterial growth and create a “green house” effect.\textsuperscript{195} The clinical practice of applying the drape on the skin so that no air bubbles or pockets of fluid are trapped under the drape also causes complications. The
removal of the plastic adhesive drape can also be difficult for patients with fragile skin, in elderly people, or for patients with certain diseases and other risk factors. In these cases, removing the adhesive drape increases the risk of damaging the skin and thereby increases the risk of wound infections. Grove et al. describe that to be effective as a sterile barrier between the surgical wound and the surrounding skin, it’s important that the plastic adhesive drape remains securely stuck to the skin, including at the wound edge, during the whole surgical procedure. For practical reasons the plastic adhesive drape is often drawn away from the edge of the wound before wound sealing. In their study, Alexander et al. concluded that separation of the plastic adhesive drapes from the skin was associated with a six-fold increase in SSI compared with surgical procedures in which the plastic drape was left in place. The data in study II showed that even though the plastic drape seemed to remain intact during surgery, it did not reduce wound contamination. At wound closure there was P. acnes growth in 70.6% of the subcutaneous tissue in the plastic adhesive group compared to 67.2% in the bare skin group.

Microbial Skin Sealant: Recolonization and Wound Contamination

No differences in bacterial cultures were found between the patients in the microbial skin sealant group versus the patients in the bare skin group (study III). Bacterial recolonization of the skin as well the contamination of the subcutaneous tissue consisted of both CoNS and P. acnes. There was no S. aureus growth in any of the intraoperative cultures. The four samplings carried out during incision showed CoNS growth in 4.4% (6/135) of samples, and P. acnes growth in 7.4% (10/135) both on the skin and in the subcutaneous tissue. At wound closure, CoNS growth on the skin and in the subcutaneous tissue was shown in 9% (12/133) of samples, and P. acnes growth in 13.5% (18/133).

125/135 patients were followed up two months postoperatively (study III), see Figure 3. Of these, 21/125 (16.8%) had been treated with antibiotics for a verified or suspected SSI at the harvest site, 7/61 (11.5%) from the skin sealant group compared with 14/64 (21.9%) from the bare skin group. The difference was not considered statistically significant (p=0.120). In the wound infections at the vein harvesting site, S. aureus growth was shown in 13/21 (62%) patients, and in 4 of these samples, Enterobacteriaceae sp (n=3), beta-haemolytic streptococci group G (n=1), enterococci (n=1), and CoNS (n=1) was also found in addition to S. aureus. Mixed cultures were found in 3 samples as follows: beta-haemolytic
streptococci group B, *S. lugdunensis* and diphtheroid rods; beta-haemolytic streptococci group G and *Enterobacteriaceae sp*; and, *Enterobacter aerogenes*. In 5/21 (23.8%) patients treated with antibiotics no bacterial samples were taken. There were no differences in patient characteristics or surgical factors between patients in the microbial skin sealant group versus patients in the bare skin group (Table 5).

**Table 5** Comparison of patients’ characteristics and surgical factors of the patients with leg wound infections

<table>
<thead>
<tr>
<th></th>
<th>Bare skin n=14</th>
<th>Microbial skin sealant n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean)</td>
<td>61.9</td>
<td>68.4</td>
</tr>
<tr>
<td>Gender, male/female (n)</td>
<td>10 / 4</td>
<td>6 / 1</td>
</tr>
<tr>
<td>BMI (mean and range)</td>
<td>27.2 (22-35)</td>
<td>26.7 (19-33)</td>
</tr>
<tr>
<td>Current smoker (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Diabetes (n)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Duration of vein harvesting min (mean &amp; range)</td>
<td>122.8 (37-254)</td>
<td>117.6 (40-260)</td>
</tr>
<tr>
<td>Wound closure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after vein harvesting (n)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>after ECC (n)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Vein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with surrounding tissue (n)</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>conventional (n)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Subcutaneous suture (n)</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Incision length, cm (mean &amp; range)</td>
<td>39.2 (27-61)</td>
<td>37.6 (23.3-45)</td>
</tr>
<tr>
<td>Positive bacterial sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>within 30 days of surgery (n)</td>
<td>7*</td>
<td>3**</td>
</tr>
<tr>
<td>within 60 days of surgery (n)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*2 missing, **3 missing

**Discussion**
In study III, virtually no intraoperative bacterial growth was found, and no differences were seen between the groups. The pretreatment with microbial
Preoperative skin preparation

Skin sealant reduces skin bacteria contamination of the wound and acts as a protective barrier. The difference between this product and the plastic adhesive drapes is that it penetrates into deeper skin layers and may immobilize resident bacteria. In the present study, no advantage was found in using the microbial skin sealant, so a cost and efficacy analysis of the product would need to be carried out before routinely using it in clinical practice. Nonetheless, the microbial skin sealant is left on the skin after surgery and this protective barrier may reduce the risk of surgical wound contamination postoperatively. There was a minor, insignificant trend towards a lower SSI rate seen in study III. However, in this study, SSI was not the primary end-point and there were a limited number of patients. Further research is necessary, with SSI as the end-point, to determine whether use of microbial skin sealant reduces the incidence of SSI at the saphenous vein harvest site. This is supported by findings of a recent Cochrane review which reported a significant difference in the SSI rate when using microbial sealant. However, the trials were not considered to be of high quality so further research is needed.

In CABG surgery, the most common conduits for coronary revascularization are provided by harvesting the saphenous vein from the patient’s leg. The most common technique for harvesting usually involves a long incision but it can also be performed with skin bridges or via endoscopic vein-harvesting techniques. In study III, all saphenous vein harvesting was performed following the traditional technique, with a long continuous incision starting above the ankle and continuing until the adequate length of vein was attained.

The incidence of leg wound infections of 16.8% (study III) is in line with the findings of Swenne et al. who showed that SSI occurred in up to 18% of patients after saphenous vein graft harvesting. Furthermore, the positive intraoperative microbiologic findings were not the same pathogen causing the leg wound infection that was verified in 16/21 patients. Unfortunately, five infected patients that were treated with antibiotics were not confirmed with positive bacterial cultures. Their infection symptoms were treated but bacterial samples were not taken, so the microbe that caused the infection is unknown.

The questions to examine are when and how did the surgical wound contamination take place? In the wound infections at the vein harvesting site, S. aureus grew in 62% of the samples. However, the results from study III indicate that no S. aureus contamination occurred intraoperatively. So what happens postoperatively? There are currently no
shared best practice guidelines for postoperative wound care. Before the patient leaves the OR, the OR nurse covers the closed wound with a sterile dressing. The aim of the dressing is to protect the wound by acting as a barrier until the skin is restored. It is of paramount importance that the dressing remains completely and tightly stuck against the skin, without any creases, otherwise there is a risk for the wound being contaminated once patients arrive on the ICU or the ward. Additionally, a Cochrane review recommends further research into whether the dressing of wounds is necessary beyond 48 hours after surgery.214

One risk factor to consider is cross-transmission from hand contamination between staff and patients that can happen in the OR, intensive care unit (ICU) and on the ward. Tammelin et al. found that of the healthcare workers investigated, half acquired *S. aureus* on their hands due to transmission from patients or the environment, and half acquired it by self-inoculation from their nose.215 Healthcare workers colonized with *S. aureus* have been identified as sources for *S. aureus* contamination causing SSI,51,216 and there is an increased risk from colonization on their hands.217 Nose picking is also associated with *S. aureus* nasal carriage and transmission.53

It's also possible that patients can contaminate themselves and it has been shown that nosocomial *S. aureus* infections do originate from the patient's own flora. 45,47,52,125,218,219 In Sweden, preoperative nasal carriage of *S. aureus* is not routinely screened in cardiac surgery patients, although it is well-known that 20-30\% of patients carry *S. aureus* in the anterior nares. To prevent patient self-inoculation, local antibiotic treatment, using mupirocin, can be used to eliminate nasal *S. aureus*. The use of mupirocin has not been applied worldwide due to concerns about the development of antibiotic resistance 220 as well as inconsistent evidence that mupirocin reduces SSI.44 However a Cochrane Review describes that in approximately 80\% of infections, the *S. aureus* strain isolated from the nares was identical to that isolated from the infected site. The review proposes administration of intranasal mupirocin to those patients who are nasal carriers of *S. aureus*.52 Courville et al reported cost efficiencies from both a “treat-all patients” strategy as well as a “screen-and-treat” strategy. Both showed lower costs and greater benefits compared with a “no-treatment” strategy in patients undergoing joint arthroplasties treated with nasal mupirocin for *S. aureus* decolonization.42 One weakness of III as well as study II was the lack of identification of nasal carriers of *S. aureus* and CoNS preoperatively. Given the high *S. aureus* growth (62\%), strain typing of
positive bacterial cultures from the nose may have helped to detect whether some of the SSI at the vein harvesting site was caused by the patient’s own flora. It would have been interesting to screen both patients’ and healthcare workers’ hands and noses to investigate a) if they were carriers and b) if the strains were harmful or resistant to antibiotics. Irrespective of this, strict compliance to hygiene routines is necessary to avoid cross-contamination.

**Gender Differences**

The comparison between the genders (study II) of the number of positive bacterial samples of *P. acnes* on the skin, showed significantly more positive cultures from the skin of men at all time-points except at 300 and 360 min. The subcutaneous wound tissue cultures showed significant differences between the genders at 60, 120, and 180 minutes and at wound closure. In males, the bacterial recolonization on the skin showed the same amount of *P. acnes* at wound closure as preoperatively on the ward (Table 6). In terms of gender differences in bacterial growth of CoNS on the skin at wound closure, women had less growth than men 48% (12/25) versus 65.7% (71/108).
Table 6 Comparison between gender in numbers of positive bacteria samples of *P. acnes* on the skin and in subcutis reported as numbers and percentage of positive cultures and mean of semi-quantify bacteria growth ranged; 0=no growth, 1=growth of <10 colonies, 2=growth of the primary streak on the agar plate), 3=growth in the secondary streak, to 4=growth in the tertiary streak.

<table>
<thead>
<tr>
<th>Skin samples</th>
<th><em>P. acnes</em></th>
<th><em>P-value</em></th>
<th>Subcutaneous samples</th>
<th><em>P. acnes</em></th>
<th><em>P-value</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td><strong>Men</strong></td>
<td></td>
<td><strong>Women</strong></td>
<td><strong>Men</strong></td>
<td></td>
</tr>
<tr>
<td>The ward</td>
<td>9/26</td>
<td>91/109</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(34.6%)</td>
<td>(83.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[1.8]</td>
<td>[3.7]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 4% chlorhexidine soap</td>
<td>9/26</td>
<td>89/109</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(34.6%)</td>
<td>(81.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[1.5]</td>
<td>[3.7]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 0.5% chlorhexidine solution in 70% ethanol</td>
<td>1/26</td>
<td>71/108</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3.8%)</td>
<td>(65.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[3]</td>
<td>[2.6]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>0/26</td>
<td>67/109*</td>
<td>&lt; 0.001</td>
<td>1/26</td>
<td>75/109</td>
</tr>
<tr>
<td></td>
<td>(61.5%)</td>
<td>(63.8%)</td>
<td></td>
<td>(3.8%)</td>
<td>(68.8%)</td>
</tr>
<tr>
<td></td>
<td>[2.8]</td>
<td>[2]</td>
<td></td>
<td>[1]</td>
<td>[2.1]</td>
</tr>
<tr>
<td>120 minutes</td>
<td>1/24</td>
<td>68/104*</td>
<td>&lt; 0.001</td>
<td>0/24</td>
<td>66/104</td>
</tr>
<tr>
<td></td>
<td>(4.2%)</td>
<td>(65.4%)</td>
<td></td>
<td>(1.4%)</td>
<td>(63.5%)</td>
</tr>
<tr>
<td></td>
<td>[2]</td>
<td>[2.9]</td>
<td></td>
<td>[1]</td>
<td>[1.9]</td>
</tr>
<tr>
<td>180 minutes</td>
<td>2/15</td>
<td>42/55*</td>
<td>&lt; 0.001</td>
<td>1/15</td>
<td>39/55</td>
</tr>
<tr>
<td></td>
<td>(13.3%)</td>
<td>(76.4%)</td>
<td></td>
<td>(6.7%)</td>
<td>(70.1%)</td>
</tr>
<tr>
<td></td>
<td>[1]</td>
<td>[2.8]</td>
<td></td>
<td>[2]</td>
<td>[1.8]</td>
</tr>
<tr>
<td>240 minutes</td>
<td>0/4</td>
<td>11/16*</td>
<td>0.013</td>
<td>0/4</td>
<td>11/16</td>
</tr>
<tr>
<td></td>
<td>(68.8%)</td>
<td>(68.8%)</td>
<td></td>
<td>(68.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[3.2]</td>
<td>[2]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 minutes</td>
<td>0/2</td>
<td>5/8</td>
<td>0.475</td>
<td>0/2</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(62.5%)</td>
<td></td>
<td>(75%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>[2.8]</td>
<td></td>
<td>[1.7]</td>
<td></td>
</tr>
<tr>
<td>360 minutes</td>
<td>0/1</td>
<td>2/2</td>
<td>0.223</td>
<td>0/1</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td></td>
<td>(50%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>[2.5]</td>
<td></td>
<td>[2]</td>
<td></td>
</tr>
<tr>
<td>At skin closure</td>
<td>3/26</td>
<td>91/109*</td>
<td>&lt; 0.001</td>
<td>2/26</td>
<td>91/109</td>
</tr>
<tr>
<td></td>
<td>(11.5%)</td>
<td>(83.5%)</td>
<td></td>
<td>(7.7%)</td>
<td>(83.5%)</td>
</tr>
<tr>
<td></td>
<td>[2.3]</td>
<td>[3.5]</td>
<td></td>
<td>[1.5]</td>
<td>[2.4]</td>
</tr>
</tbody>
</table>
**Discussion**

In study I, no differences in CoNS bacterial growth was detected between the genders. In study II looking at bacterial growth on the skin and in subcutaneous tissue, *P. acnes* was significantly more common in men than in women. After skin preparation with 0.5% chlorhexidine solution in 70% ethanol, 65.7% of the samples from men were positive, compared to 3.85% from women. At skin closure on men, bacterial growth was shown on 83.5% of the samples both on the skin and in the subcutaneous tissue. At the same time-point, skin closure, the women showed bacterial growth on 11.5% of samples on the skin and 7.7% in the subcutaneous tissue. Can this be explained by hormonal differences? In view of the results regarding *P. acnes* growth, it was unfortunate that no anaerobic sub-culturing of the samples was carried out in study I.

**Chlorhexidine Susceptibility Among *S. epidermidis* Isolates**

The *S. epidermidis* isolates obtained from postoperative infections (PJI's and cardiothoracic surgery infections) displayed decreased susceptibility to chlorhexidine to a statistically significant higher extent than commensals (Mann-Whitney; p=0.0001). In study II, decreased susceptibility to chlorhexidine was rare among *S. epidermidis* isolates obtained from the skin following pre- and perioperative disinfection. An increase in MIC values between 24 and 48 hours' incubation was seen in 13 cases, in 8 of these from full susceptibility to decreased susceptibility. In 5 cases with decreased susceptibility an additional increase in MIC value was noted following further incubation.

**Detection of QAC Genes**

PCRs were used to detect five QAC resistance genes; *qacA/B, smr, qacG, qacH, and qacJ* (Table 7). The *qacA/B* gene was present in 62/143 isolates (43%), *smr* in 8/143 (6%), and *qacH* in one isolate (0.7%). None of the isolates were positive for *qacG* or *qacJ*. Two isolates were positive for both *qacA/B* and *smr*. There was a statistically significant difference in the prevalence of *qacA/B* between *S. epidermidis* isolates from clinical infections compared with commensals (including study II) (Fisher; p<0.0001). This difference was also seen when comparing *S. epidermidis* isolates from deep cardiothoracic postoperative infections with those obtained from the skin of patients who had undergone disinfection with 0.5% chlorhexidine solution in 70% ethanol (Fisher; p=0.001) as well as when comparing commensals with PJI isolates (Fisher; p=0.03).
Table 7 Prevalence of QAC resistance genes in *S. epidermidis* isolates regarded as commensals, obtained from prosthetic joint infections and cardiothoracic surgical site infections as well as isolates sampled following routine preoperative skin preparation with alcohol and chlorhexidine (study II)

<table>
<thead>
<tr>
<th>Skin samples</th>
<th>qacA/B</th>
<th>smr</th>
<th>qacH</th>
<th>qacJ</th>
<th>qacG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commensals (n=24)</td>
<td>6 (25%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wrist (n=11)</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nares (n=13)</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Studie II (n=27)</td>
<td>5 (19%)</td>
<td>2 (7%)</td>
<td>1 (4%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Samples PJI and SWI infections

<table>
<thead>
<tr>
<th></th>
<th>qacA/B</th>
<th>smr</th>
<th>qacH</th>
<th>qacJ</th>
<th>qacG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosthetic joint infections (n=61)</td>
<td>32 (52%)</td>
<td>3 (5%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sternal Wound infection (n=31)</td>
<td>19 (61%)</td>
<td>3 (10%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Correlation Between Presence of QAC Genes and Decreased Susceptibility to Chlorhexidine**

A decreased susceptibility to chlorhexidine was found more frequently among isolates carrying *qacA/B* genes compared to those not harboring *qacA/B* genes, mean MIC value of 0.0036 and 0.00056 mg/L, respectively (Fig. 1). This difference was statistically significant (Mann-Whitney; p=0.0001). Presence of the *smr* gene did not correlate to a decreased susceptibility to chlorhexidine (Mann-Whitney; p=0.9997) compared with commensals (2/24) and isolates from study II (1/27). A strong statistical association between multi-drug resistance (MDR) and presence of *qacA/B* genes was also noted (p<0.0001).

**Discussion**

In study IV, the *S. epidermidis* isolated from the skin following preoperative skin preparation, which included three showers with chlorhexidine soap and subsequent disinfection with chlorhexidine in alcohol, did not display a higher prevalence of genes encoding resistance against QAC than commensals. In addition, these isolates did not display multi-drug resistance.

This would suggest that preoperative strategies to reduce postoperative infections by using chlorhexidine do not seem to facilitate selection of
isolates with decreased susceptibility to chlorhexidine. Chlorhexidine susceptibility does not explain the inefficiency of current preoperative disinfection with chlorhexidine in alcohol. These strains more likely represent residues of the commensal flora not completely eradicated by the disinfection procedure. Reasons why CoNS was still present on the chest after skin disinfection can only be speculated. Guidelines state that Chlorhexidine solution in alcohol should be applied to the skin in ample amounts for at least 2 minutes and then the skin should be air-dried.\textsuperscript{23,138,188} Is it compliance with these guidelines that is inadequate? In addition to the chemical effect in skin preparation, the mechanical cleaning process is also important in the removal of skin bacteria. No research has been performed in this area and there is a lack of evidence based guidelines for the most effective skin preparation technique. However, one interesting finding was the decreased susceptibility to chlorhexidine among isolates carrying \textit{qacA/B} genes in strains that caused deep infections in thoracic surgery as well as their strong association with MDR. Hospitalized patients may contract these strains during their hospital stay since otherwise healthy individuals or at least patients without any recent contact with healthcare do not carry these specific strains.\textsuperscript{65}

Is the concentration of chlorhexidine optimal for reaching a therapeutic dose level? The isolates carrying \textit{qacA/B} genes may be more affected by a higher concentration which can cause the cytoplasmic contents to precipitate, resulting in cell death. The use of 2\% chlorhexidine concentration has been shown to reduce both deep and superficial infections.\textsuperscript{81}

Accordingly, the implementation of infection control measures and adherence to basic hygiene instructions is crucial in order to prevent bacterial transmission.\textsuperscript{221} SSI following cardiothoracic surgery probably represents nosocomial strains of bacteria that successively accumulate resistance genes, including genes encoding resistance to QAC.
When designing study I, a valid and adequate method for obtaining bacterial samples from the skin and the subcutaneous tissue was sought from previous studies. In an old study, Johnston et al. described using Rodac plates when they studied recolonization of the skin, and Hambreus et al. compared different skin sampling methods using different pads, Rodac plates, and swabs. Hambreus et al. also stated that the pad method was the most efficient method. These pads, (PVA) 2 x 7 cm, were used previously for intraoperative bacterial samplings in a cardiothoracic study. However, sterile PVA pads are no longer manufactured. Further, wet pads compared to dry showed a ten-fold higher yield of bacteria. For that reason, all Rayon swabs used in studies I, II, and III were moistened with sodium chloride 0.9% before samplings were performed. Since there is no gold standard for bacterial sampling, it became necessary for us to evaluate the best sampling method (study I). Three different methods were used and evaluated: Rodac Plate (a contact plate, imprint Trypticase Soy Agar (TSA) plates); Rayon Swab (COPAN); and, an E-swab (a flocked nylon fiber swab). Although the ESwab is designed to optimize specimen collection, the results from study I showed that the Rayon swab was the most effective sampling method.

The strength of the design of study I is that, although there is only a small study population, the participants are their own control. The study was conducted in an OR department and was designed to replicate clinical reality as far as possible. There was a risk for contamination in both studies I and II, so the skin area exposed was very small. There was however one practical limitation. When samples were collected the plastic adhesive drape had to be lifted. So to avoid contamination, all subcutaneous samples were collected from the upper end of the wound where the drape remained adhered to the skin during the entire surgical procedure. Study II was performed as a randomized controlled trial (RCT), which provides a robust design for underpinning the results and also for controlling the internal validity of the study. However, RCT studies are unfortunately often expensive and take time to carry out, as was the case with the present study.

In study I, the same researcher (OR nurse) performed all skin samplings, while in the clinical trials (II and III) different OR nurses and surgeons performed the bacterial samplings. To decrease the risk of bias, the samplings were performed in a standardized way and in a pre-determined
area. That different people performed the samplings can be seen as both a strength and a limitation of the study. All the OR nurses employed in this department (n=14) performed bacterial samplings throughout the study, which may affect the reliability of the samples. On the other hand, this is a clinical study and in this regard it reflects the reality of the OR department. A research nurse was present in the OR during the interventions which was an advantage for increasing the internal reliability of the study in terms of timekeeping and managing the sampling protocol. Finally, no sedimentation plates were used to collect airborne microorganisms in studies II or III. It would have been interesting to compare the amount of CFU/m³ in the OR environment with study I, and also compare the growth in the plates with the bacterial growth from the surrounding skin in the present studies. For patients with skin covered with plastic adhesive drape until the time of sampling, airborne contamination is less likely to have occurred. In the bare skin group (study II), the colonization may have been caused by exogenous sources. However Bitkover et al. found no correlation between airborne and wound contamination, and concluded that non-airborne bacterial spread was predominant.164

In preparing the patients’ skin with 0.5% chlorhexidine in alcohol, the same OR nurse prepared both the chest and leg (for patients enrolled in both studies II and III). This consistency ensures the same technique was used for skin disinfection - using a forceps and swab - for both the chest and leg areas. This strengthens the report and increases the reliability of the data. In addition, all OR nurses in study III were educated in how to handle the skin sealant following a standardized procedure, and were provided hands-on instruction prior to the first application.

Leg wound infection was defined as wound complication requiring antibiotic treatment in study III. The definition of postoperative surgical wound infections was based on CDC criteria- diagnosis of superficial or deep SSI, made by a physician, requiring antibiotic treatment. Many confounding factors can affect to the identification of SSI. It can be complicated to recognize and diagnose a harvest site infection, but by antibiotic treatment as outcome with positive bacterial cultures reduces the risk of bias. This study is in line with the result of Swenne et al.149

Unfortunately, 5 patients were antibiotica treated by symptoms of infection whithout confirmed bacterial samplings and it may have affected in the internal reliability. There is also a risk of recall bias in the twon-month telephone follow-up.224
FUTURE CONSIDERATIONS

The most effective way to inhibit postoperative wound infections is to prevent transmission of bacteria into the surgical wound. The SSI causing bacteria originates from different sources. In this thesis, the focus has been on the patient’s recolonization intraoperatively. In the bare skin groups, it’s difficult to know if the CoNS found on and in the patient were her/his own, however it’s most likely that *P. acnes* is an endogenous bacteria from the patient himself.

The skin flora can be reduced through preoperative strategies including preoperative showers, agents such as chlorhexidine soap, and skin preparation in the operating room with antiseptic solutions such as chlorhexidine in alcohol. Products available on the market, such as adhesive drapes and skin sealant, are sold to surgical care providers to further reduce the risk of wound contamination. It is of great importance that these strategies and products are tested by independent researchers in randomized controlled clinical trials before the products are launched into clinical practice. It is also important that healthcare workers are critical regarding new products and demand independent research evidence before using them. There are also ethical considerations such as the relationship between cost and effect in the decision to adopt a new intervention or strategy.\(^{225}\)

The results of study II are an example of how results from an independent researcher can lead to the development of new evidence-based products. In this case Mölnlycke Health Care is developing a new sterile thoracic sheet without plastic adhesive drape (Fig. 16) based on this research.

![Photo](image.jpg)

**Fig. 16 Sterile thoracic sheet without plastic adhesive drape**
Considerable progress has been made in surgery and infection control since the discovery of the link between microorganisms and infections 150 years ago. The preventive work to reduce the number of postoperative wound infections is still important today. Patient safety work means protecting the patient from harm when receiving health care.226 Nowadays, there are new challenges such as the increased numbers of surgical procedures, new surgical methods with foreign materials, more invasive procedures, and a more morbid and fragile patient population.227 One major concern is increasing antibiotic resistance in common pathogens.228 This leads to greater demands for surgical care providers to identify and be compliant with practices for preventing wound infections. The preventive strategies and procedures of the OR nurse are therefore of great importance in protecting the patient against medical harm.229 They lead to decreased postoperative infections and safe intraoperative care for the patient.
FUTURE RESEARCH

The results from this thesis give rise to the following suggestions for future research.

- In contrast to earlier studies the results from studies I and II showed considerable *P. acnes* and CoNS bacterial growth on the chest after the preoperative showers with chlorhexidine soap. This finding warrants further studies such as comparing the efficiency in reducing bacterial growth of different skin preparations measuring as well as the effect on the rate of SSI.

- There was significantly more *P. acnes* growth on the chest in men compared to women. Further trials need to investigate this gender difference. Do men require different preoperative skin preparation when undergoing cardiac surgery? Further research is needed.

- There is also a need for an RCT study to compare the efficacy of Ioban adhesive skin drapes in reducing bacterial growth compared with plastic adhesive drape.

- The work of the OR nurse needs to be informed by evidence-based guidelines, but there is currently a lack of evidence-based research in this area, so there is a great demand here. For example, no studies have systematically compared which preparation technique, when using chlorhexidine solution in alcohol, is most effective on the sternal area.

- Currently, guidelines say that the patient’s skin should be washed for at least 2 minutes with ample chlorhexidine solution. Studies are needed to evaluate the time aspect of chlorhexidine’s chemical effect in eliminating *P. acnes* versus CoNS on the chest.

- Another interesting study to carry out would be a RCT study comparing the effectiveness between 0.5% and 2% chlorhexidine in alcohol in eliminating *P. acnes* and CoNS on the chest.

- The results in study III, showing a 16.8% incidence of postoperative wound infections after saphenous vein graft harvesting,
need further research. This would require a larger RCT study comparing bare skin and microbial skin sealant. Another study of interest would be to compare the traditional technique of vein harvesting versus other technologies, with SSI as the end-point. It would also be of great interest to compare the effect of microbial skin sealant versus bare skin on the chest regarding the level of bacterial growth.

- The most common bacteria causing the infections in study III was *S. aureus*. Further research should be conducted into the impact of positive nasal carriers in cardiac surgery patients with SSI as the end-point. Patients would need to be randomized into muopricin or placebo groups. Furthermore, it would be useful to map out the point prevalence of nasal carriers of *S. aureus* and *S. epidermidis* among the staff in the OR, ICU, and ward. Are specific clones present? Are they resistant?
CONCLUSIONS

- Plastic adhesive drape on patients chest did not reduce the bacterial recolonization or wound contamination

- *P. acnes* colonized male patients more often than females

- *P. acnes* is not affected by disinfection with 0.5% chlorhexidine in ethanol

- There seems to be almost no bacterial growth intraoperatively on patients leg regardless of the use of microbial skin sealant and bare skin

- SSI in leg wounds are mainly caused by *S. aureus*, a different bacterial species than observed intraoperative

- A decreased sensitivity to chlorhexidine and presence of QAC resistance genes were common among *S. epidermidis* which caused deep postoperative wound infections

- The isolates from skin after disinfection with 0.5% chlorhexidine in alcohol were sensitive to chlorhexidine
SAMMANFATTNING PÅ SVENSKA

Vårdrelaterade infektioner utgör en av de största utmaningarna i sjukvården. Idag drabbas ca 10 % av alla patienter av en infektion. Kostnaden beräknas till ca 3.7 miljarder SEK/per år. Infektioner i sårområdet efter ett kirurgiskt ingrepp är en av de vanligaste typerna av vårdrelaterade infektioner. Sveriges Kommuner och landsting har som mål att genom nationella patientsäkerhetsarbeten minska postoperativa sårinfektioner från dagsläget 10 % till 5 %. Postoperativa sårinfektioner är en allvarlig komplikation efter ett kirurgiskt ingrepp. Särinfektionerna orsakar mänskligt lidande, förlängd sjukdomstid och kan leda till döden. De vanligast förekommande bakterierna som kan leda till allvarliga infektioner inom thoraxkirurgi är koagulasnegativa stafylokocker (KNS) och Staphylococcus aureus. KNS finns normalt på huden hos alla människor. Postoperativa sårinfektioner som orsakas av bakterier från patientens egen hudflora kallas för endogen smitta. På och i kroppen finns fler bakterier än våra egna celler och fem miljoner bakterier lever permanent i genomsnitt på varje cm² på huden. Om operationssåret förörenas från patientens normala hudflora är risken för infektion förhöjd vid infektionskänslig kirurgi såsom ortopedi, hjärt-kärlkirurgi och implantationskirurgi.

Idag finns det olika produkter på marknaden som påstår sig fördröja rekoloniseringen av bakterier på huden och minska risken för att operationssåret kontamineras. En sådan infektionsförebyggande produkt är en incisionsfilm av plast, vilken täcker operationsfältet och som sedan kirurgen skär igenom. Teoretiskt skulle plasten kunna åstadkomma en ”växthusmiljö” på undersidan, vilken i så fall snarast skulle påskynda bakterietillväxt och rekolonisationen av huden. Vidare skulle incisionsplasten snarast öka särkontaminationen, åtminstone om man drar bort plasten från huden i slutet av operationen innan huden sys ihop, vilket mycket ofta förekommer av praktiska orsaker. Ytterligare en produkt för att minska föröreningarna i såret av hudbakterier är microbiell förseglning, så kallad huddättningsfilm. Teoretiskt finns fördelen jämfört med incisionsplast att filmen tränger ner i huden och immobiliserar även djupt belägna bakterier.

En operationssjuksköterska ansvarar för att systematiskt planera och organisera arbetet i samband med ett kirurgiskt ingrepp. Genom att följa hygieniska och aseptiska principer och förebygga smitta och smittspridning
bidrar hen till att patienterna får en god och säker perioperativ omvårdnad. Forskningsfrågan känns idag än mer angelägen då den ökande förekomsten av antibiotikaresistenta bakterier gör det nödvändigt att arbeta utifrån evidensbaserade riktlinjer.


**Delarbete I**

En jämförande pilotstudie av 10 försökspersoner, 5 män och 5 kvinnor, som simulerade hjärtpatientens pre- och intraoperativa förberedelser. På försökspersonernas vänstra sida av bröstkorgen fästets 8 stycken plastfilmer och på den högra sidan av bröstkorgen var det endast bar hud. Jämförande bakterieodlingar gjordes under 6 timmar med tre olika odlingsmetoder i syfte att validera det känsligaste odlingsinstrumentet. Resultatet visade att efter 30 min fanns bakterieväxt på huden som varit täckt med plastfilm men inte på den delen av huden som var bar. På den bara huden återfanns bakterieväxt efter 60 min. I samtliga odlningar växte KNS. Det totala odlingsantalet under de 6 timmarna som studien pågick visade signifikant fler positiva bakterieodlingar på hud med plastfilm jämfört med bar hud, 31 % vs. 7.5 %, p<0.001. Vidare framkom att den mest optimala odlingsmetoden var med Rayon swab i jämförelse med Rodac kontaktplatta och ESswab. Resultatet från delarbete I låg sedan till grund för hypotes och urvalsstorlek av delarbete II.

**Delarbete II**

En singel-blindad randomiserad kontrollerad studie med 140 patienter som genomgick elektiv hjärtkirurgi. Patienterna var randomiserade till incisionsfilm (bröstkorgen täckt med plastfilm) eller till bar hud. Bakteriella prover togs preoperativt och intraoperativt varje timme under operationen (på huden och i operationssåret) tills operationssåret var
hoppytt. Resultat visade att efter desinfektion med 0,5 % klorhexidin lösning i 70 % alkohol minskade KNS medan det av Propionibacterium acnes (P. acnes) fanns växt i mer än 50 % av odlingarna. P. acnes förekom i signifikant högre grad hos män jämfört med kvinnor. Intraoperativt vid 120 minuter förekom ett större antal positiva odlingar av P. acnes på huden under incisionsfilmen, 63 %, jämfört med bar hud, 44 %, p=0.034 och för KNS, 45 % under incisionsfilmen mot 24 % vid bar hud, p=0.013

En signifikant skillnad i bakterieväxt i operationssåret återfanns vid sårslutning: incisionsfilm 14.7 % mot bar hud 4.4 %, p=0.044.

**Delarbete III**

En singel-blindad randomiserad kontrollerad studie med 140 patienter som genomgick kännsäkrsloperation. Patienterna var randomiserade till hudtätningsfilm (benen ”rollat” med hudtätningsfilm) eller till bar hud. Bakterieodlingar togs preoperativt och intraoperativt vid fyra tillfällen på huden och i operationssåret. En infektionsuppföljning gjordes 2 månader postoperativt. Resultatet av bakterieodlingarna visade ringa växt av KNS och P. acnes både på bar hud och med hudtätningsfilm. Inga skillnader finns i bakterieväxt mellan bar hud och hudtätningsfilm. Av de 140 patienterna drabbades 21 patienter av en postoperativ sårinfektion, 14 med bar hud och 7 med hudtätningsfilm, p=0.120

**Delarbete IV**

En deskriptiv studie i syfte att undersöka fenotypiska och genotypiska metoders känslighet för klorhexidin hos KNS. 143 olika kliniska bakterieisolat av KNS insamlades från patienter som drabbats av infektion efter insättande av ledprotes eller efter hjärtoperation och från hud på patienter som desinficerats inför hjärtoperationen samt från friska försökspersoner. QacA/B-genen återfanns i 62/143 isolat (43 %); 52 % av isolaten från protesinfektioner och 61 % av infekte rade hjärtsjukurfigiska patienter. Bland isolaten från de friska försökspersonerna återfanns QacA/B genen i 25 % och i 19 % på hud inför hjärtsjukurgi.

Sammanfattningsvis visade resultaten från de fyra delstudierna att en incisionsfilm på patientens bröst under hjärtkirurgi inte minskar den bakteriella rekoloniseringen eller kontamineringen. Den desinfekterande effekten av 0.5 % klorhexidinsprit tycks vara sämre på männens hud jämfört med kvinnor och män koloniseras i högre grad av P. acnes än kvinnor. Oavsett om patienten har bar hud eller en hudtätningsfilm över
operationssåret på benet så finns ingen skillnad i den bakteriella rekoloniseringen eller kontamineringen. För övrigt är bakterieväxten på benet ringa under operationen men trots det drabbades 16.8 % av patienterna av en postoperativ sårinfektion. Det förefaller som att en minskad känslighet för klorhexidin och närvaro av QAC resistenta gener är vanligt förekommande bland *S. Epidermidis* i de fall den orsakar djupa sårinfektioner. Bakterieisolat från hud efter desinfektion med 0.5 % klorhexidinsprit visar sig däremot vara känsliga för klorhexidin.
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Vetenskaplig uppsats för licentiatexamen/Academic essay.

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Doktorsavhandling/Doctoral thesis with focus on Occupational Therapy.

Vetenskaplig uppsats för licentiatexamen/Academic essay.

* Seriens namn var tidigare (nr 1–24) ”Örebro Studies in Caring Sciences”.


