**Study protocol**

**Official title:** Prophylactic Treatment of Breast Implants With a Solution of Gentamicin, Vancomycin and Cefazolin Antibiotics for Women Undergoing Breast Reconstructive Surgery: a Randomized Controlled Trial (The BREAST-AB Trial)

**NCT:** NCT04731025

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This document relates to the ClinicalTrials.gov study record above.

Trial Protocol

# Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Short Title: Local Antibiotics for Breast Implants

Acronym: The Breast-AB Trial

By

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## Trial synopsis

**Title**: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

**Lay title**: Local Antibiotics for Women Undergoing Breast Reconstruction Surgery with Implants

**Acronym:** The BREAST-AB Trial

**Trial design**: A multi-center, investigator initiated, 1:1 randomized, double blind, placebo-controlled trial

**Intervention**: Application of gentamicin, vancomycin and cefazolin in a saline solution onto the implant and the dissected breast pocket used for breast reconstructive surgery

**Objective**: To determine the efficacy of local antibiotics in decreasing all-cause implant explantation

**Inclusion criteria**: Age ≥ 18, female, signed informed consent, breast reconstruction with implants including immediate/delayed reconstructions, bilateral/unilateral reconstructions and with or without flap reconstruction

**Exclusion criteria**: Pregnancy, breast feeding, known allergy towards any of the applied antibiotics, known anaphylactic reaction towards the same class of antibiotics as used in the trial, known allergy towards neomycin, known impaired renal function with GFR < 60 ml/min, participation in investigational drug trials and projects concerning disinfecting agents in the implant pocket and myasthenia gravis disease

**Primary outcome**: All-cause explantation of the breast implant within 180 days after the breast reconstruction surgery

**Secondary outcomes:**

- Time to explantation (days)
- All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Infection-specific revision surgery: Revision surgery due to clinically suspected deep surgical site infection with surgical access to the breast implant pocket or clinical signs of an infection in the breast implant pocket found intraoperatively within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)

**Tertiary outcomes:** Assessed for patients undergoing unilateral breast reconstruction

- Time from surgery to discharge (days)
- Re-admission within 180 days after the surgery (Y/N)

**Long-term outcomes**: All-cause incision of the fibrous capsule and capsular contracture after 5, 10 and 15 years

**Sample size**: A total number of 1274 breasts undergoing breast reconstruction will be included in the trial. Assuming that 27 % of the patients undergo bilateral breast reconstruction, this entails 1003 included patients

**Trial duration**: 6 years

**Randomization**: Stratified randomization according to the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous or scheduled radiotherapy within the follow-up period (yes/no)

All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast. Combining these factors gives a total of 14 randomization strata per trial site. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum

**Treatment**: The intervention treatment will consist of 1000 mg vancomycin, 2 mL of 40 mg/mL gentamicin and 1000 mg cefazolin in a 500 mL sterile isotonic (9 %) saline solution. The placebo solution will consist of 500 mL of sterile isotonic (9%) saline. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (in total 150 ml) and use it to wash the dissected implant pocket. Another 50 ml syringe will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket

**Clinical follow-up**: The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site

**Blinding:** The patients, surgeons and data assessors will be blinded to the treatment allocation throughout the trial period. The coordinating sponsor-investigator will be responsible for monitoring adverse events. The trial coordinating unit will have access to the randomization sequences. They will not take part in any treatment of the participants or analysis of data. In the case of emergency unblinding the trial coordinating unit can always be contacted

**Safety**: Treatment-related adverse events will be reported and assessed continuously throughout the trial period

**Trial Steering Committee**

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An independent biostatistician who will be appointed in the statistical analysis plan (SAP) before the assessment committee receives the data. The SAP will be made publicly available before inclusion of the last patient, and before unblinding.

| Site Investigators | Clinical Trial Site |
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**Cefazolin**

Cefazolin "MIP"
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**Gentamicin**
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# 1. Introduction

The incidence of breast cancer in Danish women is approximately 4700 per year.[1] Many women choose to undergo breast reconstruction with an implant following a mastectomy.[2] Unfortunately, implant-based breast reconstructions are associated with high complication rates. Postoperative infection of the breast and implant is one of the most severe short-term complications affecting around 5-10 % of the women.[3–7] Clinically infected implants must be surgically removed and the recovery period that follows is long and agonizing for the women. Subsequent attempts to reconstruct the breast are often postponed for several months or abandoned altogether.

Many strategies to prevent complications associated with bacterial contamination of the breast implant have been attempted.[8–13] According to a survey made by the American Society of Plastic Surgeons, the most widely followed approach is to apply antibiotics directly on the breast implant and the dissected tissue pocket to eliminate bacterial contamination during the surgery.[14] Although the use of local antibiotics on breast implants is now widespread, the treatment regimen has never been investigated in a randomized controlled trial.[15] This protocol will describe a randomized controlled trial that will investigate the effect of antibiotics applied locally on the implant and in the breast implant pocket on the incidence of infection that leads to explantation of the implant. The protocol has been designed in accordance with the SPIRIT 2013 Statement guidelines for protocol content.[16]

## 1.1. Bacterial contamination of the breast implant

The most prevalent bacterial agents associated with breast implant infections are similar to those of the breast duct and skin flora which suggests that these are possible sources of contamination.[17] The most common microorganisms found on infected breast implants are *Staphylococcus epidermidis* and *Cutibacterium acnes* (previously known as *Propionibacterium acnes*). Other bacteria that have been identified on breast implants are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.[18,19]

Previous studies propose that bacterial colonization of breast implants sometimes occur without any immediate clinical manifestations.[20,21] Instead, bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant known as capsular contracture, affecting approximately 10-20 % of the patients.[22,23]

## 1.2. Local administered antibiotics

Local administration of antibiotics can achieve a high local concentration with a low systemic uptake[24] and thereby, minimize the systemic side effects while achieving high antibiotic penetrance. A high local concentration can ensure optimal effect of the antibiotics at the surgical site,[25] and thereby decrease the rate of postoperative surgical site infections, while potentially minimize the risk of antibiotic resistance. Local antibiotics also have the benefit of being independent from the tissue vascularization to achieve peak concentration as opposed to systemic antibiotics, which is an advantage during larger surgeries where the vascularization can be compromised.[25]

Studies have shown that the concentration of locally applied antibiotics in the surgical drain output is high during the first 24 hours[24] and after 72 hours, the concentration is negligible. Therefore, it is assumed that the potential side effects to the medication will occur within the first 72 hours and previous studies have not reported side effects to the local treatment.[26]

In 2001, Adams et al recommended an antibiotic regimen consisting of gentamicin, cefazolin combined with either bacitracin or vancomycin.[27] Internationally, this irrigation regimen has become the most commonly used for local breast pocket and implant irrigation.[28] In this trial we will investigate the combinations of gentamicin, cefazolin and vancomycin for irrigation for the breast pocket and breast implant.

### 1.3. Pre-clinical data

Preclinical data from in vitro models suggest that the combination of Gentamicin, Cefazolin and Vancomycin is the most efficient treatment against the bacterial species most commonly associated with breast implants.[27,29] Animal studies suggest that the local application of these antibiotics is safe.[30–33]

### 1.4. Clinical data

**Current Evidence – a systematic review**

The regimen of local antibiotics for breast implants and the dissected implant pocket has been widely applied in humans[14], but few studies have investigated the clinical effect. In May 2020, we searched scientific literature databases including Embase, Cochrane, Pubmed and Web of Science. We used the following search terms (((breast) AND (implant OR expander OR augmentation OR reconstruction)) AND (irrigation OR antibiotics OR antibacterial OR antiinfective OR antimicrobial OR disinfection OR bacitracin OR gentamicin OR vancomycin OR cefazolin OR neomycin)) AND (infection OR "capsular contracture" OR "capsular contraction" OR capsulitis). We included studies and reviews investigating the effect of any local antibiotics for irrigation of the implant and/or implant pocket in women undergoing implant-based breast reconstruction or cosmetic breast augmentation. Studies that did not list outcomes that were relevant for the primary and secondary outcomes of the BREAST-AB trial were excluded. The search identified 1697 studies of which 17 studies were included after title/abstract and full text screening. Seven review articles,[15,34–39] two prospective studies[40,41] and eight retrospective studies[41–49] were identified. No randomized controlled trials were identified. Most of the included studies included solely reported on patients undergoing cosmetic augmentation. Two studies included patients undergoing cosmetic breast augmentation and breast reconstruction, but they did not stratify the outcome.[48,49]

Two studies found a significant decrease in the infection rate when applying local antibiotics compared to a control group,[42,43] whereas one study found no significant decrease.[44] These studies were limited by the relatively small study populations and a poorly defined outcome and none of the studies were blinded. The rate of capsular contracture was found to be significantly decreased in two studies,[44,45] two studies found no significant decrease,[43,46] whereas one study found a significant increase in the capsular contracture rate.[41] However, all studies investigating

capsular contracture were limited by a short follow-up period for this long-term outcome. No adverse events have been reported in any of the included studies, and the local antibiotics were generally considered well-tolerated. See appendix 1 for a table of characteristics of the included studies.

### 1.5. Rationale

Administration of antibiotics directly on to the breast implant and dissected implant pocket will give a high concentration of the antibiotics where they are needed which may prevent bacterial contamination of the implant. This may decrease the rate of postoperative infections that lead to explantation of the implant and thereby improve the outcome for the patients.

### 1.6. Hypothesis

Local administration of gentamicin, cefazolin and vancomycin on the breast implant will decrease the rate of postoperative clinical infections compared to placebo.

## 2. Experimental design

### 2.1. Trial design

This trial is an investigator-initiated, randomized, double-blind and placebo-controlled clinical phase III trial. The triple antibiotic solution or placebo solution will be applied directly onto the implant used for breast reconstruction and the implant pocket. The included subjects who undergo bilateral reconstruction will be randomized to the triple antibiotic solution to one of their breasts and placebo to the contralateral breast. Those who undergo unilateral reconstruction will be randomized to the triple antibiotic solution or the placebo solution. See 4.2 for a more detailed description of the randomization. The triple antibiotic solution will consist of 1 g Vancomycin, 1 g Cefazolin and 80 mg Gentamicin diluted in 500 mL of saline.[27] The placebo solution will consist of 500 mL of saline. See section 5 for more information on the trial treatment.

### 2.2. Outcomes

#### 2.2.1. Primary outcome

All-cause explantation of the breast implant within 180 days after the breast reconstruction surgery

Definition

All-cause explantation will be defined as explantation and discarding of the implant. Replacement of an expander with a permanent implant and replacement of a permanent breast implant with a new permanent breast implant due to cosmetic revisions such as asymmetry, implant malposition, change of size or implant rotation will not be counted as an explantation.

Rationale

The rationale for applying local antibiotics is to decrease the risk of severe complications associated with the presence of bacteria such as deep surgical site infection that leads to

explantation and discarding of the implant. Postoperative infection that leads to explantation of the implant will sometimes occur simultaneously with other complications where the cause of explantation may be unclear. Therefore, all-cause explantation is a logical and meaningful primary outcome.

The reason for excluding from the primary outcome: explantations of permanent implants followed by replacements with a new permanent implant for cosmetic reasons, is that such revisional surgery is not considered a proxy for severe complications that may be associated with a deep surgical site infection.

### 2.2.2. Secondary outcomes

The secondary outcomes will include:

- Time to explantation (days)
- All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)
- Infection-specific revision surgery: Revision surgery due to clinically suspected deep surgical site infection with surgical access to the breast implant pocket or clinical signs of an infection in the breast implant pocket found intraoperatively within 180 days after the breast reconstruction surgery (Y/N)

<u>Definition</u>

Time to explantation will be defined as the number of days between the breast reconstruction and the implant explantation surgery. The breast reconstruction surgery will be defined as the surgery where they received the allocated treatment. Surgical site infection will be defined according to the CDC classification of surgical site infetion[50] leading to antibiotic treatment with oral or intravenous antibiotics administered after the surgery. Infection-specific revision surgery will follow the same definition as the CDC classification of surgical site infetion[50] and may involve a positive culture, pus, redness of breast, fever or other signs of infection around the breast implant.

<u>Rationale</u>

Time to explantation is important to determine the relation to the breast reconstruction surgery and the etiology of the event that leads to explantation of the implant. In some cases, the reconstructed breast may be upheld despite complications associated with bacteria by revisional surgery and therefore revisional surgery is an outcome of importance. Local antibiotics may decrease the incidence of postoperative surgical site infection requiring antibiotic treatment. Postoperative swelling and redness are to be expected after a larger surgery and can be difficult to

distinguish from signs of infection. Therefore, surgical site infection that leads to antibiotic treatment is a logical outcome.
Revision surgery due to clinically suspected deep surgical site infection is an important secondary outcome to help isolate the effect of the intervention. Some of the explantations in the primary outcome (all-cause explantation) are expected to result from ischemia and necrosis of the mastectomy flaps due to impaired perfusion, rather than from infection. The intervention (triple antibiotic irrigation of the implant and pocket) is designed to reduce the risk of bacterial contamination and subsequent deep surgical site infection. It is not expected to influence perfusion-related complications. To better separate these mechanisms and to estimate the effect of the intervention on infection-related complications, all primary outcome events will undergo blinded adjudication to determine whether the indication for revision surgery was consistent with deep surgical site infection involving the implant pocket. This adjudicated endpoint will serve as an important secondary outcome to isolate the component of the primary outcome that the intervention could plausibly modify.

### 2.2.3. Tertiary outcomes

The tertiary outcomes will be assessed for patients undergoing unilateral breast reconstruction. The tertiary outcomes will include:

- Time from the breast reconstruction surgery to discharge (days)
- Re-admission within 180 days after the breast reconstruction surgery (Y/N)

Definition

Time to discharge will be defined as the amount of days between the breast reconstruction and the day of discharge. The breast reconstruction surgery will be defined as the surgery where the patient received the allocated treatment.

Rationale

The rationale for excluding bilateral patients from the tertiary outcomes is that all bilateral patients will receive placebo on one breast and the intervention on the contralateral breast. Therefore, patient related outcomes are only applicable for patients who undergo unilateral breast reconstruction. Postoperative infection that occurs during the hospital admission can prolong the admission period. Application of local antibiotics may shorten the admission period by decreasing the rate of postoperative infection occurring during the hospital admission. Infection that occurs after discharge can cause re-admission to the hospital. Local antibiotics may decrease the infection rate after discharge that require hospitalization.

### 2.2.3 Additional follow-up

The trial will include additional long-term outcomes focused on all-cause incision of the fibrous capsule around the breast implant, capsular contracture, Baker classification[51] and quality-of-life. See Gantt chart figure 1.

Definition

Capsular contracture and the Baker classification grade will be obtained from the National Patient Registry and the patients' medical journals after 5, 10 and 15 years. The BREAST-Q questionnaire[52] will be used to assess patient-reported outcomes. The patients will be contacted and asked to fill out the questionnaire with 5 year-intervals after the surgery.

Rationale

Previous studies suggest that bacterial contamination of the breast implant can occur without immediate clinical manifestation.[20,21] Instead, the bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant called capsular contracture, affection 10-20 % of the patients.[22,23] The use of local antibiotics could potentially decrease the rate of capsular contracture by minimizing the bacterial contamination of the implant. Therefore, capsular contracture is a meaningful long-term outcome.

The application of local antibiotics may decrease the risk of postoperative complications and thereby decrease the risk of undergoing revision surgery. This in turn may lead to improved patient satisfaction and quality of life. Breast-Q is a validated tool used to quantify patient satisfaction and health-related quality of life after breast reconstruction surgery.[52]

Patients may be included in additional exploratory substudies at the time of implant explantation (e.g. expander removal). The exploratory substudies will be applied for in separate protocols to be approved by the relevant authorities and they will not interfere with this trial.

### 2.3. Setting and locations

The trial will be a nationwide multi-center trial with enrollment of patients from the following Danish hospitals:

- Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen
- Department of Plastic Surgery, Herlev and Gentofte Hospital, Borgmester Ib Juuls Vej 1, 2730 Herlev
- Department of Plastic Surgery, Zealand University Hospital, Sygehusvej 10, 4000 Roskilde
- Department of Plastic Surgery, Odense University hospital, J. B. Winsløws Vej 4, 5000 Odense
- Department of Plastic Surgery, Aarhus University Hospital, Palle Juul-Jensens Boulevard 35, 8200 Aarhus
- Department of Plastic Surgery, Aalborg University Hospital, Søndre Skovvej 3, 9000 Aalborg

All sites have clinical experience and expertise in performing implant-based breast reconstructions.

### 2.4. Number of Subjects

The trial will include patients until a total number of 1274 breast reconstructions according to our power calculation. We estimate that this number will be distributed on approximately 1003 patients provided that approximately 27% of patients undergo bilateral procedures. A total of 637 breasts will be allocated to placebo and 637 breasts will be allocated to treatment with the local antibiotic solution. The statistical considerations behind the sample size calculation is elaborated in section 8.1.

### 2.5. Trial Duration

We plan to begin inclusion in January 2021 at Rigshospitalet and Herlev Hospital. The other trial sites will begin enrollment thereafter according to the plan outlined below. We expect to begin inclusion at Zealand University Hospital in spring 2022, Odense and Aarhus University Hospital in autumn 2022, Aalborg University Hospital in spring 2023 followed by South-West Jutland Hospital and Hospital Little Belt in the autumn 2023. Additional trial sites may be applied for during the trial period if we do not meet our expected aim for included patients. See Gantt chart in figure 1.

We expect to include the 1003 patients over a 6-year period with planned completion June 2026, hence the last follow-up after 180 days will be completed in December 2026. Currently, 700 women undergo reconstruction with implants each year in Denmark.[53] Therefore, we assume that it will be feasible to include approximately 334 patients per year.

| Trial site | Inclusion period | Expected no. of included patients |
|---|---|---|
| Rigshospitalet | Jan 2021 – June 2026 | 222 |
| Herlev Hospital | Jan 2021 – June 2026 | 360 |
| University Hospital Zealand | Feb 2022 – June 2026 | 183 |
| Odense University Hospital | Oct 2022 – June 2026 | 97 |
| Aarhus University Hospital | Sep 2022 – June 2026 | 111 |
| Aalborg University Hospital | Feb 2023 – June 2026 | 30 |
| **Total** | | **1003** |

## 3. Subjects eligibility

All trial candidates will be evaluated for suitability by a medical doctor with expertise in the field of breast reconstruction surgery. All potential participating patients will receive oral information by the medical doctor and all information material will be given to the patient before the written informed consent form is signed. See participant timeline in figure 2.

### 3.1. Inclusion criteria

The patients must fulfill all the following criteria to be eligible for inclusion in the trial:

- Age ≥ 18 years
- Biologically female

- Signed informed consent
- Scheduled for breast reconstruction with implants or expanders including:
    a. Immediate or delayed reconstructions
    b. Bilateral or unilateral reconstructions
    c. With or without simultaneous flap reconstruction

### 3.2. Exclusion criteria

Patients are considered ineligible if any of the following criteria is fulfilled:

- Pregnancy
- Breast feeding
- Known allergy towards Vancomycin, Gentamicin and Cefazolin
- Known anaphylactic reaction towards other beta-lactam antibiotics or aminoglycosides
- Known allergy towards neomycin
- Known impaired renal function with GFR < 60 mL/min
- Participation in investigational drug trials and projects concerning disinfecting agents in the breast implant cavity
- Myasthenia Gravis

### 3.3. Pregnancy

Fertile women with child-bearing potential must provide a negative urine HCG prior to inclusion in the trial.

## 4. Enrollment

The patients will be registered in the trial after providing written consent (via the written consent form or a digital signature). The registered patients are considered enrolled in the trial when they have received the treatment. Each step of the enrollment procedure is described below.

### 4.1. Registration

All patients scheduled for a preoperative visit concerning a breast reconstruction procedure will be screened for eligibility and recorded in the individual trial site's screening log (appendix 2). No personal data will be recorded in the screening log. The following variables will be registered in the screening: screening number, screening date, initials of the person conducting the screening, age of the patient, if available date of pre-operative visit and surgery, eligibility of the patient yes/no and if "no", reason for non-eligibility. All patients who are considered eligible and have provided a written consent will be registered in the trial with a letter code for each site (e.g. RH for Rigshospitalet) combined with a record ID. The record ID will be assigned in sequential order as subjects are registered (1, 2, 3). Registration will include date of registration, central registration number, unilateral or bilateral reconstruction, type of surgery (immediate or delayed reconstruction) and radiotherapy status. The identification number remains constant throughout the trial.

### 4.2. Randomization and treatment assignment

Registered subjects will be randomized to placebo or the trial drugs on the day of surgery or the day before, and they will be considered enrolled in the trial when they have received the trial treatment. The randomization number will be the same as the record identification number. All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast (Investigational Product Dosage and Administration, section 5). See figure 3.

We will use a stratified randomization to ensure that potential risk factors which could confound the outcome are evenly distributed in the placebo and intervention group. The randomization strata will be generated by the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous radiotherapy and/or planned radiotherapy within the follow-up period (yes/no)

When the three factors are combined, we get a total of 14 randomization strata per trial site. See appendix 3 for an overview of the 14 randomization strata. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum. The fixed block size of two will not increase the risk of the investigator anticipating the allocation because the investigators are blinded to the treatment throughout the trial.

A member of the trial coordinating unit will access the computer-generated allocation sequence via RedCap The allocation will be registered in a REDCap module only available for members of the trial coordinating unit, who are unblinded.

#### 4.2.1. Treatment assignment in two-stage breast reconstruction

Most implant-based breast reconstructions are performed in a single surgery with a permanent breast implant. During the surgery, the surgeon evaluates the quality and vitality of the dissected skin flaps before inserting the breast implant. In some patients, the skin quality is not considered suitable to allow for insertion of the permanent implant. These patients will be reconstructed in two stages, where an expander implant is used in the first surgery. The expander implant is used to expand the tissue before it can be replaced with a permanent implant after approximately 3 to 9 months of expansion. During the first surgery where the expander implant is inserted, the patient will be assigned to treatment according the randomization stratum. The allocation sequence number will be registered. At the second surgery where the expander is replaced with the permanent implant, the same treatment allocation will be used (e.g., if the right breast received treatment with the antibiotic solution in the first surgery, the right breast will receive treatment with the antibiotic solution in the second surgery). See figure 3.

### 4.2.2. Treatment assignment if a unilateral patient later becomes bilateral

In some cases, a unilateral patient can switch to the bilateral set-up. An example could be that a patient develops unilateral breast cancer, undergo mastectomy and reconstruction and then later in the trial period decide to undergo prophylactic risk-reducing mastectomy and reconstruction of the other breast. In this case, the patient would be assigned to either placebo or the trial drug in the first surgery. Then, when the patient undergo the second prophylactic surgery on the other breast, she will be allocated to receive placebo, if she had received antibiotics in the first surgery and vice versa, if she had received placebo in the first surgery she would be allocated to antibiotics on the other breast in the second surgery.

### 4.3. Registration failures

Registered subjects who are ineligible for randomization will be recorded as screening failures and they will be registered along with the reason for exclusion.

### 4.4. Discontinuation from the trial

Patients who withdraw their informed consent at any point during the trial period will be omitted from the trial and registered as “withdrawal of consent”. The coordinating sponsor investigator may omit participants from the trial at any point during the trial period due to safety of the participant. There will be no additional follow-up or data collection from these patients. The trial treatment is administered as a single dosage and therefore, exclusion will not have any effect on the trial treatment. The data analysis in the end of the trial will be performed on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent.

### 4.4.1. Registration of dropouts

A designated representative at each trial site will be responsible for contacting the trial coordinating unit in case an included subject withdraws consent or is unable to complete the follow-up. All excluded patients will be recorded including date, central registration number, reason for exclusion and treatment allocation. Patients excluded from the trial will continue to follow the scheduled follow-up visits as a part of the standard treatment. Exclusion from the trial will not interfere with the standard treatment or entail any additional procedures or follow-up visits. Dropout rates will be monitored continuously by the sponsor-investigator.

### 4.4.2. Replacement of dropouts

Patients who drop out of the trial after enrollment (allocation to trial treatment) will not be replaced. The sample size calculation accounts for a drop-out rate of 5 %. In case of a drop-out rate of more than 5 %, we will apply imputations by chained equations and repeat the primary analysis (for the primary outcome) after imputations.

## 5. Treatment procedures

The trial drug will be administered as a single dosage during the breast reconstructive surgery. The administration procedure will be identical for the antibiotic solution and placebo. Only qualified

healthcare personnel will perform the administration of trial drugs and no self-administration will take place. All personnel that handles investigational products will be instructed by members of the trial coordinating unit.

### 5.1. Investigational drugs

Gentamicin: 40 mg/ml gentamicin sulfate, 2 mL suspension in glass ampoules containing clear, colorless suspension without visible particles. Gentamicin is a broad-spectrum, bactericidal aminoglycoside primarily targeting gram-negative rods. Gentamicin (Hexamycin) produced by Sandoz A/S can be used but other producers of the same drug may be used as an alternative.

Cefazolin: 2096,72 mg cefazolin natrium equivalent to 2000 mg cefazolin of white or almost white powder in a capped vial. Cefazolin is a bactericidal antibiotic targeting both gram-negative and gram-positive bacteria. Cefazolin produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

Vancomycin: one capped vial contains vancomycin hydrochloride equivalent to 1000 mg vancomycin as a finely grounded white powder with a pink to brown nuance. Vancomycin is bactericidal and targets gram-positive bacteria. Vancomycin (Bactocin®) produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

All investigational drugs will be purchased through each trial site's clinical pharmaceutical services and their respective purchasing agreements. The investigational drugs will be kept and prepared in accordance with the manufacturer's recommendations (see 'summery of product characteristics' for cefazolin, gentamicin and vancomycin. The investigational drugs are part of the standard drug selection available at all trial sites. Therefore, we will not account for the overall stock of medicine. However, we will account for the use of investigational medicine for each patient enrolled in the trial, including batch-number, expiration date, patient registration number and date of administration.

### 5.2. Preparation of the drug solutions

The preparation of the trial drug solution will take place in a medication room on the trial site on the day of the surgery or day before the surgery. A designated trained nurse will be responsible for the preparation and labelling of the trial drug solution. To minimize errors, it will be double checked. The labelling will include the trial identification number and marking of which breast the treatment will be applied to (right or left). The manufacturing nurse will contact the trial coordinating unit to confirm his/her identity as investigational drug manufacturer and obtain instruction as to how to allocate the trial treatment. The communication between the Trial coordinating unit and the manufacturing nurse will be looped to prevent mistakes regarding the allocation. A designated person will deliver the prepared solutions to the operation room, and the surgeon and the scrub nurse will be blinded for the allocation. A local SOP describing the preparation of the trial drugs will be compiled for each trial site (See appendix 4 for the SOP).

#### 5.2.1. The antibiotic solution

The antibiotic solution will contain 1000 mg vancomycin, 80 mg gentamicin and 1000 mg cefazolin. Two syringes of 20 mL sterile saline will be drawn from an infusion bag containing 500 mL of

sterile isotonic (9%) saline. The drawn saline will be infused in the capped vials containing cefazolin and vancomycin to dissolve the powder. The entire content of the vial containing vancomycin (20 mL) will be drawn back into the syringe and reinfused in the infusion bag. Only 10 mL of the cefazolin solution will be drawn from the capped vial containing the dissolved cefazolin. The 10 mL will also be reinfused in the infusion bag. The remaining content the capped vial will be discarded as medical waste. Hereafter, the 2 mL gentamicin suspension will be drawn into a syringe and infused in the infusion bag already containing vancomycin and cefazolin. See figure 4 for an illustration of the treatment preparation.

#### 5.2.2. The placebo solution

The placebo solution will consist of 500 mL of sterile isotonic (9%) saline contained in a similar infusion bag.

Both the antibiotic solution and the placebo solution will be achromatic and will be indistinguishable from one another to ensure blinding of the health care personnel administering the drugs.

### 5.3. Investigational product administration

The assigned solution will be administered in an enclosed infusion bag. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (entailing 150 ml) and use it to wash the dissected implant pocket. Another 50 ml will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket. The rest of the content in the infusion bag will be discarded as medical waste. Patients undergoing unilateral breast reconstruction will receive the assigned solution in one infusion bag marked either left or right. The patients undergoing bilateral breast reconstruction will be assigned to the antibiotic solution to one breast and placebo solution to the contralateral breast, and the allocation sequence will determine which breast (right or left) gets which solution. The assigned solution bags will be marked right and left. See appendix 5 for SOP.

### 5.4. Dosage adjustments

The dose of investigational products will be fixed. The drug will be administered as a single dose, and one time only. No continuous administration will occur. It will not be possible to adjust the treatment after the administration of the treatment.

### 5.5. Concurrent medication

All included patients will be treated according to the local guidelines for breast reconstruction surgery at each trial site. The trial intervention will not interfere with any treatment procedures or administration of medication.

### 5.6. Blinding

The trial will double-blind so that the patients, site investigators and data monitors will be blinded to the allocation. Only the designated nurses and the members of the trial coordinating unit (who provide the randomization number and treatment allocation) are not blinded to the allocation.

The unblinded persons will not in any way be part of the treatment, clinical evaluation of outcomes or data assessment.

The intervention drug will be prepared in infusion bags that will be indistinguishable to the placebo solution (infusion bags with saline). Both solutions are colorless and identical in appearance without any identifying features, and therefore we do not anticipate any risk of unintentional unblinding. The designated manufacturing nurse will contact the trial coordinating unit to receive the allocation by telephone. The randomization number and the treatment allocation will be provided by the trial coordinating unit. An emergency telephone number to the trial coordinating unit will be available to access the treatment allocation of individual patients in the case where emergency unblinding is necessary. If unblinding should occur, it will be documented via the case report form. The unblinded patient will not be excluded from the trial.

The patients will remain blinded until the end of the additional follow-up period. The rationale for keeping the patients blinded is to minimize bias when assessing the long-term outcomes. The patients can be unblinded upon request if they withdraw their consent to participate in the trial.

#### 5.6.1. Ensuring blinding

The randomization number will ensure that the allocation is given during both surgeries in situations where the intervention treatment is repeated, for instance in two stage breast reconstruction (see section 4.2.1.) and if a unilateral patient becomes bilateral (see section 4.2.2.). During the first surgery, the registration number which equals the randomization number will be registered in the case report form. During the second surgery, the manufacturing nurse will contact the trial coordinating unit to obtain the treatment allocation and members of the trial coordinating unit (who are unblinded) will look up the patient and inform the manufacturing nurse of the treatment allocation. The manufacturing nurse will not take part in any treatment procedures.

## 6. Data collection

The site investigators, along with members of the trial coordination unit, will be responsible for trial related data collection and entry. The individual trial site investigator may delegate assignments to designated doctors, scholarship students or nurses registered in the site-specific delegation log. Limited trial specific data will be entered into a numbered case report form (see appendix 6) at the time of inclusion and the surgery. This along with the screening log will be the only source data and all additional data will be obtained from the patients' medical records. Data will be entered directly into REDCap.

### 6.1. Variables

All enrolled patients (i.e. patients who have been assigned to treatment) will be entered into the database. An overview of included variables is provided below.

#### 6.1.1. Pre-surgery variables

<u>Trial related variables</u>

Study ID

Site (location)

Unilateral or bilateral breast reconstruction

Immediate or delayed breast reconstruction

Prophylactic or cancer (including carcinoma in situ)

Radiation therapy status (Y/N)

Name

CPR number

Date and name of data collector

Patient demographics

Height (cm)

Weight (kg)

Smoking (never, former, active)

Alcohol consumption (units per week)

ASA classification (class I-VI)

Race

Comorbidities

Prescription medications

Oral or intravenous antibiotic treatment within 2 months up to the surgery (Y/N, name and dose of antibiotic)

Prior breast surgery (Y/N, type of surgery, date)

Radiation therapy (dose and fraction)

Chemotherapy (type, dose, duration, frequency and no of cycles)

Antihormonal therapy (type, dose and duration)

Antibody therapy (type, dose and duration)

### 6.1.2. Surgical variables

Trial related variables

Date and time of surgery

Randomization number

Direct-to-implant or expander breast reconstruction

Deviations from the protocol

Date and time of treatment administration

Date and name of the data collector

Surgery characteristics

Mesh (Y/N)

Drain (Y/N)

Operative time (hours, minutes)

Implant type (brand, volume, texture, serial no.)

Implant placement (prepectoral or subpectoral)

Type of mastectomy procedure (nipple sparring KHAC10 or skin sparring KHAC15)

Type of reconstruction (KHAE00, KHAE05)

Thickness of mastectomy flap (mm)

Pre- and perioperative medications

VAC (Y/N)

### 6.1.3. Post-surgery variables

Characteristics

Date of discharge

Time to drain removal

Post-operative medication

Hematoma (Y/N)

Mastectomy flap necrosis (Y/N)

Nipple-areola-complex necrosis (Y/N)

Seroma (Y/N)

Explantation (Y/N)

Date of explantation

Indication of explantation

Surgical site infection (Y/N)

Bacterial agent (culturing/PCR)

Revisional surgery with incision of the fibrous capsule (Y/N)

Date of revisional surgery

Indication of revisional surgery

Baker grading

Local adverse event

Severity of event

Time of event

Treatment/action taken

Exclusion/loss to follow-up

Reason for exclusion

### 6.2. Clinical follow-up

The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site. There will no trial specific clinical follow-up visits. The patients will be instructed to contact the local treatment site if they should experience adverse events after they have been discharged. Additionally, the patients will be instructed to contact us if they receive relevant treatment related to the reconstructed breast at another hospital than their primary treatment site or via their general practitioner within 180 days after the surgery.

### 6.3. Data quality and security

Each variable is clearly defined in the case report form. Each data field will be provided with a definition of the variable, category for categorial variables and units for continuous variables.

All relevant trial documents including the signed consent form for each patient will be stored in the trial master file in a secure, locked place at each individual site. Only the site investigator and designated personnel will have access. The trial has been approved by the Danish Data Protection Agency. The files will be stored for 25 years, after which they will be destroyed.

## 7. Assessments of safety and harm

Women receiving breast cancer treatment and subsequently undergo breast reconstructive surgery are in high risk of experiencing adverse events in relation with the surgical treatment and cancer. These adverse events will not be registered as trial drug-adverse events.

The trial drugs are widely used internationally, and the adverse reaction profile for each drug is well-defined. (See 'Summery of Product Characteristics' section 4.8 for each drug). Previous studies show, that only low levels of locally administered antibiotics enter the bloodstream and therefore, the systemic side effects to the trial drugs are expected to be negligible.[54]

### 7.1. Expected adverse events unrelated to the trial drug

The complication rate following breast reconstruction is relatively high, and certain adverse events are to be expected after surgical resection of the breast and reconstruction with an implant.

Several postoperative adverse events are likely to occur in the included patients due to the surgery and patient comorbidities and these events will not be registered as adverse events related to the interventional drugs. The following adverse events are expected in the included patients and will not be registered as adverse events to the trial treatment:

- Surgery specific complications: surgical site infection, implant malposition, expander deflation, expander port malfunction, implant/expander exposure, implant/expander rupture, wound dehiscence, hematoma, flap necrosis, seroma, capsular contracture, nerve damage, pain and lymphedema.
- Infectious disease including sepsis without signs of surgical site infection and fever without signs of surgical site infection.
- Cardiovascular disease including stroke, acute myocardial infarction, heart failure, arrythmia, cardiac arrest, pulmonary embolism, DVT and coagulopathy.
- Gastrointestinal disease including diarrhea, nausea, vomiting, gastroenteritis, colitis and ileus.
- Adverse events related to the kidneys and urinary tract including uremia, proteinuria, hematuria, interstitial nephritis, upper and lower urinary tract infection and pre-renal and post-renal kidney failure.
- Liver- and biliary tract disease including increase in serum liver enzymes levels, increase in bilirubin and alkaline phosphatase, hepatitis, liver cirrhosis, cholecystitis and pancreatitis.
- Respiratory disease including dyspnea, pleural effusion, pneumonia, pharyngitis, sinusitis and rhinosinusitis.
- Neurological disease including seizures, dizziness, impaired vision and vertigo.
- Metabolic disease including hyperglycemia and hypoglycemia.
- Immunological disease including thrombocytopenia, leukopenia, granulocytopenia, agranulocytosis, pancytopenia, leukocytosis, granulocytosis, anemia and polycythemia.
- Psychiatric disease including depression.
- Cancer recurrence.

### 7.2. Adverse events possibly related to the trial drug

The presumed relation to the trial drug will be evaluated using the ‘Summery of Product Characteristics’ for Gentamicin, Cefazolin and Vancomycin.

Examples of events that are likely to be related to the trial drugs are:

Erythema multiforme

Urticaria

Angioneurotic edema

Toxic epidermal necrolysis

Steven Johnsons' syndrome

Anaphylactic shock

Red man syndrome (appearing after a maximum of 10 minutes after administration)

Acute tinnitus

Acute deafness

Drug induced-acute kidney injury

Myasthenia gravis-like syndrome

Local adverse events (incl. surgical site infection, skin irritation, erythema, delayed wound healing, itching)

### 7.3. Adverse Event Reporting

Local antibiotics therapy is considered safe.[24] Previous studies have shown that the serum level of antibiotics after local application is low,[26] and therefore the risk of systemic organ toxicity is low. Gentamicin, cefazolin and vancomycin have all been approved for marketing for many years and have a well-known systemic adverse reaction profile. The combination of gentamicin, vancomycin and cefazolin has been used for local breast pocket irrigation for many years and is considered safe.[36] Due to the extremely low systemic uptake when using locally administered antibiotics, the event of systemic adverse events following the trial intervention is considered very unlikely.

Adverse events related to the study drug will be assessed continuously by the co-investigator at each study site during the admission period (typically 72 hours). The trial drugs have a short half-life period of maximum 6 hours and will only be administered one time during the surgery. Therefore, it is considered very unlikely that adverse events related to the trial drugs should occur after discharge. However, adverse events will be monitored and reported for 14 days after administration of the study drug. If the patients experience adverse events, the site investigator will be responsible for registering the adverse event directly in the case report form. The patients will be instructed to contact the local treatment site if they should experience adverse events after discharge from the hospital. The site investigator will report all adverse events to the coordinating sponsor-investigator who will be responsible for monitoring the safety of the trial. Each adverse event will require the investigator to fill in the AE form including the following variables: patient identification number, description of event, onset and end of event, severity, relation to the intervention, action taken and outcome.

Any adverse events will be treated according to the local guidelines.

#### 7.3.1. Adverse Events (AE) and Adverse Reactions (AR)

Any event that occurs after administrations of the trial drug regardless of the relation to the trial drug will be defined as an adverse event. Adverse reactions will be defined as events that are related to the trial drug.

### 7.3.2. Serious Adverse Events (SAE) and Serious Adverse Reactions (SAR)

For each recording of adverse event, the event will be evaluated as to whether it was a serious adverse event. A SAE will be defined as an AE that is life threatening, results in death, requires prolonged hospitalization or results in significant disability.

The site investigator at each trial site will be responsible for contacting the coordinating sponsor-investigator in case of serious adverse events within 24 hours of awareness. The site investigator will record the event in the case report form.

All serious adverse reactions will be reported yearly to the Danish Medicines Agency and the Regional Ethics Committee by the sponsor-investigator during the study period of three years and 6 months. After last patient last visit, serious adverse events will no longer be reported annually to the Danish Medicines Agency and The Regional Ethics Committee, since the effect of the study drug is considered negligible after 30 hours, and it is unlikely that any serious adverse events related to the study drug would occur after three days.

During the long-term follow-up, serious adverse events will not be reported in the annual safety report (ASR). Serious adverse events will be registered in the trial and included in a final clinical study report after the last long-term follow-up. See Gantt chart figure 1.

### 7.3.3. Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is an unexpected and serious presumed reaction to the trial drug. Section 4.8 in the 'Summery of Product Characteristics' for each drug (cefazolin, gentamicin and vancomycin) will be used as the reference safety information to determine whether or not the serious adverse event is unexpected. The sponsor-investigator will be responsible for that all relevant information about SUSARS, which are fatal or life threatening, is recorded and reported to the Regional Health Ethics Committee and the Danish Health and Medicines Authority as soon as possible, and no later than 7 days after the sponsor-investigator has been informed of such an event. No later than 8 days after the reporting, the sponsor-investigator is responsible for informing the Regional Health Ethics Committee and the Danish Medicines Agency of relevant treatment initiated by the co-investigator or a doctor at the trial site. Any other SUSAR must be reported to the Regional Health Ethics Committee and the Danish Medicines Agency no longer than 15 days after the sponsor-investigator has been informed. All trial investigators will be informed by the coordinating sponsor-investigator in the event of a SUSAR. See Gantt chart figure 1.

### 7.3.4. Severity of Adverse Events

The severity of each adverse event suspected to be related to the trial drug will be graded accordingly

- Mild: transient symptoms, with no interference in normal daily activity
- Moderate: persistent symptoms, resulting in moderate inhibition of daily activity
- Severe: persistent symptoms, resulting in severe inhibition of daily activity

### 7.3.5. Relationship of AE to Trial Intervention

For each AE suspected to be related to the trial drug, the probability will be rated accordingly

- Probable: there is good reason and adequate documentation to assume causal relationship
- Possible: a causal relationship is likely and cannot be dismissed
- Unlikely: the event is most likely related to an etiology other than the intervention
- Unknown: causality is not assessable

### 7.3.6. Adverse Reactions reporting during long-term follow-up

The annual reporting of SAR will not apply during the long-term follow-up period. Serious adverse reactions that we learn of during the long-term follow-up period of 5, 10 and 15 years will be recorded and included in a final clinical study report after the last long-term follow up.

SUSARS will be reported continuously to the Danish Medical Agency throughout the long-term follow-up period. See Gantt chart in figure 1.

## 8. Statistical considerations

### 8.1. Sample size

The trial will be powered towards the primary outcome. Previous data[3] do not suggest that there is an overrepresentation of bilateral infections compared to the unilateral infection rate which suggests that infections may be randomly distributed. Therefore, we do not assume that infections are correlated between breasts within the same patient. However, due to the paired design in the patients that undergo bilateral reconstruction, any correlation between the individual patient's breasts will increase the statistical power of this trial. The independent sampling unit of this trial will be 'breast', and the trial will be powered towards 'number of breasts', so that the final number of included patients will depend on the proportion of patients undergoing bilateral breast reconstruction. The incidence of the primary outcome is reported at 10%. With an α-level of 0.05, the trial will have a power of 0.90 to detect an absolute risk reduction of 5% if 1158 'breasts' are included. We will include patients, until a total number of 1274 breasts have completed the follow-up period of 180 days to account for a dropout rate of 10%. We estimate that 1003 patients will be included in the trial if approximately 27% of the patients undergo bilateral breast reconstruction.

### 8.2. Statistical analysis plan

The statistical analyses will be conducted on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent. Categorical variables will be presented as frequencies whereas normally distributed continuous variables will be presented as mean ± SD,

and as median (25th percentile-75th percentile) if non-normally distributed. Differences in endpoints (including the primary outcome) between the treatment allocations will be analyzed using multivariable logistic regression with a generalised estimating equation (GEE) that takes clustering into account adjusted for stratification variables. The stratified randomization approach will ensure an even distribution of known outcome risk factors between the placebo group and the treatment group. In case of missingness greater than 5% for the primary outcome, we will apply multiple imputations by chained equations and repeat the primary analysis as sensitivity analysis. A statistical significance level of 0.05 will be applied throughout. The open source statistical program "R" (http://www.r-project.org) will be used for data treatment and statistical analysis. A separate statistical analysis plan (SAP) will describe the detailed analysis plan, and it will be made publicly available before inclusion of the last patient, and before any unblinding of data.

### 8.3. Registration of changes

Changes to the original statistical analysis plan will be recorded in the sponsor's trial master file before unblinding.

### 8.4. Rationale for model selection

Based on extensive simulations published in the separate SAP, we have selected a multivariable logistic regression with a generalised estimating equation (GEE) with an exchangeable correlation structure to analyze the primary outcome. The multivariable logistic regression model is chosen as the primary outcome is binary. The model can adjust for the randomization strata and other potential confounders to control for residual imbalances in the baseline parameters. Using a generalized estimating equation (GEE) is necessary to account for correlation between the two breasts nested within a patient undergoing bilateral reconstruction. The GEE method models the population treatment effect which has a more appealing interpretation than random effects models which are subject specific/within subject interpretation. The GEE would likely gain more efficacy from the unilateral patients with no clustering whereas the random effects model could become unstable due to singularity and random effects variances near or equal to zero.

## 9. Ethical considerations

This trial will be conducted in accordance with EU and national legislation on medical research in capable patient volunteers. Eligible subjects shall provide oral and written informed consent to participate in the trial. The informed consent can be withdrawn by the participant at any time during the trial after which the patient will convert to receiving treatment as determined by local guidelines. All data on trial participants will be protected according to the General Data Protection Regulation (GDPR), the data protection law and the Danish Health Act. The project is approved by the Danish Data Protection Agency and is to be approved by the Danish Medicines Agency and the Regional Committee on Health Research Ethics. The trial will be conducted according to national and international standards of good clinical practice (GCP) and will be monitored by the regional GCP unit.

### 9.1. Ethical justification

The trial will investigate the beneficial effects and potential side-effects of applying gentamicin, vancomycin and cefazolin locally onto the breast implant during breast reconstruction surgery. This may limit bacterial contamination of the implant and thereby decrease the risk of postoperative infection which is associated with a poor outcome for the patients. Therefore, participation in this trial could benefit the individual participant.

Inclusion in the trial may benefit the individual subject by decrease the risk of undergoing explantation of the implant, minimizing the risk of postoperative infection, minimize the hospitalization period and may as well improve the outcome for future women undergoing implant-based breast reconstructive surgery. Alternatively, we may find that the local antibiotics do not have a clinically relevant effect and perhaps negative side-effects that should be explored further.

The trial drug regimen is widely used internationally[14] but the potential positive and/or negative effects of using local antibiotics on breast implants have never been tested against placebo in a randomized trial. See appendix 1 for a review of the current literature.

Though the drug regimen has not been tested in a randomized controlled trial, the drugs have been applied by plastic surgeons for several years, and no adverse events has been reported.[42,44,48,55] Therefore, the drug regimen is expected to be of minimal risk to the subjects in the trial. Moreover, the systemic levels of antibiotics after locally applied antibiotics has been shown to be much lower than the levels seen with systemic antibiotics and the trial treatment consists of a single dose, and therefore we do not expect systemic adverse reactions to the trial drugs.[36]

Currently, no clinical guidelines exist in Denmark regarding the prophylactic use of local antibiotics to breast implants, and the treatment depends on the local approach at each hospital and the individual surgeon's preference. Allocation to placebo in this trial is therefore considered ethically acceptable. Application of local vancomycin on the breast implant has been used routinely at the Department of Plastic Surgery, Herlev and Gentofte Hospital, in the past years. This treatment regimen is not based on any evidence and is scientifically unjustified. Therefore, we find it ethically acceptable to include patients from Herlev and Gentofte Hospital in the trial. All eligible patients from Herlev and Gentofte Hospital will receive this information before providing consent and specific written patient information material will be provided for these patients. Patients at Herlev and Gentofte Hospital who are not included in the trial will receive local vancomycin during the breast reconstruction surgery.

The results from this trial could change the guidelines for breast reconstruction surgery on an international level and be used to provide patients with evidence-based treatment.

### 9.2. Informed consent

Registered patients will receive information about the trial in their e-boks. They will be informed that the trial coordinating unit will contact them to provide oral information about the project after they have had time to read the information material about the trial. The contact information for registered patients will be passed on to the trial coordination unit from the treatment site.

Recruitment of trial participants will be carried out at the time of the preoperative patient visit or by telemedicine (i.e. telephone or a secure video connection) by a medical doctor with relevant expertise. All patients are encouraged to bring a third party (i.e. relative or partner) to this appointment. The responsible medical doctor can assign a designated nurse or medical student with relevant expertise to provide the patient with both oral and written information concerning the trial during this visit as the medical doctor has limited time available at the initial pre-operative consultation. The investigator carrying out the recruitment will be responsible for obtaining the informed consent (either by a digital consent or a written consent form) from the patients prior to any protocol-related activities. The conversation will take place in a private room behind a closed door. Participation in the trial will not influence the choice of treatment. The patient and the responsible medical doctor must personally provide a dated signature digitally or on a consent form. The informed consent can be withdrawn by the patient at any time and without explanation, after which the patient will receive the standard treatment according to the local guidelines. No personal data will be collected patients before informed consent is obtained.

### 9.2.1. General considerations

We will strive to provide the patients with at least 24 hours of consideration, but in some patients this will not be possible. For instance, when it comes to patients undergoing primary breast reconstruction, the breast reconstruction is performed during the same surgery as the cancer surgery and the patients are treated in an accelerated cancer treatment course. Due the short period of time from planning to carrying out the surgery, the decisions regarding possible use of implants or expander for reconstruction are often made a few hours before the surgery. In these cases, the patients will have at least 2 hours of consideration.

## 10. Direct access to source data/documentation

The site investigator will permit direct access to source data blinded for treatment allocation for monitoring, audits and reviews by the Health Ethics Committee, Good Clinical Practice unit, Danish Medicines Agency and other regulatory authorities.

## 11. Data management

All data management will be conducted according to guidelines of the Danish Data Protection Agency. The data will be kept in REDCap. The database will be maintained for 25 years from the last patient visit and anonymized when the approval from the Danish Data Protection Agency terminates.

## 12. Quality control and quality assurance

The trial will be conducted according to the approved protocol and will comply to standard procedures for quality control and quality assurance. The investigator at each trial site will report any deviations from standard protocol to the coordinating sponsor-investigator either by direct contact or via the case report form.

The trial conduct, data generation, data documentation and reporting will be in accordance with ICH-GCP guidelines and the trial will be monitored by the national Good Clinical Practice (GCP) unit. Monitoring will be conducted upon initiation of the trial, during the trial and at termination of the trial.

The sponsor-investigator, local trial site investigators and the trial coordinating unit are responsible for maintaining up-to-date accrual information, enrollment status and safety data. It is the responsibility of the sponsor-investigator to ensure oversight of trial related activities and on a yearly basis, report trial progression, enrollment status and safety data to the Trial Steering Committee (see Trial Steering Committee Charter). The sponsor-investigator will submit all relevant documents to the board members for scientific progress review. The sponsor-investigator is responsible for ensuring that access to clinical trial data is consistent with data protection principles and in accordance with the patients' informed consent provided in relation to their participation in the clinical trial.

The trial steering committee will be responsible for data monitoring and quality control of the data extracted from the patients' medical journals at the long-term follow-up after 5, 10 and 15 years.

## 13. Finance and insurance

The trial is funded by a project grant in surgical research of 2,975,000 DKK from the Novo Nordisk Foundation. The grant budget will cover a PhD salary, a part-time post-doc salary, and medicine and materials required for the trial. See attached research budget. The grant is disbursed to Professor Tine Engberg Damsgaard on behalf of Rigshospitalet and administered by the Financial Department of the Centre of Head and Orthopedics, Rigshospitalet. None of the researchers have any financial disclosures or relation to the Novo Nordisk Foundation. The Novo Nordisk Foundation have had no part in the design of the study, and they will not participate in the reporting of the results. The study participants will not be reimbursed for participation in the study. The trial participants are insured according to the Danish patient compensation scheme.

## 14. Publication plan

The trial is registered on EudraCT. The trial protocol will be registered at ClinicalTrials.gov before enrollment of the first patient. The protocol will be published prior to unblinding of the results in a methodology article including the statistical analysis plan. The main article will include the primary and secondary outcomes prespecified in the SAP. The tertiary and long-term outcomes will be included in subsequent articles. The manuscripts will be used to report the results of the trial to the scientific community and will adhere to the CONSORT guidelines.[56] The members of the trial coordinating unit will be co-first authors and the coordinating sponsor-investigator will be senior/last author and corresponding author. All site investigators and members of the Trial Steering Committee will be co-authors. Other co-authorships will be decided by contribution according to the ICMJE authorship guidelines[57] depending on personal involvement. All publications will refer to the trial group which will include all contributing parties to the BREAST-AB trial. The manuscripts will be submitted to peer-reviewed international journals and both

positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be disseminated to the public but will not be shared directly with participating patients. See Gant chart in figure 1.

## 15. Tasks and Responsibilities

Trial Coordinating Unit: Protocol development, daily management in the trial period, contact to Good Clinical Practice monitoring unit, contact to trial sites, data dictionary development, responsible for providing randomization sequences for each trial site, provide deidentified data to the trial steering committee for safety monitoring, available for unblinding the treatment allocation of individual patients in the case of an emergency, instructing health care personnel involved in the trial, data collection and management.

Coordinating Sponsor-Investigator: Overall responsibility for protocol development, funding, budget overview, ethical approval, trial registration, trial oversight (including enrollment trends and safety), contact to Good Clinical Practice monitoring unit, potential recruitment of additional sites, and dissemination and presentation of results.

Trial Steering Committee: Clinical and scientific advising to the sponsor-investigator, evaluation and recommendations based on yearly reports regarding enrollment trends, progress and safety of the trial, counseling regarding scientific reporting of data, data quality control during long-term follow-up. See 'Trial Steering Committee Charter' for a more thorough description of the tasks and responsibilities of the committee.

Site investigators: Responsible for site-specific enrollment, evaluation and reporting of eligible patients not included, education of personnel at trial sites, reporting of site-specific issues or challenges to the coordinating sponsor-investigator, inclusion of patients, responsible for obtaining informed consent, contact to the regional Good Clinical Practice monitoring unit, trial related data entry in the case report form

Clinical personnel: Preparation and administration of the trial drugs.

Good Clinical Practice-unit: See section 12.

Data Assessment Committee: Assessment and analysis of data.

## 16. Contact information

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## 18. Appendices

Appendix 1: Systematic review

Appendix 2: Screening log

Appendix 3: Randomization strata

Appendix 4: Standard operating form for preparation of investigational drugs

Appendix 5: Standard operating form for administration of investigational drugs

Appendix 6: Case report forms

Appendix 7: Medicine account

Appendix 8: Trial Steering Committee Charter

Figure 1: Gantt chart

Figure 2: Participant timeline

Figure 3: Patients

Figure 4: Preparation of treatment