BReast Ecology Assessment in the STudy of local MicroFlora - Study Protocol

Dana Mihaela Jianu,¹ Anca Streinu-Cercel,² Alexandru Blidaru,³ Maria Filipescu,⁴ Ioan Petre Florescu,⁵ Ioana Berciu,⁶ Oltjon Cobani,⁷ Olga Dorobăț,⁸ Ștefan Adrian Jianu,⁹ Oana Streinu-Cercel,^{10,*} Floria Stăniceanu,¹¹ Adrian Streinu-Cercel¹²

Abstract

Introduction Recent articles have described an endogenous breast flora, particularly in the nipple ducts, with potential implications in the outcome of aesthetic breast surgery. To characterize the ecology of the breast, we designed a study to assess the microbial species identified on the breast skin and parenchyma in patients undergoing breast surgical interventions.

Methods After obtaining informed consent and background data on concurrent diseases, previous contact with the hospital system and prior use of antibiotics, samples are collected preoperatively from three areas of the breast skin, bilaterally: the inframammary fold, the areola and the axilla, prior to decontamination. These samples will serve as positive controls and will aid in characterizing the normal breast skin flora. After preoperative decontamination, samples are again collected, to check for any residual bacterial flora and the nipple is sealed with Tegaderm (3M, USA) and betadine ointment, to reduce any putative bacterial load. Intraoperatively, samples are collected from: a) the incision line (dermal level): 1. superficially, 2. medium depth in the breast parenchyma, 3. deep parenchyma, and b) axillary parenchyma (where possible), together with a bioptic fragment. Postoperatively, a second nipple sample is collected. For secondary breast augmentation surgeries, capsular biopsy is also performed (where relevant), and the implants undergo sonication, to allow biofilm identification.

In the laboratory, all samples are cultured on blood agar incubated with CO₂, cystine lactose electrolyte deficient medium and Sabouraud gentamicin-chloramphenicol agar. For positive culture samples, the number of colonies and their morphologic characteristics are reported. Identification will be carried out with MALDI-TOF and VITEK (bioMérieux, France), yielding automated antibiotic sensitivity profiles. For all germs with sensitivity profiles differing from the wild-type strain, E-tests will be performed. Follow-up information on the postoperative evolution will be collected and analyzed for potential factors predictive of good evolution.

Discussion This study will provide important information about the microflora of the breast skin, its sensitivity profile, and the degree of contamination of the nipple ducts and parenchyma, if any, addressing a scientific hypothesis insufficiently explored so far.

Received: February 10, 2013; accepted: February 26, 2013

¹MD, PhD, Assoc.Prof., Carol Davila University of Medicine and Pharmacy, Bucharest; ProEstetica Medical Center, Bucharest; Vice-president of the Romanian Aesthetic Surgery Society (RASS); National Secretary of the International Society of Aesthetic Plastic Surgery (ISAPS); Historian and Parliamentarian of the European Association of Societies of Aesthetic Plastic Surgery (EASAPS); ²MD, PhD, Lect., Dept. of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest; National Institute for Infectious Diseases "Prof.Dr. Matei Balș"; ³MD, PhD, Prof., Dept. of Surgery, Carol Davila University of Medicine and Pharmacy, Bucharest, Dept. of Oncologic Surgery, Institute of Oncology "Prof. Dr. Al Trestioreanu", Bucharest; ⁴MD, ProEstetica Medical Center; ⁵MD, PhD, Scientific Researcher, 1st degree, Prof., Dept. of Plastic and Reconstructive Surgery, Carol Davila University of Medicine and Pharmacy, Bucharest, Bagdasar-Arseni Clinical

Emergency Hospital; ⁶MD, Assist.Lect., Dept. of Microbiology, Carol Davila University of Medicine and Pharmacy, Bucharest; National Institute for Infectious Diseases "Prof.Dr. Matei Balș"; ⁷MD, ProEstetica Medical Center; ⁸MD, PhD, Prof., Dept. of Microbiology, Carol Davila University of Medicine and Pharmacy, Bucharest; National Institute for Infectious Diseases "Prof.Dr. Matei Balș"; ⁹MD, PhD, MBA, General Surgery, ProEstetica Medical Center; ¹⁰MD, Assis.Lect., Dept. of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest; National Institute for Infectious Diseases "Prof.Dr. Matei Balș"; ¹¹MD, PhD, Prof., Dept. of Pathology, Carol Davila University of Medicine and Pharmacy, Bucharest; Clinical Hospital Colentina, Bucharest; ¹²MD, PhD, Prof., Dept. of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest; National Institute for Infectious Diseases "Prof.Dr. Matei Balș".

Introduction

Microbiology textbooks describe in a wealth of detail the microflora of different relevant areas of the human body, such as the skin, the gastrointestinal tract, genital tract, etc. However, to our knowledge, so far the breast has not been described to have a resident flora. Recent data have been published in medical literature, suggesting that such a flora may indeed exist and that it might pose a risk of colonization of surgical devices placed in the area, generating biofilms.¹⁻³ We aim to perform a study to provide an answer to this research question.

To differentiate between isolated bacteria and actual resident flora, certain criteria need to be met. First, the species identified need to be different from those of the surrounding areas, to suggest residency rather than carry-over. Second, the habitat needs to be rich in nutrients, meeting bacterial survival needs and granting exemption from the local anti-infective protection of the host.⁴ Third, the species isolated need to be non-pathogenic and consistently identified over a relatively large sample of subjects. And fourth, the germs need to perform a role in that particular area. Provided all these criteria are met, we can then proceed to describe a local resident flora. Our intent is to clarify the issue of the putative breast flora through this study.

Aims of the study

We aim to identify the microbial species that make up the microflora of the breast skin, ductal tissue and parenchyma. We also intent to characterize the antimicrobial sensitivity profile for the identified microflora and calculate the rate of postoperative infections and capsular

contracture at time points of primary and secondary interest of 6 months and 1 year, respectively. Dependent on study data, we will also schedule a reinterpretation of the data on capsular contracture at 2 years post surgical intervention.

A parallel analysis will compare antimicrobial sensitivity profiles against those expected for each patient, as calculated based on the Carmeli score.^{5,6,7}

Methods

Study design

We present the study protocol for a prospective cohort study of the local microflora of the breast. Adult female patients undergoing breast surgery will be included in the study. Patients presenting any of the following criteria will be excluded from the study: coagulation issues, cardiac, kidney or liver failure, uncontrolled high blood pressure, any contraindications for general anesthesia or surgery, acute infection, severe or uncompensated psychiatric disease (see full protocol for details).

Fifty consecutive patients will be included in the study, after providing written informed consent. For each patient, a thorough medical history is collected along with a complete preoperative work-up, including: complete blood count, urea, creatinine, fasting plasma glucose, alanine aminotransferase, aspartate aminotransferase, serum proteins, serum ionogram, ionic calcium, magnesium, iron, sodium, kalium, international normalized ratio (INR), activated partial thromboplastin time (aPTT), HIV, HBV and HCV serology, plus 12-lead ECG, blood pressure and heart rate, cardiopulmonary X-ray and breast ultrasound.

All patients are required to fill out a standardized questionnaire assessing: age at the time of the surgical intervention, number of prior pregnancies, number of prior births, history of breastfeeding, history of mastitis during the past 5 years, sexual contact within the past 48 hours, oro-areolar contact within the past 48 hours, history of surgical intervention, hospital admission and antibiotic use within the past 6 months, history of breast surgery, use of contraceptive methods, history of diabetes, HIV infection, hematologic disease, chronic

*Corresponding author: Oana Streinu-Cercel, MD, Assistant Lecturer, Department of Infectious Diseases, Carol Davila University of Medicine and Pharmacy, 37 Dionisie Lupu Street, Bucharest, 020022, Romania; National Institute for Infectious Diseases "Prof. Dr. Matei Balș"; oanastreinucercel@gmail.com

obstructive pulmonary disease (COPD), chronic hepatitis, cirrhosis, and history of previous medication during the past 6 months: chemotherapy, antiretrovirals, immuno-suppressive drugs, cortisone.

Standardized data is collected by the investigator for each patient: type of intervention, primary or secondary intervention, surgical incision site, type of implant used, prosthesis volume, interpretation of preoperative breast ultrasound. For secondary surgery, the cause for reintervention and the presence or absence of capsular contracture are also documented.

Swab collection

Samples will be collected using sterile Amies Agar Gel Swabs (Copan Diagnostics Inc., USA) from the skin pre- and postoperatively and from the ductal area and the breast parenchyma intraoperatively. Preoperatively, samples will be taken from three different areas of the skin, bilaterally: the inframammary fold, the nipple and the axilla, before and after decontamination. When applicable, the nipple is sealed with Tegaderm (3M, USA) and betadine ointment, to reduce any putative bacterial load, and swabbed for culture postoperatively. During the surgical procedure, samples are collected from: a) the incision line (dermal level): 1. superficially, 2. medium depth in the breast parenchyma, 3. deep parenchyma, and b) axillary parenchyma (when within swab reach). A tissue sample is collected for a microbiology and pathology exam. For secondary procedures with extraction of breast implant(s), the prostheses are collected in a sterile container and transported to the laboratory within the following 6 hours. Also, an extra swab sample will be collected if relevant fluid is present surrounding the implant, along with an extra tissue biopsy if a capsule is identified.

Laboratory procedures

Each swab sample is plated on the following media: COS (Columbia agar plus 5% sheep blood), CLED (cysteine lactose electrolyte-deficient) agar, and SGC2 (Sabouraud gentamicin-chloramphenicol 2) agar (all plates, bioMérieux, France) and incubated at $35 \pm 2^\circ\text{C}$ for 24-48 hours. Blood agar is incubated in BD GasPak EZ (Becton, Dickinson and Company, USA). At 24 hours, bacterial growth is assessed

and morphological characteristics of the isolated colonies are described. At 48 hours, the final colony counts for blood agar and CLED are reported. Sabouraud plates will be read at 24 hours and again at 7 days in case of negative results. For each type of colony identified a Gram-stained saline preparation is examined through immersion microscopy. For Gram-positive cocci from colonies with morphology suggestive for *Staphylococcus* spp, rapid identification through latex agglutination (Staphytect plus, Oxoid, Diagnostic Reagents, UK) is performed. Culture isolates are subcultured for VITEK (bacterial metabolism and growth identification through fluorescence and/or colorimetry) antibiotic sensitivity profiling, and MALDI-TOF (mass spectrometry) identification is carried out (both, bioMérieux, France). E-tests are performed for all isolates with sensitivity profiles differing from the wild-type strain on VITEK profiling.

For all tissue samples, a pathology exam is performed, including histochemistry and hematoxylin-eosin, periodic acid-Schiff (PAS) and Gram staining. For all extracted breasts implants, normal saline is added in the sterile containers under a laminar flow hood and sonication is performed. The resulting fluid is plated, 100 μL on each of the following solid media: COS, chocolate agar and CLED and 3-4 mL in thioglycolate broth with resazurin (bioMérieux, France). Blood and chocolate agar are incubated with 5% CO_2 , CLED and broth are incubated in normal atmosphere, all at $35 \pm 2^\circ\text{C}$. Agar growth is interpreted at 5 days and broth growth at 10 days. Results are reported according to the laboratory standard operating procedures (SOP).

Follow-up

Patients will be discharged the second day after surgery and will come in for reevaluation every other day during the first week, and then at ten days, 1, 3 and 6 months and 1 year. At each follow-up visit, patients will fill out a satisfaction survey and the investigator will look for signs of infection, local edema, and findings suggestive of capsular contracture (if present, skin and/or incision site cultures may be repeated and results compared to the baseline results for the preoperative and intraoperative samples).

Discussion

In the light of new data on the human microbiome, it has become apparent that our bodies are made up of substantially more bacterial cells than human cells.

Given the soaring research interest in this field, it is increasingly important to describe the flora of the human body in a wide array of physiologic and pathologic conditions, to better understand the relationship between bacterial, fungal and possibly viral commensal species and their hosts and whether human health can be improved by controlling the microbiome.⁸

This study aims to identify the breast microflora, on different depth levels, and characterize its antimicrobial sensitivity profile. The results of this study have the potential to reshape the way breast surgery is viewed by plastic surgeons, general surgeons, oncology surgeons, gynecologist and also infectious diseases practitioners.

The comparison of microflora antimicrobial sensitivity profiles against those expected for each patient will allow a validation of the Carmeli score in surgical patients and, possibly, a new labeling for surgical wound classification in aesthetic breast surgery.

Conflicts of interest All authors – none to declare.

Author contribution All authors had equal contributions.

References

1. Bartsich S, Ascherman JA, Whittier S, Yao CA, Rohde C. The breast: a clean-contaminated surgical site. *Aesthet Surg J* 2011;31(7):802-6. [[CrossRef](#)] [[PubMed](#)]
2. Del Pozo JL, Tran NV, Petty PM, et al. Pilot study of association of bacteria on breast implants with capsular contracture. *J Clin Microbiol* 2009;47(5):1333-7. [[CrossRef](#)] [[PubMed](#)] [[FullText](#)]
3. Netscher DT, Weizer G, Wigoda P, Walker LE, Thornby J, Bowen D. Clinical relevance of positive breast periprosthetic cultures without overt infection. *Plast Reconstr Surg* 1995;96(5):1125-9. [[CrossRef](#)] [[PubMed](#)]
4. Hemsell D. Gynecologic infection. In: Hoffman B, Schorge J, Schaffer J, et al., eds. *Williams Gynecology*, Second Edition. McGraw-Hill; 2008.
5. Streinu-Cercel A. [Classification of severe acute infections. Therapeutic implications]. *Therapeutics, Pharmacology and Clinical Toxicology* 2008;XII(1):8-10.
6. Streinu-Cercel A. [Levofloxacin, an original fluoroquinolone for treating pneumonia]. *Infectio.ro* 2009;V(1):38-9.
7. Carmeli Y, Eliopoulos GM, Samore MH. Antecedent treatment with different antibiotic agents as a risk factor for vancomycin-resistant *Enterococcus*. *Emerg Infect Dis*. 2002;8(8):802-7. [[CrossRef](#)] [[PubMed](#)] [[FullText](#)]
8. NIH HMP Working Group, Peterson J, Garges S, et al. The NIH Human Microbiome Project. *Genome Res*, 2009;19(12):2317-23. [[CrossRef](#)] [[PubMed](#)] [[FullText](#)]

Please cite this article as:

Jianu DM, Streinu-Cercel A, Blidaru A, Filipescu M, Florescu IP, Berciu I, Cobani O, Dorobăț O, Jianu ȘA, Streinu-Cercel O, Stăniceanu F, Streinu-Cercel A. BREast Ecology Assessment in the STudy of local MicroFlora - Study Protocol. *GERMS* 2013;3(1):14-7.
doi:10.11599/GERMS.2013.1029