Wound Infection Day

# Strategies for Infection Prevention, Bioburden Management, and Antimicrobial Stewardship


- Supported by an educational grant from

URGO FOUNDATION
OUR COMMITMENT TO YOUR HEALTH

# The Foundation

URGO



FONDATION URGO

NOTRE ENGAGEMENT POUR VOTRE SANTÉ

# THE URGO FOUNDATION

Created in 2010, the URGO Foundation's purpose is to contribute to improving the quality of life of patients around the world.

To this end, its mission is to support and train healthcare professionals in the following areas:

- **Healing**
- **Pain management**
- **Access to first aid**





# THE URGO FOUNDATION

In the U.S., our focus has been on leg ulcers and lymphedema, wound debridement, and infection management. This program will continue in the coming years, with ongoing efforts to strengthen these areas of care.









# THE URGO FOUNDATION

Since
2010

**11.7M**
*euros*

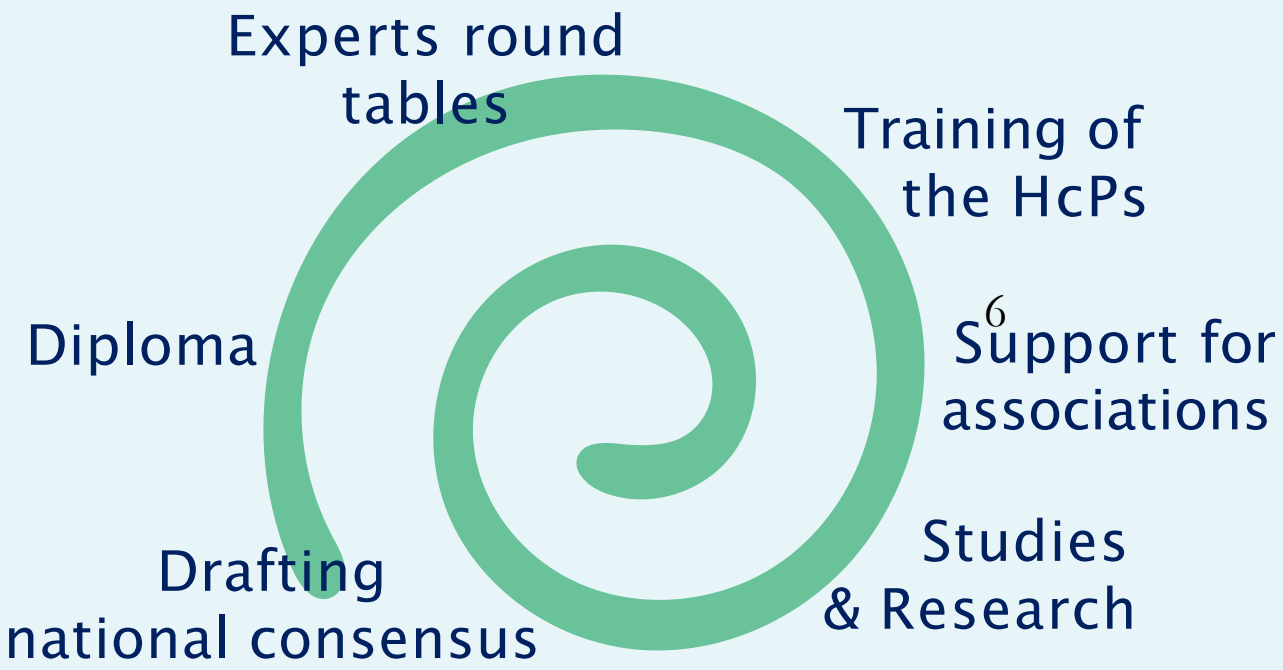
**25**
*countries*

**291**
*projects*

**216K**
*trained healthcare professionals, patients & caregivers*

**>1M**
*people who have been made aware*

In these areas of intervention, the URGO Foundation acts in **various ways:**



# *JOIN US!*

www.fondation-urgo.org

# Faculty

- **Sujay Dutta, MD**
  Attending Physician, Infectious Diseases and Wound Care Medicine Center for Advanced Wound Healing, Los Robles Regional Medical Center (Thousand Oaks, CA)
- **Lisa Gould, MD, PhD, FACS**
  Staff Surgeon, South Shore Health (Weymouth, MA)
- **Michael K. Mansour, MD, PhD**
  Division of Infectious Diseases, Massachusetts General Hospital; Harvard Medical School (Boston, MA)
- **Paul Nee, MD, FIDSA, CWSP**
  Regional Medical Director for Wound Care and Hyperbaric Medicine, Hudson Valley and Connecticut; Director of Antimicrobial Stewardship at Danbury/New Milford Hospital, Northwell Health (Danbury, CT)

# Faculty Disclosures

- **Sujay Dutta, MD** has disclosed no relevant financial relationship with any ineligible company (commercial interest)
- **Lisa Gould, MD, PhD, FACS**
  Advisory Board: Solventum, Medical Surgical Business; AVITA Medical, Inc
- **Michael K. Mansour, MD, PhD**
  Advisory Board: GenMark/Roche Diagnostics; Vericel; Consultant: C-POLAR; Patents Held: Neutrophil Therapeutics; Medical Writer: UpToDate; Unrestricted Grant: ThermoFisher Scientific
- **Paul Nee, MD, FIDSA, CWSP** has disclosed no relevant financial relationship with any ineligible company (commercial interest)

# Disclosures

- The faculty have been informed of their responsibility to disclose to the audience if they will be discussing off-label or investigational use(s) of drugs, products, and/or devices (any use not approved by the U.S. Food and Drug Administration)
  - Applicable CME staff have no relationships to disclose relating to the subject matter of this activity
  - This activity has been independently reviewed for balance

This CME activity includes brand names for participant clarity purposes only.
No product promotion or recommendation should be inferred.

# Learning Objectives

- Outline the patient-centric drivers of wound infection today
- Assess levels of wound contamination in inpatient and outpatient settings to determine and implement appropriate contamination and infection management strategies
- Examine the emerging threat of *Candida auris* and strategies for its prevention, treatment, and eradication from wounds and healthcare surfaces
- Explain the role of antimicrobial agents — including antibiotics, antimicrobial barriers, and antimicrobial-preserved cleansers — and their appropriate use in managing wound bioburden while supporting antimicrobial stewardship
- Evaluate emerging technologies for slough, debris, and microbial removal — including hypochlorous acid-based cleansers and charged fiber technology — and the relationship between effective slough removal and infection control



# Patient-Centric Drivers of Wound Infection


Sujay Dutta, MD, MS
Attending Physician, Infectious Diseases and Wound Care Medicine Center for Advanced Wound Healing
Los Robles Regional Medical Center
Thousand Oaks, CA

# Host Factors Promoting Wound Infection

1) Age
2) Diabetes mellitus
3) Cancer chemotherapy
4) Immunomodulatory agents
5) Corticosteroids
6) Malnutrition
7) Cigarette smoking
8) Other immunodeficiencies

# Age and Chronic Wound Infection

85% OF PATIENTS WITH CHRONIC WOUNDS ARE OVER 65

SKIN INTEGRITY DECLINES WITH AGE

DECLINE IN INNATE IMMUNITY

A) DECREASED NEUTROPHIL FUNCTION

B) DECREASED MACROPHAGE FUNCTION

C) IMPAIRED COOPERATION BETWEEN KERATINOCYTES AND DENDRITIC CELLS

* "INFLAMMAGING": PROLONGED CHRONIC INFLAMMATION AND DELAYED PROLIFERATIVE PHASE (FIBROBLAST SENESCENCE) + DELAYED ANGIOGENESIS = CHRONIC COLONIZATION AND INFECTION

# Diabetes Mellitus: Types 1 and 2

- Multiple levels of impairment
- Increases risk of all infections (especially skin/soft tissue, and bone/joint in older adults)
- Hyperglycemia impairs the function of neutrophils, macrophages, and T-cells: inability to clear infection
- High glucose in tissues: increased bacterial growth
- Vasculopathy due to DM: increased hypoxia, immune cell delivery
- Prolonged hyperglycemia favors chronic inflammation (less effective in infection control)

# Which country in the world has the highest prevalence of type 2 diabetes?

POLL

A. USA
B. Pakistan
C. India
D. Saudi Arabia

# Which country in the world has the highest prevalence of type 2 diabetes?

POLL

A. USA

B. Pakistan

C. India

D. Saudi Arabia

# Which country has the lowest prevalence of diabetic foot ulcers?

POLL

A. Sweden
B. Australia
C. China
D. France

# Which country has the lowest prevalence of diabetic foot ulcers?

POLL

A. Sweden

B. Australia

C. China

D. France

# Cancer Chemotherapy

- Neutropenia and bone marrow suppression
- Neutrophils are essential to the innate immune response
- In a neutropenic patient (as in older patients), classic signs of wound infection (erythema, calor, purulence) may not be present
- Decreased function of macrophages, T and B lymphocytes; impaired opsonization of pathogens
- Impairment and death of rapidly dividing cells, including fibroblasts, keratinocytes, and endothelial cells

# Target Therapies for Cancer

- Effect on the risk of wound infection depends on the agent
- VEGF/VEGFR inhibitors and mTOR inhibitors: directly impair wound healing, leaving surgical sites open to infection/promote dehiscence
- EGFR inhibitors: break down skin barrier, impair antimicrobial peptide production, increase water loss, pH, and create pustular rash, opening the door to infection
- Immune checkpoint inhibitors: minimal impact on wound infection and healing

# Biologic Agents: Monoclonal Antibodies

- Increasingly used in rheumatologic and dermatologic conditions
- Also used in multiple sclerosis
- Anti-TNF agents and anti-CD20 agents have the highest risk of immune impairment: impair key cytokine (TNF) and decrease antibody production
- Anti-IL-6 antibody can impair granulation, hide signs of infection, but has less impact on chronic wound infection
- Anti-IL-17 and IL-23 antibodies, with much less risk of wound infection, except mucocutaneous candidiasis

# Corticosteroids and Wound Infection

- Greater than 30-day use increases the risk of wound infection directly and impairs all phases of wound healing.
- Impaired inflammatory phase
- Impaired collagen deposition via decreased (TGF-β) and IGF production and decreased fibroblast activity
- Compromised re-epithelialization, increased skin and blood vessel fragility
- Directly impairs innate and adaptive immunity at all levels (neutrophil, macrophage, T and B cell function)

# Protein-Calorie Malnutrition

- Immune cells have rapid turnover and need high energy input, much like other cells in wound healing
- Wound healing is impaired, and the host is unable to clear colonizing bacteria and fungi in chronic wounds, increasing the risk of tissue invasion/infection
- Leads to prolonged chronic inflammation
- Impaired cell-mediated and humoral immunity — can persist even after restoration of adequate calories and protein
- Impaired phagocyte function
- Micro-nutrient deficiency impairs collagen synthesis, deposition, cross-linking, and local immune response

# Cigarette Use and Wound Infection

- Multiple adverse effects on host immunity
- Tissue hypoxia: nicotine (vasoconstrictor), CO (decreased oxygen delivery to chronic wounds), and hydrogen cyanide (inhibits aerobic metabolism even when oxygen is available — impaired cytochrome enzymes
- Impairs the migration and phagocytic ability of neutrophils and macrophages
- Impaired inflammatory and proliferative phase of wound healing

# Other Immunodeficiencies

- HIV infection: mainly affects wound infection when CD4<200 (AIDS) — impaired T cell and B cell function
- Use of highly active antiretroviral therapy (HAART) restores the ability to fight wound infection
- Chronic HIV infection promotes chronic inflammation
- Primary immunodeficiencies
- Common variable immunodeficiency (CVID) (>2 classes of Ig low, selective IgG or IgG subclass deficiencies): recurrent infections (often respiratory tract or GI infection) but also increases risk of skin and wound infections
- IVIG or SCIG replacement therapy if symptomatic

HAART = highly active antiretroviral therapy; CVID = common variable immunodeficiency.

# Clinical Pearls

- Older adults have decreased ability to mount an acute inflammatory response
- Infections in skin, soft tissue, and chronic wounds may go unnoticed as fever, erythema, and leukocytosis may not be present in older patients
- Even highly pathogenic organisms, such as *Staphylococcus aureus* and *Streptococci*, may cause infection without typical signs or only subtle signs
- Mental status change may be the first sign



# *Candida auris:* Spontaneous Evolution?

Michael K. Mansour, MD, PhD
Division of Infectious Diseases
Massachusetts General Hospital
Harvard Medical School
Boston, MA

# Outline

1. Case
2. Epidemiology and current status of the *C. auris* epidemic
3. New therapeutic approaches to drug-resistant fungi

# Case: 71y M s/p Bilateral Lung Transplant

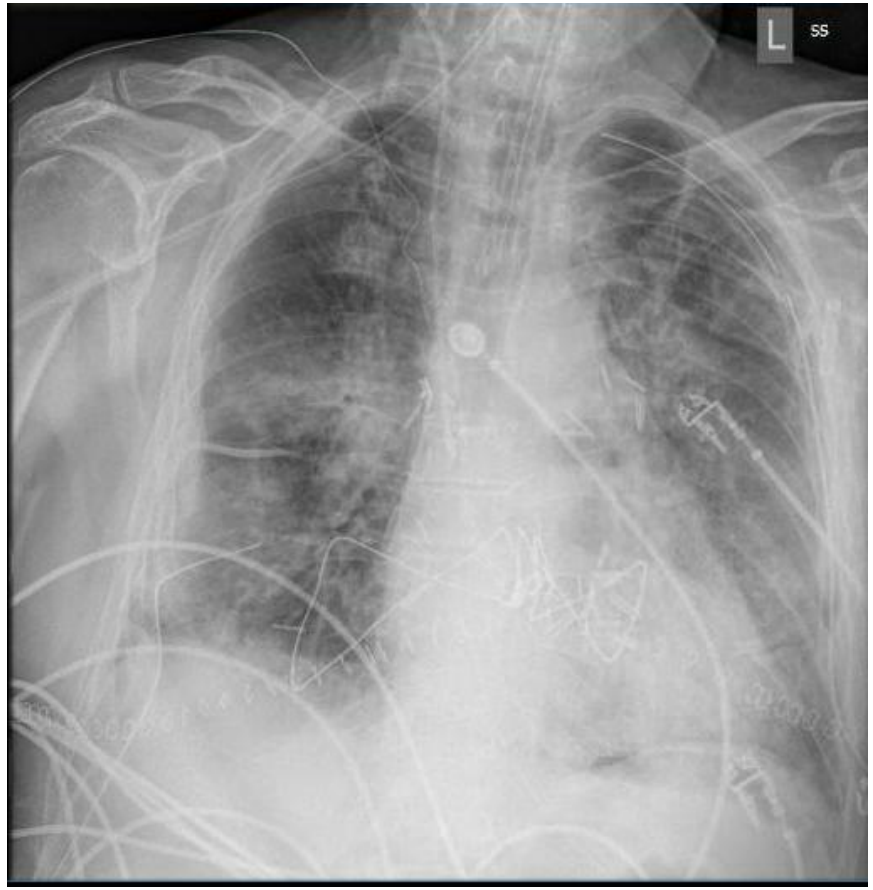
- Idiopathic pulmonary fibrosis s/p lung transplant
- Bronchoscopy of donor and recipient performed at time of transplant

# Case: Bronchoalveolar Lavage




**BAL donor bronchial washing:**
**Yeast to be identified**

**BAL patient:**
**Rare yeast to be identified**

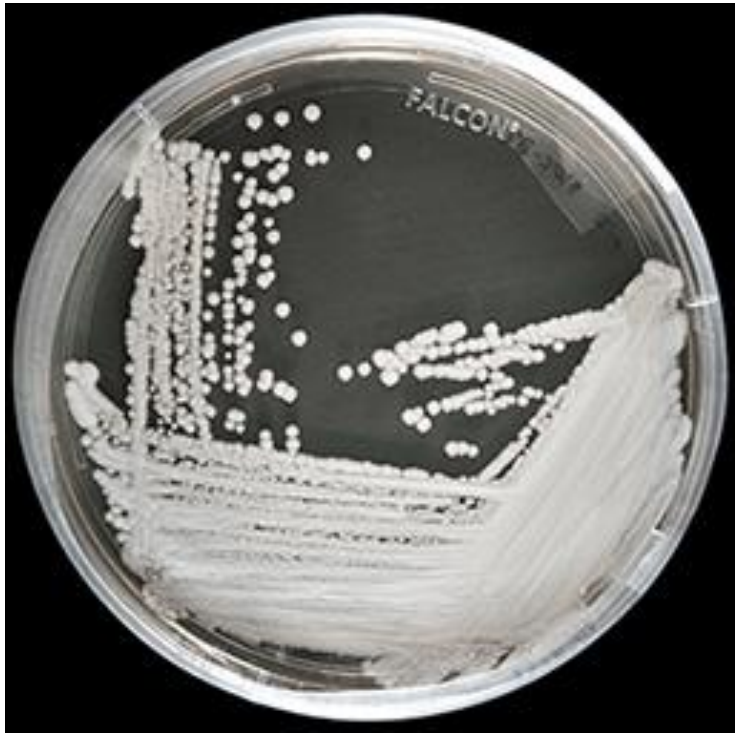



BAL = bronchoalveolar lavage.

Azar MM, et al. *Clin Infect. Dis*. 2017;65(6):1040-1042.

# Case: Hospital Course

- Day 5
  - Hypotension and respiratory arrest
  - Intubated
- Day 6
  - Fevers to 102.5, worsening hypoxemia
- Day 8
  - ECMO, CVVHD initiated, passed
- CDC confirms donor and recipient yeast = *C. auris*



****C. auris is difficult to identify****

ECMO = extracorporeal membrane oxygenation; CVVHD = continuous venovenous hemodialysis.

Azar MM, et al. *Clin Infect. Dis*. 2017;65(6):1040-1042.

# Outline

1. Case
2. **Epidemiology and current status of the *C. auris* epidemic**
3. New therapeutic approaches to drug-resistant fungi

# What are fungi?

POLL

A. Prokaryotes
B. Eukaryotes
C. Plants
D. Algae









# What are fungi?

POLL

A. Prokaryotes
B. Eukaryotes
C. Plants
D. Algae

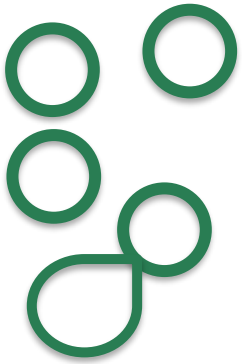


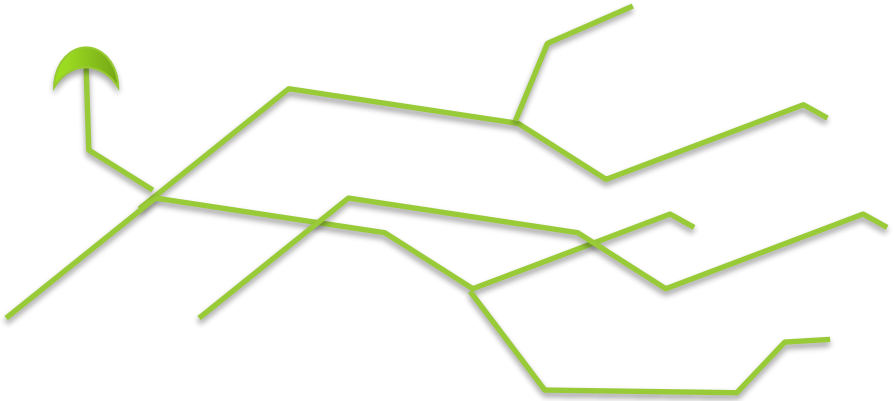






# Fungal Kingdom Overview



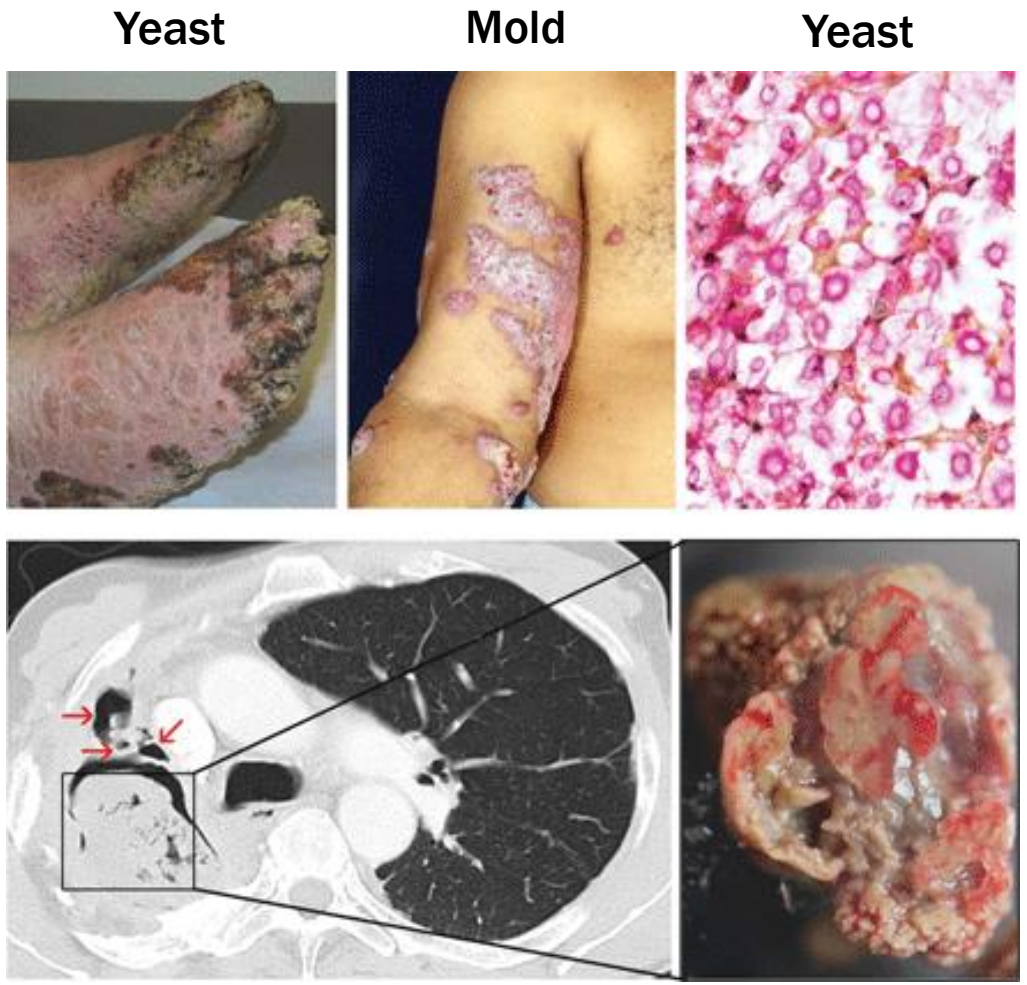


| **Yeasts** | ⟷ | **Dimorphic** | ⟷ | **Molds** |
|---|---|---|---|---|
| *Cryptococcus* | | *Blastomyces* | | *Aspergillus* |
| *Saccharomyces* | | *Histoplasmosis* | | *Zygomycetes* |
| *Candida glabrata* | | *Coccidiomycoces* | | *Exserohilum* |
| *Candida auris* | | *Candida albicans* | | Dermatophytes |

# Only a handful of fungi can infect humans. What is the most important variable that limits fungal infections?

POLL

A. Immune cells
B. Complement
C. Temperature
D. Antibodies



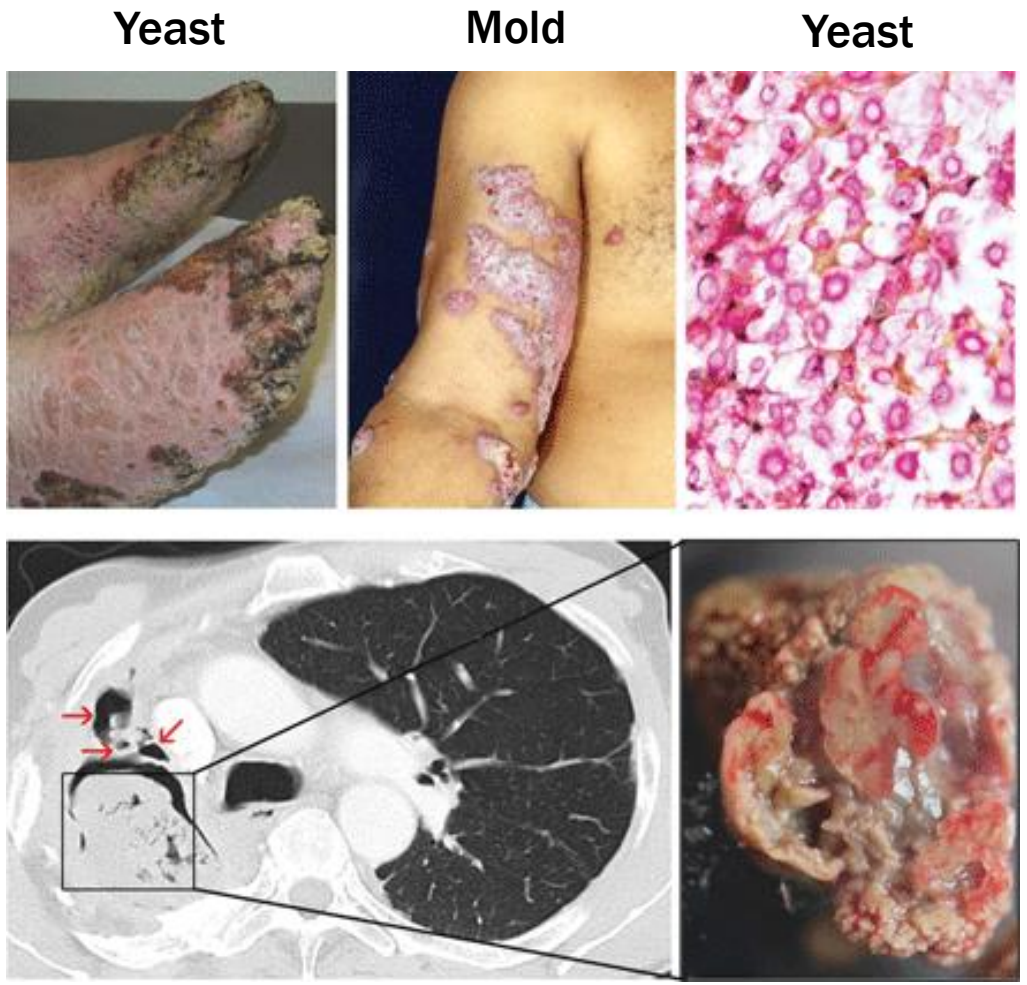

Mold

Brown GD, et al. *Sci Transl Med*. 2012;4(16)rv13.

# Only a handful of fungi can infect humans. What is the most important variable that limits fungal infections?

POLL

A. Immune cells
B. Complement
C. Temperature
D. Antibodies




Mold

Brown GD, et al. *Sci Transl Med*. 2012;4(16)rv13.

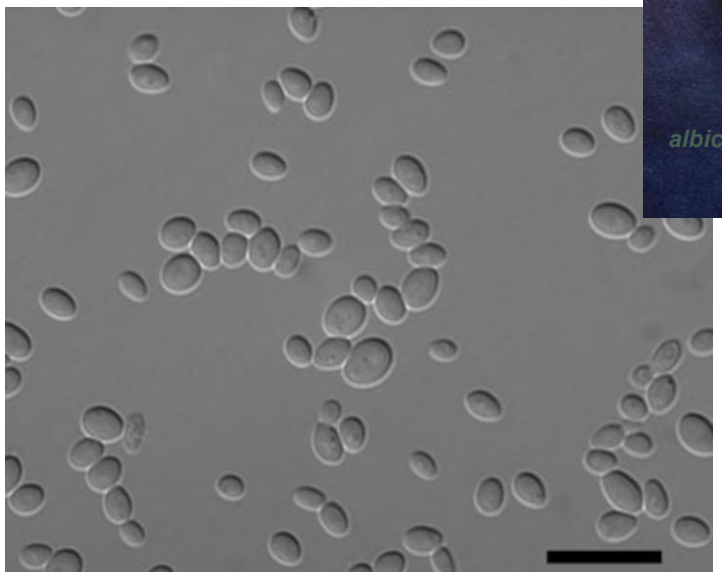
POLL

# Where was *C. auris* first identified?

A. Bloodstream infection
B. Open chronic wound
C. Outer ear infection
D. Stool microbiome





Courtesy of Dr. Sarah Turbett, MGH Microbiology Lab.
Satoh K, et al. Microbiol Immunol. 2009;53(1):41-44.

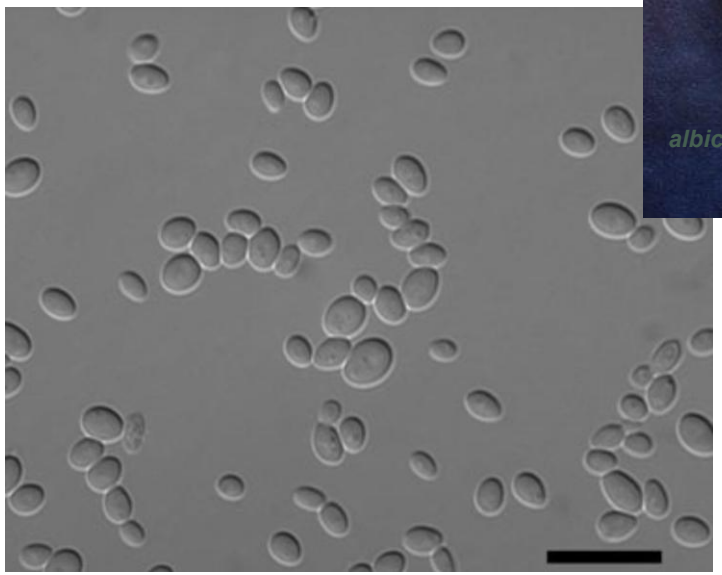
POLL

# Where was *C. auris* first identified?

A. Bloodstream infection
B. Open chronic wound
C. Outer ear infection
D. Stool microbiome





Courtesy of Dr. Sarah Turbett, MGH Microbiology Lab.
Satoh K, et al. Microbiol Immunol. 2009;53(1):41-44.

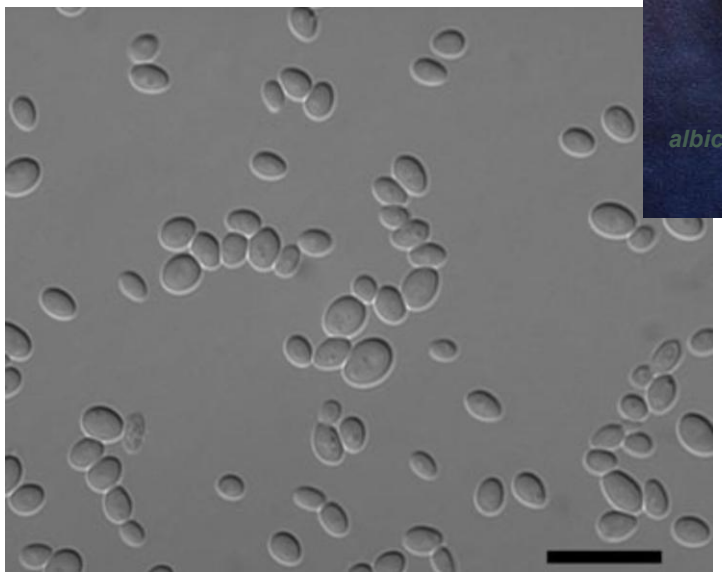
# Where Did *C. auris* Come From?

2009

External ear canal of a 70y Japanese Female at Tokyo Metropolitan Geriatric Hospital





**Latin = *aureum***

**Anatomically = *auricle***

***Candida auris***

Courtesy of Dr. Sarah Turbett, MGH Microbiology Lab.
Satoh K, et al. *Microbiol Immunol.* 2009;53(1):41-44.

# How Is *C. auris* Identified?

- Very difficult, poses challenges to hospital microbiology labs

- Conventional testing often misidentifies to related species
- Clues to suspect *C. auris*: no hyphae

- Using ribosomal or internal transcribed spacer (ITS) sequencing shows homology to:
  - 85%-87% to *C. haemulonii*
  - 81% to *C. pseudohaemulonii*

- Both form pseudohyphae, unlike *C. auris*

ITS = internal transcribed spacer.

Satoh K, et al. *Microbiol Immunol.* 2009;53(1):41-44.

# The Spread of *C. auris*

- 2009
- 15 cases of *C. auris* identified from 3 hospitals in S. Korea
- Otitis media
- Risk factors
  - 46% ENT surgical procedures
  - 100% broad spectrum antimicrobials

| Patient, isolate | Hospital | Source | Identification by D1/D2 domain and ITS sequence analysis | Vitek 2 YST result[a] |
|---|---|---|---|---|
| 9: 9 | C | Ear | *Candida* sp. nov.[b] | *C. haemulonii* (98) |
| 10: 10 | C | Ear | *Candida* sp. nov. | *C. haemulonii* (94) |
| 11: 11 | C | Ear | *Candida* sp. nov. | *C. haemulonii* (99) |
| 12: 12 | C | Ear | *Candida* sp. nov. | *C. haemulonii* (91) |
| 13: 13 | C | Ear | *Candida* sp. nov. | *C. haemulonii* (89) |
| 14: 14 | C | Ear | *Candida* sp. nov. | *C. haemulonii* (95) |
| 15: 15 | C | Ear | *Candida* sp. nov. | *C. haemulonii* (89) |
| 16: 16 | C | Ear | *Candida* sp. nov. | *C. haemulonii* (99) |
| 17: 17 | D | Ear | *Candida* sp. nov. | *C. haemulonii* (99) |
| 18: 18 | D | Ear | *Candida* sp. nov. | *C. haemulonii* (95) |
| 19: 19 | D | Ear | *Candida* sp. nov. | *C. haemulonii* (91) |
| 20: 20 | D | Ear | *Candida* sp. nov. | *C. haemulonii* (99) |
| 21: 21 | E | Ear | *Candida* sp. nov. | *C. haemulonii* (99) |
| 22: 22 | E | Ear | *Candida* sp. nov. | *C. haemulonii* (98) |
| 23: 23 | E | Ear | *Candida* sp. nov. | *C. haemulonii* (99) |

Kim MN, et al. *Clin Infect Dis*. 2009;48(6):e57-e61.

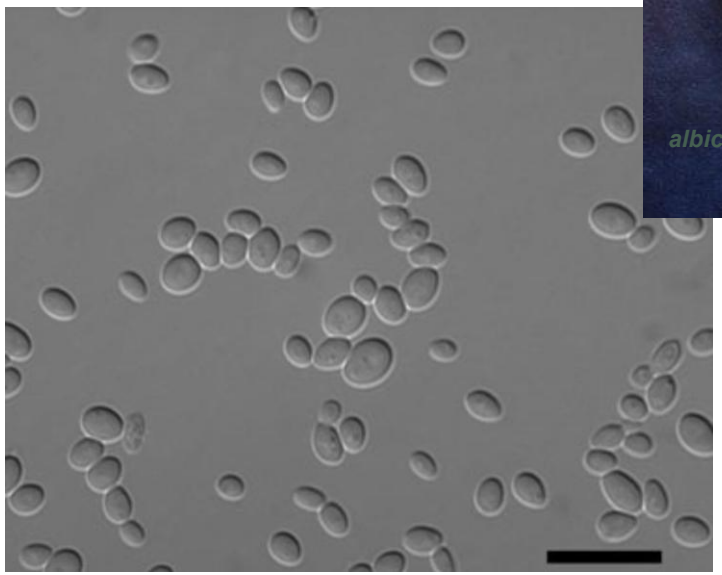
POLL

# What are *C. auris* features?

A. Only found on skin
B. Multidrug resistant
C. Never form hyphae
D. All of the above





Courtesy of Dr. Sarah Turbett, MGH Microbiology Lab.
Satoh K, et al. Microbiol Immunol. 2009;53(1):41-44.

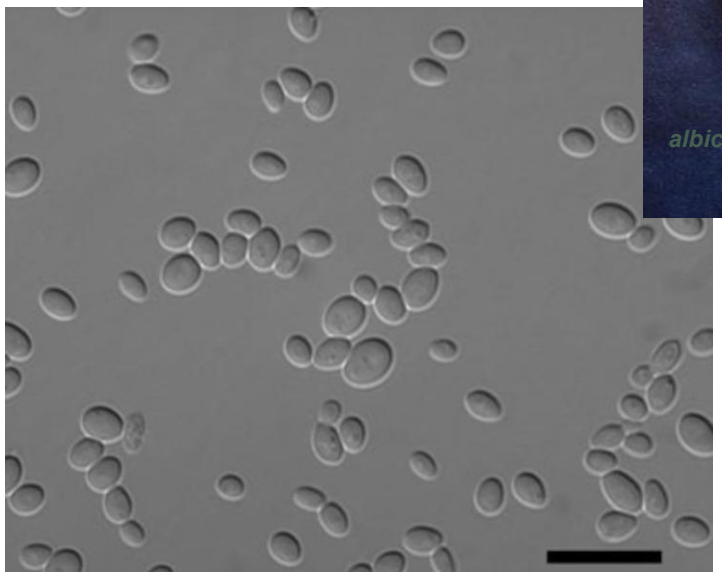
POLL

# What are *C. auris* features?

A. Only found on skin
B. Multidrug resistant
C. Never form hyphae
D. All of the above





Courtesy of Dr. Sarah Turbett, MGH Microbiology Lab.
Satoh K, et al. Microbiol Immunol. 2009;53(1):41-44.

# *C. auris* Are Multidrug Resistant

- Antifungal susceptibility shows resistance across multiple antifungal classes
  - Azole, amphotericin
  - Echinocandin: most likely to be sensitive

- Outcomes
  - Prolonged positive cultures
  - 1 patient required 2 months of IV antifungal treatment

Kim MN, et al. *Clin Infect Dis*. 2009;48(6):e57-e61.

# Otitis, or Is *C. auris* More Invasive?

- 2011
- 3 *C. auris* fungemia reported in pediatric and adult patients in South Korea
- Risk factors:
  - Indwelling catheter
  - High acuity
  - Broad spectrum antimicrobials, TPN

- The invasive *C. auris* are invariably multidrug resistant

Lee WG, et al. *J Clin Microbiol.* 2011;49(9):3139-3142.

# *C. auris* Fungemia = High Mortality

## Patient Comorbidity

- 6 (50%) diabetes
- 5 (42%) chronic kidney disease
- 2 (17%) chemotherapy
- 1 (8%) HIV

## Hospital Risks

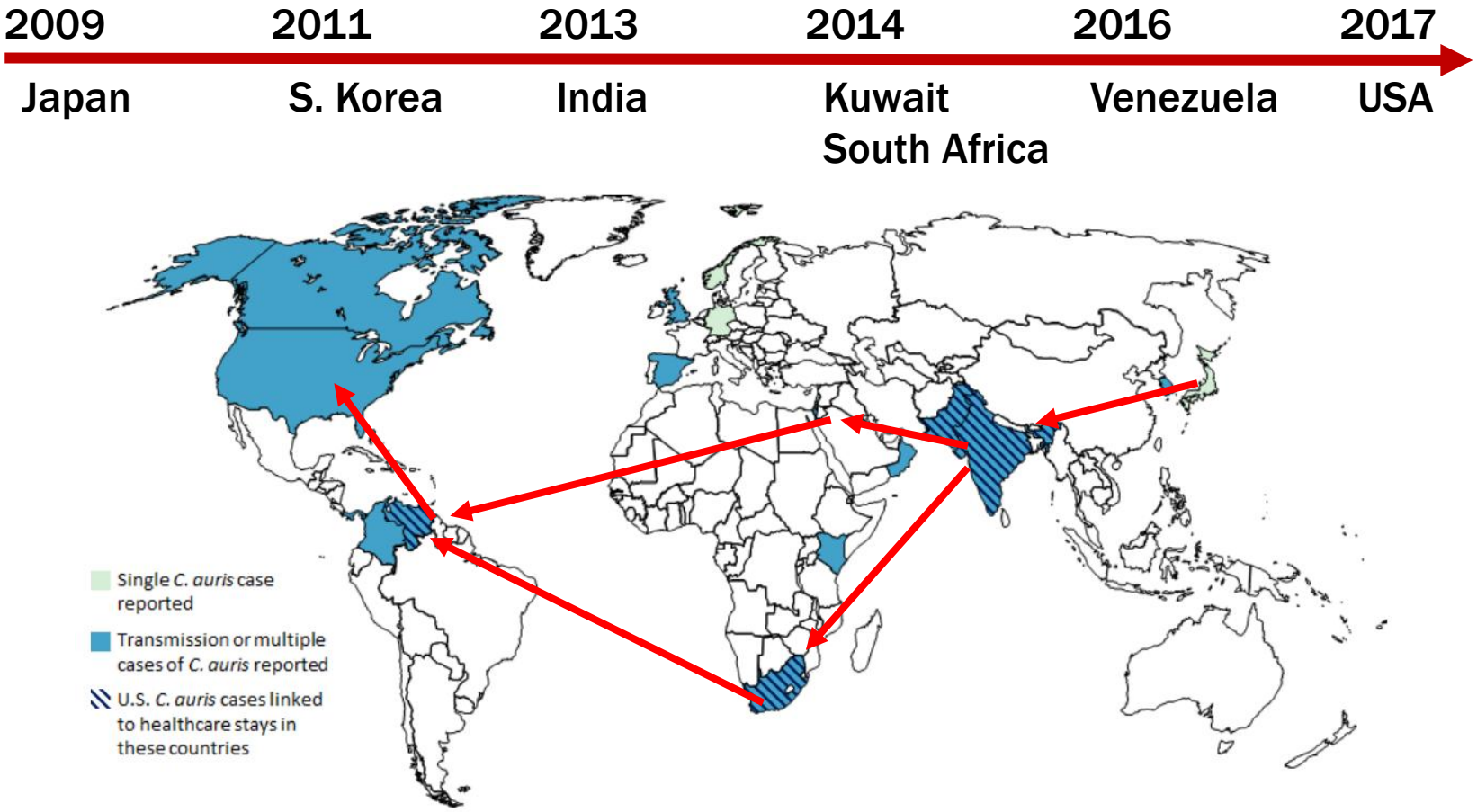
- 83% urinary catheter
- 75% on broad spectrum abx
- 58% ICU
- 50% neutropenic
- 42% CVC

- Overall
  - Significant comorbidities
  - Lots of catheters
  - High acuity

**Mortality: 50%-70%**

Chowdhary A, et al. *Emerg Infect Dis*. 2013;19(10):1670-1673.

# *C. auris* Reported Globally




Emara M, et al. *Emerg Infect Dis*. 2015;21(6):1091-1092. Magobo RE, et al. *Emerg Infect Dis*. 2014;20(7):1250-1251. Calvo B, et al. *J Infect*. 2016;73(4):369-374. U.S. Centers for Disease Control and Prevention (CDC). Published April 24, 2024. Accessed Sept. 23, 2026. https://www.cdc.gov/candidiasis/hcp/clinical-overview/index.html

# CDC Sounds Alarm

- 2017, USA
  - 2013-2016
  - CDC clinical alert issued in June 2016
  - 7 cases identified
    - Only 1 with travel outside the U.S.
  - Majority of patients with severe immunodeficiency



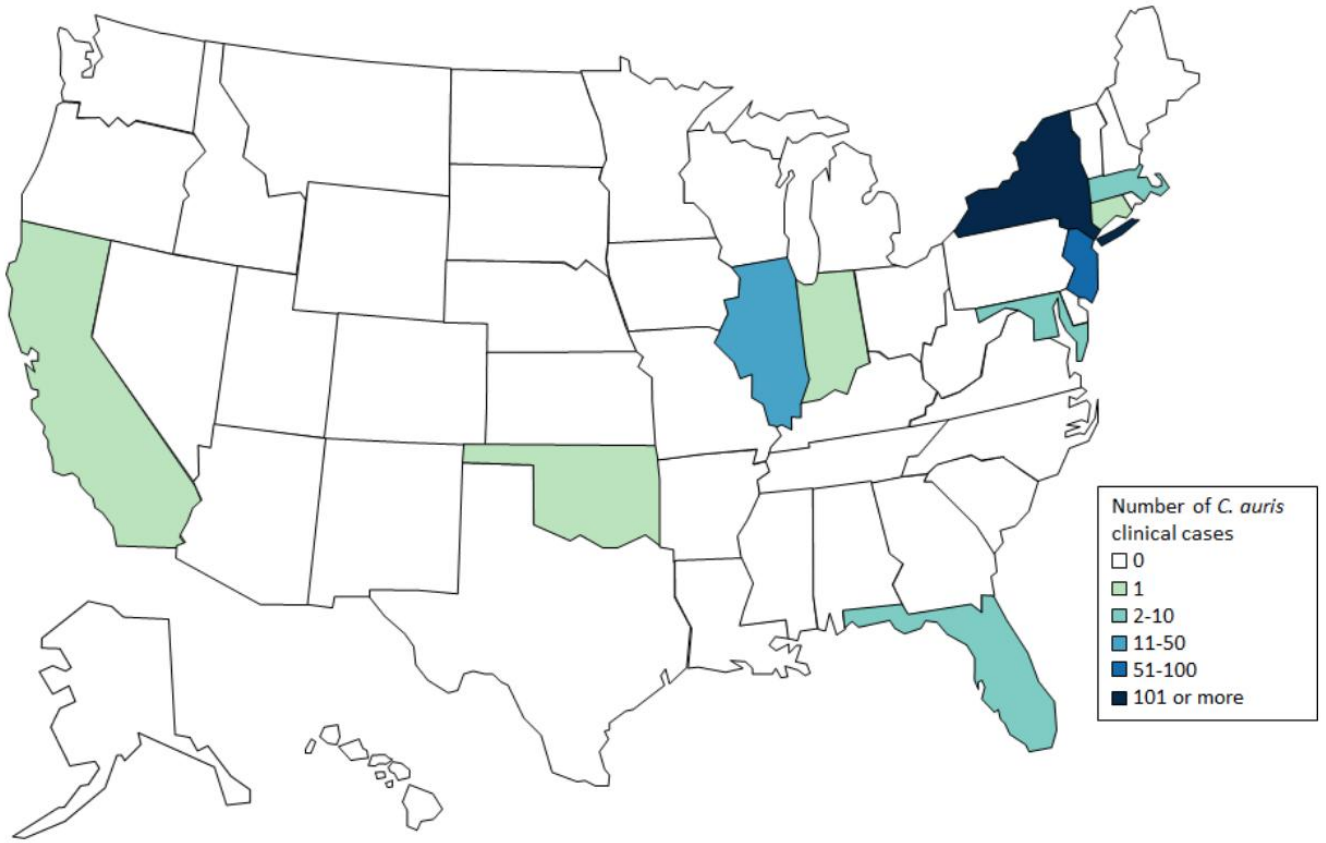

**Dr. Tom Chiller, CDC**

| Isolation month/year | State | Site of *C. auris* isolation | Underlying medical condition(s) | Outcome[1] |
|---|---|---|---|---|
| May 2013 | New York | Blood | Respiratory failure requiring high-dose corticosteroids | Died |
| July 2015 | New Jersey | Blood | Brain tumor and recent villous adenoma resection | Died |
| April 2016 | Maryland | Blood | Hematologic malignancy and bone marrow transplant | Died |
| April 2016 | New York | Blood | Hematologic malignancy | Died |
| May 2016 | Illinois | Blood | Short gut syndrome requiring total parenteral nutrition and high-dose corticosteroid use | Survived |
| July 2016 | Illinois | Urine | Paraplegia with long-term, indwelling Foley catheter | Survived |
| August 2016 | New York | Ear | Severe peripheral vascular disease and skull base osteomyelitis | Survived |

Vallabhaneni S, et al. *Am J Transplant*. 2017;17(1):296–299.

# Superbug *C. auris* Identified in 122 People across 7 States, CDC Says

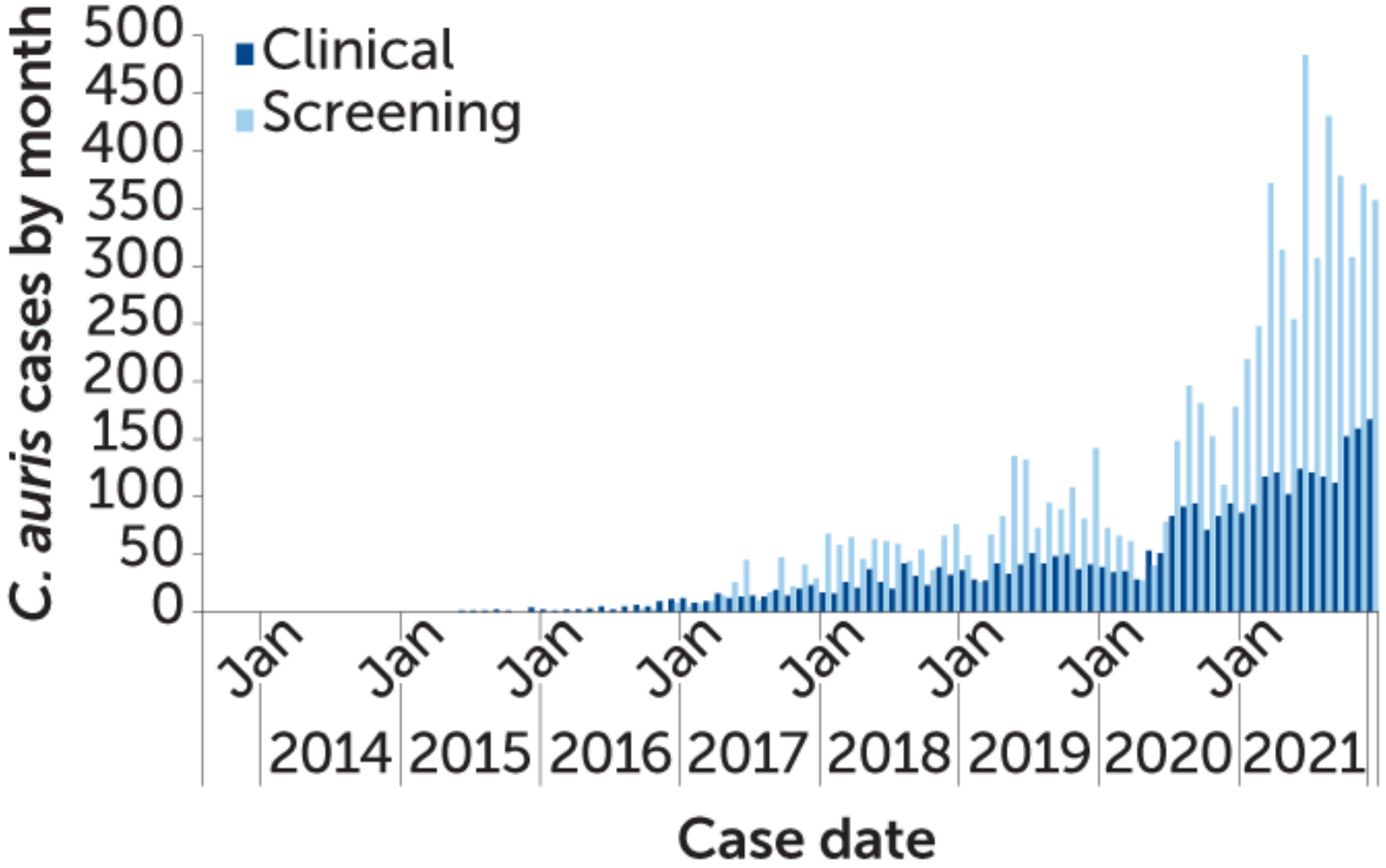
CNN, May 2017



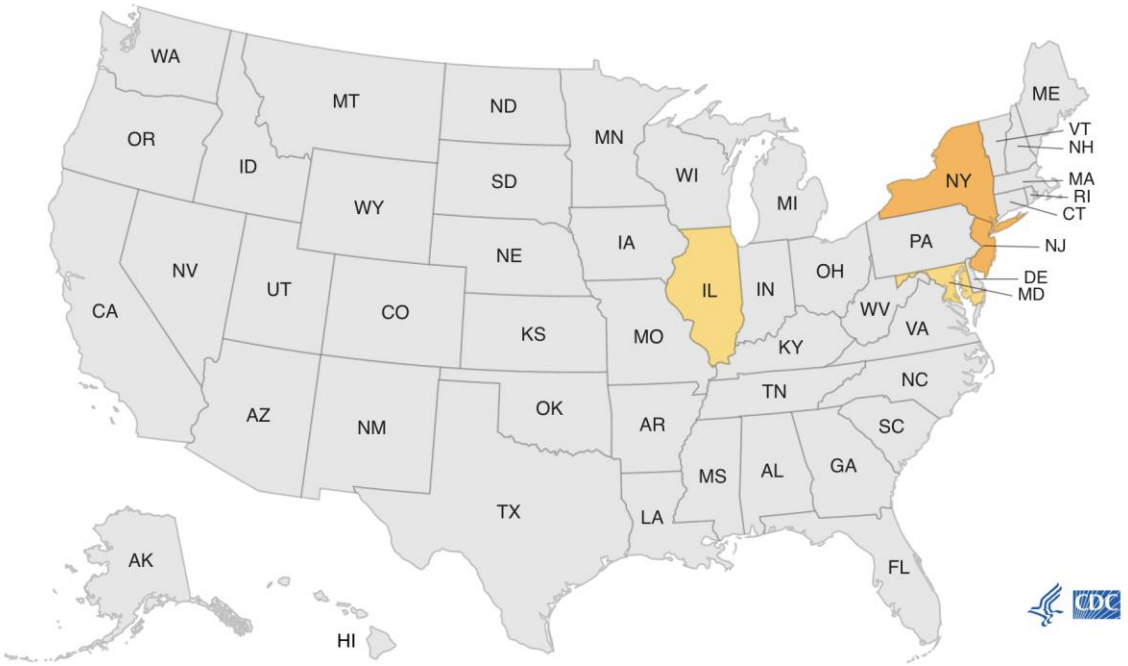

**174 total cases in 10 states**

U.S. Centers for Disease Control and Prevention (CDC). Published April 24, 2024. Accessed Sept. 23, 2026. https://www.cdc.gov/candidiasis/hcp/clinical-overview/index.html

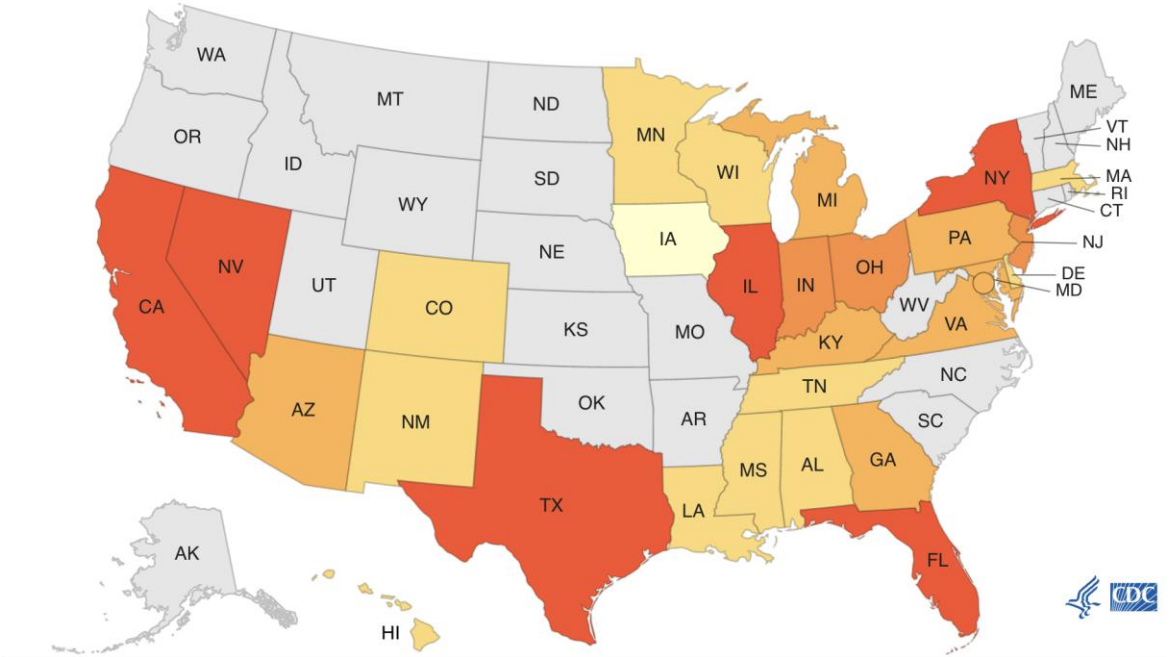
# *C. auris* Cases Now on the Rise Again



# Current Status




2016-2019




2022

# *C. auris* Persists

- … in patients and the hospital environment
- Surveillance of patients weeks following infection show persistent *C. auris* in axillae, groin, rectum

- Following standard hospital room cleaning
  - "samples taken from the mattress, bedside table, bed rail, chair, and window sill all yielded *C. auris*"

Vallabhaneni S, et al. *Am J Transplant*. 2017;17(1):296-299.

# *C. auris* Shuts Down ICU

COMMENTARY

## The Unexpected and Troubling Rise of *Candida auris*

News

### Intensive care unit closed after new deadly superbug emerges in the UK

share



Health officials are concerned about the emergence of a new infection CREDIT: PA

In the UK

- >200 cases
- Multiple services shut down
- Outbreaks often linked to a single ill patient

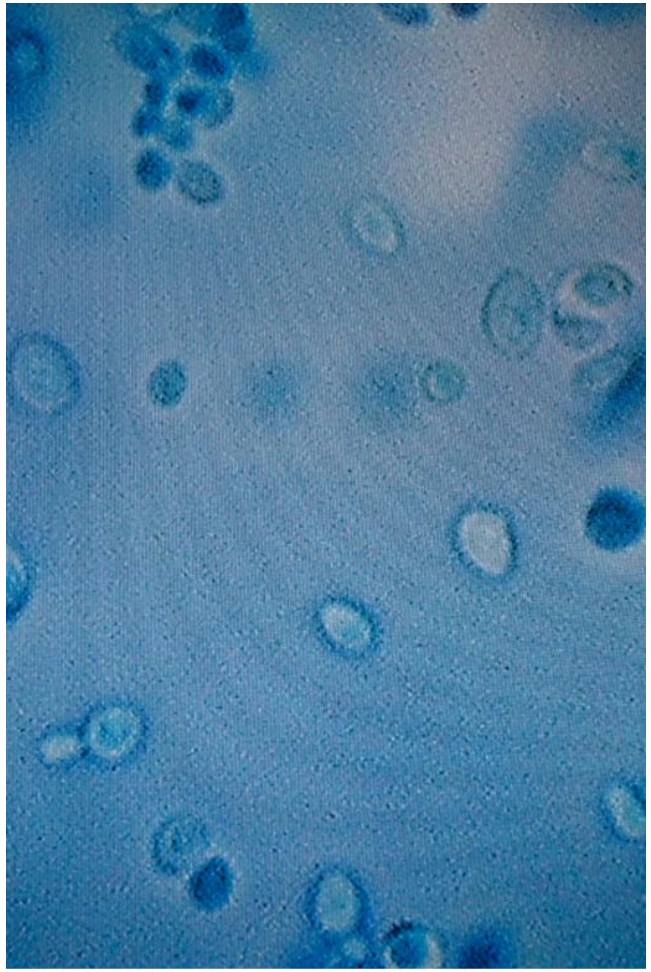
Schelenz S, et al. *Antimicrob Resist Infect Control*. 2016;5(1):35.

# *The New York Times*

TIMES INSIDER

## Candida Auris: The Fungus Nobody Wants to Talk About



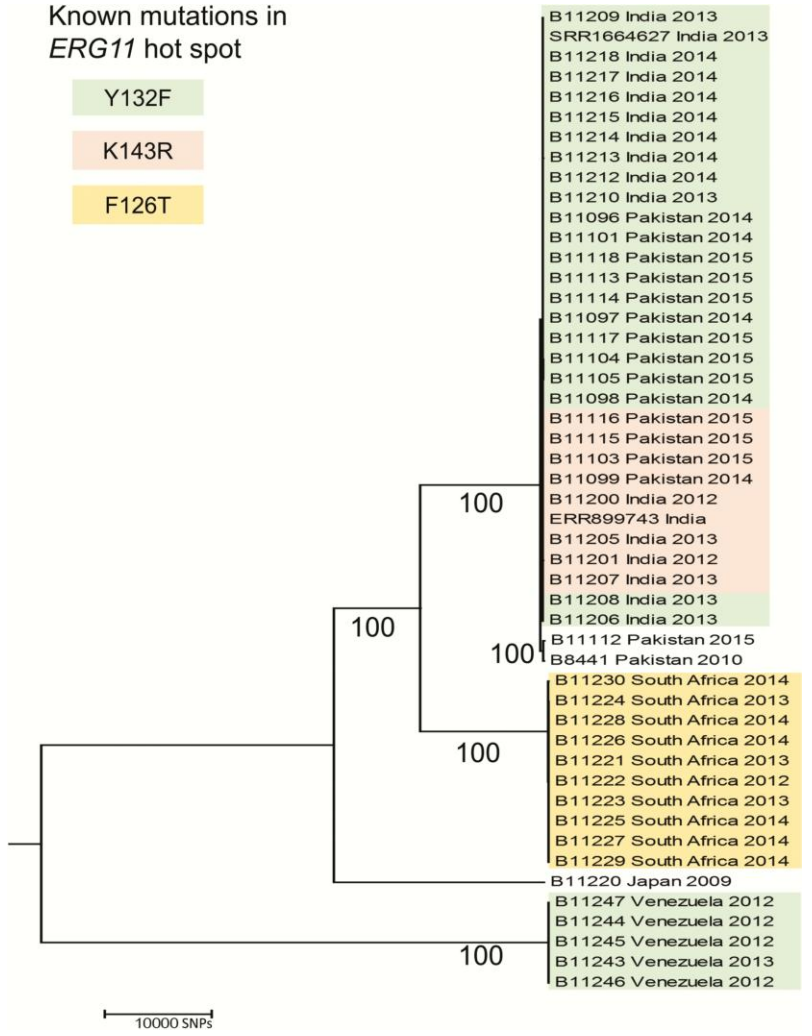


elissa Golden for The New York Times

# *C. auris* Emergence… Revealed

- 54 *C. auris* isolates collected by the CDC
  - India, Pakistan, Venezuela, Japan, and South Africa
  - Whole genome sequencing at the Broad Institute
- **Susceptibility:** 41% resistance >2 classes
- **Mortality:** 59%

Sequencing conclusion:
*C. auris* segregates into distinct clades




Lockhart SR, et al. *Clin Infect Dis*. 2017;64(2):134-140.

# *C. auris* Reported Globally

| 2009 | 2011 | 2013 | 2014 | 2016 | 2017 |
|---|---|---|---|---|---|
| Japan | S. Korea | India | Kuwait<br>South Africa | Venezuela | USA |




Emara M, et al. *Emerg Infect Dis*. 2015;21(6):1091-1092. Magobo RE, et al. *Emerg Infect Dis*. 2014;20(7):1250-1251. Calvo B, et al. *J Infect*. 2016;73(4):369-374. U.S. Centers for Disease Control and Prevention (CDC). Published April 24, 2024. Accessed Sept. 23, 2026. https://www.cdc.gov/candidiasis/hcp/clinical-overview/index.html

# *C. auris* Reported Globally

| 2009 | 2011 | 2013 | 2014 | 2016 | 2017 |
|---|---|---|---|---|---|
| Japan | S. Korea | India | Kuwait<br>South Africa | Venezuela | USA |




Emara M, et al. *Emerg Infect Dis*. 2015;21(6):1091-1092. Magobo RE, et al. *Emerg Infect Dis*. 2014;20(7):1250-1251. Calvo B, et al. *J Infect*. 2016;73(4):369-374. U.S. Centers for Disease Control and Prevention (CDC). Published April 24, 2024. Accessed Sept. 23, 2026. https://www.cdc.gov/candidiasis/hcp/clinical-overview/index.html

# Did *C. auris* Exist before 2009?

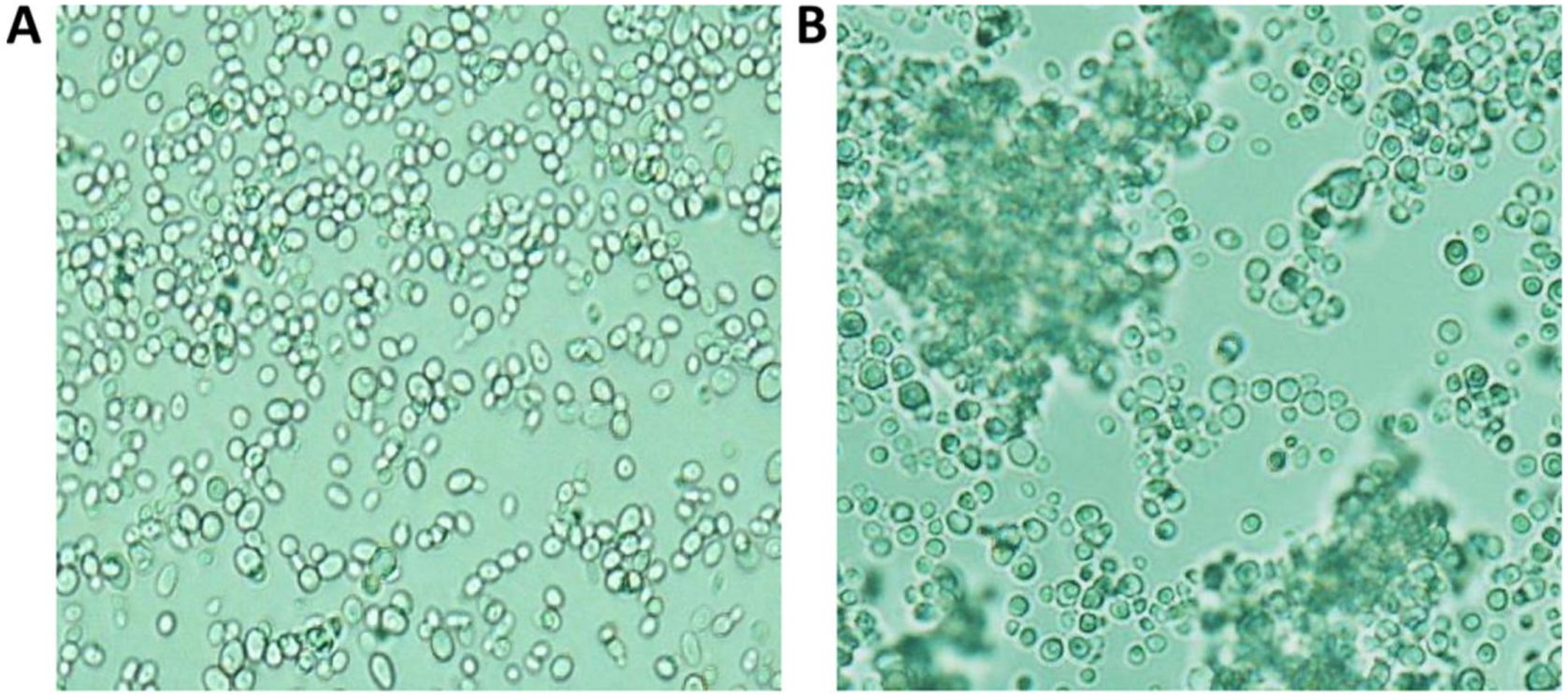
- International SENTRY database
  - 15,271 *Candida* isolates
  - 2004-2015
  - Only 4 *C. auris* isolates from 2009, 2013, 2014, 2015
  - 1 single *C. auris* identified before 2009

- Synchronous emergence of clonal *C. auris* strains at different global sites, NOT contiguous spread starting in 2009

Lockhart SR, et al. *Clin Infect Dis*. 2017;64(2):134-140.

# *C. auris* — Clues to Pathogenesis

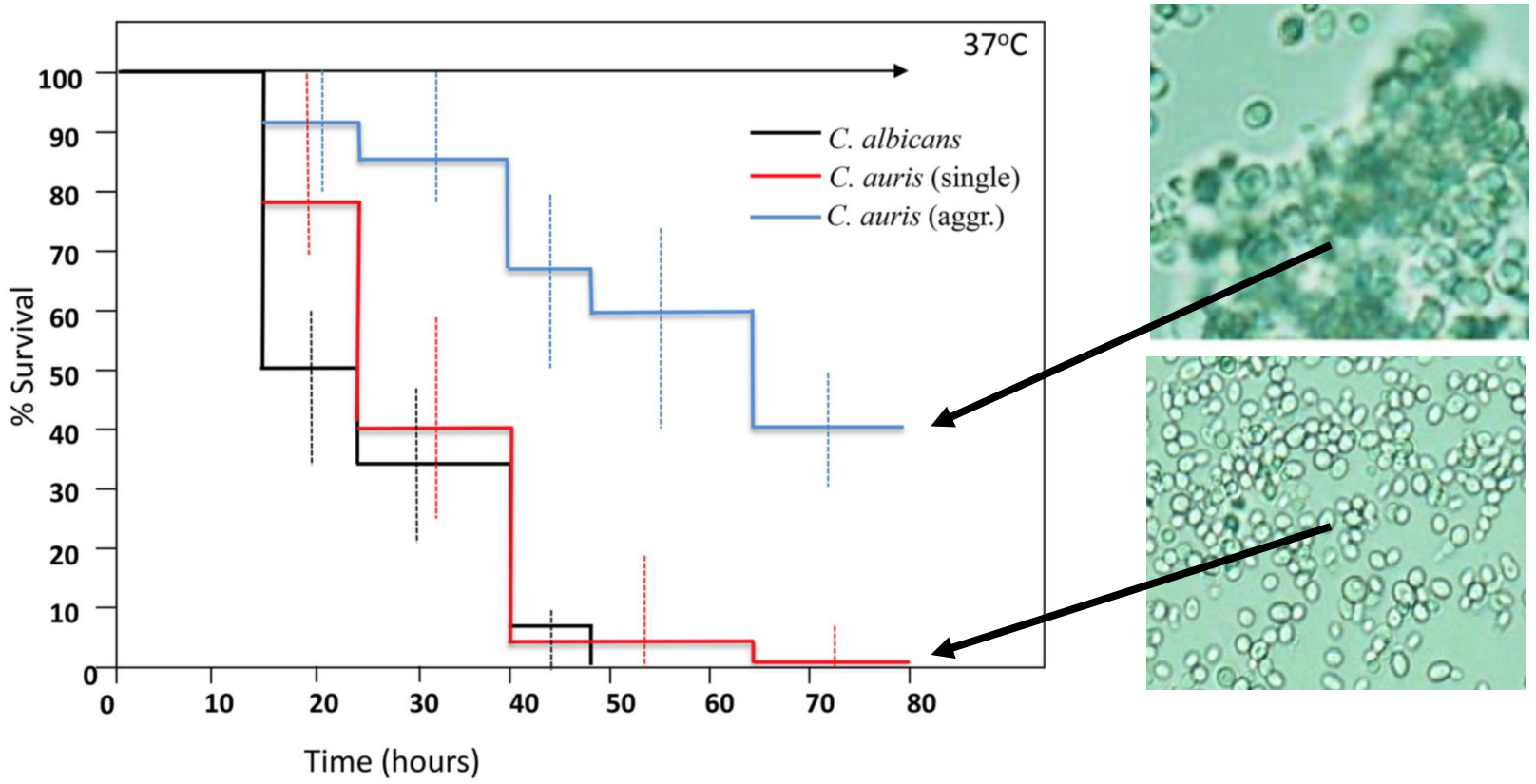
- Striking observation made when attempting to culture
  - Aggregated vs single cell growth




Borman AM, et al. *mSphere*. 2016;*1*(4):e00189–16.

# *C. auris* — Clues to Pathogenesis

- Aggregated forms of *C. auris* cause less mortality as compared to single cell phenotype





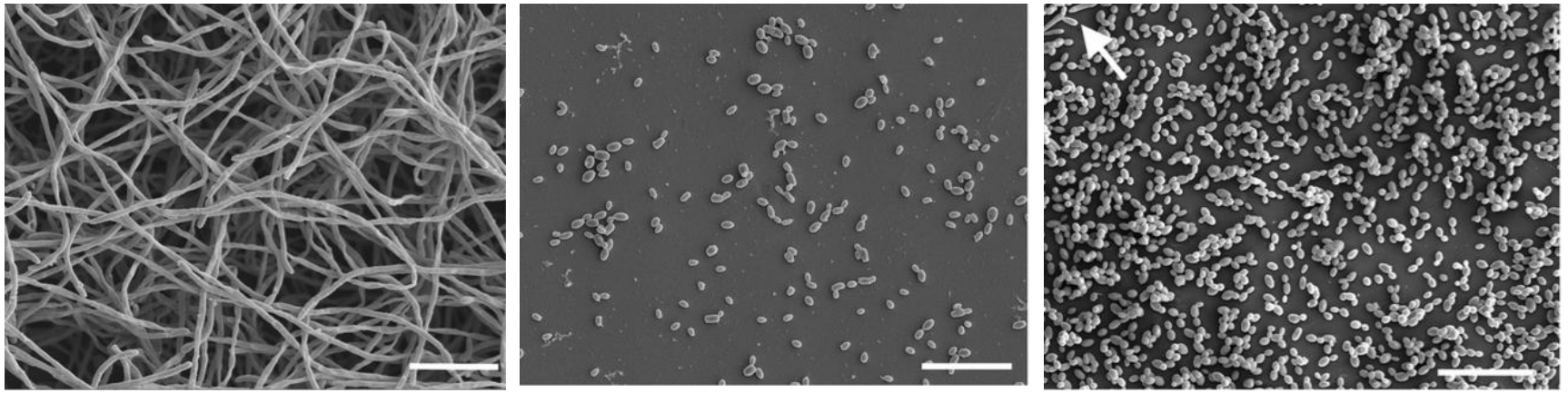

**Invertebrate *Galleria mellonella* model**

Borman AM, et al. *mSphere*. 2016;*1*(4):e00189–16.

# *C. auris* Biofilm May Enhance Resistance

- Aggregates and single cell strains cultured and imaged to determine biofilm production
- **Biofilm partially explains the drug resistance**

*C. albicans* *C. glabrata* *C. auris*



Sherry L, et al. *Emerg Infect Dis.* 2017;23(2):328–331.

# Outline

1. Case
2. Epidemiology and current status of the *C. auris* epidemic
3. **New therapeutic approaches to drug-resistant *C. auris***

# *C. auris* Is Highly Drug Resistant

## Resistance in the U.S.

1




**87.6%**
Azoles

2




**33.7%**
Polyenes

3



**1.7%**
Echinocandins

- 33% multidrug resistant
- 2 pan-resistance found in 2019

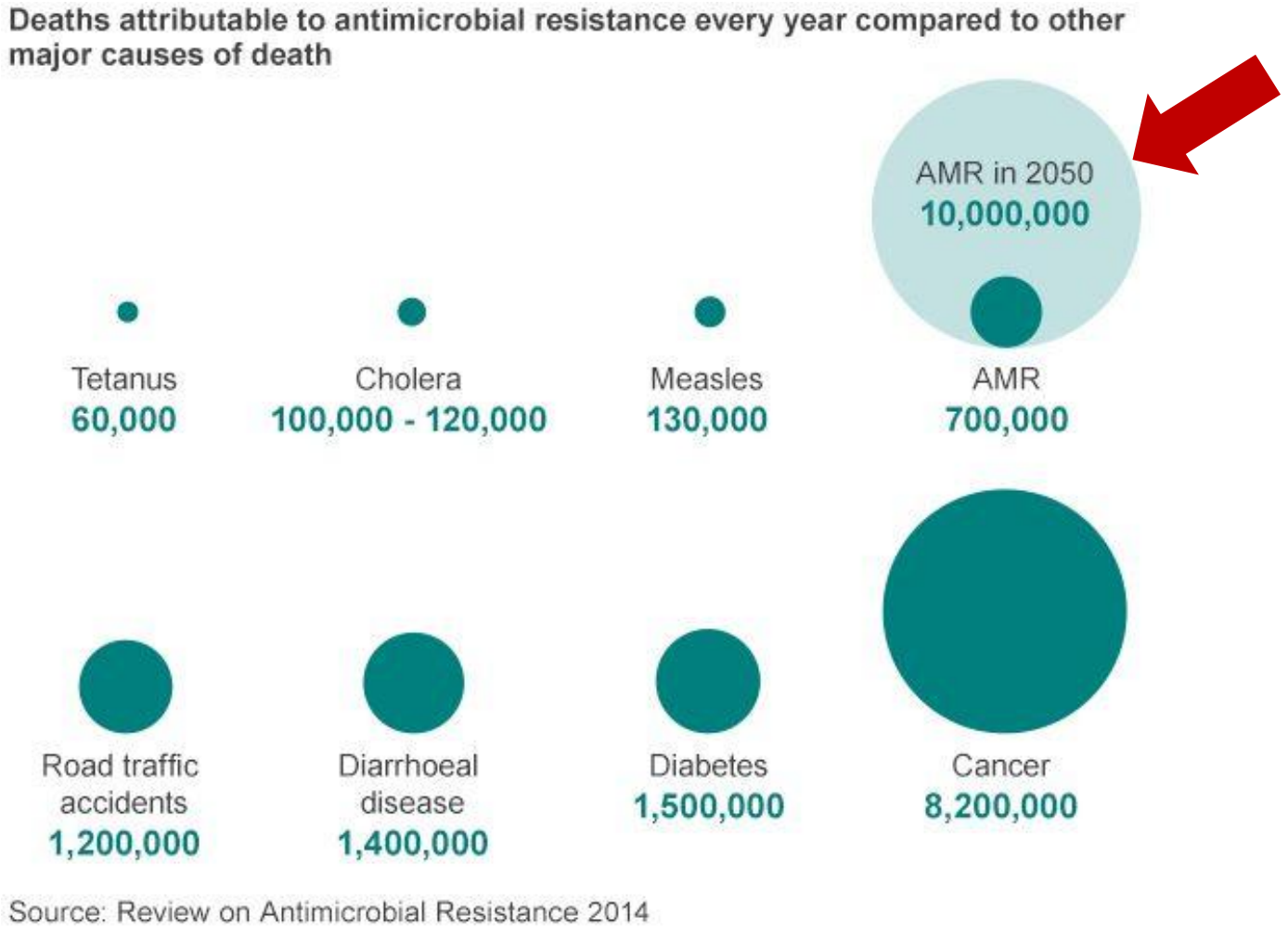
Courtesy of the CDC.

# Drug-Resistant Pathogen = Higher Mortality

*They can be*

1. Bacteria
2. Fungi
3. Viruses
4. Parasites

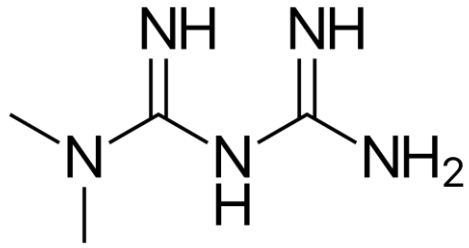
**Resistant** to one or more drugs used for treatment



# *Example*: Metformin (a Biguanide)

- Helps control blood sugar level
- Primary therapy option for Type 2 diabetes
- Most widely used oral diabetes medication



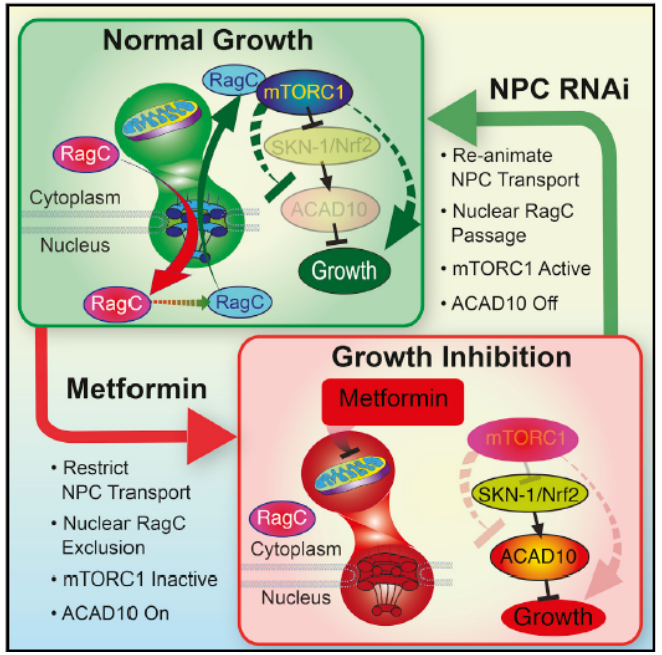
# Metformin and Growth-Inhibition

Article

Cell

## An Ancient, Unified Mechanism for Metformin Growth Inhibition in *C. elegans* and Cancer

### Graphical Abstract




### Authors

Lianfeng Wu, Ben Zhou, Noriko Oshiro-Rapley, ..., Steven P. Gygi, Bin Zheng, Alexander A. Soukas

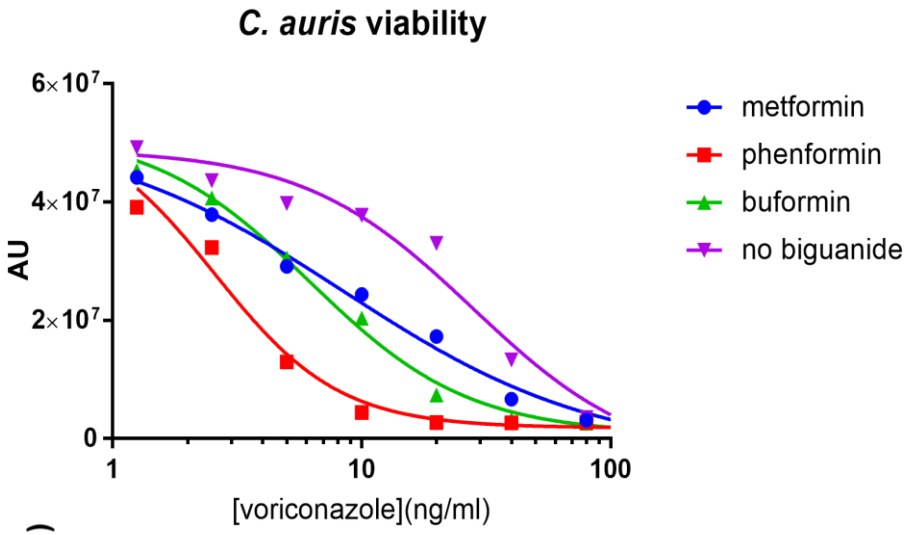
### Correspondence

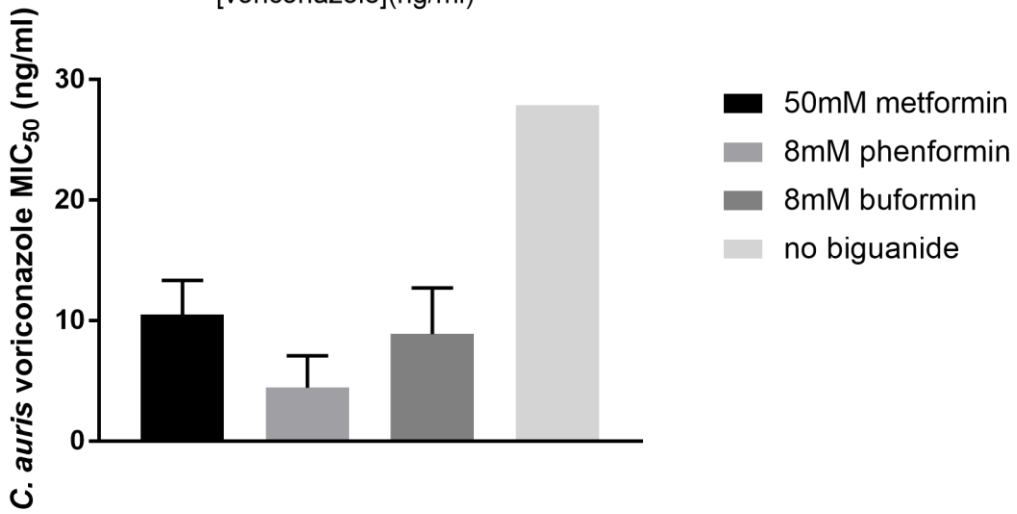
asoukas@mgh.harvard.edu

### In Brief

Metformin both suppresses cancer cell growth and promotes organismal longevity through a key transcriptional target that is induced through inhibition of mitochondrial respiration and modulation of mTOR signaling.

# Metformin Enhances Anti-Fungal Activity






Sam Steigler

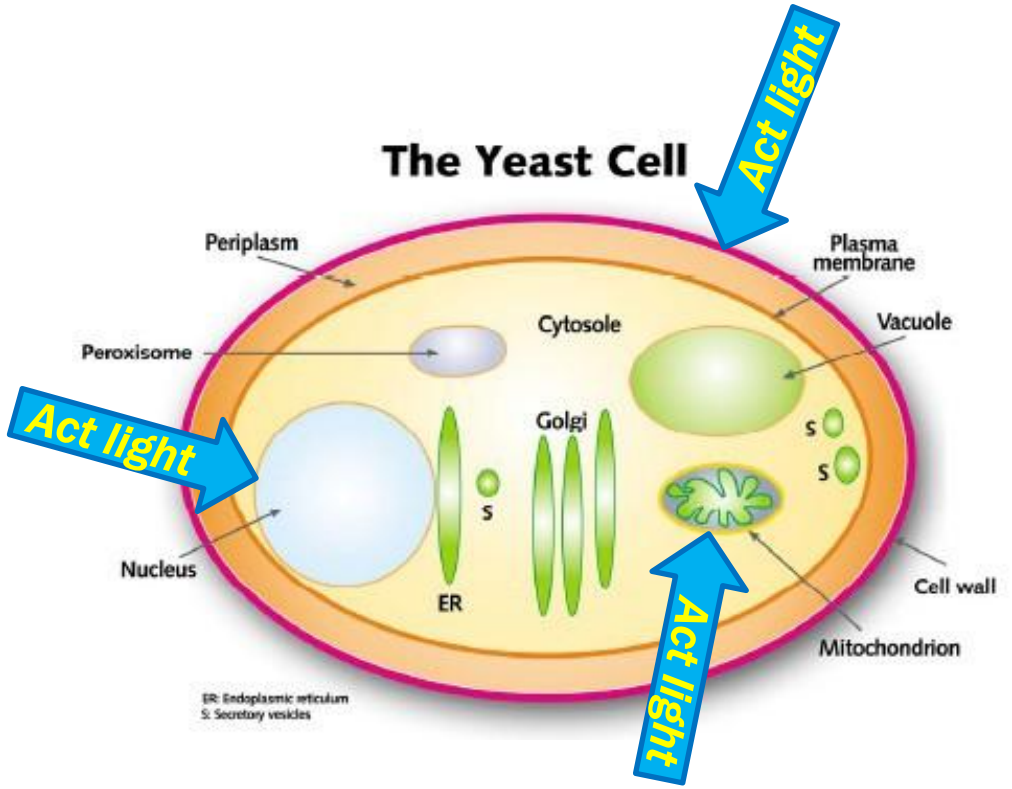


Linda Xu

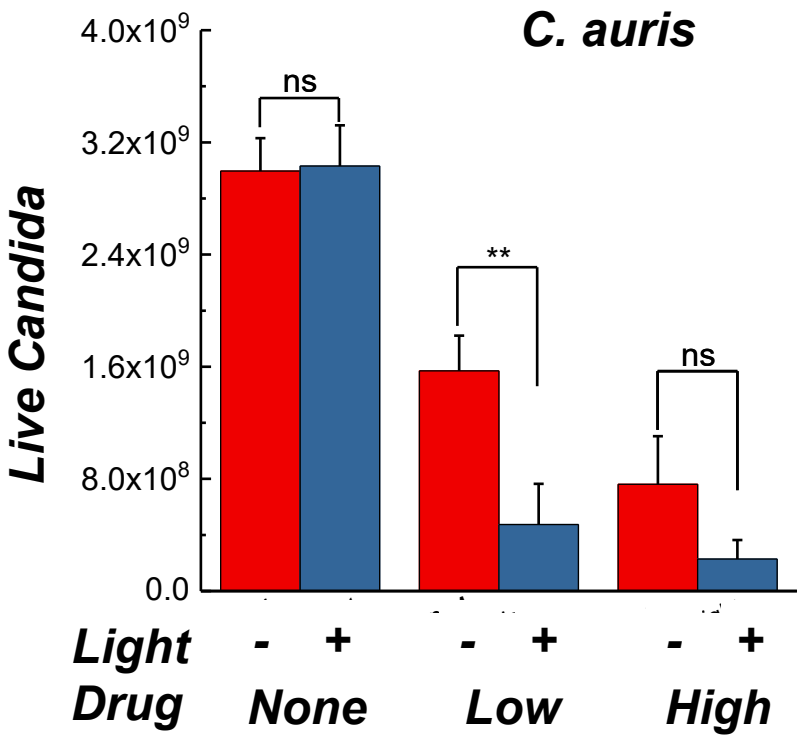

Media
Voriconazole
Metformin
Vori+Met

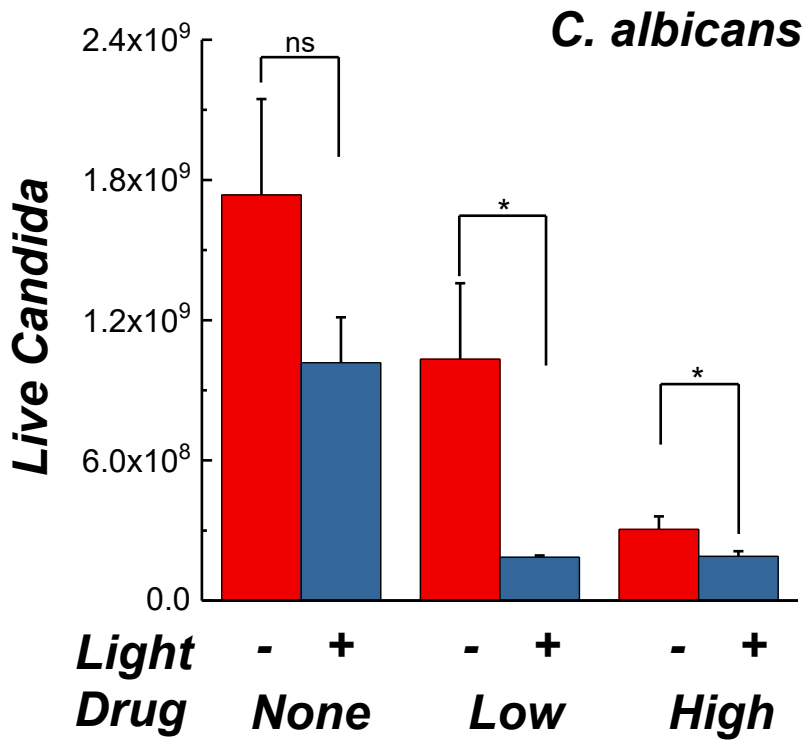
# Example 2: Activated Light Therapy





# Activated Light Delivers near Complete Fungal Sterilization



# Example 3: Polymers with Antifungal Properties

| Antifungal polymer | Membrane | Cell wall | Intracellular targets | Toxicity | Reference |
|---|---|---|---|---|---|
| Synthetic antimicrobial peptides | Does not appear to permeabilise fungal cell membranes | — | Binds to nucleic acids | Dependent on hydrophobic region length | Ramamourthy et al. (2020) |
| Synthetic antimicrobial peptides | Membrane permeabilisation | — | Likely | Low haemolytic activity | Dodou Lima et al. (2020) |
| (Nylon-3 copolymers) | Membrane permeabilisation | — | — | Low toxicity, dependent on hydrophobic region length | Liu et al., 2015 Rank et al. (2018) |
| PHMB | Membrane permeabilisation | Cell wall target | Nucleus, binds to DNA/RNA | Low | Elsztein et al., 2011 |
| POGH | Membrane permeabilisation likely | — | Likely | Slightly | Luo et al. (2017) |
| PQ-1 | Membrane permeabilisation | Prevents conidia germination | Likely | — | Codling., 2003 Kilvington et al. (2013) |
| PEI | Membrane permeabilisation | — | Likely Cell membrane depolarisation, binds to nucleic acids | Slightly, dependent on hydrophobic region length | Azevedo et al. (2014) |
| Chitosan | Membrane permeabilisation | — | Nucleus, binds to DNA/RNA | Low toxicity | Palma-Guerrero et al., 2008 |
| PHMB derivatives (PHMG-P PHMGH) | Membrane permeabilisation | Cell wall target | Likely | Low toxicity but severe toxicity when inhaled | Choi, Kim and Lee, (2017) Olmedo et al. (2018) |
| (N-(2-hydroxypropyl)-3-trimethylammonium chitosan chlorides) HTCC | Membrane permeabilisation | — | Likely | Low toxicity | Hoque et al. (2016) |
| Quaternary ammonium chloride derivatives of chitosan | Membrane permeabilisation | — | Likely | Low toxicity | Jung et al. (2020) |

Ntow-Boahene W, et al. *Front Bioeng Biotechnol*. 2021;9:780328.

# Summary of mechanisms of action for polymers with antifungal properties

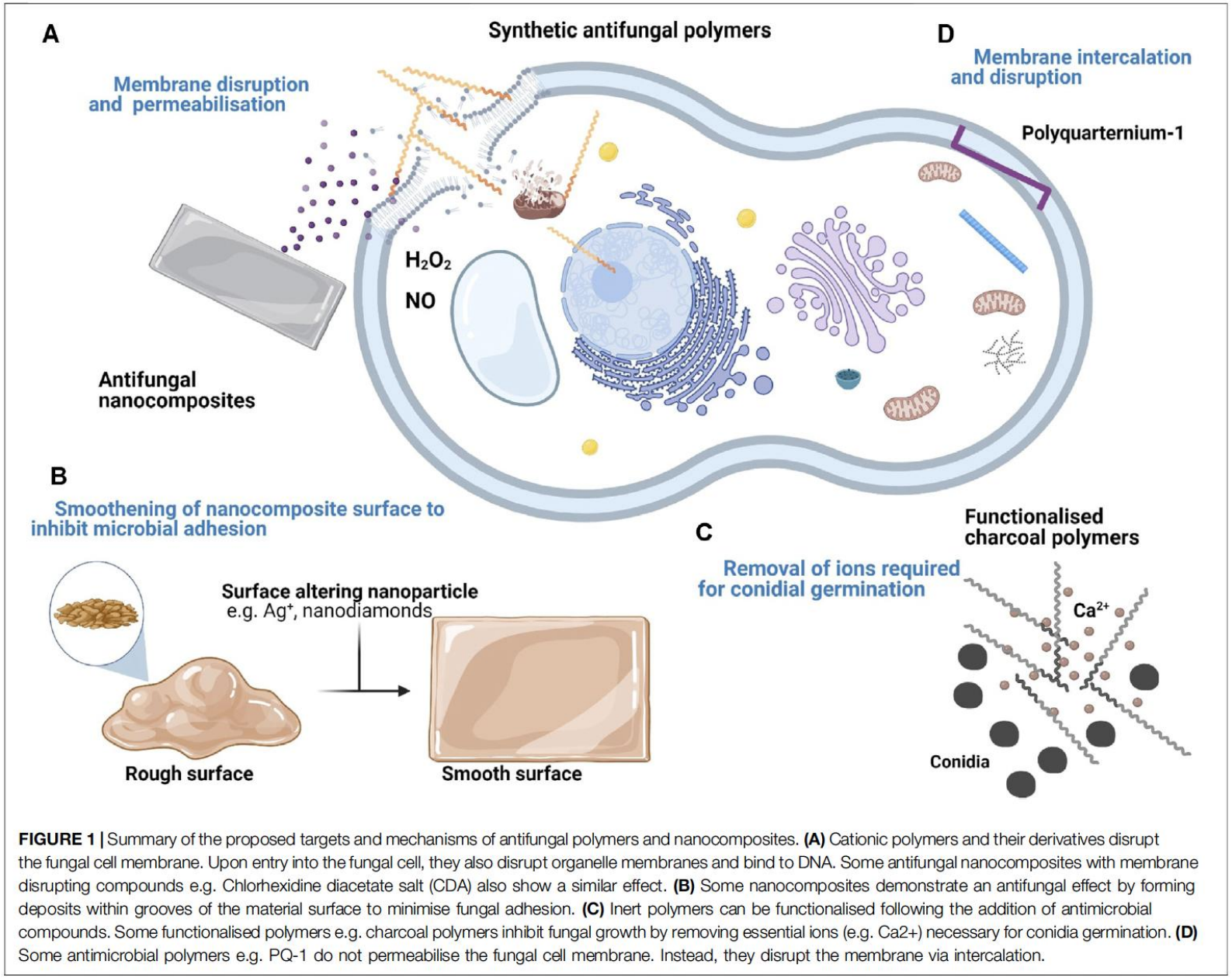
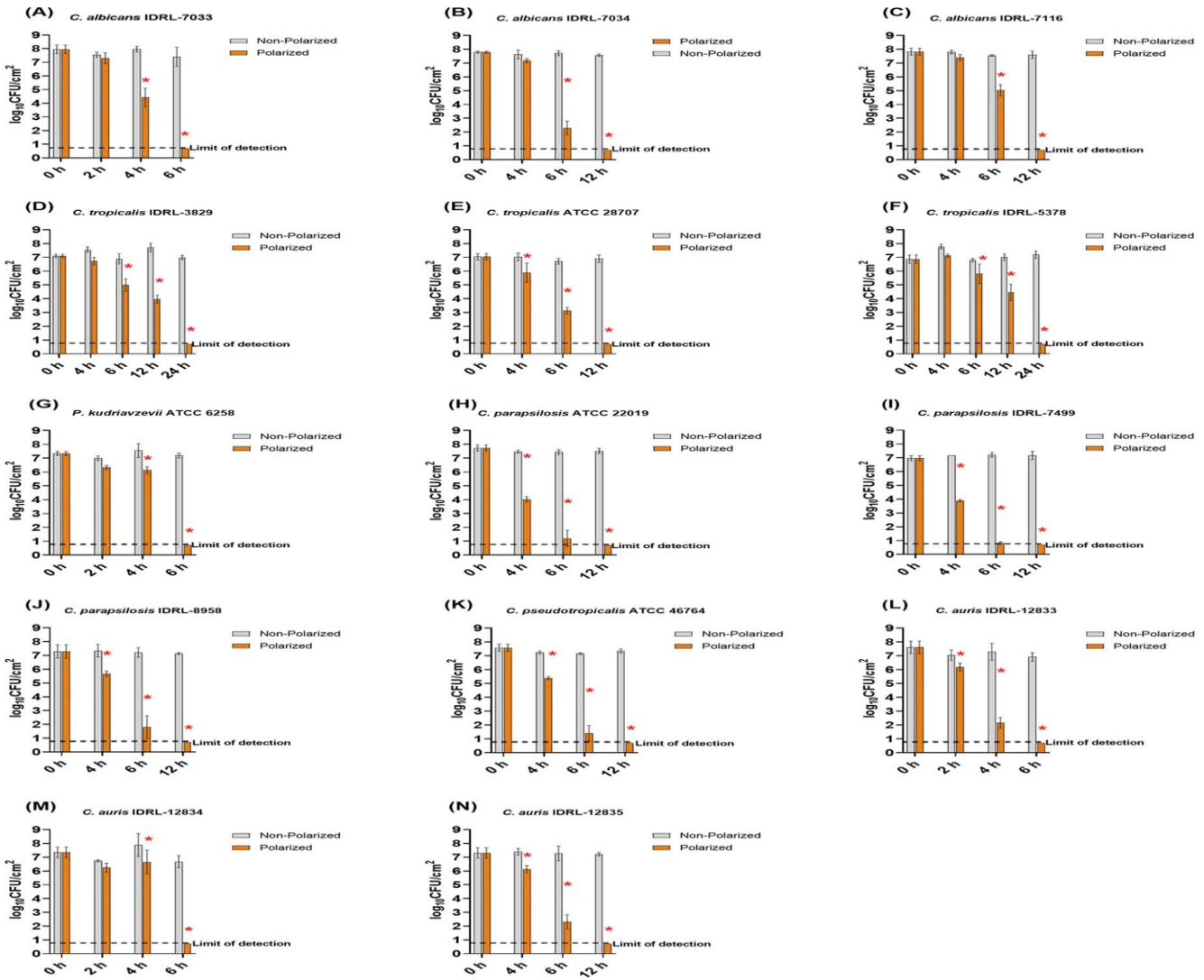



**FIGURE 1 |** Summary of the proposed targets and mechanisms of antifungal polymers and nanocomposites. **(A)** Cationic polymers and their derivatives disrupt the fungal cell membrane. Upon entry into the fungal cell, they also disrupt organelle membranes and bind to DNA. Some antifungal nanocomposites with membrane disrupting compounds e.g. Chlorhexidine diacetate salt (CDA) also show a similar effect. **(B)** Some nanocomposites demonstrate an antifungal effect by forming deposits within grooves of the material surface to minimise fungal adhesion. **(C)** Inert polymers can be functionalised following the addition of antimicrobial compounds. Some functionalised polymers e.g. charcoal polymers inhibit fungal growth by removing essential ions (e.g. Ca2+) necessary for conidia germination. **(D)** Some antimicrobial polymers e.g. PQ-1 do not permeabilise the fungal cell membrane. Instead, they disrupt the membrane via intercalation.

Ntow-Boahene W, et al. *Front Bioeng Biotechnol*. 2021;9:780328.

# Example 4: Hypochlorous Agents

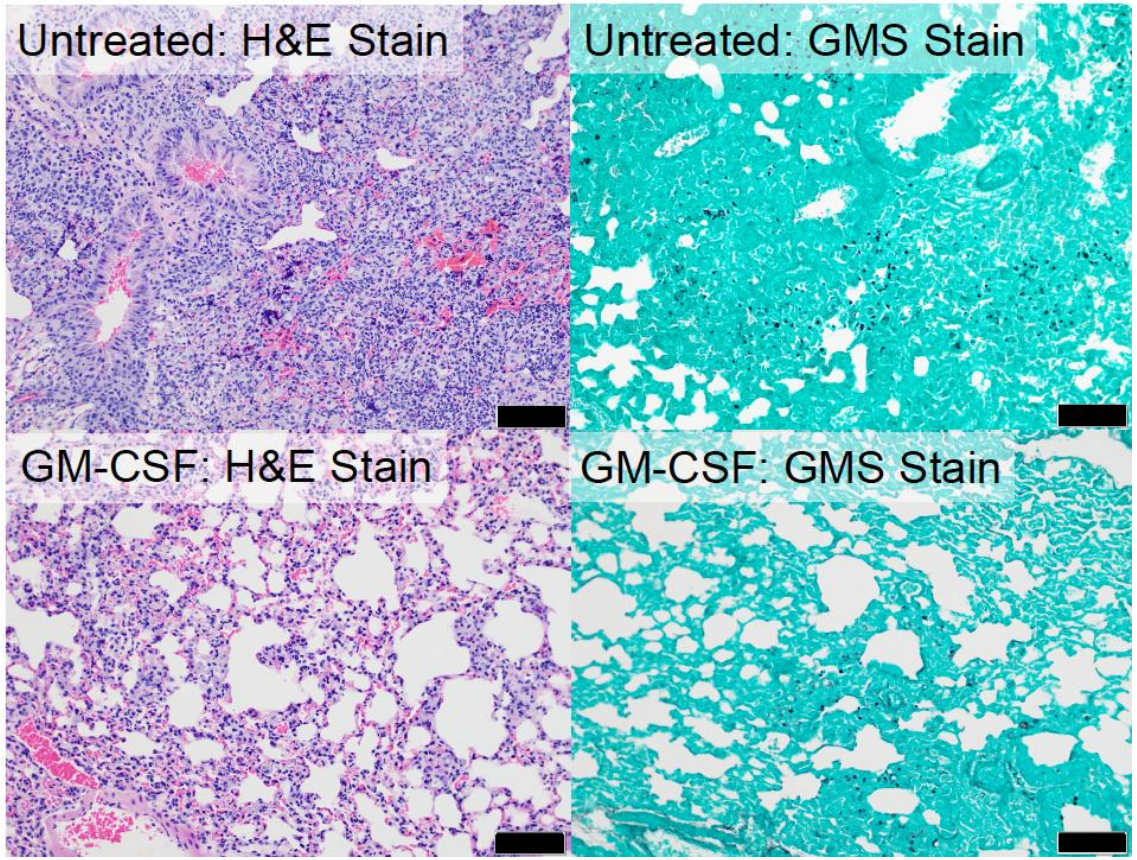
- Stabilized hypochlorous acid (HOCl)
- Active agent with broad anti-fungal activity
- Neutrophils, our first responders, generate similar molecules for the elimination of fungal species




Kletzer J, et al. *Antimicrob Agents Chemother*. 2023;67(1):e0116622.

# Example 5: Other Future Agents and Concepts

- Can we augment the immune system?
- Controversial, but supporting evidence is building
- Certain cytokines (FDA approved) can increase immune activity resulting in clearance of fungi (and likely bacteria)




Mansour MK, augmentation of neutrophils against fungal pathogens, unpublished data

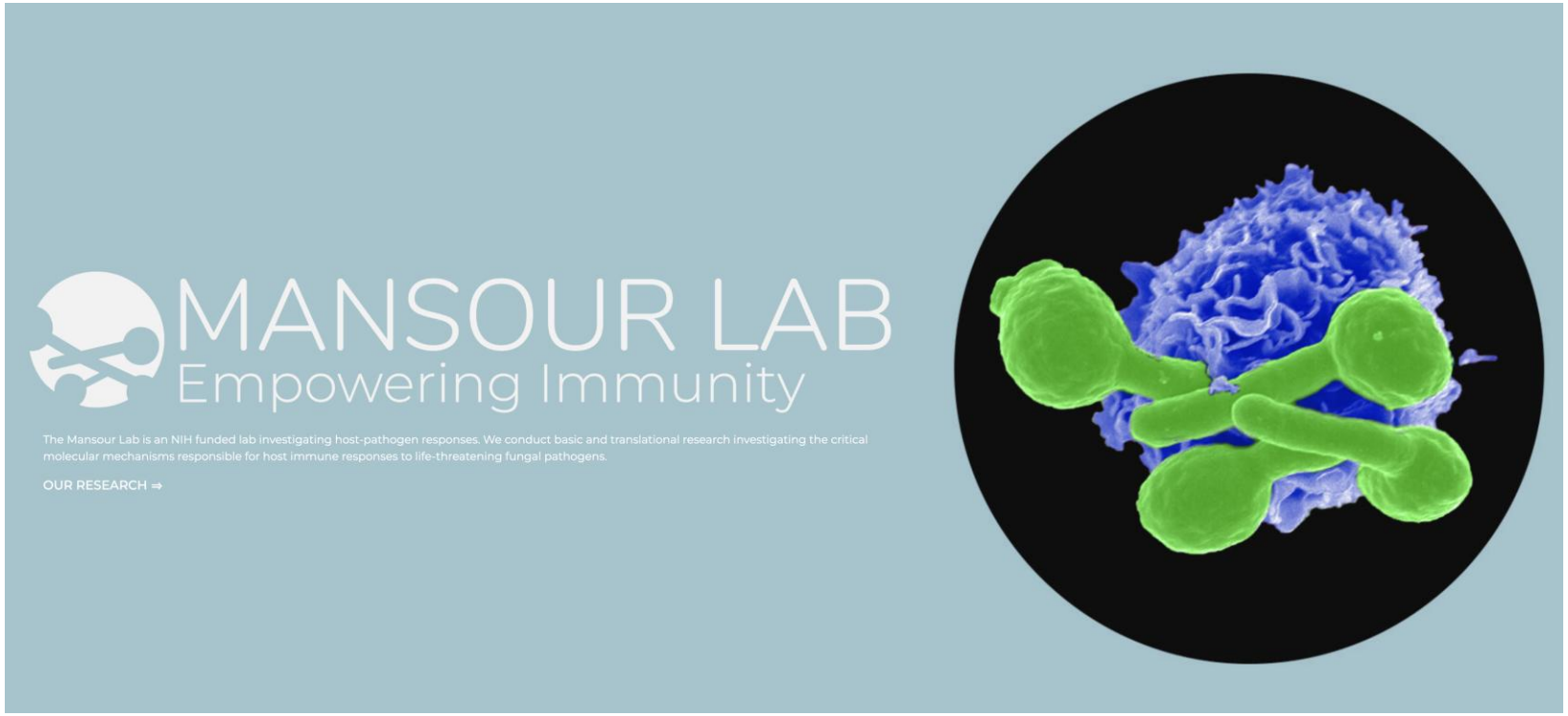
# *Candida auris* — Conclusions

- *C. auris* has proven to be a challenge to diagnose
- Highly resistant, often towards 2 or more classes of antifungals
- *C. auris* has emerged in a synchronous fashion
- Very rare evidence of *C. auris* prior to 2009 suggesting selective pressure has resulted in global emergence
- Persists in the environment and on patients

# Thank you, and visit the Mansour Lab!

## Biology, Diagnostics, and Therapeutics

www.mansourlab.com





## From Bioburden to Resistance:

# Antimicrobial Stewardship in Wound Care


Paul Nee, MD, FIDSA, CWSP

Regional Medical Director for Wound Care and Hyperbaric Medicine

Director of Antimicrobial Stewardship at Danbury/New Milford Hospital

Northwell Health

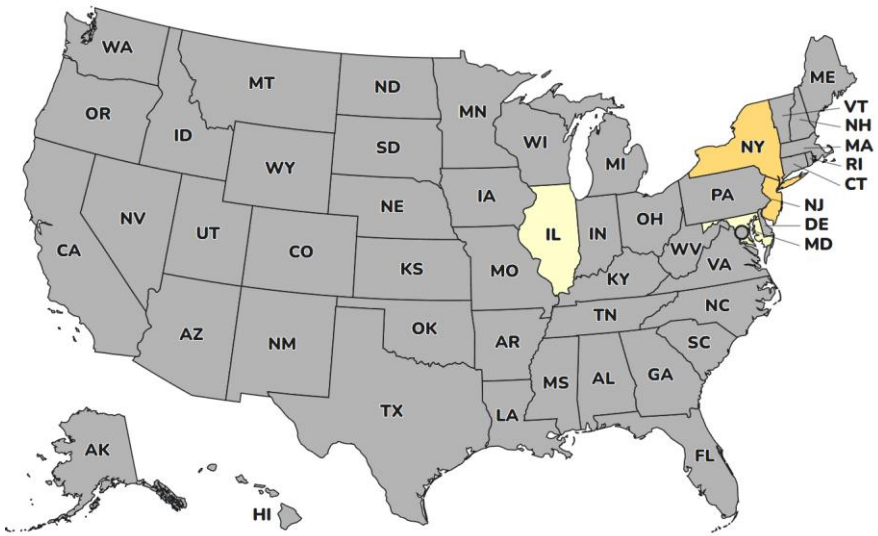
# Semi-urgent text from nurse manager:

The Infection Prevention (IP) nurse is on her way to the center to review a case of multidrug-resistant (MDR) *Pseudomonas* isolated from a patient. Patient has been seen weekly for the previous 4 weeks. The state has called, done genetic testing, and wants a "report" on the patient. No recent antimicrobial, but was admitted to another hospital system a few weeks previously.

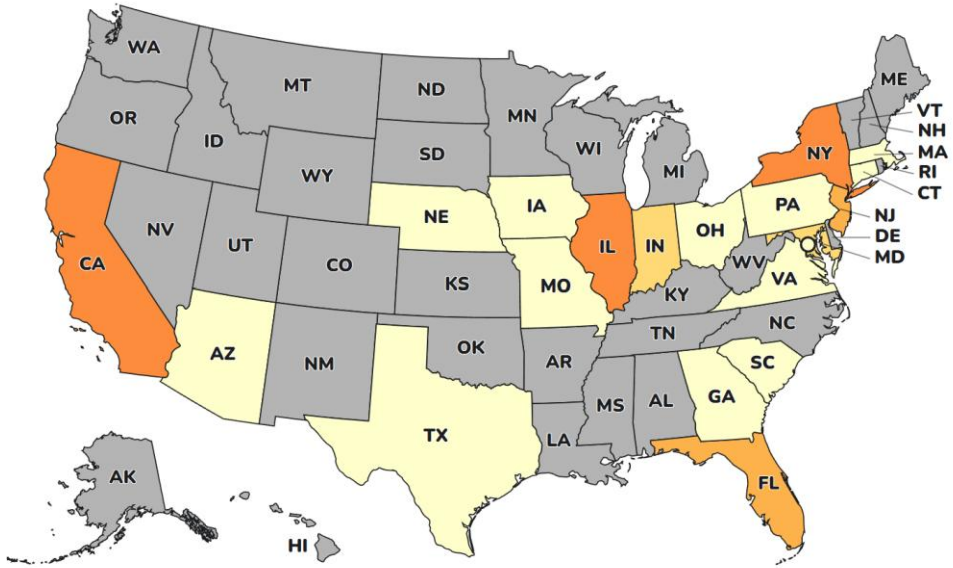
3 wks later: IP RN returning to the center. *C. auris* is isolated from a bone biopsy of a patient with chronic refractory osteomyelitis.

"We could have big problems if we do not get ahead of this."

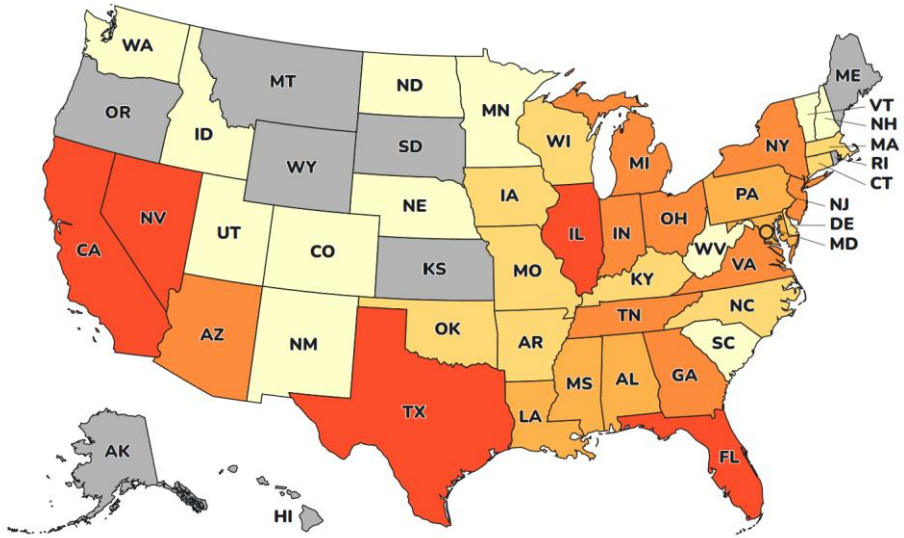
IP = infection prevention; MDR = multi-drug resistant.




2016




2020




2024

**C. *auris* over 8 yrs**

U.S. Centers for Disease Control and Prevention (CDC). Published March 2026. Accessed July 2026. https://www.cdc.gov/candida-auris/tracking-c-auris/index.html?2024=2024

# Antimicrobial Resistance: A Global Crisis

- 4.95 million antimicrobial resistance (AMR)-associated deaths globally
- AMR among top public health threats
- Projected increase through 2050 to 8 million

- AMR is not a future problem — it's a now problem
  - 3 million resistant infections in U.S. – 48,000 deaths
  - Increasing in Gram-negative rods (GNR) resistance
  - Rising *C. auris*

AMR = antimicrobial resistance; GNR = Gram-negative rods.

Naghavi M, et al. *The Lancet*. 2024;404(10459):1199-1226. U.S. Centers for Disease Control and Prevention (CDC). Published Feb 2025. Accessed July 2026. https://www.cdc.gov/antimicrobial-resistance/data-research/facts-stats/index.html?utm_source=chatgpt.com

# Approximately what percentage of bacterial isolates are multidrug-resistant DFUs?

POLL

A. 10%

B. 25%

C. 50%

D. 75%

# Approximately what percentage of bacterial isolates are multidrug-resistant DFUs?

POLL

A. 10%
B. 25%
C. **50%**
D. 75%

## Yang, et al meta-analysis:

21 studies from 2010-2023

2633 of 4885 isolates were MDR (50.8%)

- 12% MDR Staph
- 38% MDR GNR

## Henig, et al

2012-2015: 364/648 (56%) had >= 1 MDRO

- 46.2% of patients with MDRO infection had received antibiotics during the prior 3 months
- MDRO predicted DFI recurrence

# Antimicrobial Resistance Strategies

## Antimicrobial Stewardship

Optimization of antimicrobials

Right duration/dose

Treating infection, not colonization

## Infection Prevention

Hand hygiene

Isolation

Environmental cleaning

## Monitor/ Surveillance

Resistance patterns

Antibiograms

Regional changes

**For Wound Care:**

**Debridement**

**Topical antiseptics**

# Clinical Signs and Symptoms

"The inability of standard-of-care assessment to detect wounds with significant bacterial burden undoubtedly contributed to inconsistent and haphazard antimicrobial prescribing practices for chronic wounds."

Serena TE, et al. *Adv Wound Care (New Rochelle).* 2022;11(12):639-649.

# Antibiotic Prescriptions

- Patient expectations
- Diagnostic uncertainty
- Fear of missing an infection
- “Better safe than sorry”
- Antibiotic prescription provides an immediate reduction in clinician anxiety

# Which statement BEST reflects the relationship between antibiotic duration and clinical outcomes?

POLL

A. Longer antibiotic courses generally reduce treatment failure but increase adverse events

B. Antibiotic-associated adverse events are primarily determined by drug choice rather than duration

C. Extending antibiotic duration beyond what is clinically necessary may increase adverse events without improving infection-related outcomes

D. Once an antibiotic is appropriately indicated, duration has little impact on antimicrobial stewardship

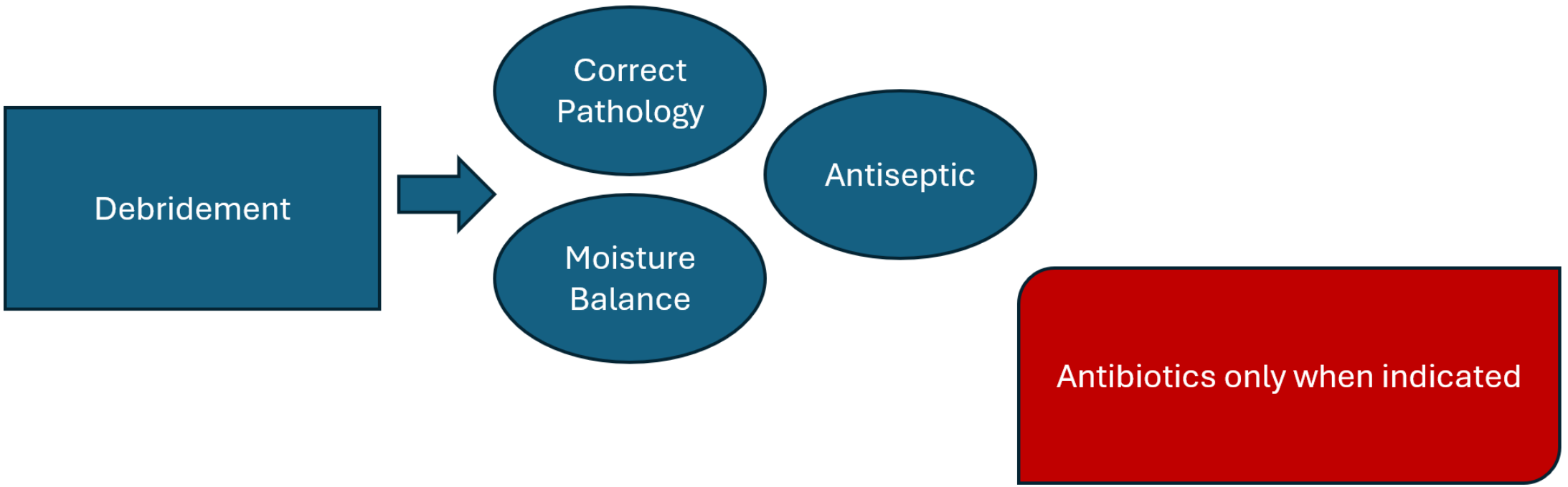
- **Risks: Not just antimicrobial resistance**
- **Just-in-case scenario: Use of prophylactic antibiotics does not improve healing rates**
  - Chantelau E, Tanudjaja T, Altenhöfer F, et al. Antibiotic treatment for uncomplicated neuropathic forefoot ulcers in diabetes: a controlled trial. *Diabet Med*. 1996;13(2):156-159.
  - O'Meara S, Al-Kurdi D, Ologun Y, Ovington. Antibiotics and antiseptics for venous leg ulcers. *Cochrane Database of Systematic Reviews* 2014, Issue 1.
- **6 vs 12 wks abx for OM — no difference in outcome**
  - Adverse effects from abx 15% vs 45%
- **Surgical site infection prophylaxis**
  - ≥72 hrs: ≈80% greater odds of AKI
  - ≈3.6-fold odds of *C. difficile*
  - No additional reduction in SSI

POLL

# Following sharp debridement of a chronic wound with substantial slough and suspected biofilm, which strategy BEST reflects antimicrobial stewardship principles?

A. Initiate systemic antibiotics because biofilm is presumed to remain after debridement
B. Apply an antimicrobial dressing routinely until complete wound closure to prevent biofilm recurrence
C. Continue appropriate physical removal of slough/debris between debridements, and use topical antimicrobial therapy only when the clinical assessment supports a need for microbial control
D. Avoid further debridement because disruption of biofilm may disseminate bacteria into surrounding tissue

**Debridement is the primary treatment for wound biofilm. Antiseptics are adjunctive therapies that help delay biofilm re-establishment. Antibiotics alone do not reliably eradicate mature biofilm.**



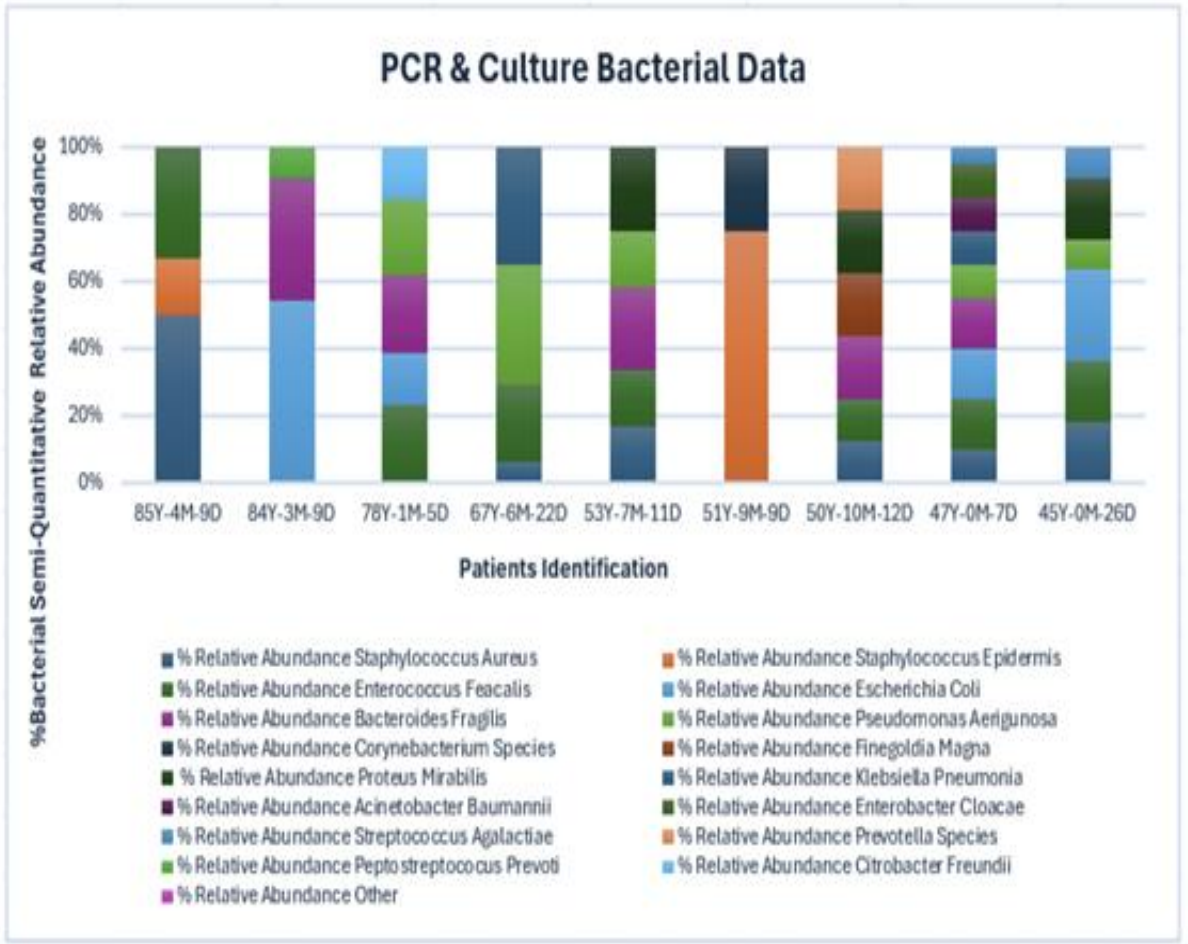

Schultz G, et al. *Wound Repair Regen*. 2017;25(5):744-757.

# Slough Removal

| Mechanism | Examples | What It Does | Stewardship Relevance |
|---|---|---|---|
| **Sharp/surgical** | Curette, scalpel, scissors | Immediate physical removal of slough/devitalized tissue; disrupts biofilm | Source control |
| **Mechanical** | Monofilament pads, gauze/mechanical irrigation, hydrosurgical/ultrasonic approaches | Physically separates/removes adherent debris and slough | Removal without antimicrobial exposure |
| **Autolytic** | Hydrogels, moisture-retentive dressings | Uses endogenous enzymes/phagocytes to liquefy devitalized tissue | Selective but slower |
| **Enzymatic** | Collagenase ointment | Proteolytic cleavage of devitalized collagen | Selective ongoing debridement |
| **Polyabsorbent fibers** | Negatively-charged fibers | Sequester/retain slough and debris in dressing | **Ongoing physical removal between debridements** |
| **Biologic** | Sterile larvae | Proteolytic enzymes + ingestion of liquefied necrotic tissue/microorganisms | Debridement without systemic antibiotics |

# Slough Is Also an Infection-Control Problem

**Figure 1: Culture and PCR Results in Chronic Wounds Used Dressings per Patient**




## Observational Study to Assess Bacterial Load in Used Wound Dressings Following Use of Highly Charged Fiber Dressings* to Remove Slough

Yadwinder Dhillon, MD; Kelsey Resler, NP; Noreen Rana; Kelly Perez; Jessica Aragon; Cameron Fridell; Jennifer Onorato; Natalie Wilkinson, NP; Ishnoor Sandhu; Sara Ashraf; Debashish Chakravarthy, Ph.D.; Rose Yaghi, DHA. M.S.

### INTRODUCTION

Chronic wounds are commonly colonized by diverse bacterial populations that complicate healing and increase the risk of infection. Real-world data on Highly Charged Fiber (HCF) dressings*, microbial capture, and antimicrobial barrier properties remain limited. The high-density negative charges at wound pH in these dressings are known to be negative, and some germs are also negatively charged under those conditions.

This exploratory study aimed to demonstrate that the dressings would not "pull out" germs from the wound but instead repel them, tending to leave them behind. We explored, through a clinical study coupled with germ-detection technologies, whether the dressing removes germs when applied to a wound, or whether the opposite is true.

### METHODS

HCF dressing was consistently applied to patients for two weeks per the instructions for use, with periodic changes as required. At week 2, dressings were collected and submitted to two independent laboratories for complementary analyses. One laboratory performed culture-based testing to identify *viable bacteria*, while another conducted PCR-based testing to detect both *viable and non-viable bacteria*.

This dual approach allowed differentiation between organisms that persisted (as recoverable microbes in cultures) and those that were effectively reduced or eradicated following real-life contact with the silver-based dressing (PCR can detect both live and dead germs).

The data presented are semi-quantitative, with PCR results for species reported as categorical values for gene copy number detected /mL, with high: $>10^8$, moderate:> $10^{\wedge 6}$-$10^{\wedge 8}$, and low: $10^{\wedge 4}$-$10^{\wedge 6}$. However, to be able to do between patients descriptive analysis and comparison the categorical viable culture results and the PCR results were converted based on a semi-quantitative ordinal score with (3) was used; for the bacterial species showing high abundance, code (2) was used; for the bacterial species showing moderate abundance, code (1) was used; for the bacterial species showing low abundance, and code (0.5) for the detected bacterial species. Then, from the ordinal score obtained, the % semi-quantitative relative abundance of each species was calculated: the ratio of the code presence of the specific bacterial species to the patient's total bacterial presence was computed and plotted in a stacked colored bar graph. These results showed a semi-quantitative comparison of targeting between patients for species abundance and detection, rather than quantitative data on numerical abundance for each species.

### RESULTS

This study demonstrated that HCF dressings collected during slough removal contained a wide spectrum of clinically relevant organisms, as detected by both culture and PCR. Among the most frequently detected species (and their levels) seen post culture/PCR were *Enterococcus faecalis* (High), *Proteus mirabilis* (High), *Staphylococcus aureus* (High), *Finegoldia magna* (*Peptostreptococcus magnus*) (High), *Prevotella* species (High), *Corynebacterium* species (High), *Peptostreptococcus anaerobius* (High), *Peptostreptococcus prevotii* (Detected/Low), and *Streptococcus agalactiae* (Group B) (Low). The detection of both facultative anaerobes and resistant organisms, including *Staphylococcus aureus*, highlights the dressing's germ-capturing impact across a diverse microbial community.

### RESULTS

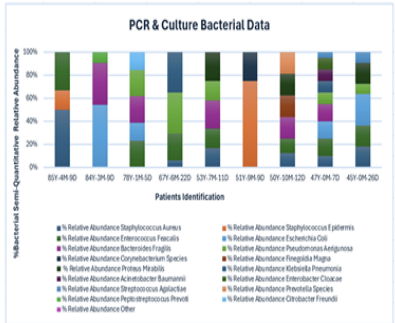
Table 1 summarizes the % semi-quantitative bacterial species presence in the (n=9) patients based on their Gram staining and metabolism. Gram-negative bacteria were the most captured species, accounting for 60.4% in total, with 42.7% facultative anaerobes and 17.7% anaerobes. Gram-positive bacteria accounted for 39.5%, with 35.9% facultative anaerobes, and 3.6% anaerobes.

**Table 1: Relative % Abundance of Culture and PCR Bacterial Species by Categories**

| Species Categories | Bacteria | % Semi-Quantitative Relative Abundance in (n=9 Patients) |
|---|---|---|
| Gram⁺ / Aerobic & Facultative Anaerobes | Staphylococcus epidermis, Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium species, and Enterococcus faecalis | 35.9% |
| Gram⁺ / Anaerobic | Peptostreptococcus prevotii and Finegoldia magna | 3.6% |
| Gram⁻ / Aerobic & Facultative Anaerobes | Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus mirabilis, and Citrobacter Freundii | 42.7% |
| Gram⁻ / Anaerobic | Prevotella species and Bacteroides fragilis | 17.7% |

Figure 1 summarizes the culture analysis results (viable bacteria) and the PCR results for (viable and non-viable bacteria). From the stacked bar colors, it can be summarized that each patient's wounds contained multiple bacterial species, with varying % semi-quantitative relative abundances both within and between patients.

**Figure 1: Culture and PCR Results in Chronic Wounds Used Dressings per Patient**




### DISCUSSION

These semi-quantitative results, based on PCR analysis of viable and non-viable bacteria and culture analysis of viable bacteria, support the hypothesis that HCF dressing effectively reduces bacterial load during routine clinical use, removing a substantial proportion of wound-associated microorganisms. These data on bacterial presence and reduction, which facilitate slough removal, show that HCF dressings simultaneously reduce bacterial load. Table 1 and Figure 1 show that HCF dressings captured various bacterial species, including Gram-negative and Gram-positive, as well as facultative anaerobic and anaerobic species. These data revealed the broad-spectrum antibacterial activity of the HCF dressing against various bacterial species found in chronic wounds.

It is known that bacterial surfaces may carry a negative charge due to the presence of acidic components such as teichoic acids in Gram-positive species and lipopolysaccharides in Gram-negative species, which might suggest electrostatic repulsion from the negatively charged dressing fibers, which would be a problem, as germs will tend not to be "picked up" by the dressing and instead accumulate in the wound. However, this highly negatively charged fiber matrix appears to adhere strongly to slough, which is generally positively charged, as shown by visual observations and previously published studies (RCT) [1-5].

Therefore, as slough serves as the primary habitat for bacterial colonization, when slough is removed, germs, deeply embedded and on the surface of slough, are also removed, in either viable or nonviable form. It also appears that, as is well known, PCR is far more accurate at identifying a broader range of species from the wound than culturing alone.

### LIMITATION

Because culture and PCR results target different biological signals (viable bacteria versus DNA from viable and non-viable bacteria) and are categorical, we converted them into an ordinal score dataset for descriptive purposes. The results of this study are semi-quantitative and do not provide absolute quantitative measures of the total or of each bacterial species present.

### CONCLUSION

By capturing both viable and non-viable bacteria, the HCF dressing provides not only desloughing and cleansing benefits but also demonstrates potential utility as a non-invasive medium for microbial removal and surveillance in chronic wounds. The germ-removal analysis shows that capturing and removing slough-associated microorganisms may extend the therapeutic window by lowering bioburden when the dressing is used on highly contaminated wounds.

### REFERENCES

1. Meaume, S, Dissemond, J., Addab, A. Evaluation of two fibrous wound dressings for the management of leg ulcers: results of a European randomised controlled trial (EARTH RCT). J Wound Care 2014; 23: 3, 105-116.
2. Lazarenko, et al. The role of a silver-releasing lipido-colloid contact layer in overuse leg ulcers presenting inflammatory signs suggesting heavy bacterial colonization: Results of a randomized controlled study. Wounds. 2008;20(6):158-66.
3. Dalal, S., Sigal, I., Addab, A. et al Clinical evaluation of a dressing with poly absorbent fibres and a silver matrix for managing chronic wounds at risk of infection: a non-comparative trial. J Wound Care. Vol 25, No 9, September 2016
4. Dissemond J, Dietlein M, Kelleter I, Bobbert T et al. Use of a TLC-Ag dressing on 2270 patients with wounds at risk or with signs of local infection: an observational study. Journal of Wound Care. 2020 Mar;29(3):162-173.

# Slough Removal — Negatively-Charged Fibers

- Absorbs exudate
- Interacts with/sequesters proteinaceous debris
- Traps slough/debris

**Basic science:**
**Polyacrylate fibers**

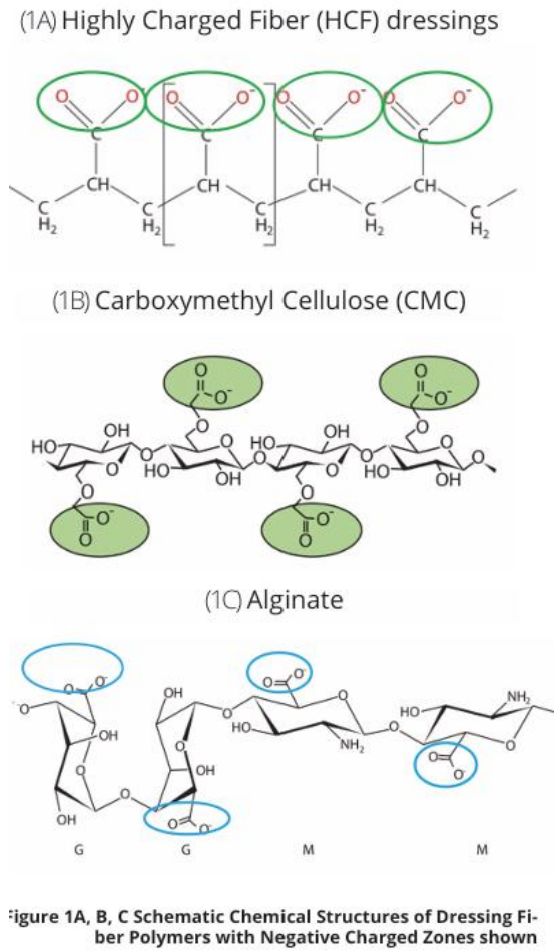



Figure 1A, B, C Schematic Chemical Structures of Dressing Fiber Polymers with Negative Charged Zones shown

**Physical wound cleansing — not antimicrobial killing.**

# What Are We Actually Removing? Evidence from Used HNCFD-Ag Dressings

| BIOLOGICAL CATEGORY | REPRESENTATIVE PROTEINS | BIOLOGICAL RELEVANCE |
|---|---|---|
| NEUTROPHIL/INFLAMMATORY | MPO, ELANE, PRTN3, DEFA3, AZU1, LTF | Neutrophil activation, inflammation, host defense |
| CHRONIC INFLAMMATORY | S100A8, S100A9, S100A12, LCN2 | Persistent inflammation and tissue injury |
| PROTEOLYTIC | MMP1, MMP8, MMP9, ELANE, PRTN3, CTSB, CTSD | Extracellular matrix degradation, tissue injury, chronic wound persistence |
| FIBRIN/SLOUGH-ASSOCIATED | FGA, FGB, FGG | Fibrin/fibrinogen-rich exudate and wound-bed material |
| ECM/TISSUE | FN1, COL1A1, COL1A2, COL3A1, COL4A1, COL6A1 | Structural tissue and matrix remodeling |
| COMPLEMENT | C3, C4A, C4B, CFB, CFH | Innate immunity and inflammatory activity |
| EXUDATE/PLASMA | ALB, TF, HP, HPX, SERPINA1, ORM1 | Protein-rich wound fluid and plasma-derived material |
| IMMUNOGLOBULINS | IGHG1, IGHG2, IGHG3, IGHG4, IGHA1, IGKC | Immune-derived exudate |
| MICROBIAL | Bacterial proteins from S. aureus, P. aeruginosa, S.epidermis, C.albicans, S. pyogenes, C. tetani | Microbial-derived material/biomass |

## Proteomic Characterization of Wound Slough Captured in Used Highly Negatively Charged Fiber Dressings-Silver (Ag)* Over Two Weeks

*Rose Yaghi, DHA, M.S; Debashish Chakravarthy, PhD; Yadwinder Dhillon, MD; Noreen Rana; Michael Espinoza*

### BACKGROUND

The chronic wound microenvironment is characterized by high levels of inflammatory mediators, pro-inflammatory macrophages, elevated reactive oxygen species, matrix metalloproteinases, and microbial colonies, which sustain the inflammatory phase. Persistent inflammatory proteins and microbial colonies delay chronic wound healing (Rodrigues et al., 2019; Razieyva et al., 2021).

Highly negatively charged fiber dressings (HNCFD) are designed to sequester and attract wound exudate, slough, and biomolecules over time via a physical, adhesion-type mechanism, and have been proven to be a valuable matrix for desloughing (Meaume et al., 2014; Lazareth et al., 2008; Dalac et al., 2016; Dissemond et al., 2020). This study aimed to perform a proteomic analysis of slough captured by the HNCFD- Ag which may provide future insights into patients' wound-healing status based on host inflammatory and microbial proteins captured within the dressing.

### METHODS

This prospective, single-site, observational feasibility and molecular characterization study aimed to characterize the proteomic profile of wound slough collected from 10 chronic wound patients whose wounds were managed with HNCFD-Ag. Used dressings were collected after 2 weeks of standard clinical treatment and analyzed using LC-MS/MS (Liquid Chromatography-Mass Spectrometry) DIA (Data-Independent Acquisition).

We analyzed proteomic outputs and clinical descriptors throughout the study to explore the relationship between wound etiologies and proteomic dressing analysis. We also compared the results to previous studies where slough analysis was described (Townsend et al., 2024)

### RESULTS

- Proteomic analysis of the HNCFD-Ag used in n=10 patients with chronic wounds revealed diverse host and microbial proteins captured within the wound slough and sequestered by the dressings.
- The proteomic analysis presented 4,683 protein groups, 39,951 peptides, 2,252-3,102 proteins/dressing, and 1,118 proteins detected across all 10 dressings.
- The protein profile captured by HNCFD-Ag included multiple components of the chronic wound environment (Table1).
- Microbial-associated proteins were detected across all 10 dressings, with microbial protein variability between patients (Figure 1).

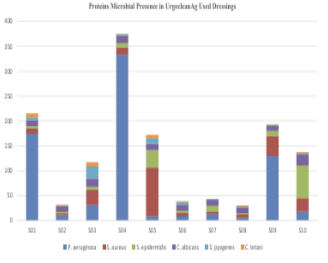
### RESULTS

Table 1: Major Biological Components Captured

| BIOLOGICAL CATEGORY | REPRESENTATIVE PROTEINS | BIOLOGICAL RELEVANCE |
|---|---|---|
| NEUTROPHIL/INFLAMMATORY | MPO, ELANE, PRTN3, DEFA3, AZU1, LTF | Neutrophil activation, inflammation, host defense |
| CHRONIC INFLAMMATORY | S100A8, S100A9, S100A12, LCN2 | Persistent inflammation and tissue injury |
| PROTEOLYTIC | MMP1, MMP8, MMP9, ELANE, PRTN3, CTSB, CTSD | Extracellular matrix degradation, tissue injury, chronic wound persistence |
| FIBRIN/SLOUGH-ASSOCIATED | FGA, FGB, FGG | Fibrin/fibrinogen-rich exudate and wound-bed material |
| ECM/TISSUE | FN1, COL1A1, COL1A2, COL3A1, COL4A1, COL6A1 | Structural tissue and matrix remodeling |
| COMPLEMENT | C3, C4A, C4B, CFB, CFH | Innate immunity and inflammatory activity |
| EXUDATE/PLASMA | ALB, TF, HP, HPX, SERPINA1, ORM1 | Protein-rich wound fluid and plasma-derived material |
| IMMUNOGLOBULINS | IGHG1, IGHG2, IGHG3, IGHG4, IGHA1, IGKC | Immune-derived exudate |
| MICROBIAL | Bacterial proteins from S. aureus, P. aeruginosa, S.epidermis, C.albicans, S. pyogenes, C. tetani | Microbial-derived material/biomass |

### RESULTS

Figure 1: Microbial-associated protein profile across S01-S10




### CONCLUSION

Proteomic analysis of the HNCFD-Ag used throughout this study was feasible as part of a reasonable experimental process, enabling characterization of host and microbial protein presence in the microenvironment of n=10 patients with chronic wounds. The results generally align with previous slough analysis studies by Townsend et al., 2024, which used slough collected directly from wounds. **The dressings captured a complex molecular signature of the chronic wound environment, including inflammatory, proteolytic, fibrin/slough-associated, extracellular matrix, immune, exudative, and microbial-associated proteins.** A shared core proteome across all 10 dressings, together with substantial patient-to-patient variation, suggests that the dressing captures both **common and wound-specific biological components.** These preliminary findings suggest that non-invasive HNCFD may help heal chronic wounds, given its ability to sequester a broad profile of proteins related to the inflammatory immune response and microbes. It also shows that, despite its negative charge, the dressing can capture negatively charged germs, likely because they are removed with the slough, which adheres strongly to the dressing. This slough analysis shows that the dressing has an innate ability to capture and remove slough-associated material and help extend the therapeutic window, an effect that has been observed in several clinical studies (Dissemond, et al., 2020; Dalac, et al., 2016; Meaume, et al., 2014; Meaume, et al., 2012).

### REFERENCES

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound Healing: A Cellular Perspective. Physiological Reviews, 99(1), 665–706. https://doi.org/10.1152/physrev.00067.2017

Razykova, A., Kim, Y., Zhuldybekova, Z., Kanysheva, S., Jiee, L., & Kapanov, A. (2021). Immunology of Acute and Chronic Wound Healing. Biomolecules, 11(5), 700. https://doi.org/10.3390/biom11050700

Meaume, S., Dissemond, J., Jaldaa, A., Senet-Hoh, M., Vissier, M., Gerrgy, T., Perrau, C., Chahin, N., Alvis, C., Peter, J., Tarsa, D. & Richter, S. (2014). Evaluation of new fibrous wound dressings for the management of leg ulcers: results of a European multicentre randomized trial. EWMA POSITS. Journal of wound care, 23(3), 107–114. https://doi.org/10.12968/jowc.2014.23.3.107

Ioannidis, I., Meaume, S., Puglia-Ginsberg, M.-L., Zamboulis, P., Capadoc, T.-L., Segard, A., Perrau, J.-L., Guerandel, A., & Richter, S. (2016). The first of a fixed following triple-enabled Crimean Layer in Venous Leg Ulcers Presenting Inflammatory Signs Suggesting Deep Bacterial Colonization: Results of a Randomized Controlled Study. Advances in compression of clinical research and practice, 2(2), 113–116.

Dalac, F., Vigo, J., Jaldaa, A., Chahin, N., Saint-Cyprien, C., Jandyal, S. & Richter, S. (2016). Clinical evaluation of a dressing with polyabsorbent fibers and active matrix for managing chronic wounds at risk of infection: a non-comparative trial. Journal of wound care, 25(5), 317–326. https://doi.org/10.12968/jowc.2016.25.5.317

Dissemond, J., Ehrsten, M., Kröhnert, S., Dulau, S., Schneemann, G., Becker, Z., Thomsen, J., Müller, S., Richter, S., & Meaume, S. (2020). Use of a TLC-Ag dressing on 2179 patients with wounds at risk or with signs of local infection: an observational study. Journal of Wound care, 29(3), 130–139. https://doi.org/10.12968/jowc.2020.29.3.130

Townsend, E. C., Ghoreishi, Z. A., Nadeima, M., Kim, B., Mahto, M., Ogamdah, T., Zhang, G., Gaurav, T., Schafer, C., Ghosn, A. J. E., & Kalan, L. R. (2024). What a tough! Defining the proteomic and microbial composition of slough and its implications for wound healing. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society, 32(5), 763–778. https://doi.org/10.1111/wrr.13197

Meaume, S., Vloemans, J., Moneib, H., Gerbaux, O., Dompmartin, A., Boukovac, I. M., Legrouyer, T., Tarsa, D., & Richter, S. (2014). Management of chronic venous leg ulcers treated with an innovative silver-based wound dressing. Journal of wound care, 23(7), 347–360. https://doi.org/10.12968/jowc.2014.23.7.347

**Used dressing analyses provide evidence that highly negatively-charged fiber/silver (HNCFD-Ag) physically captures complex wound-associated material, including host proteins and microbial-associated material, during routine wound management.**

# Slough Formation Is Continuous

- SHARP DEBRIDEMENT
  - Immediate removal
  - Biofilm disruption
  - Source control
- Days between visits…
  - POLYABSORBENT FIBERS
    - Absorb
    - Sequester
    - Retain
    - Remove at dressing change







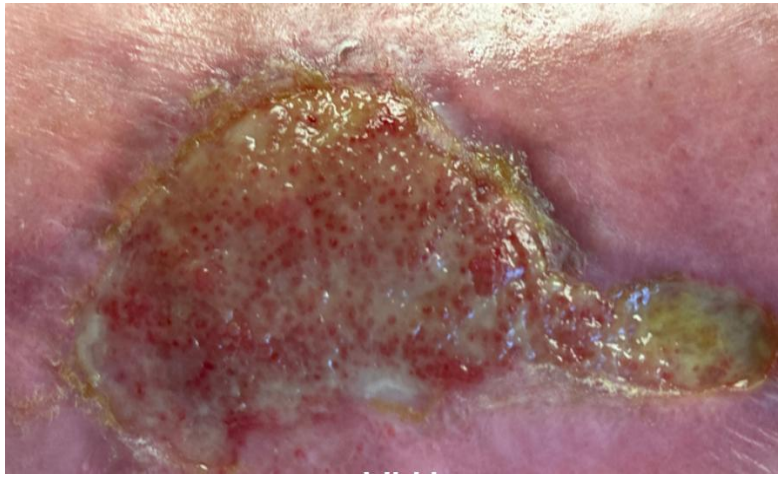


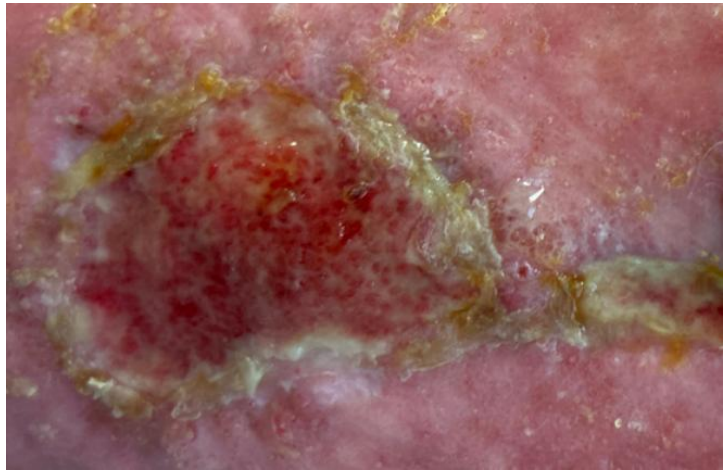
## Earth-RTC

| 6 wks | Polyabsorbent fiber | Hydrofiber |
|---|---|---|
| Slough reduction | **65.3%** | 42.6% |
| Wounds debrided | **52.5%** | 35.1% |
| Wound area reduction | 36.9% | 35.4% |

Meaume S, et al. *J Wound Care*. 2014 Mar;23(3):105-6,108-11, 114-6.

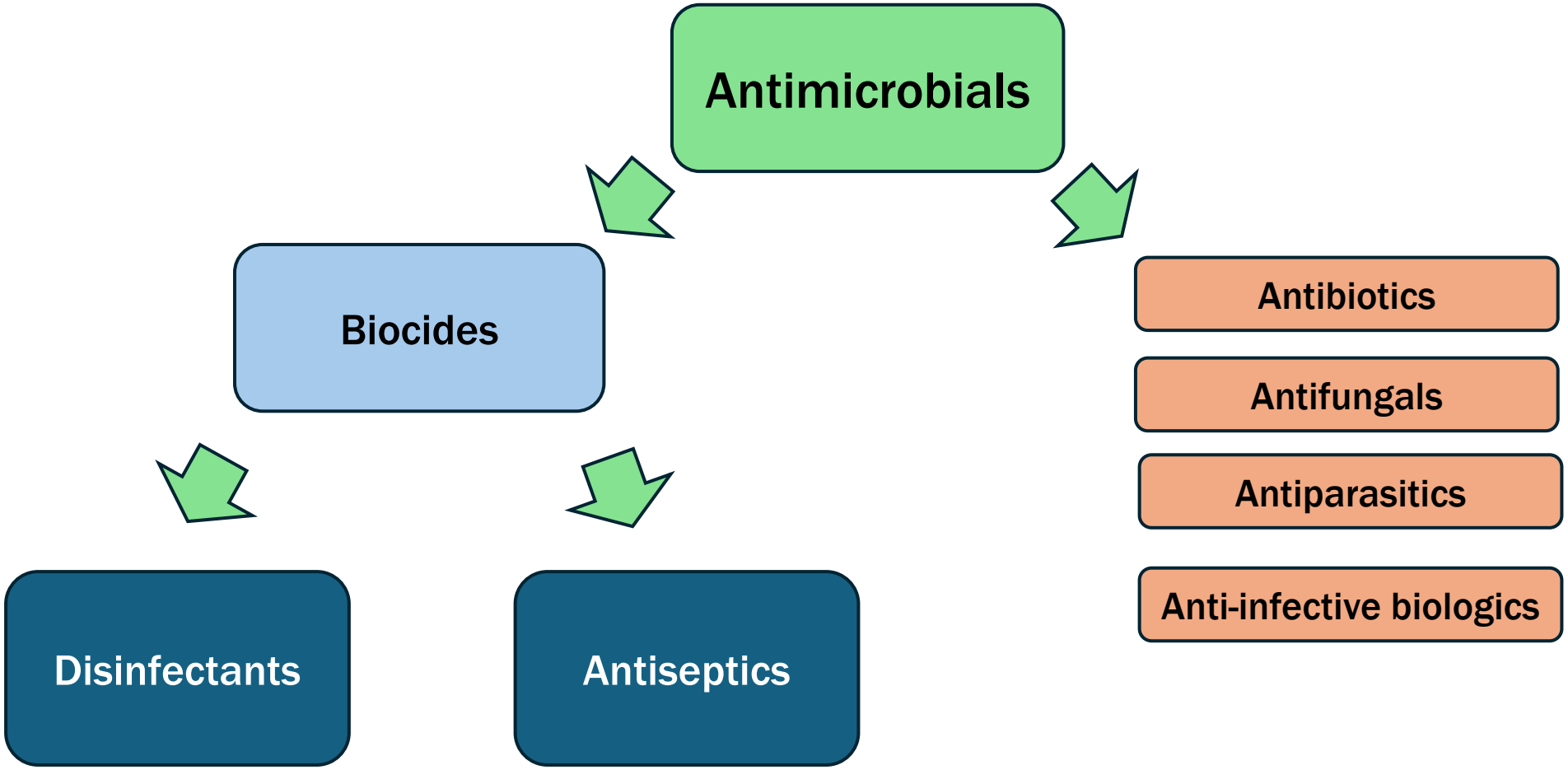








# Stewardship Is Not Just about Antibiotics




Swanson T, et al. *J Wound Care.* 2022;31(Sup12):S10-S21. Idensohn P, et al. *J Wound Care*. 2026;35(5A):S1-S40.

# The Stewardship Blind Spot

- Many clinicians assume
  - Topical = safe
  - Topical = no resistance
- Reality
  - Topical antibiotics create selection pressure

Efficacy data/evidence: limited in chronic wounds

***Acute Wounds Are Different***

“Judicious use of topical antiseptics should be considered as reasonable alternative to topical antibiotics.”



Swanson T, et al. *J Wound Care*. 2022;31(Sup12):S10-S21.

# Resistance to Topical Antibiotics

- Fusidic acid and MRSA in New Zealand
  - Williamson, et al: Increased use of topical fusidic acid increased 7.8%-37.4% in MRSA isolates over 11 yrs
- Topical gentamicin usage→ resistance *Pseudomonas*
  - Burns Units – Snelling, et al
- Mupirocin resistance → various studies from 3.2% to more than 31%

# Topical Antibiotics — Where to Use

| What about the biofilm and topical antibiotics | Unclear as to dose and level delivered in the wound. This MAY lead to resistance and/or have effect on biofilm formation | Judicious use |
| --- | --- | --- |
| • Gentamicin: sub-MIC exposure has increased biofilm formation or induced changes<br>• Mupirocin: sub-MIC level can induce biofilm formation<br>• Metronidazole-evidence against selected anaerobes | • Higher concentrations needed due to biofilm | • Localized MRSA<br>• Mupirocin-sensitive infection<br>• Malodor (metronidazole)<br>• Selected Gram-negative infections-carefully… |

# Mechanism of Action and Resistance of the Antiseptics

| | Action | Resistance | Tissue Toxicity | BBWC |
|---|---|---|---|---|
| Iodine | Free iodine disrupts membranes | Rare | PV-I: Mod<br>Cadexomer: Low/mod | YES |
| CHG/PHMB | Membrane disruption and proteins | Reduced susp CHG/Rare PHMB | CHG-Mod<br>PHMB-low | PHMB-YES |
| HOCl | Oxidative damage | Rare | Very low | YES |
| Sodium Hypochlorite (Dakin's) | Oxidative damage | Rare | Dose-dependent | YES |
| Silver | Multiple | Reported but rare | Reported but rare | Dressing |
| Acetic Acid | pH disruption | Rare | Dose-dependent | Yes PsA biofilm |

# Antibiotics vs Antiseptics

| | Antibiotics | Antiseptics |
|---|---|---|
| Definition | Agents that selectively inhibit or kill microorganisms through specific microbial targets | Broad-spectrum chemical agents that reduce or eliminate microorganisms through multiple non-specific mechanisms |
| Spectrum of Activity | Narrow or organism-specific | Typically broad |
| Resistance | Common and well documented | Uncommon/rare |
| Effect on Microbiome | May significantly alter host microbiome | Usually localized effect with less systemic impact |
| Stewardship Concerns | High | Moderate to low |
| Examples | Gentamicin, mupirocin, metronidazole | HOCl, PHMB, povidone-iodine, Dakin's solution |

# What about MDR Organisms?

| Pathogen | Antiseptics |
|---|---|
| MRSA | HOCl, PHMB, iodine, CHG active |
| ESBL | HOCl, PHMB, iodine active |
| CRE | HOCl, PHMB, iodine active |
| *C. auris* | CHG, iodine, PHMB, HOCl active in vitro |
| MDR *Pseudomonas* | Acetic acid, iodine, PHMB, HOCl active |

Harikrishna K R Nair, et al. *Wounds Int.* Published Oct. 2023. Accessed July 2026. https://woundsinternational.com/consensus-documents/use-of-wound-antiseptics-in-practice/

# Pure HOCl Solution + HOCl-Based Gel: Activity Against Mature *P. aeruginosa* Colonies

- **Pure HOCl solution → HOCl-based gel achieved >6-log$_{10}$ reduction** in a 48-hr *P. aeruginosa* colony on a wounded porcine explant model
- **Additive or synergistic effect** of sequential HOCl solution + gel

## Assessment of synergistic treatments using wound solutions and antimicrobial gels to evaluate the degree of inactivation of microbial colonies.

**Rebecca Catton[1], MSc, Fergus Watson[1], PhD, Rui Chen[1], PhD, Debashish Chakravarthy[2], PhD, Steven L Percival[1], PhD.**
1.Centre of Excellence in Biofilm Science and Technologies (CEBST), 5D Health Protection Group Ltd, Accelerator Building, 1 Daulby Street, Liverpool. L7 8XZ, UK
2. Urgo Medical North America, 100 Lexington Street, Suit 400, Fort Worth, TX 76102

### BACKGROUND

Microbial colonies in wounds create substantial therapeutic challenges by forming protective extracellular matrices that dramatically complicates wound healing. Some wound cleansing solutions with antimicrobial preservatives can provide microbial colony disruption on an immediate basis, while antimicrobial gels offer the potential of prolonged germ removal efficacy.

This study addresses how synergistic combination therapy with sequential applications of antimicrobial preserved wound solutions and antimicrobial gels can overcome potentially work alone or together, and enhance clinical outcomes.

### METHOD

A 48-hour age *P. aeruginosa* AATCC 15442 colony was established on a wounded porcine explant model, then submerged in *Hypochlorous acid solution or Saline for 10 minutes before being placed into a *Poloxamer gel, a *Hypochlorous acid gel or Saline for a further 4 hours. Also, samples were placed in a Hypochlorous acid gel for 10 minutes, then in Saline for 4 hours. Samples were then neutralized for 5 minutes, sonicated for 30 minutes, and enumerated to determine CFU/cm$^2$. A log$_{10}$ reduction value was calculated from an untreated 48-hour control.

### METHOD

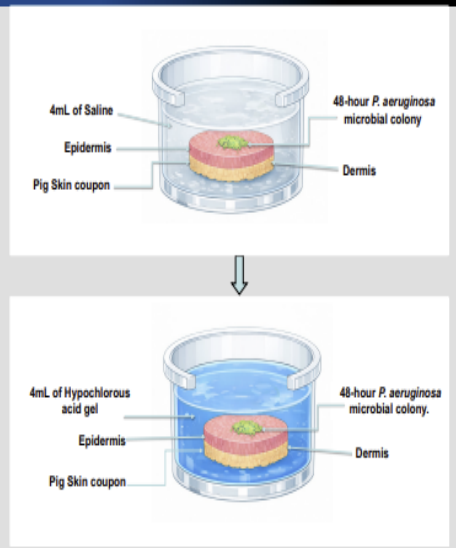



**Figure 1.** Schematic demonstrating the methodology set up of pig skin sample, with a 48-hour *P. aeruginosa* colony in place first placed into Saline for 10 minutes and then placed into hypochlorous acid gel for 4 hours.

### DISCUSSION

Improved activity was observed for Hypochlorous acid solution and Saline, which produced a 3.59 log$_{10}$ reduction. A combination of Saline followed by Hypochlorous acid gel achieved a >4 log$_{10}$ reduction. The most successful synergistic treatment was the use of Hypochlorous acid solution followed by Hypochlorous acid antimicrobial gel which demonstrated a >6 log$_{10}$ reduction. The poloxamer gel demonstrated lower germ colony inactivation, achieving <2 log$_{10}$ reduction.

### RESULTS

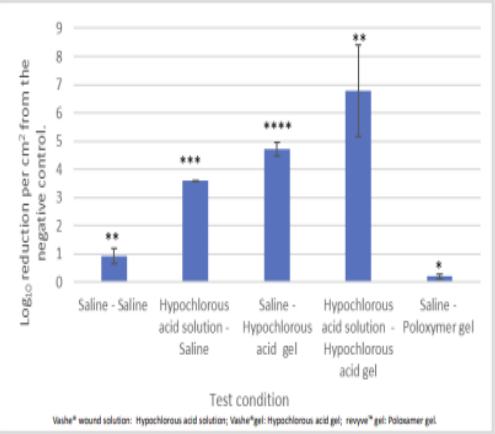



**Figure 2.** Log10 reduction (Log$_{10}$ CFU/cm$^2$) of 48-hour *P. aeruginosa* colonized porcine explant soaked in Saline or Hypochlorous acid for 10 minutes before being placed into Saline (control), Hypochlorous acid gel or poloxamer gel for 4 hours. Significance levels: *$p$<0.05, **$p$ < 0.01, ***$p$ < 0.001, ****$p$ < 0.0001. Student's t-test comparing to the untreated control.

### CONCLUSIONS

The results suggest there is a synergistic or additive effect between the Hypochlorous acid antimicrobial solution and Hypochlorous acid antimicrobial gel in the inactivation of mature colonies of germs within a wounded pig skin model.

# HOCl-Based Gel:
# Rapid Broad-Spectrum Antimicrobial Activity

| Organism | Time to Kill | % Reduction |
|---|---|---|
| *Enterococcus faecalis* | 30 seconds | ≥99.9999% |
| *Enterococcus faecium* | 30 seconds | ≥99.9999% |
| *Escherichia coli* | 30 seconds | ≥99.9999% |
| *Staphylococcus aureus* | 30 seconds | ≥99.9999% |
| *Staphylococcus aureus* | 30 seconds | 99.999% |
| *Acinetobacter baumannii* | 30 seconds | ≥99.9999% |
| *Aeromonas caviae* | 30 seconds | ≥99.999% |
| *Aeromonas hydrophila* | 30 seconds | ≥99.999% |
| *Candida albicans* | 30 seconds | ≥99.9999% |
| ***Candida auris*** | **30 seconds** | **≥99.9999%** |
| *Enterobacter aerogenes* | 30 seconds | ≥99.999% |
| *Enterococcus faecium* | 30 seconds | ≥99.999% |
| *Escherichia coli* | 30 seconds | ≥99.9999% |
| *Klebsiella oxytoca* | 30 seconds | ≥99.9999% |
| *Klebsiella pneumoniae* | 30 seconds | ≥99.9999% |

| Organism | Time to Kill | % Reduction |
|---|---|---|
| *Micrococcus luteus* | 30 seconds | ≥99.9999% |
| *Propionibacterium acnes* | 30 seconds | 99.9999% |
| *Proteus mirabilis* | 30 seconds | ≥99.9999% |
| *Pseudomonas aeruginosa* | 30 seconds | ≥99.999% |
| *Pseudomonas paraeruginosa* | 30 seconds | ≥99.999% |
| *Serratia marcescens* | 30 seconds | ≥99.9999% |
| *Staphylococcus aureus* | 30 seconds | ≥99.9999% |
| *Staphylococcus epidermidis* | 30 seconds | ≥99.999% |
| *Staphylococcus haemolyticus* | 30 seconds | ≥99.999% |
| *Staphylococcus hominis* | 30 seconds | 99.999% |
| *Staphylococcus saprophyticus* | 30 seconds | 99.999% |
| *Stenotrophomonas maltophilia* | 30 seconds | ≥99.999% |
| *Streptococcus pyogenes* | 30 seconds | ≥99.9999% |
| ***Clostridium difficile*** | **5 minutes** | **99.99%** |

# *C. auris* Case

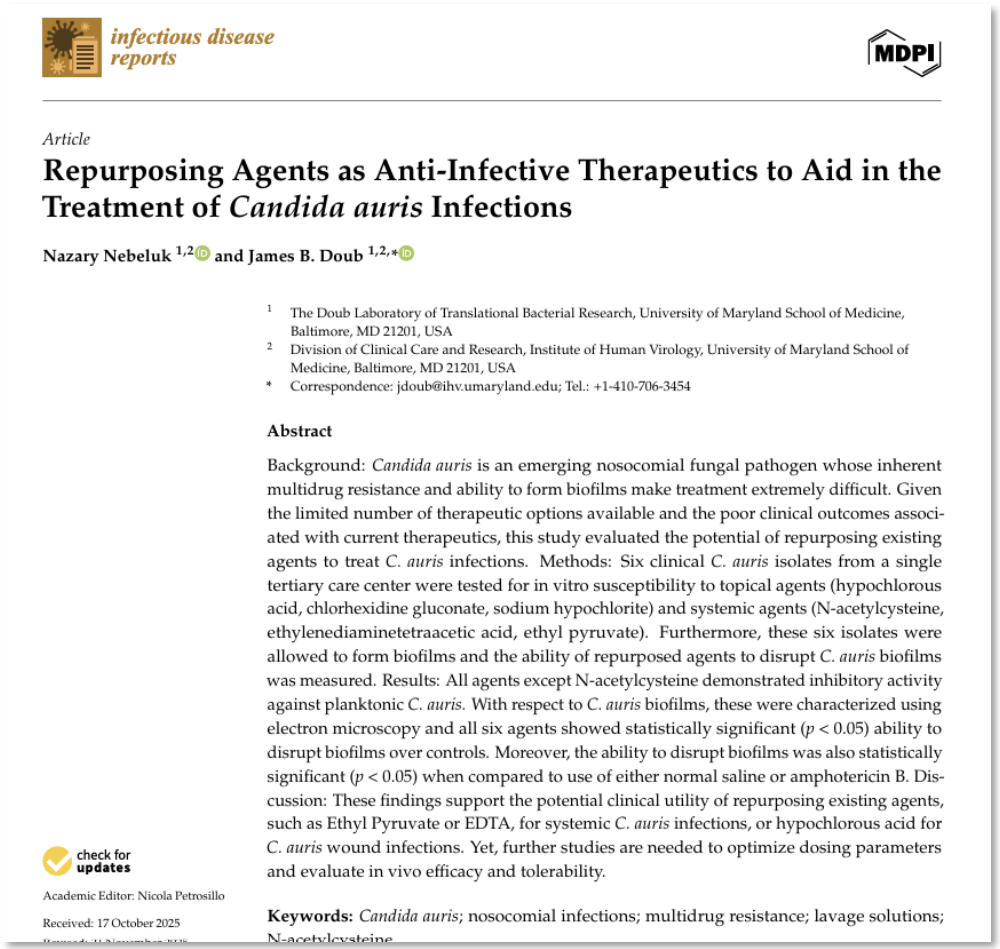
- Reviewed case with wound care staff, treating podiatrist, and Infection Prevention
- Patient seen as last patient of the day
- Contact precautions initiated
- Treatment discussion
- Infected bone was removed: Clean margins

PVP-I, chlorhexidine, NaOCl, HOCl acid *in vitro* activity

Nebeluk N, Doub JB. *Infect Dis Rep*. 2025;17(6):144. Kean R, et al. *Int J Antimicrob Agents*. 2018;52(5):673-677.

# Nebeluk & Doub Study: HOCl Evaluated Against Clinical *C. auris* Isolates

**Nebeluk & Doub, *Infectious Disease Reports*, 2025**



*infectious disease reports*

MDPI

*Article*

## Repurposing Agents as Anti-Infective Therapeutics to Aid in the Treatment of *Candida auris* Infections

Nazary Nebeluk [1,2] and James B. Doub [1,2,*]

[1] The Doub Laboratory of Translational Bacterial Research, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[2] Division of Clinical Care and Research, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD 21201, USA
* Correspondence: jdoub@ihv.umaryland.edu; Tel.: +1-410-706-3454

**Abstract**

Background: *Candida auris* is an emerging nosocomial fungal pathogen whose inherent multidrug resistance and ability to form biofilms make treatment extremely difficult. Given the limited number of therapeutic options available and the poor clinical outcomes associated with current therapeutics, this study evaluated the potential of repurposing existing agents to treat *C. auris* infections. Methods: Six clinical *C. auris* isolates from a single tertiary care center were tested for in vitro susceptibility to topical agents (hypochlorous acid, chlorhexidine gluconate, sodium hypochlorite) and systemic agents (N-acetylcysteine, ethylenediaminetetraacetic acid, ethyl pyruvate). Furthermore, these six isolates were allowed to form biofilms and the ability of repurposed agents to disrupt *C. auris* biofilms was measured. Results: All agents except N-acetylcysteine demonstrated inhibitory activity against planktonic *C. auris*. With respect to *C. auris* biofilms, these were characterized using electron microscopy and all six agents showed statistically significant ($p < 0.05$) ability to disrupt biofilms over controls. Moreover, the ability to disrupt biofilms was also statistically significant ($p < 0.05$) when compared to use of either normal saline or amphotericin B. Discussion: These findings support the potential clinical utility of repurposing existing agents, such as Ethyl Pyruvate or EDTA, for systemic *C. auris* infections, or hypochlorous acid for *C. auris* wound infections. Yet, further studies are needed to optimize dosing parameters and evaluate in vivo efficacy and tolerability.

check for updates

Academic Editor: Nicola Petrosillo

Received: 17 October 2025

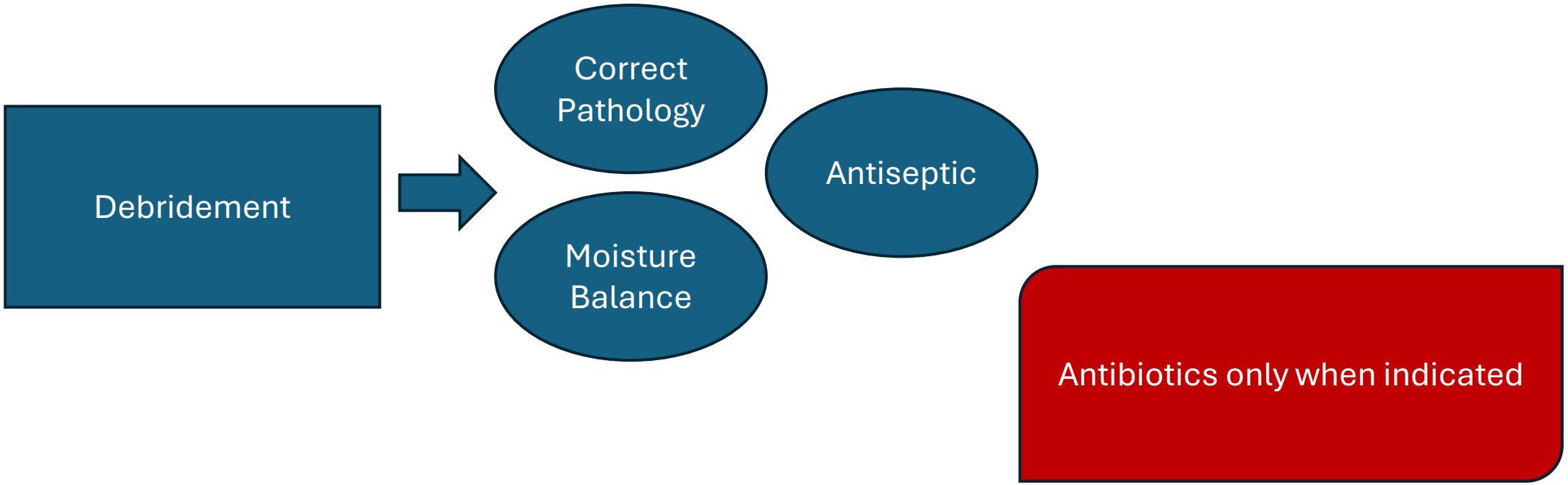
**Keywords:** *Candida auris*; nosocomial infections; multidrug resistance; lavage solutions; N-acetylcysteine

6 clinical *C. auris* isolates:
3 from chronic wound infections; 3 from deep-seated infections.

- Planktonic activity: HOCl inhibited all 6 isolates
- Biofilm disruption: Repurposed agents produced significantly greater biofilm disruption than saline or amphotericin B lavage ($p<.05$)
- Authors' interpretation: HOCl may have potential utility as an adjunct for *C. auris* wound infections; further in-vivo/clinical study is needed

# Stewardship for Chronic Wounds

- Debridement is the primary treatment for wound biofilm. Antiseptics are adjunctive therapies that help delay biofilm re-establishment. Antibiotics alone do not reliably eradicate mature biofilm




Schultz G, et al. *Wound Repair Regen*. 2017;25(5):744-757.

POLL

A patient with a diabetic foot infection has undergone adequate source control and is clinically improving on appropriate antibiotics. Which factor provides the LEAST justification for extending antibiotic therapy?

A. Persistent evidence of active soft-tissue infection
B. Residual infected bone that cannot be completely resected
C. Inadequate source control
D. The wound remains open and has not completely healed

POLL

**A patient with a diabetic foot infection has undergone adequate source control and is clinically improving on appropriate antibiotics. Which factor provides the LEAST justification for extending antibiotic therapy?**

A. Persistent evidence of active soft-tissue infection
B. Residual infected bone that cannot be completely resected
C. Inadequate source control
D. The wound remains open and has not completely healed

# Referred → MRSA SSTI/OM








75y patient transferred for gangrene; uncontrolled DM. Underwent revascularization and amputation. Returned 6 wks later, recommended for BKA

OM = osteomyelitis; DM = diabetes mellitus; BKA = below-knee amputation.

# Referred → MRSA SSTI/OM











# HOCl in Complex and High-Risk Wounds: Clinical Translation

ReprintsDesk | 7/2/2026 11:55:54 AM

ORIGINAL INVESTIGATION

## Hypochlorous Acid as an Adjunctive Treatment for Periocular Necrotizing Fasciitis

*David R. Jordan, M.D. and Pushpinder Kanda, M.D., PH.D.*

*Department of Ophthalmology, University of Ottawa, Ottawa, Ontario, Canada*

***Purpose:*** To report the outcomes of a small case series of patients with periorbital necrotizing fasciitis (PONF) treated with high-dose intravenous antibiotics and dilute, pure hypochlorous acid (HOCL) irrigated through affected tissues as well as sprayed topically, with no tissue debridement.

***Methods:*** In this single-center, retrospective chart review, 6 patients were identified between May 1, 2022, and May 1, 2024, with PONF who had HOCL used during their treatment. The primary outcome measures included successful eradication of infection, acceptable aesthetic result with minimal or no reconstruction required, and little or no loss of vision. Results of treatment were compared with those PONF patients in the literature treated more traditionally with antibiotics and debridement, and those treated with antibiotics alone.

***Results:*** Of the 6 patients identified with PONF, all 6 were treated with dilute, pure HOCL (0.033%). The median age of the patients was 51 (range 32–73). The female-to-male ratio was equal. The median follow-up time since the first presentation was 158 days (range 30–180). *Streptococcus pyogenes* was the most common causative organism, found in 80% of cases. No patient underwent tissue debridement in the periocular area. All patients recovered with minimal sequelae. No patient reported loss of vision on their final follow-up visit. There were no patients who required enucleation or exenteration and no patients died because of PONF.

***Conclusions:*** The authors describe a simple method of introducing dilute, pure HOCL (0.033%) without hypochlorite or sodium hypochlorite as impurities via irrigation and topical spray into the treatment plan for PONF. There was little if any loss of tissue with this treatment in this series. The outcomes compared favorably to those outcomes in the literature of PONF patients treated more traditionally with antibiotics and debridement, and those patients treated more conservatively with antibiotics alone. HOCL irrigation and spray techniques appear to be beneficial in the management of PONF and decrease the need to debride, allowing faster disease resolution and contributing to favorable outcomes.

(*Ophthalmic Plast Reconstr Surg* XXX;XX:00–00)

Necrotizing fasciitis (NF), commonly known as flesh-eating disease, is a rapidly spreading infection of the deeper layers of skin, subcutaneous tissues, and fascia anywhere in the body that may also involve the periorbital area (periorbital necrotizing fasciitis [PONF]).[1–11] Clinical presentation includes tissue swelling, erythema, bullae, followed by ischemic necrosis of tissue layers that may involve dermis to deep musculature.[2,10,11] Systemic effects can include extensive capillary leakage, hypotension, organ failure, and even death.[12–15]

The pathogenesis and resultant tissue destruction seen with NF stem from the effects of a variety of surface and secreted virulence factors (toxins), enzymes (e.g., hyaluronidase and collagenase) as well as the host's response.[2,3,10,11,14,15] Exotoxins are polypeptide proteins secreted by bacteria that come in a wide variety, are unusually resistant to eradication, and can disseminate through the body, potentially causing serious harm, while endotoxins (generally less potent) are lipopolysaccharide protein complexes produced at the time of cell death.[3,11,12,14,16,17] Some exotoxins are known as "superantigens" as they have the ability to corrupt the normal humoral immune response, resulting in immunosuppression in the face of active infection.[2,10,11,14–19] For example, neutrophil function can be compromised through inhibition of hypochlorous acid (HOCL) production and reductions in migratory and phagocytic activity as well as diapedesis.[20] The immune system dysfunction blunts the ability to target invasive microbes, which then continue to replicate with the additional release of exotoxins, superantigens, and more enzymes.[16,17] Superantigens can also be potent immunostimulators, causing widespread, nonspecific T-cell activation, and massive pro-inflammatory cytokine and chemokine release throughout the body, following which the cascade of events seen clinically as toxic shock syndrome may occur, including capillary leakage, hypotension, shock, multiorgan failure, and possibly death.[10,11,14,15,19]

Although antibiotic treatment and early aggressive surgical debridement of necrotic tissue are widely advocated (and frequently necessary) for limb and trunk NF as well as PONF, Passemard et al.'s[7] recent review reported that surgical debridement was rarely necessary in PONF. The natural course of PONF differs significantly from disease elsewhere and can be attributed to 1) the rich blood supply of the periocular tissues, 2) thinner eyelid skin allowing better visibility for early infection and earlier treatment, and 3) the orbital septum integrity acts as a potential barrier to slow the posterior spread.[1,3,5–7,21–24] A more conservative medical approach with delayed or no surgical debridement for PONF has been reported by several

Accepted for publication November 30, 2025.
The authors have no financial or conflicts of interest to disclose.

- This **retrospective case series** evaluated dilute, pure hypochlorous acid (HOCl; **0.033%**) as an adjunctive irrigation and topical spray treatment in **6 patients with periocular necrotizing fasciitis (PONF)** treated between May 2022 and May 2024
- All 6 patients received HOCl during treatment; median age was 61 yrs, and median treatment duration was **21 days**. Importantly, **no patient required tissue debridement in the periocular area**; all recovered with minimal sequelae, and no patients required enucleation or exenteration, or died from PONV





# Resistance GNRs







# HOCl in Complex and High-Risk Wounds: Clinical Translation

19y Female: Complicated angioinvasive coinfection with fungus and mold, which was managed with a multidisciplinary approach of intensive medical management, aggressive surgical debridement, and NPWT with instillation of HOCl solution.







CASE REPORT

## An Intensive Multidisciplinary Approach in Management of Extensive Nonuremic Calciphylaxis of the Bilateral Lower Extremities with Angioinvasive Fungus and Mold

Jeffrey L. Roberson, MD*, Zoya Butt, MD*, Stephanie Florez-Pollack, MD†, Eric Morgan, MD‡, Misha Rosenbach, MD†, Benjamin M. Braslow, MD‖ and Jay A. Yelon, DO‖

Management of infected wounds related to calciphylaxis poses a significant clinical challenge with high morbidity and mortality. Given no definitive management guidelines exist specific to nonuremic calciphylaxis, multiple modalities including sodium thiosulfate, antibiotics, hyperbaric oxygen therapy, and surgical debridement with wound care must be considered. When occurring over a large surface area, standard daily dressing changes are especially labor intensive, inefficient, and ineffective. Negative pressure wound therapy with instillation and dwell time offers broad wound coverage with ongoing therapeutic benefit. We present the case of a previously healthy 19-year-old woman who was transferred for tertiary level care of extensive nonuremic calciphylaxis wounds of the bilateral lower extremities complicated by angioinvasive coinfection with fungus and mold that was managed with a multidisciplinary approach of intensive medical management, aggressive surgical debridement, and negative pressure wound therapy with instillation of hypochlorous acid solution. Ultimately, she achieved full granulation and wound coverage with skin grafting. Large area, infected wounds related to nonuremic calciphylaxis can be successfully managed with multidisciplinary medical management, aggressive surgical debridement, and negative pressure wound therapy that can instill and dwell hypochlorous acid solution.

### BACKGROUND

Calciphylaxis is a rare disease characterized by calcification of arterioles and capillaries in dermal and subcutaneous adipose tissue that ultimately results in skin ischemia and necrosis.[1,2] Carrying a roughly 50% six-month survival rate, the true pathogenesis remains elusive; however, the characteristic skin lesions are believed to be caused by reduced blood flow from vessel calcification and fibrosis that ultimately leads to tissue infarction.[3]

Disorders that predispose to the development of calciphylaxis include end-stage renal disease (ESRD), chronic inflammation, and deficiencies of inhibitors of vascular calcification. In the setting of ESRD, abnormal phosphate and calcium metabolism, along with uremic toxins, are thought to trigger smooth muscle cell transformation to osteoblast-like cells. These osteoblast-like cells initiate the process of calcific arteriopathy.[4] In a cohort of 89 patients in the United Kingdom with calciphylaxis, the majority of patients were diabetic and engaged in hemodialysis. Bacteremia occurred in 26% of patients and only 17% achieved complete wound healing.[5]

Nonuremic calciphylaxis (NUC) is a less common entity. Risk factors include female sex, obesity, systemic corticosteroid use, warfarin, and systemic diseases including primary hyperparathyroidism, malignancy, alcoholic liver disease, and connective tissue diseases. The diagnosis carries a mortality rate of 52% with the most common cause being sepsis,[6,7] though other studies and expert opinion suggest a better prognosis than calciphylaxis in the setting of ESRD.

Current treatments available for calciphylaxis in the absence of hyperparathyroidism[8] include sodium thiosulfate (STS) infusions, hyperbaric oxygen therapy (HBOT), and surgical debridement. While the true mechanism of STS is unclear, it has been proposed that it provides calcium-chelation, inhibits vascular calcification, induces a high anion-gap acidosis, and/or provides anti-oxidative effects.[9] However, studies have varied regardings STS' true efficacy and effect on disease course.[10]

Multiple case reports have suggested a role for HBOT in calciphylaxis with a goal of restoring tissue oxygen tension. Increased oxygen tension is hypothesized to bolster fibroblast proliferation, collagen production, and angiogenesis in order to aid in the healing of large wounds. While a small case series demonstrated that some patients' wounds improve with HBOT, the results have been inconsistent and limited by access to treatment, cost, and claustrophobia.[11]

*Department of Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA; †Department of Dermatology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA; ‡Department of Pathology & Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA; ‖Division of Trauma, Surgical Critical Care, and Emergency Surgery, Department of Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA

Conflicts of interest statement: None applicable.

Address Correspondence to: Jeffrey L. Roberson, MD, Department of Surgery, Hospital of the University of Pennsylvania, 3400 Spruce Street, 4 Maloney, Philadelphia, PA 19104. Email: jeffrey.roberson@pennmedicine.upenn.edu

© The Author(s) 2022. Published by Oxford University Press on behalf of the American Burn Association. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

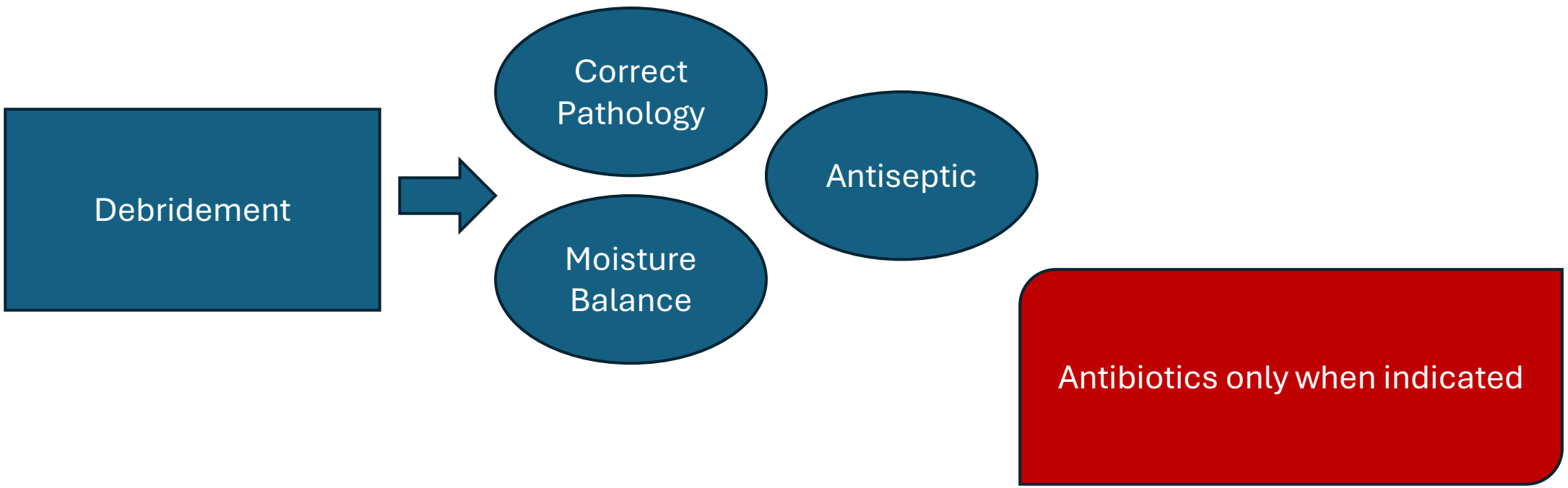
https://doi.org/10.1093/jbcr/irac158



Downloaded from https://academic.oup.com/jbcr/article/44/1/218/6780044 by Reprints Desk user on 03 August 2026

# Stewardship for Chronic Wounds

- Debridement is the primary treatment for wound biofilm. Antiseptics are adjunctive therapies that help delay biofilm re-establishment. Antibiotics alone do not reliably eradicate mature biofilm.




Schultz G, et al. *Wound Repair Regen*. 2017;25(5):744-757.

# Stewardship Approach in Wound Care

- Resistance is here
- Debridement is source control
- Source control between visits
- Use antibiotics when they are needed
- Topical antiseptics support stewardship principles
- Topical antimicrobials are a blind spot and can lead to resistance

# Contamination vs Infection




## Lisa Gould, MD, PhD, FACS

Plastic Surgeon
Adjunct Clinical Associate Professor
Brown University Warren Alpert Medical School
Providence, RI

# Some Infections Are Obvious

55y Male, non-diabetic noticed a “lump” 5 days prior to admission. He presented with rapidly progressing erythema and pain in the left thigh during the past 24 hrs



# Surgical Emergency

## Time Is Tissue

- Early necrotizing soft tissue infection
  - Surgical disease
- Subsequent topical wound care; healed without sequelae
- No graft required



**Erythema cleared quickly**



Nawijn F, et al. *World J Emerg Surg*. 2020 Jan 8;15:4.

# Some Are Not So Obvious

- 50y Male with diabetes; long-term 5$^{th}$ met head ulcer, neuropathy but noted some pain in his foot



# Some Infections Are Obscure

- 51y Male w/poorly managed T2DM (HgA1c=10.3 and presenting BG=410)
  - Patient noted small red spot, possibly related to trauma
  - 2 wks later noted increased edema and redness

Patient goes to Urgent Care, and you are on duty. What is your treatment?

A. Recommend immediate admission
B. Compression wrap
C. Rest, Ice, Elevate
D. Treat empirically with oral antibiotic



# Decisions in the Hospital

- 4 days later, he presented to the hospital
- Markedly increased pain, difficulty ambulating
- WBC 10.7, ESR 30, CT scan non-diagnostic
- The surgical team was consulted



- Immediate operative debridement with second look 2 days later



**Final wound 16x7x2cm.**

**Wound culture: MSSA**

MSSA = methicillin-susceptible *Staphylococcus aureus.*

# Some leave you wondering if you missed something…

- 72y Female on home HD for ESRD, well-controlled T2DM, and neuropathy; developed plantar ulcer over past 1 month
- Debridement and offloading in clinic



- Returned 2 wks later reporting pain and increased drainage
- Bone is palpable but not overtly exposed





HD = hemodialysis; ESRD = end-stage renal disease.

# The next most important step in this patient's management is

POLL

A. Admit with urgent consult to podiatry
B. Debride and treat with topical antimicrobial wound care
C. Culture
D. Start empiric antibiotics

# The next most important step in this patient's management is

POLL

A. Admit with urgent consult to podiatry
B. Debride and treat with topical antimicrobial wound care
C. Culture
D. Start empiric antibiotics

# And Then There's Yellow Stuff



# We *Thought* We Knew…



&



= Slough



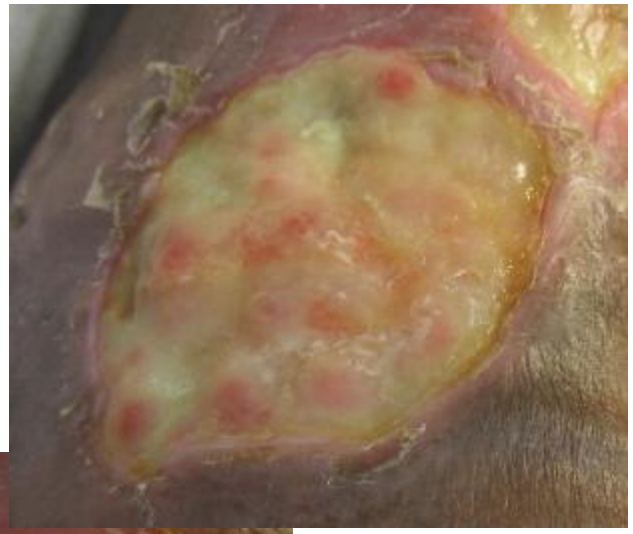
= Fat



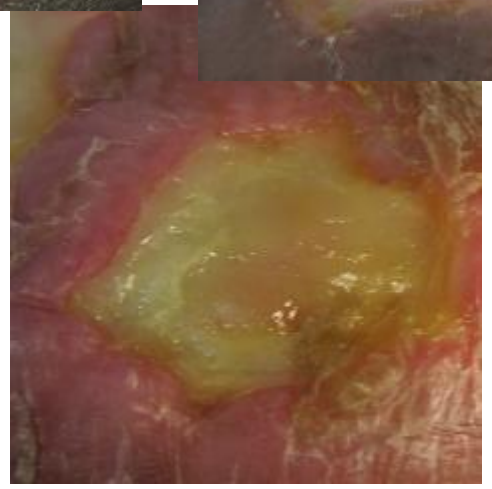
= Fibrin

# What About Other “Yellow Stuff”











# Definition of Slough (Merriam Webster)

- Noun: a place of deep mud or mire, swamp
- Adjective: a state of moral degradation or spiritual dejection
- Verb: to become shed or cast off; to cast off one's skin; to separate in the form of dead tissue from living tissue





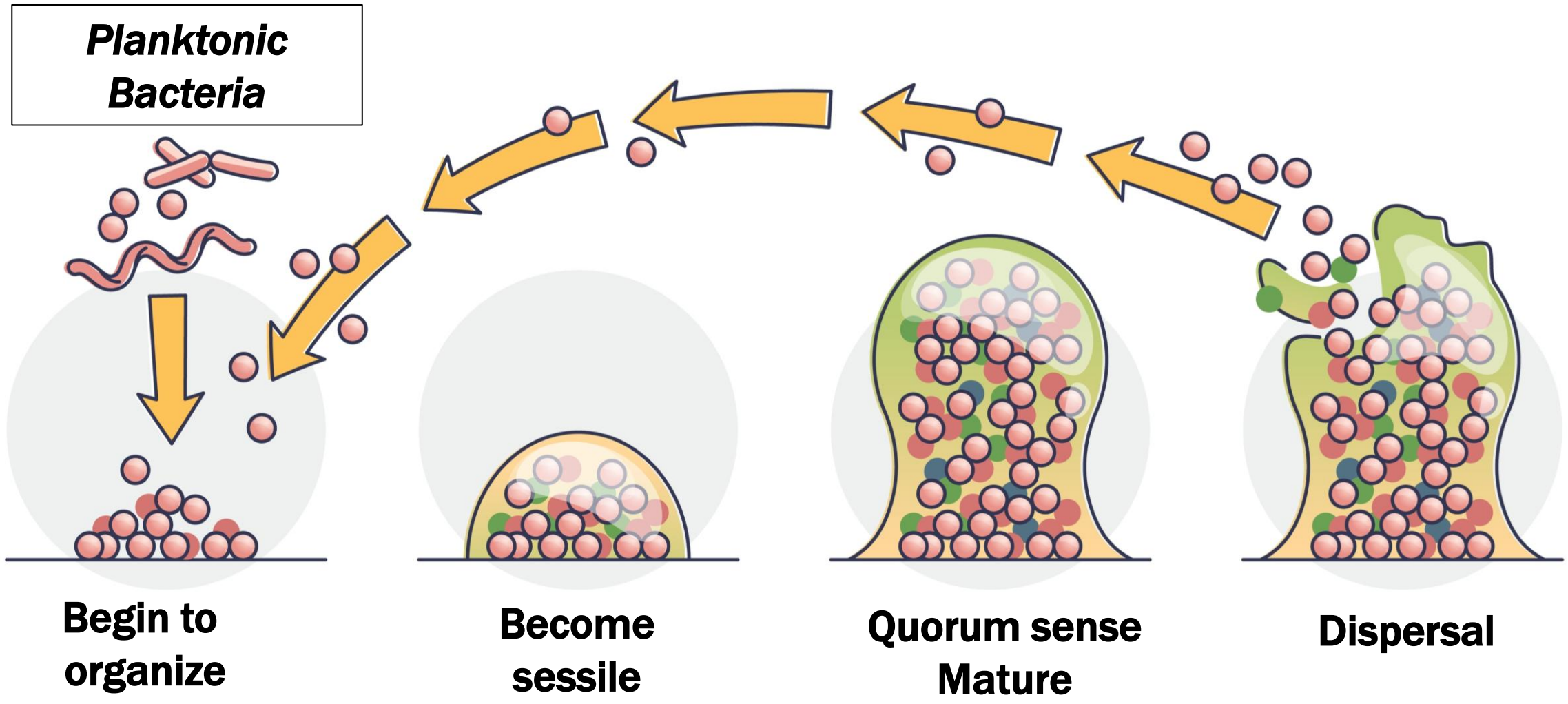


# What is the major component of slough?

POLL

A. Dead tissue
B. Neutrophils
C. Bacteria
D. Proteins
E. All of the above

# What about Biofilm?




Bjarnsholt T, et al. *APMIS*. 2021;129(10):598-606.

# Is It Slough, Biofilm, or Infected with Planktonic Bacteria?

## Peels off easily






## Requires curette





## Needs a scalpel

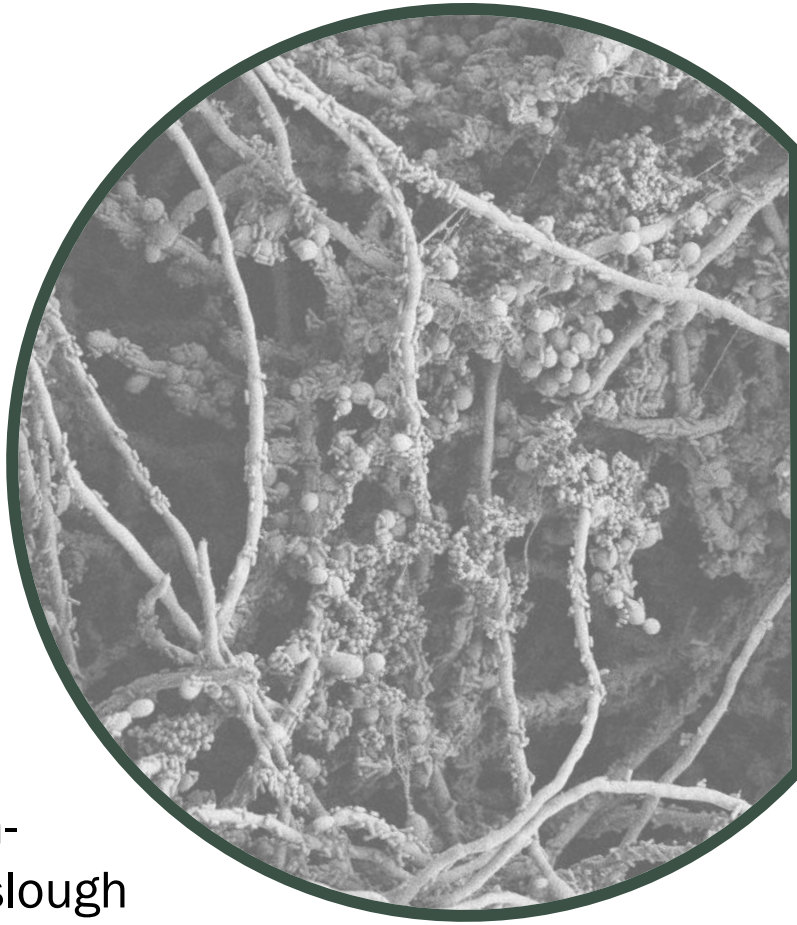




# The Slough Project

International Wound Infection Institute

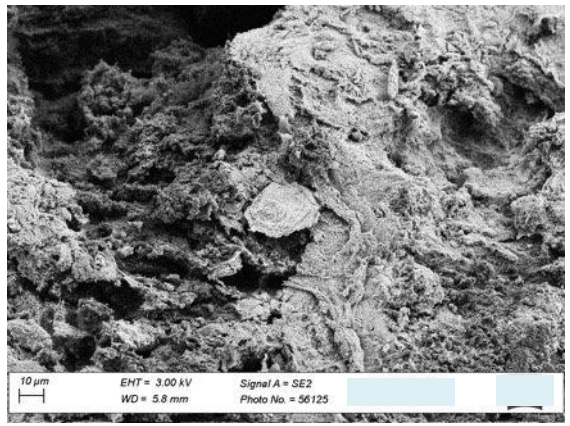
**Dr. Lindsay Kalan, University of Wisconsin, USA**
**Dr. Greg Schultz, University of Florida, USA**
**Dr. Thomas Bjarnsholt, University of Copenhagen, Demark**
**Dr. Matt Malone, Western Sydney University, Australia**
**NP Terry Swanson, South West Healthcare, Australia**
**Dr. Karen Ousey, University of Huddersfield, UK**
**Dr. Steven Percival, 5D Healthcare, UK**
**IWII Committee**



- An international collaborative team collected slough samples with high-resolution photography and wound data to characterize the nature of slough
- ***The* most comprehensive assessment of the composition of the 'slough' and its relation to inflammation and biofilm bacteria.**

# Patient 1

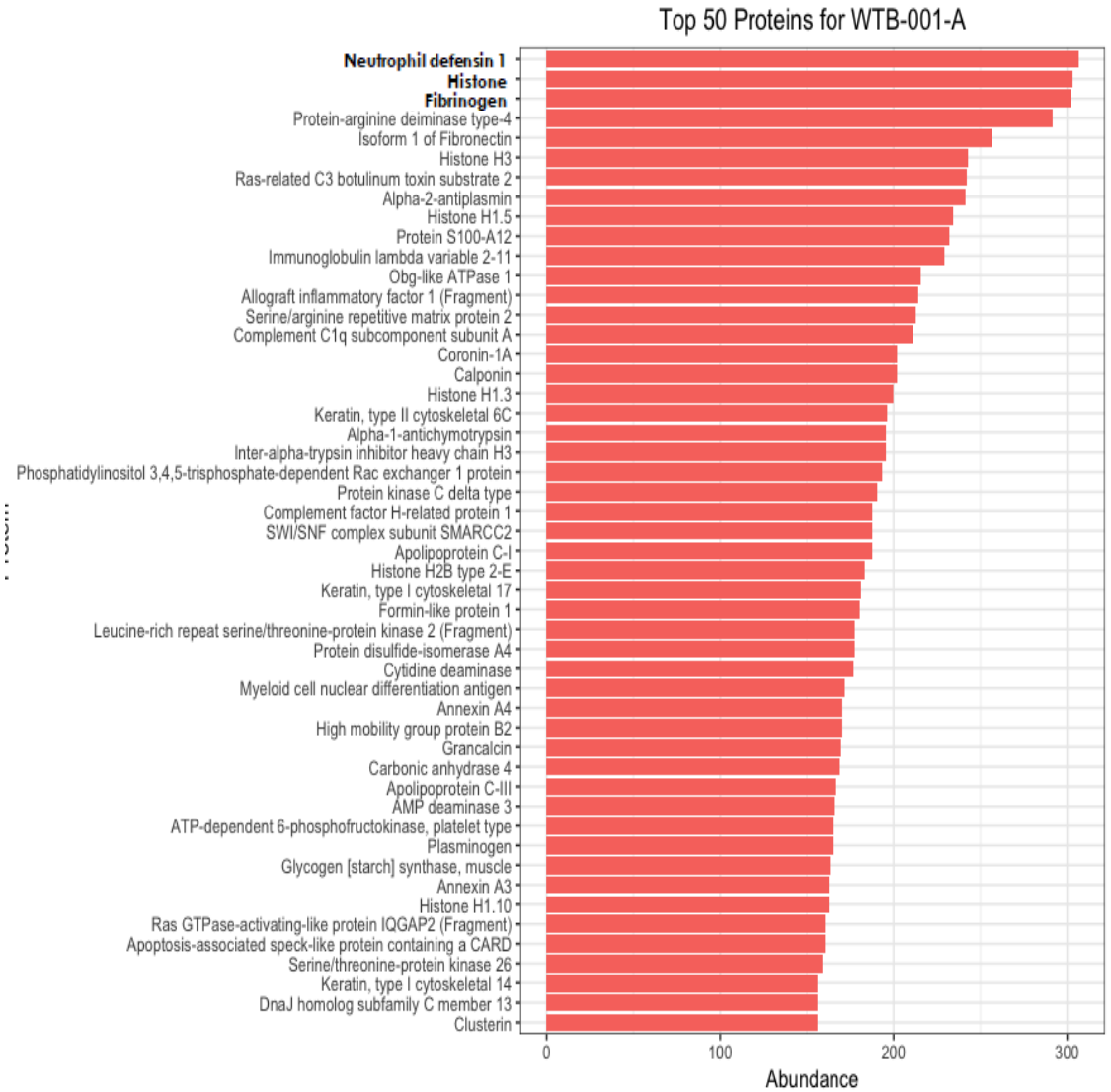
| Side | Location | Etiology | Wound Age | Length(cm) | Width(cm) | $cm^2$ | Shape | CFUs |
|---|---|---|---|---|---|---|---|---|
| Left | Medial Ankle | Lymphedema | 15 | 8.5 | 4 | 34 | Irregular; Round Oval | $1.3 \times 10^7$ |








- Loosely adherent yellow slough
- High bioburden $1.3 \times 10^7$ CFU
- Polymicrobial (4+ by culture)
- No obvious biofilm by SEM
- Amorphous material




SEM = scanning electron microscopy.

Kalan L, et al. Published May 25, 2023. Accessed July 1, 2026. https://woundsinternational.com/consensus-documents/slough-composition-analysis-and-effect-on-healing/

# Patient 7

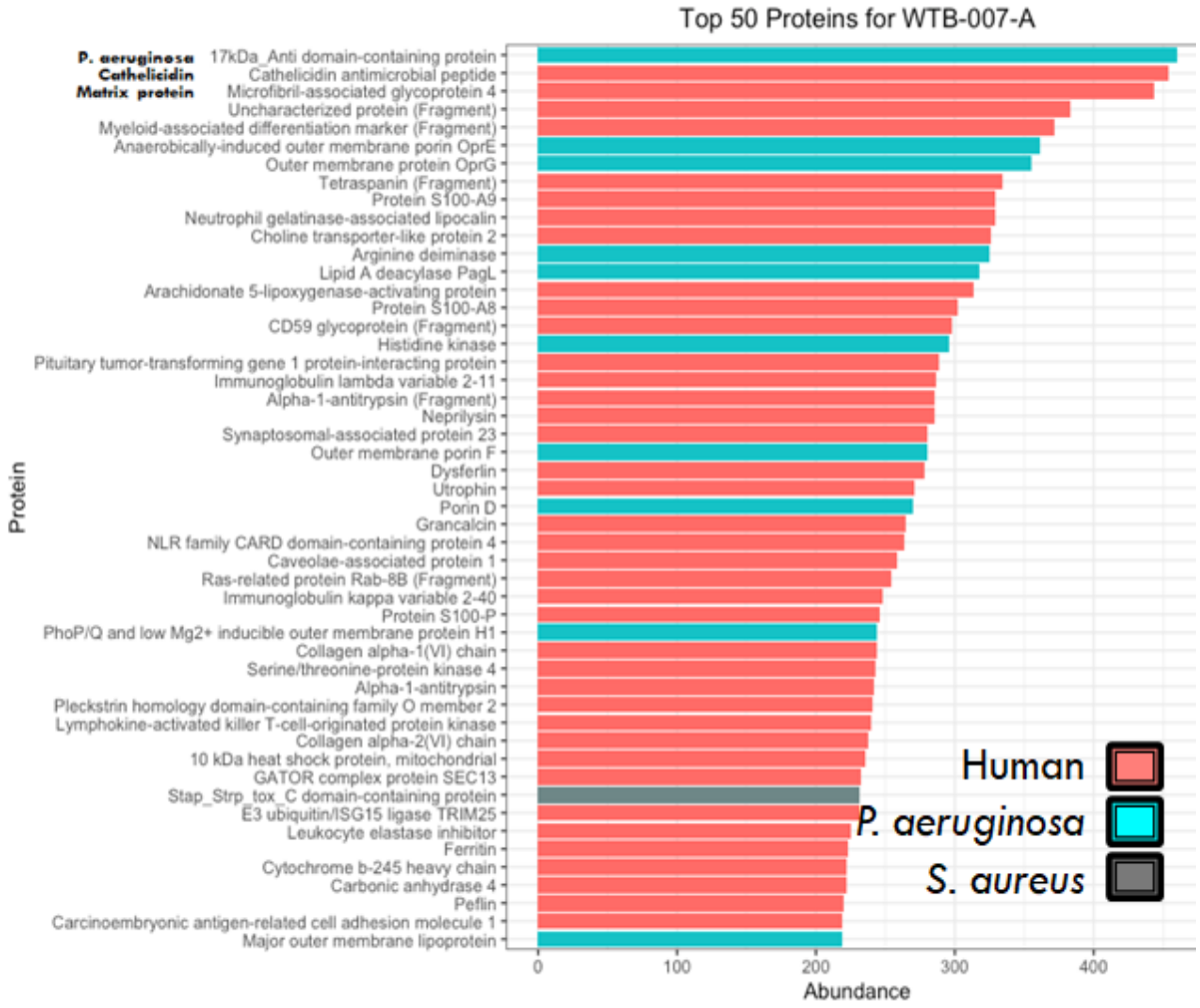
| Side | Location | Etiology | Wound Age | Length(cm) | Width(cm) | $cm^2$ | Shape | CFUs |
|---|---|---|---|---|---|---|---|---|
| Right | Lateral Ankle | Surgical wound | 1.25 | 8 | 10.4 | 93.6 | Irregular | $8 \times 10^7$ |







- Adherent
- High bioburden
- *Pseudomonas aeruginosa* cultured
- No obvious biofilm by SEM
- Proteomics identifies *P. aeruginosa* and *S. aureus*
- 16S sequencing is pending




Kalan L, et al. Published May 25, 2023. Accessed July 1, 2026. https://woundsinternational.com/consensus-documents/slough-composition-analysis-and-effect-on-healing/

# New Insights into the Role of Biofilm

## Wound Healing Is Impeded by Metabolically Active Bacteria

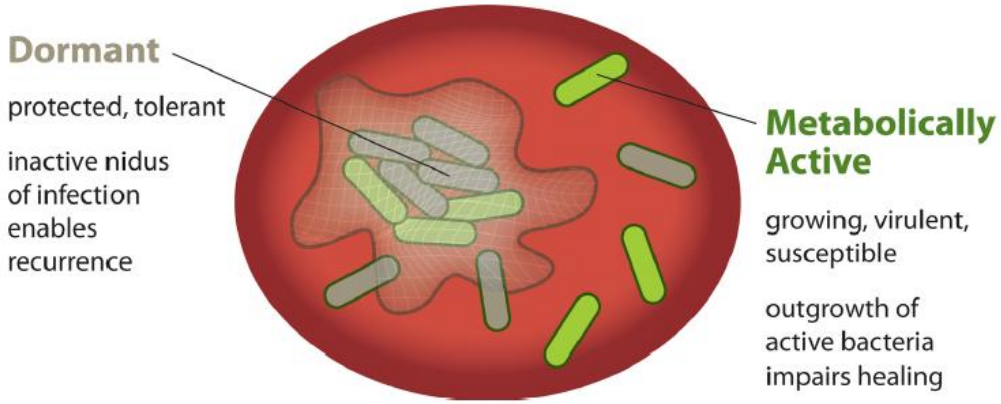
| Parameter | Result | *p*-value | | |
|---|---|---|---|---|
| | | Healing slope | Healing versus non-healing | PAR |
| Biofilm microscopy score | No association in any test | 0.25 | 0.15 | 0.19 |
| Total viable bacteria | Less healing in 2 of 3 tests when more bacteria were present | 0.94 | 0.012 | 0.012 |
| Bleach-tolerant bacteria | No association in any test | 0.053 | 0.97 | 0.69 |
| Bleach-susceptible bacteria | Less healing in all tests when more bleach-susceptible bacteria were present | 0.003 | 0.0012 | 0.004 |

**First longitudinal study characterizing presence of biofilm and rate of healing in chronic venous leg ulcers**

- **117 patients**
- **No correlation of healing with biofilm score or bleach-tolerant bacteria**
- **Reduced healing with bleach-susceptible (metabolically active) bacteria**

Stewart PS, et al. *Wound Repair Regen*. 2024;32(6):858-871.

# A Paradigm Shift and New Model: The Infectious Microenvironment




Bacterial aggregation and biofilm are a phenotype, not the cause of bacterial persistence

- Aggregate size can interfere with phagocytosis

The trajectory of infections is controlled by the metabolism of the bacteria

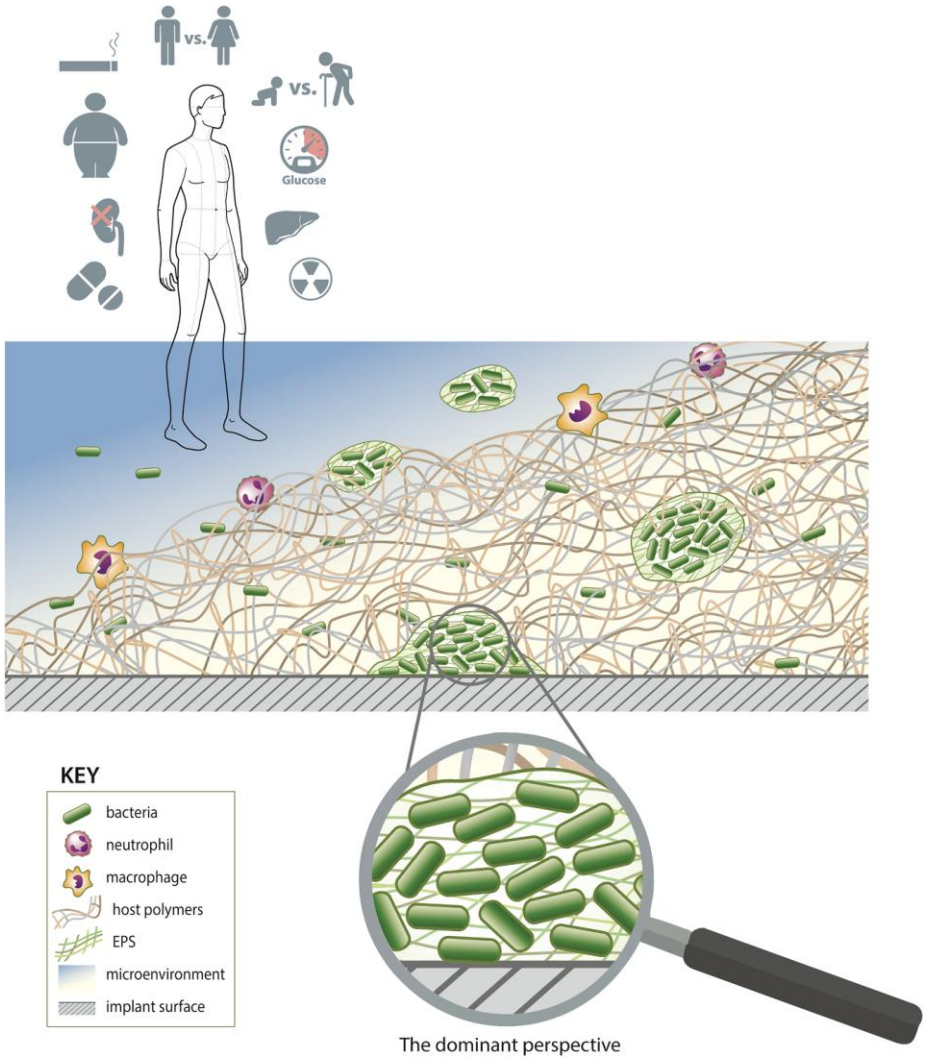
To understand Wound Infection, we need to understand the Infectious Microenvironment

1) Bacterial Metabolism
2) Inflammatory response
3) The metabolic signature of bacteria and host

The new research goal is not to grow biofilms but to mimic the microenvironment

Stewart PS, et al. *Wound Repair Regen*. 2024;32(6):858-871.

# Host-Pathogen Partnership




## Unanswered questions

- The persistence of infections in chronic wounds is attributed to biofilms, yet definitive proof remains elusive
- It is not fully understood which bacterial species or virulence factors impact wound severity
- The concept of “critical colonization,” meaning the threshold for microbial load to cause infection in wounds, is debatable

Bjarnsholt T, et al. *Cell Rep*. 2026;45(3):117014.

# Slough vs Biofilm vs Planktonic Bacteria?

- 60y Male admitted via the ED with overtly necrotic wounds, pain, and cold right foot; prior femoral to distal popliteal bypass 1 yr ago
- Underwent revision of the bypass; wound was treated with operative debridement and topical wound care
- At his first clinic visit, he presented with increased pain and erythema of the right leg with malodorous drainage





Close-up of lateral ankle

POLL

# The next most important step in this patient's management is:

A. Debridement in the clinic
B. Treat with analgesics and topical wound care
C. Wound culture using the Levine technique
D. Start empiric antibiotics

POLL

# The next most important step in this patient's management is:

A. Debridement in the clinic

B. Treat with analgesics and topical wound care

C. Wound culture using the Levine technique

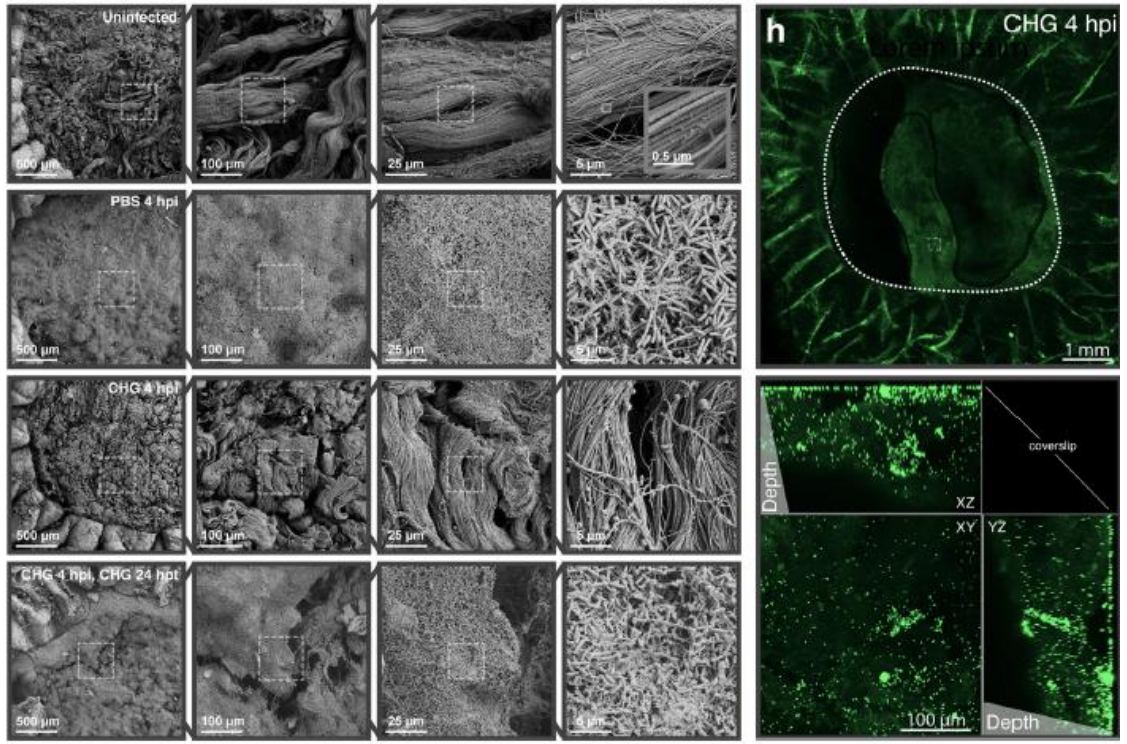
D. Start empiric antibiotics

# Why Debridement Is Critical




**WHS Fireside Chats**

- SEM vs confocal laser scanning microscopy
- Images compare different depths of infection



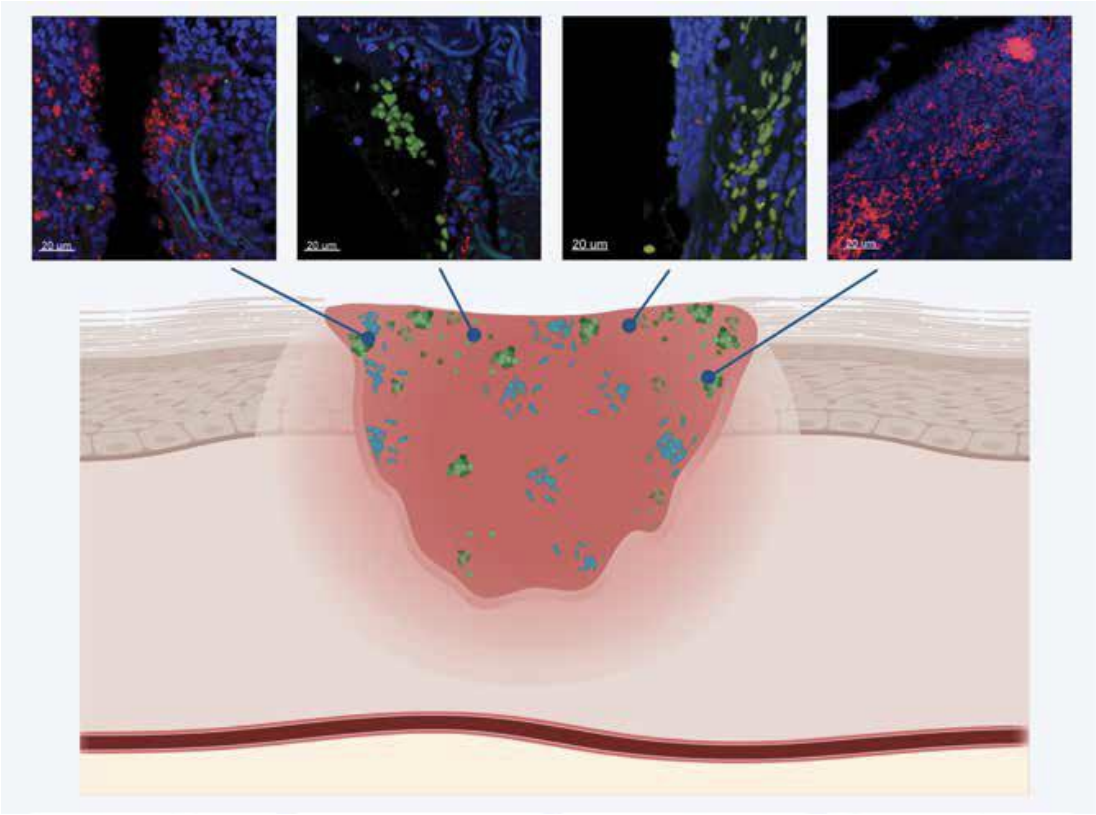

Identified reservoirs of *P. aeruginosa* in deep tissue not seen with SEM

Cheong JZA, et al. *Wound Repair Regen*. 2022;30(5):573-584.

# Proper Culture Technique for Correct Diagnosis

- Levine technique
  - 90% sensitivity, 57% specificity
- Steps
  - Clean with NS and gently scrub the surface
  - Choose the cleanest area, and avoid necrotic tissue
  - Obtain tissue fluid by pressing firmly
  - Specific location



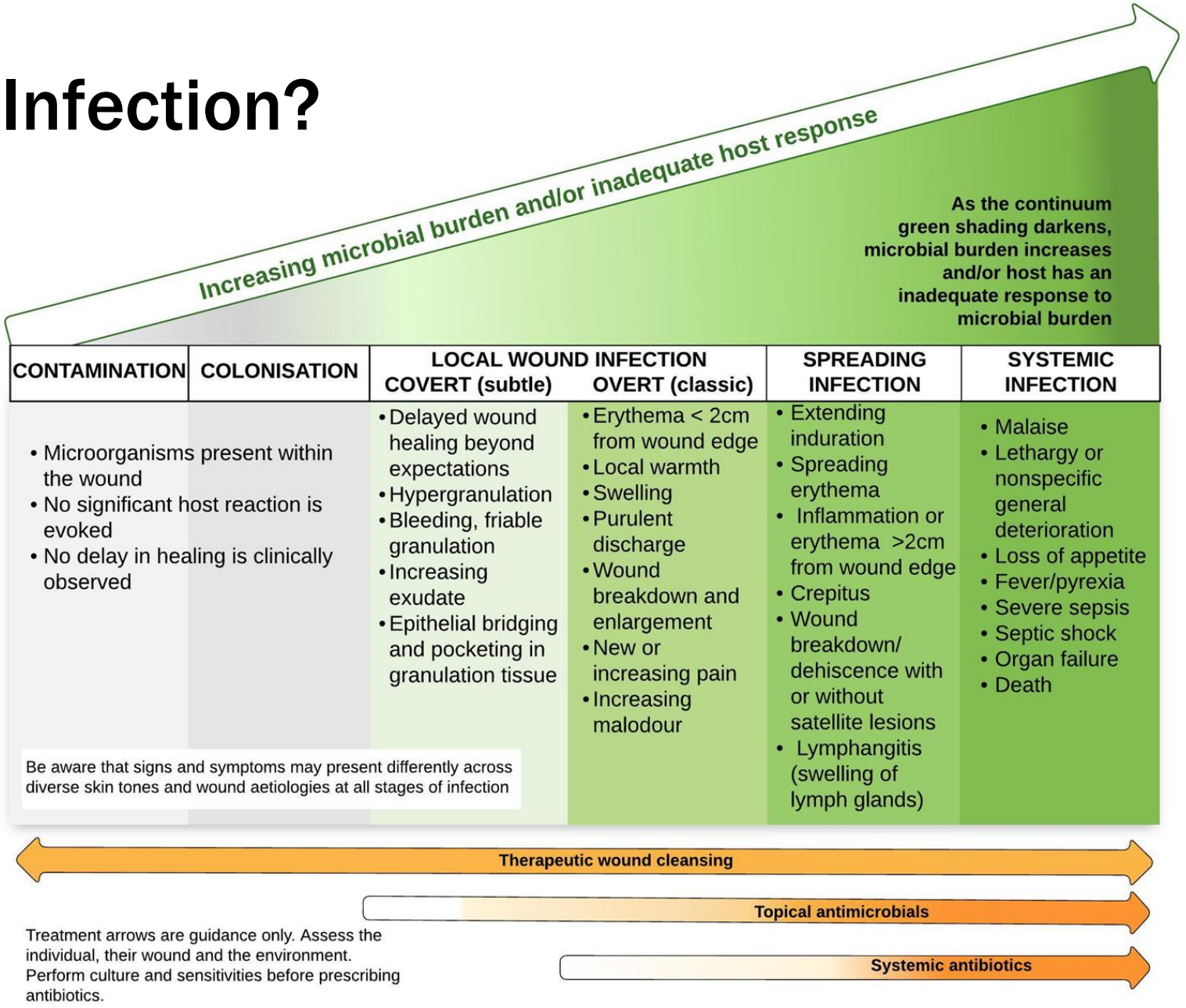
Jakobsen TH, et al. *J Wound Manag*. 2025;26(1):54-56.

NS = normal saline.

Levine NS, et al. *J Trauma*. 1976;16(2):89-94. Copeland-Halperin LR, et. al. *J Wound Care*. 2016;25(4):S4-6, s8-10.

# When or WHAT Is an Infection?

https://woundinfection-institute.com/resources/the-iwii-wound-infection-continuum-iwii-wic



# 2-wk follow-up in wound center, patient denies fevers or chills but has increased pain and has noticed some drainage

POLL

Based on the IWII wound infection continuum, this patient has



A. Colonization
B. Covert infection
C. Spreading infection
D. Systemic infection
E. None of the above: the erythema is from moisture-associated skin damage (MASD)

# 2-wk follow-up in wound center, patient denies fevers or chills but has increased pain and has noticed some drainage

POLL

Based on the IWII wound infection continuum, this patient has



A. Colonization

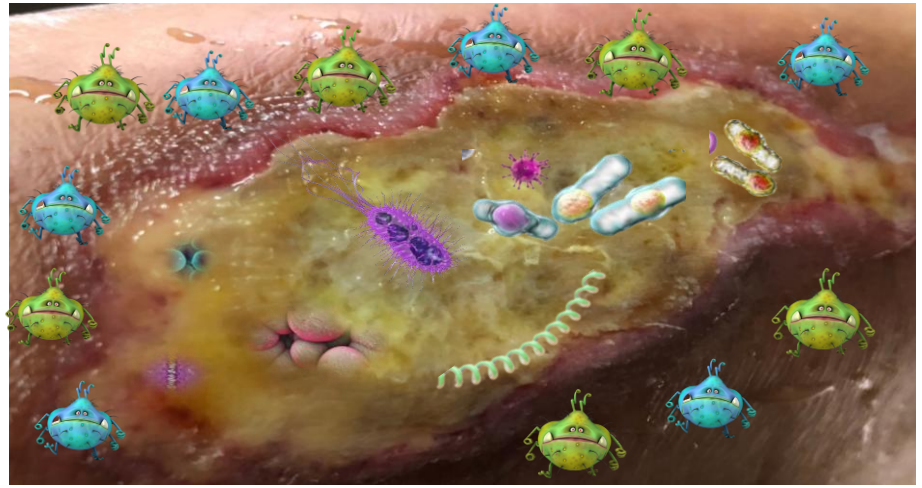
B. Covert infection

C. Spreading infection

D. Systemic infection

E. None of the above: the erythema is from moisture-associated skin damage (MASD)

# Clinical Pearls



- Wounds are a buffet for bacteria: “Clean it like you mean it”
- Infection can occur rapidly
- TIME IS TISSUE
- Debride and cleanse before culture
  - Do not send culture of necrotic tissue
- Biofilm is not visible to the naked eye
- More research is needed to understand the host-pathogen partnership
- IWII has free resources: Cleansing document, Wound Infection Continuum, Wound Infection in Clinical Practice Consensus
  - https://woundinfection-institute.com/
- EWMA local wound infection fact sheets
  - https://ewma.org/resources/infection-fact-sheets/

