First Coast ID/CM Symposium

32nd Annual Meeting

February 7-8, 2025 Hyatt Regency, Jacksonville Riverfront, Jacksonville, FL

Microbiology's Most Challenging Culture: The Diabetic Foot Wound

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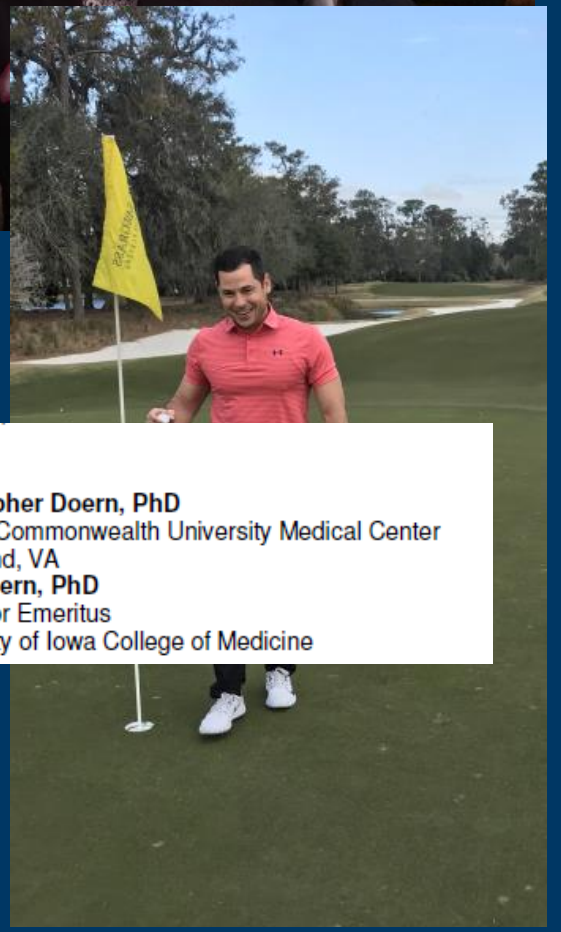
1/29/2025

25th Annual First Coast Infectious Disease/Clinical Microbiology Symposium
February 2-3, 2018



2:45 pm	**Stretch Break**	
Moderator: Noel Gomez, MMSc, UF Health Jacksonville, Jacksonville, FL		
3:00 pm	Antimicrobial Resistance and Susceptibility Testing: Preamble to the Ramblings of an Older Microbiologist	**Christopher Doern, PhD** Virginia Commonwealth University Medical Center Richmond, VA
	The Clinical Relevance of Antimicrobial Susceptibility Testing: Ramblings of a Very Old Clinical Microbiologist	**Gary Doern, PhD** Professor Emeritus University of Iowa College of Medicine

Conflicts of Interest

Advisory activities – Quidel, Karius, Roche, Cepheid, GeneCapture

Speaker’s bureau - Shionogi

Background on Diabetes

Scope of the problem

- Over 500 million people globally suffering from diabetes
  - 37 million in the US
- Estimated that 20-30% of DM patients will develop a chronic non-healing wound in their life
  - Foot ulcers often require amputation

Diabetes and Insulin

- Essential hormone for regulating blood sugar levels
- Two types – Type 1 and Type 2

Type 1 – Autoimmune disease

- Pancreas does not make insulin because the body's immune system attacks the islet cells that make insulin.
  - Genetic factors
  - Immunologic dysregulation
  - Environmental triggers

Type 2 - Acquired

- Pancreas does not make enough insulin and/or the body doesn't regulate insulin properly
  - Caused by insulin resistance – when muscles, fat, and liver don't respond as they should to insulin
    - Genetic factors
    - Obesity
    - Inactivity
    - Eating highly processed, high carbohydrate foods

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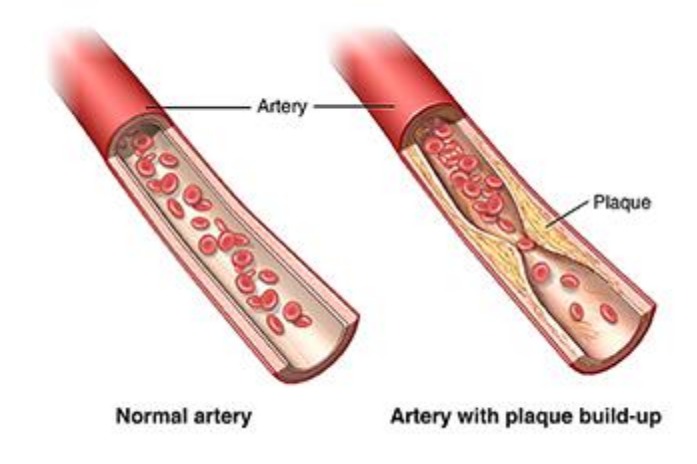
Pathophysiology of Wound Healing

Hyperglycemia

- Contributes to the development of atherosclerosis.
  - Prevents circulating nutrients from reaching wound and impairs healing



https://www.hopkinsmedicine.org/health/conditions-and-diseases/atherosclerosis

- Hyperglycemia may contribute to dysfunction of endothelial cells via pressure induced vasodilation (normally protective)

Neuropathy

- Autonomic
  - Impaired sweat gland function → dry cracked skin

- Motor
  - Increases pressure on plantar surface of foot and impairs healing

Hypoxia

- Due to poor circulation
- Hypoxic wound environment leads to poor healing

Peripheral artery disease

- Poor circulation leads to increased risk of amputation

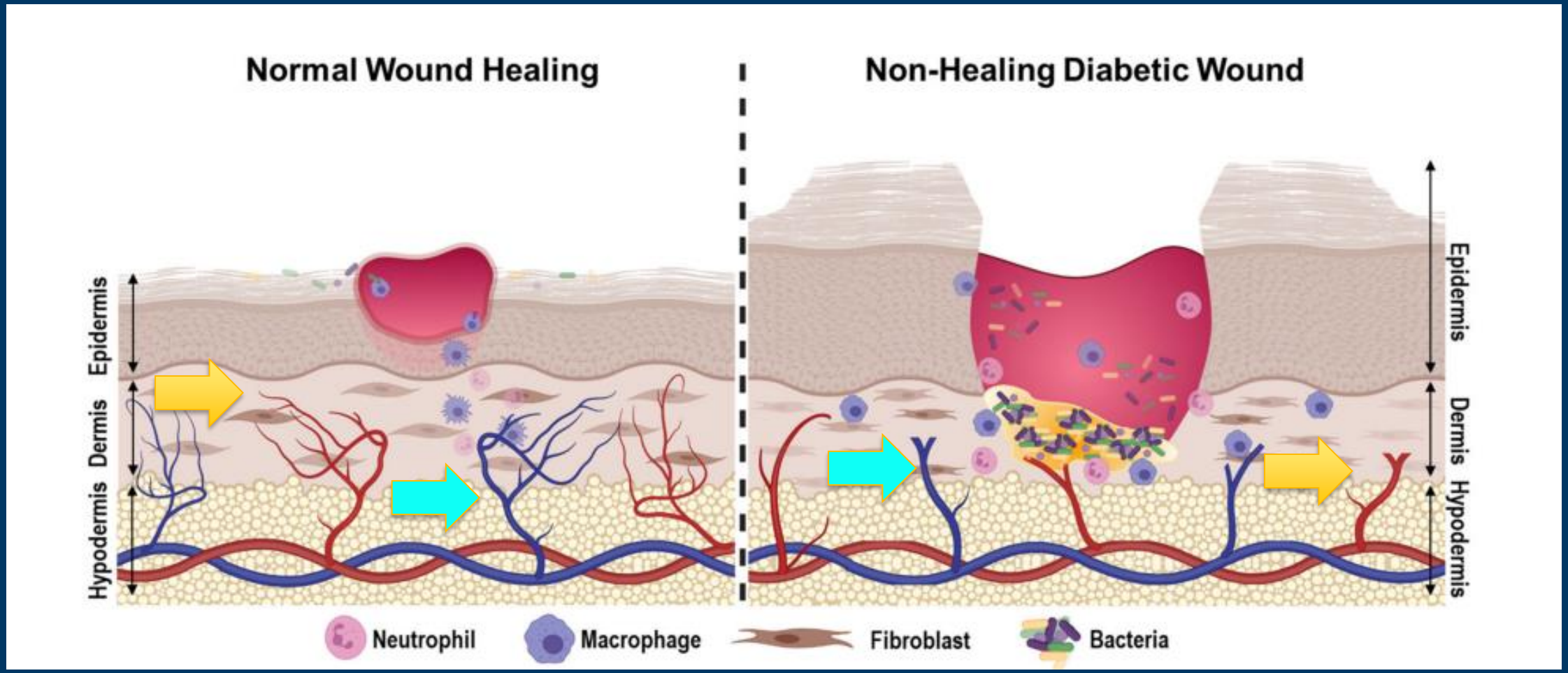
Antimicrobial Peptides

- Healthy skin produces antimicrobial peptides that fight infection
- This is impaired in diabetic wounds

Bacterial Diversity

- Diabetic skin more likely to be colonized with…
  - *S. aureus*, *Pseudomonas,* Enterobacterales

Pathophysiology of Wound Healing



Burgess et al. Medicina. 2021, 57,1072

Diabetic Foot Ulcers

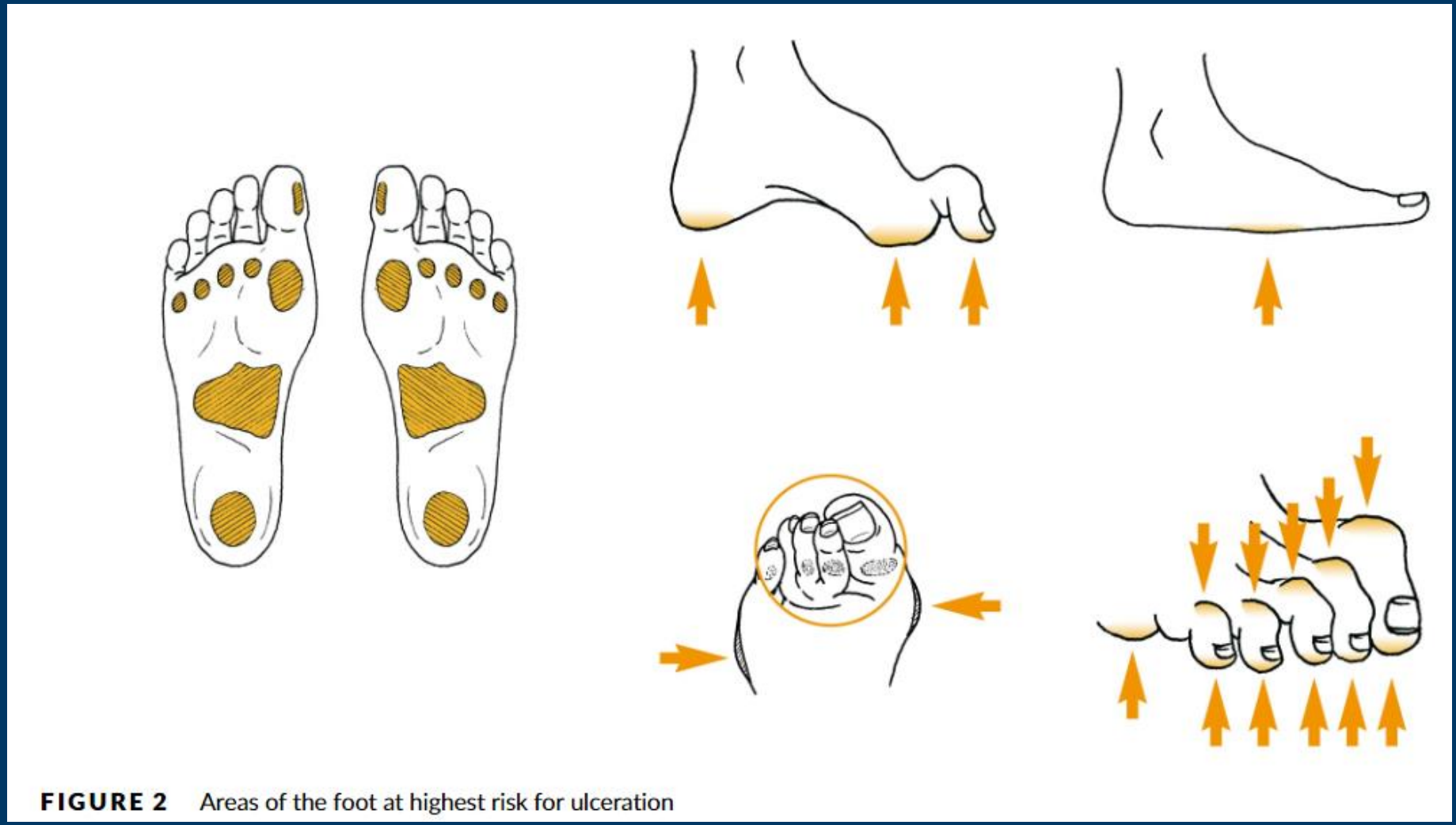


FIGURE 2 Areas of the foot at highest risk for ulceration

Schaper et al. Diabet Metab Res Rev. 2020. 36(S1)

Diabetic Wound Infections

IWGDF/IDSA

Ulcers colonized with potential pathogens.

Signs of Infection

Inflammation

- Redness
- Warmth
- Induration
- Pain/tenderness

Purulent Secretions

Challenges

1. Symptoms blunted by neuropathy or ischemia
2. Systemic symptoms often absent (pain, fever, leukocytosis)
3. Particularly challenging in mild or moderate infections

Table 1. The classification system for defining the presence and severity of foot infection in a person with diabetes.[a]

Clinical classification of infection, definitions	IWGDF/IDSA classification
No systemic or local symptoms or signs of infection	1/Uninfected
Infected: At least two of these items are present: • Local swelling or induration • Erythema >0.5 but <2 cm[b] around the wound • Local tenderness or pain • Local increased warmth • Purulent discharge	2/Mild
And, no other cause of an inflammatory response of the skin (e.g., trauma, gout, acute charcot neuro-arthropathy, fracture, thrombosis, or venous stasis)	
Infection with no systemic manifestations and involving: • Erythema extending ≥2 cm[b] from the wound margin, *and/or* • Tissue deeper than skin and subcutaneous tissues (e.g., tendon, muscle, joint, and bone)[c]	3/Moderate
Infection involving bone (osteomyelitis)	Add "(O)"
Any foot infection with associated systemic manifestations (of the systemic inflammatory response syndrome [SIRS]), as manifested by ≥2 of the following: • Temperature, > 38°C or <36°C • Heart rate, > 90 beats/min • Respiratory rate, > 20 breaths/min, *or* PaCO2 < 4.3 kPa (32 mmHg) • White blood cell count >12,000/mm^3, *or* < 4G/L, *or* >10% immature (band) forms	4/Severe
- Infection involving bone (osteomyelitis)	Add "(O)"

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Senneville et al. CID. 2023. October

How to Classify Diabetic Foot Infection?

D-FOOT international

	1	2	3
Infection severity	MILD INFECTION (IDSA[a])-PEDIS[b] 2	MODERATE INFECTION (IDSA)-PEDIS 3/3osteomyelitis	SEVERE INFECTION (IDSA)/PEDIS 4/4osteomyelitis
Characteristics	**Presence of at least two of:** ▪ Local swelling or induration ▪ Erythema > 0.5 cm ▪ Local tenderness or pain ▪ Local increased warmth ▪ Purulent discharge	▪ Local infection with erythema >2 cm **OR** ▪ Involvement structures deeper than skin and subcutaneous tissue ▪ No signs of systemic inflammatory response	▪ Temperature >38 °C or <36 °C ▪ Heart rate >90 beats/minute ▪ Respiratory rate >20 breaths/minute or PaCO2 <4.3 kPa (32 mmHg) ▪ White blood cell count >12,000/mm3, or <4,000/mm3, or >10% immature (band) forms
Antibiotics *	Oral agents	Oral or initial parenteral agents	Parenteral agents
** See recommendations of Infection Guideline for empirical antibiotic regimen for diabetic foot infection*			
Clinical presentation			

[a] **IDSA**: Infectious Disease Society of America
[b] **PEDIS**: Perfusion, Extent, Depth, Infection and Sensation

IWGDF Guidelines — IWGDF Infection Guideline

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Castater et al. Surg Clin N Am. 2023. 103. 1191-1216

Work up of a Diabetic Wound Infection

Laboratory Testing

- CBC
- CRP
- ESR

Poor sensitivity and specificity

Only 50% of diabetic patients with deep wound infections have a leukocytosis.

Imaging

- Begins with X-ray
  - Fracture, foreign body, osteolytic changes
- MRI and CT can be done
  - Diagnose osteomyelitis

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What is the role of culture?

From IDSA

In a person with suspected soft tissue DFI, consider a sample for culture to determine the causative microorganisms, preferably by aseptically collecting a tissue specimen (by curettage or biopsy) from the wound. (Conditional; Moderate)

In a person with diabetes for whom there is a suspicion of **osteomyelitis** of the foot (before or after treatment), **bone** (rather than soft tissue) samples should be obtained for culture, either intraoperatively or percutaneously. (Conditional; Moderate)

Consider a duration of up to 3 weeks of antibiotic therapy after **minor amputation** for diabetes-related osteomyelitis of the foot and **positive bone margin culture** (Conditional; Low)…

"Since all wounds are colonized (often with potentially pathogenic microorganisms), wound infection cannot be defined using only the results of wound cultures."

Pathogens of Diabetic Wound Infections

Definitions –

Infection – Virulence factors of one or more wound organisms overwhelm host resistance resulting in invasion and replication of the organisms and local tissue damage.

Contamination – Presence of bacteria on the wound surface with no multiplication of bacteria.

Colonization – Replication of organisms on the wound surface without invasion of wound tissue and with no host immune response.

Also…

Mere presence of organisms in nonviable tissue, without invasion of viable tissue, does NOT constitute wound infection.

Gardner and Frantz. Biol Res Nurs. 2008; 10(1)

DFI Pathogens

Reference	Severity of Infection	Predominant Pathogens
Armstrong et al. 1995	Unclear	*S. aureus* (51%) Anaerobes (7%)
Diamantolpoulos et al. 1998	Limb-threatening	*S. aureus* (51%) Anaerobes (21%)
El-Tahawy. 2000	Unclear	*S. aureus* (28%) Anaerobes (11%)
Goldstein et al. 1996	Mild to moderate	*S. aureus* (76%) Anaerobes (40%)
Louie et al. 1976	Uninfected to severe	*Peptococcus* (80%) *S. aureus* (35%)
Prabhakar et al. 1981	Gangrenous	*Proteus* (31%) *S. pyogenes* (46%)
Sapico et al. 1984	Scheduled for amputation	Group D Strep (41%) Anaerobes (28%)

Summary

- *S. aureus*
- Anaerobes
- Beta-hemolytic streptococci
- *Enterococcus??*

What is missing?

If you had asked me…

Pseudomonas aeruginosa

Staphylococcus aureus

Streptococcus agalactiae

Mixed enteric flora

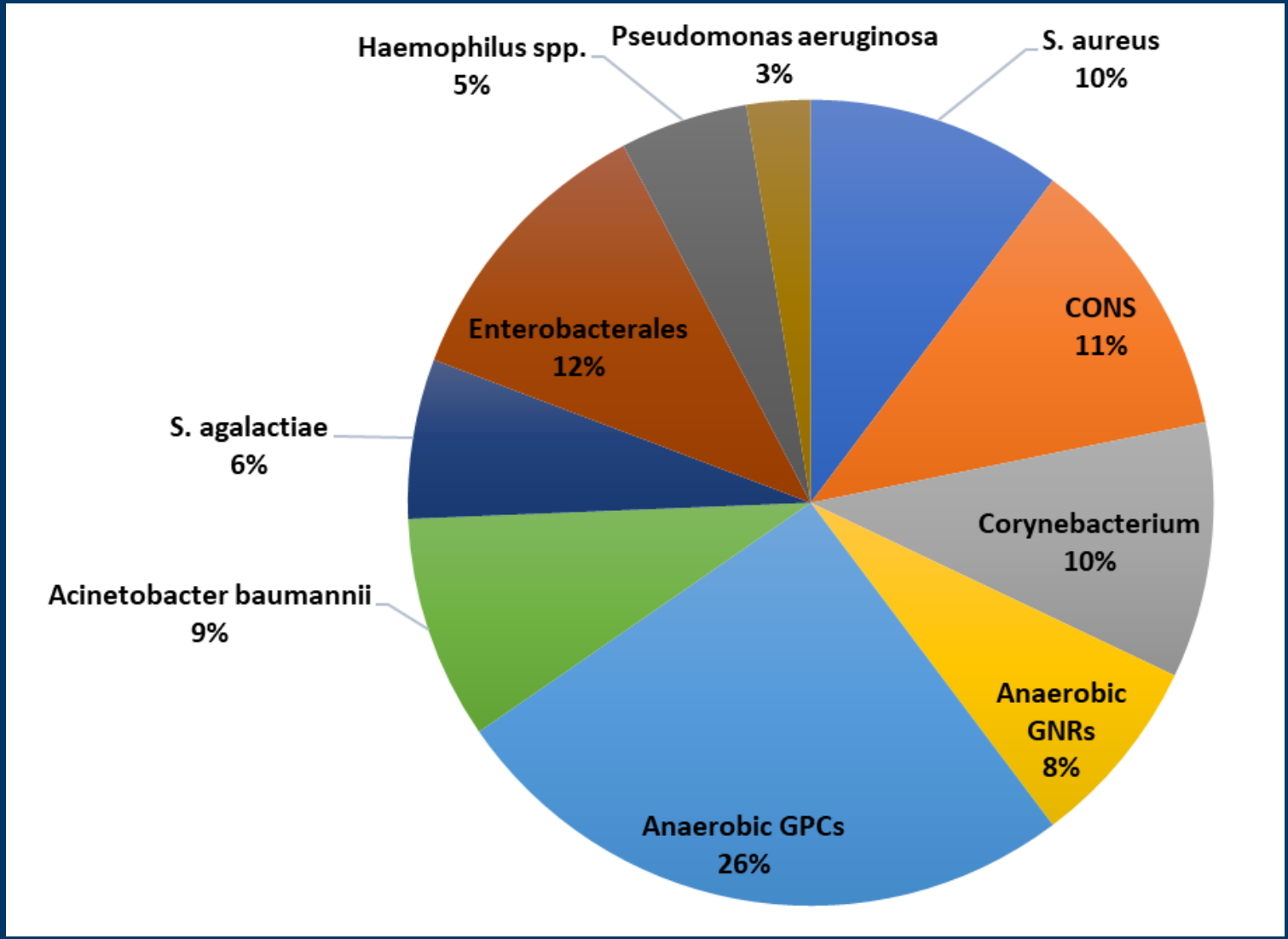
Junk

Anaerobes

Enterococcus

Microbiome of the Diabetic Wound

- Conducted in England
- 39 Newly infected patients (>18 yo)
- Tissue punch biopsy performed
- Prior topical or systemic antibiotics – excluded
- Next generation sequencing and qPCR (microbial load) were performed



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Malone et al. EBioMedicine. 2017. 142-149

What principals do we use to determine significance in a bacterial culture?

Key pathogens

Relative abundance

Gram stain

Many wound cultures

Diabetic Wound Cultures

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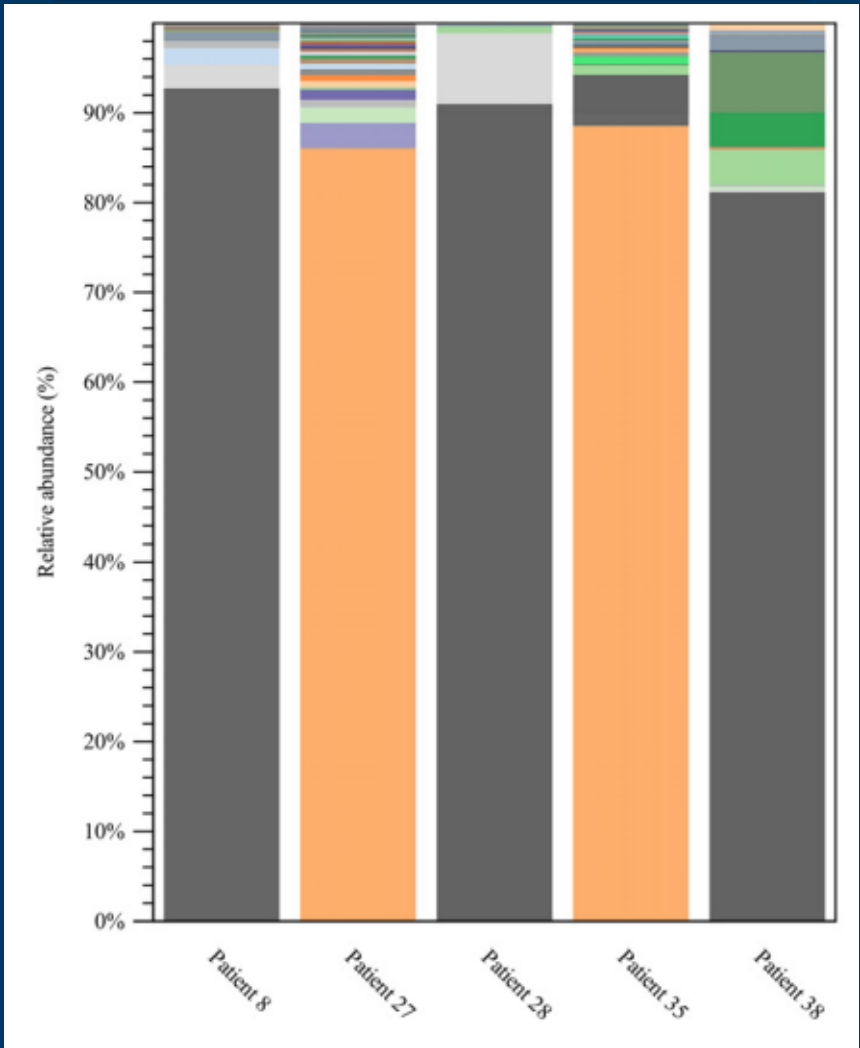
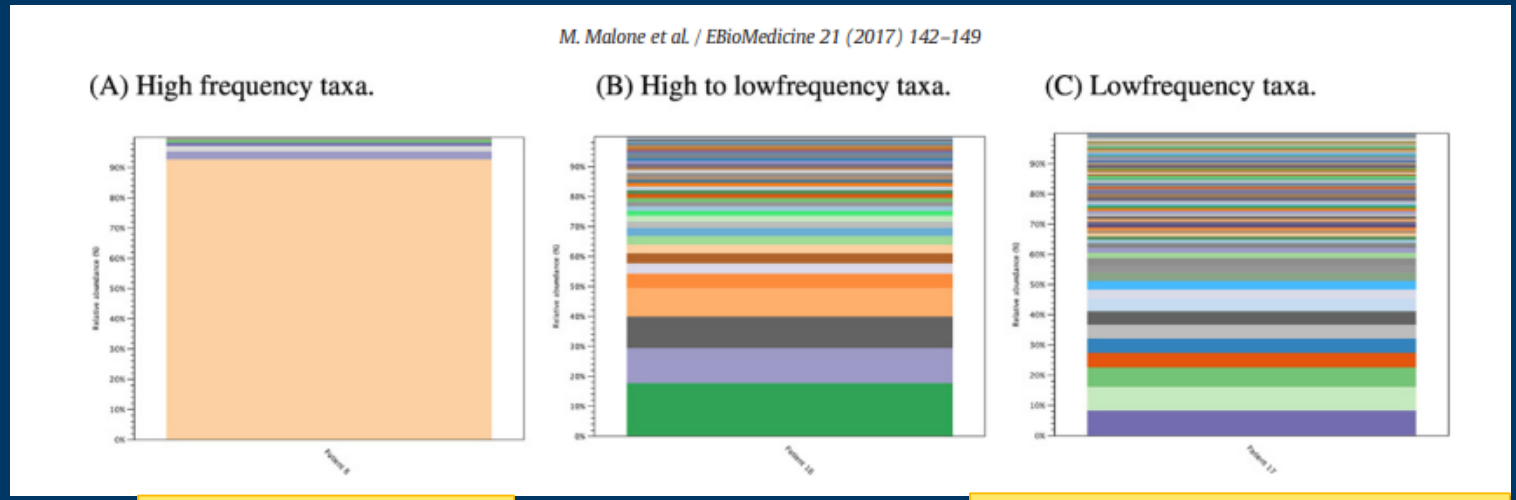
Interesting Findings from the Microbiome

Age of the Wound Matters



- < 6 month wounds
  - Dominant growth of single pathogens
  - Little bacterial diversity
  - *Staphylococcus aureus* and GBS – dominant pathogens

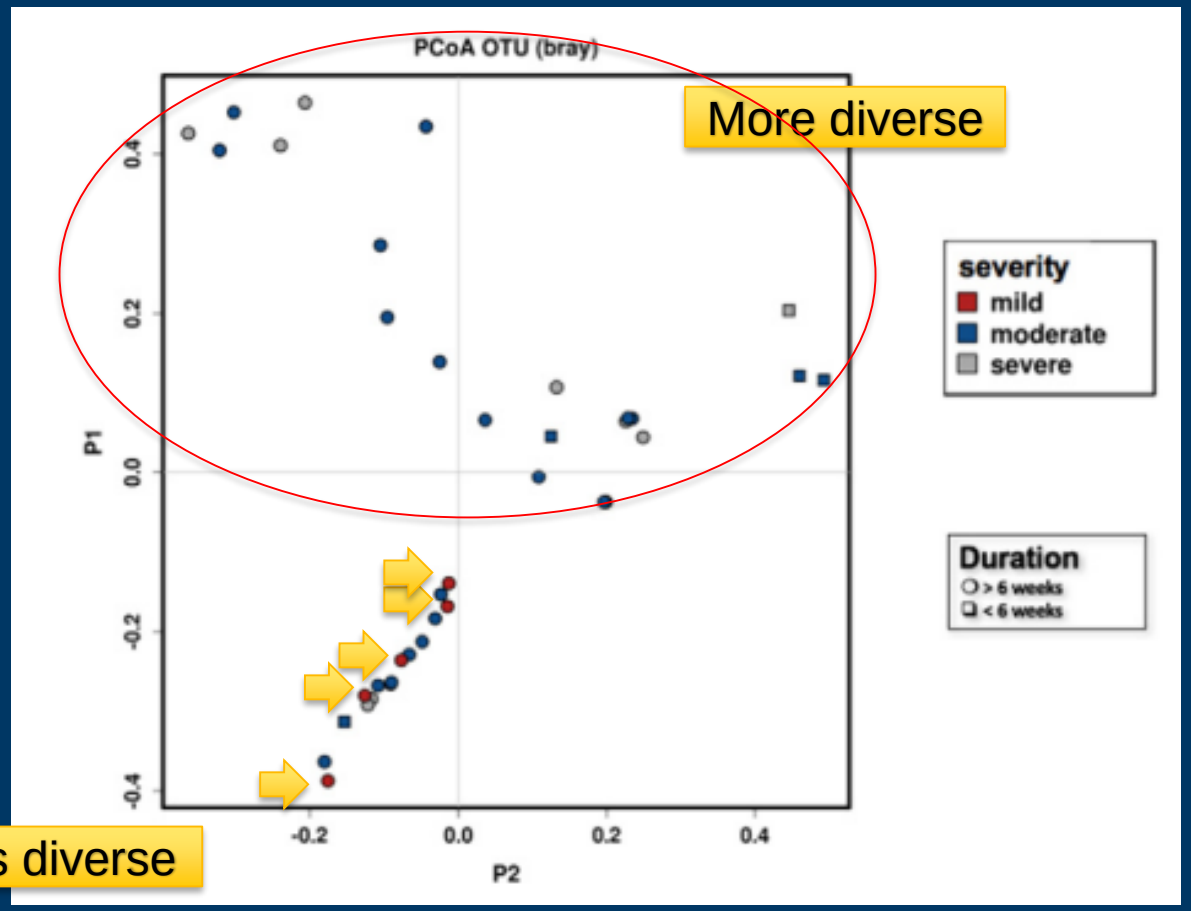


< 6 months

> 6 months
- Great diversity
- Anaerobes dominant

Malone et al. EBioMedicine. 2017. 142-149

Do pathogens vary by wound severity



<u>These data are difficult to interpret</u>

Mild Infection

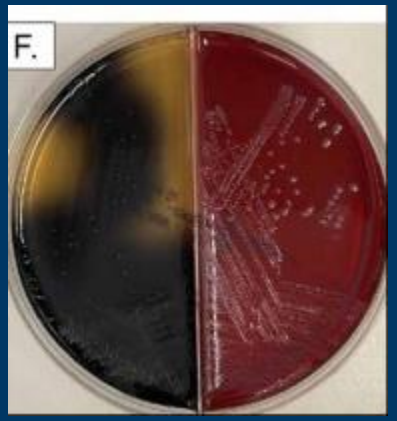
- Low diversity (fewer types of organisms)

Moderate – Severe Infection

- Higher diversity

Anaerobes

- Present across all infections equally



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Malone et al. EBioMedicine. 2017. 142-149

What does this mean?

Which culture is more "significant"

Abundant *S. aureus*
Rare mixed anaerobic bacteria

Mild

Moderate mixed anaerobic bacteria

Moderate - Severe

Are these really infections?

Microbiology of the Diabetic Foot: Specimens and processing

Preferred

Tissue biopsy from a debrided area

Bone biopsy

Suboptimal

Superficial swabs

Do not do!!!

Severed limbs and appendages

- Send us biopsy from the clean margin following amputation

Processing of Bone Specimens

- Cover with saline or broth medium
- Vortex for 10 seconds
- Remove vortexed medium
  - Use one drop for Gram stain
  - Inoculate plates with 1-2 drops
    - Quadrant streaking patter

Alternatively (If viable tissue present)

- Excise tissue and process as you would for a tissue biopsy.

If bone too large to fit in container….

- Avoid if possible
- Contact provider to see if they can assist in excising bone fragments

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Gram stain Principals

What do we know about the accuracy of the Gram stain?

Look for signs of a quality specimen

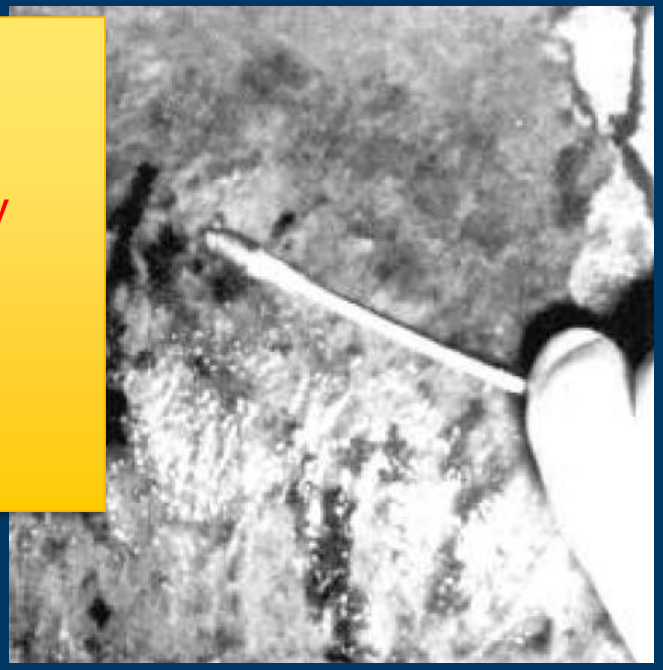
- Presence of inflammation
- Lack of epithelial cells
- Presence of organisms

Notification of results

- Organisms seen on sterile specimens???

Conclusions

1. Cultured organism quantity correlates with quantity on smear.
2. Visualization of organisms implies > 10^5 organisms.



THE JOURNAL OF TRAUMA
Vol. 16, No. 2

THE QUANTITATIVE SWAB CULTURE AND SMEAR: A QUICK, SIMPLE METHOD FOR DETERMINING THE NUMBER OF VIABLE AEROBIC BACTERIA ON OPEN WOUNDS

NORMAN S. LEVINE, M.D., LT COL, MC, ROBERT B. LINDBERG, PH.D., ARTHUR D. MASON, JR., M.D. AND BASIL A. PRUITT, JR., M.D., COLONEL, MC

From the United States Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas

TABLE I

Relationship between the Number of Viable Bacteria Counted and the Visualization of Bacteria on a Gram-Stained Smear of a Wound Swab

Number of Viable Bacteria Counted	Number of Swab Counts in This Range	Number with Visualization of Bacteria on Gram Stained Smear
$\geq 10^8$	6	6
1×10^7 to 9.9×10^7	1	0
1×10^6 to 9.9×10^6	4	4
1×10^5 to 9.9×10^5	2	0
$<10^5$	11	0

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Gram stains in patients with diabetic ulcers

- Tanzania – low resources

What is the utility of using the Gram stain to guide therapy, when culture is not available?

RESULTS

- 118 cultures of tissue biopsies yielded growth
  - 59 (50%) were polymicrobic (80% GNRs)
  - 38 (32%) – GNRs alone
  - 20 (17%) – GPs alone

- Gram stain predictive in 93% of cultures
  - Gram positives 15/20 (75%)
  - Gram negatives 31/38 (83%)

Table 3 Results of Grams stains with the corresponding matched culture result*

Gram stain result on light microscopy	Growth of Gram-negative microorganisms (single species)	Growth of Gram-positive microorganism (single species)
Gram-negative bacilli	38	0
Gram-positive cocci	2	15

*Discordancy not significant by McNemar test ($P = 0{\cdot}25$). The matched data array indicates the complementarity of Gram stains and culture.

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Abbas et al. Inter Wound Journal. 2012. 9

Treatment of Diabetic Wound Infections

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1/29/2025

Antibiotic Use in the Diabetic Ulcer

What Antibiotics Are Used?

Table 2. Empirical Antibiotic Options for Diabetic Foot Infections[31]

Non–limb threatening (generally oral outpatient therapy)
- Cephalosporins (cephalexin, cefadroxil, cefdinir)
- Fluoroquinolones (levofloxacin, moxifloxacin)
- Penicillins (dicloxacillin, amoxicillin/clavulanate)
- Linezolid
- Trimethoprim-sulfamethoxazole
- Doxycycline

Life threatening
- Ampicillin-sulbactam + aztreonam
- Piperacillin-tazobactam + vancomycin
- Vancomycin + metronidazole + ceftazidime
- Antipseudomonal carbapenem (doripenem, imipenem-cilistatin, meropenem)
- Fluoroquinolone + vancomycin + metronidazole
- Ertapenem
- Linezolid
- Tigecycline

Limb threatening
- Ampicillin-sulbactam
- Ticarcillin-clavulanate
- Piperacillin-tazobactam
- Ceftazidime + clindamycin
- Cefotaxime ± clindamycin
- Fluoroquinolone + clindamycin
- Antipseudomonal carbapenem (doripenem, imipenem-cilistatin, meropenem)
- Fluoroquinolone + vancomycin + metronidazole
- Linezolid
- Ertapenem
- Tigecycline

How well do these antibiotics work in diabetic patients?

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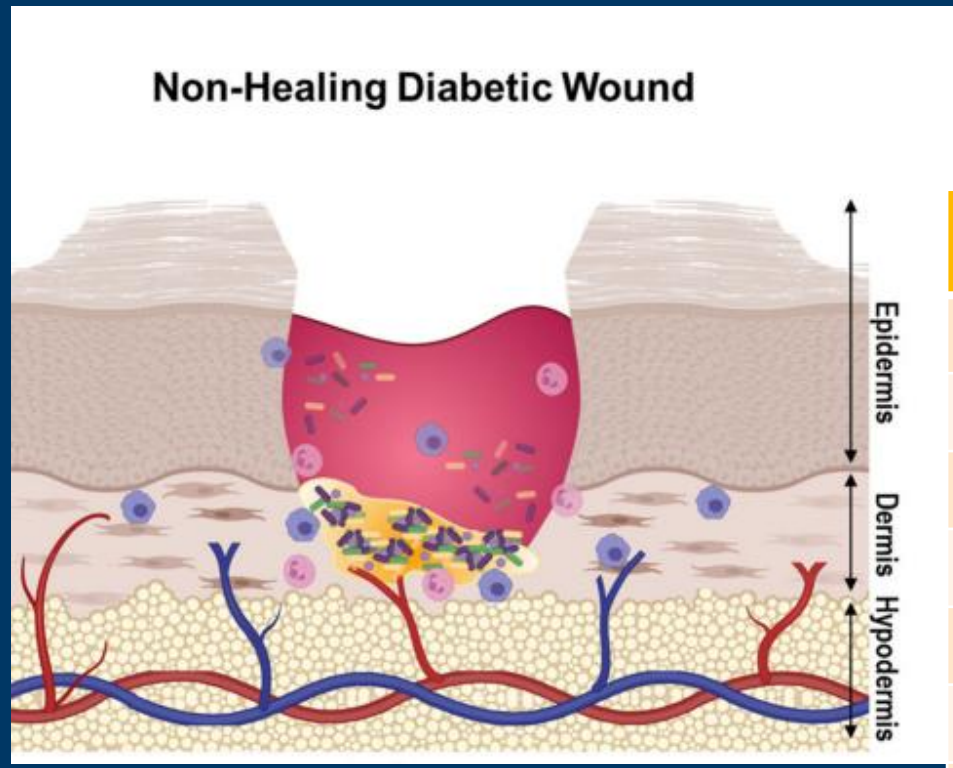
Nicolau and Stein. JAPMA. 2010. 100(1)

Pharmacokinetics of Antibiotic Use in Diabetic Foot Ulcers: **Levofloxacin**

Journal of Antimicrobial Chemotherapy (2004) **54**, 836–839
DOI: 10.1093/jac/dkh412
Advance Access publication 16 September 2004
JAC

Tissue and serum levofloxacin concentrations in diabetic foot infection patients

K. Oberdorfer[1]*, S. Swoboda[2], A. Hamann[3], U. Baertsch[3], K. Kusterer[4], B. Born[5], T. Hoppe-Tichy[2], H. K. Geiss[1] and H. von Baum[6]



METHODS

- 10 outpatients with diabetes and ulcers enrolled.
- All received oral levofloxacin.
- Levofloxacin concentration determined from wound tissue.

Patient Number	Tissue [ ] mg/kg	MIC Mg/L	Pathogens cleared?
D3	7.22	0.125	Yes
D6	2.33	<2.0	Yes
D7	15.76	<2.0	Yes
D8	23.23	<2.0	Yes
D9	15.36	No pathogens	Yes
D10	2.36	<2.0	Yes
D11	9.66	0.25	New pathogens with high levo MICs
D1	7.73	0.25	No
D2	14.14	2.0	No
D5	10.02	<2.0	Yes

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Pharmacokinetics of Antibiotic Use in Diabetic Foot Ulcers: **Tigecycline**

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2010, p. 5209–5213 Vol. 54, No. 12
0066-4804/10/$12.00 doi:10.1128/AAC.01051-10

Tissue Penetration and Pharmacokinetics of Tigecycline in Diabetic Patients with Chronic Wound Infections Described by Using *In Vivo* Microdialysis▽

Catharine C. Bulik,[1] Dora E. Wiskirchen,[1] Ashley Shepard,[2] Christina A. Sutherland,[1] Joseph L. Kuti,[1] and David P. Nicolau[1,3]*

Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, Connecticut[1]; Connecticut Surgical Group, Hartford Hospital, Hartford, Connecticut[2]; and Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut[3]

METHODS

- 8 Patients with Grade 2 or 3 DFU
- Simultaneous administration of other therapies permitted
- Measured tigecycline in uninfected thigh and wound

CONCLUSION

Tigecycline penetration into diabetic wounds does not differ from non-wound tissue.

TABLE 2. Steady-state pharmacokinetic parameters representing tigecycline concentrations in plasma, wound interstitial fluid, and uninfected thigh interstitial fluid samples[a]

Sample category	Parameter[b]						
	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-24} (μg · h/ml)	$t_{1/2}$ (h)	CL_{ss} (liters/h/kg)	V_{ss} (liters/kg)	Penetration[c] (%)
Plasma (total)	0.42 ± 0.11	1.13 ± 0.35	3.99 ± 0.75	9.73 ± 4.62	0.28 ± 0.09	3.95 ± 2.31	
Plasma (free)	0.16 ± 0.01	1.13 ± 0.35	2.65 ± 0.33				
Wound	0.16 ± 0.06	4.38 ± 3.38	2.60 ± 1.02	24.88 ± 28.67			100.00 ± 44.78
Thigh	0.18 ± 0.13	3.38 ± 3.54	2.52 ± 1.15	15.96 ± 13.2			98.94 ± 52.75

[a] Steady-state conditions consisted of a 100-mg loading dose and then 3 to 4 doses of 50 mg twice daily.
[b] C_{max}, peak concentration; T_{max}, time to reach peak concentration; CL[illegible] clearance at steady state; V[illegible] volume of distribution at steady state. Data are reported as means ± standard deviations. P values (representing statistical analysis o[illegible] $t_{1/2}$, 0.437; for percent penetration, 0.966.
[c] Percent penetration calculated as follows: $AUC_{thigh}/fAUC_{plasma} \times 10$[illegible]

Gill et al. JAC. 2022. 77(5)

Similar conclusions for Omadacycline

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Treatment and Outcome of DFU

38 patients were treated with outcomes measured

- 19 (49%) failed

Wound Severity	Number	Wound Duration	Treatment Failure Rate	Description of Microbiology
Moderate to Severe	33	> 6 weeks	15 (45%)	Polymicrobial anaerobes
Moderate	5	< 6 weeks	4 (80%)	Monomicrobial (Staph and Strep)

Antibiotic	Number	Wound Duration	Treatment Failure Rate
Narrow spectrum	9	> 6 weeks	4 (44.4%)
Broad spectrum	25	Not reported	11 (44%)

Conclusion

Treatment failure rates are >40% regardless of…
- Antibiotic used
- Wound severity or duration

The presence of … correlated with treatment failure… what was it?

Multi-drug resis… …nas

Streptococcus agalactiae

Malone et al. EBioMedicine. 2017. 142-149

What about empiric therapy?

Open Forum Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America | hivma hiv medicine association | OXFORD

Empirical Antibiotic Therapy in Diabetic Foot Ulcer Infection Increases Hospitalization

Brian M. Schmidt,[1] Keith S. Kaye,[2] David G. Armstrong,[3] and Rodica Pop-Busui[1]

[1]Division of Metabolism, Endocrinology, and Diabetes, Department of Internal Medicine, University of Michigan Health, Ann Arbor, Michigan, USA, [2]Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA, and [3]Department of Surgery, Southwestern Academic Limb Salvage Alliance (SALSA), Keck School of Medicine of University of Southern California, Los Angeles, California, USA

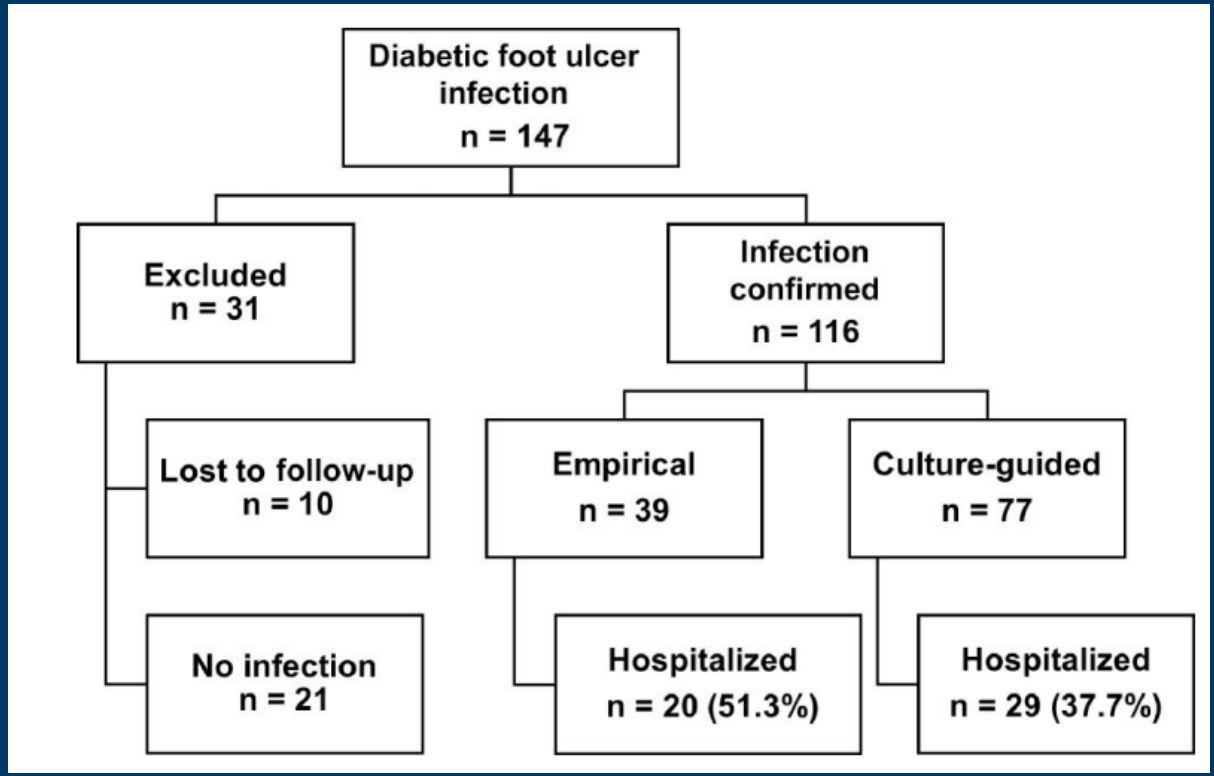
116 patients with infected DFUs

- 68% mild
- 26% moderate
- 6% severe

Treatment

- Empiric – 39
- Culture guided – 77

No demographic differences between these groups



Only noted for mild infections – which is counter intuitive.

- No difference in amputation or death.
- Could differences be due to uncontrolled variables?

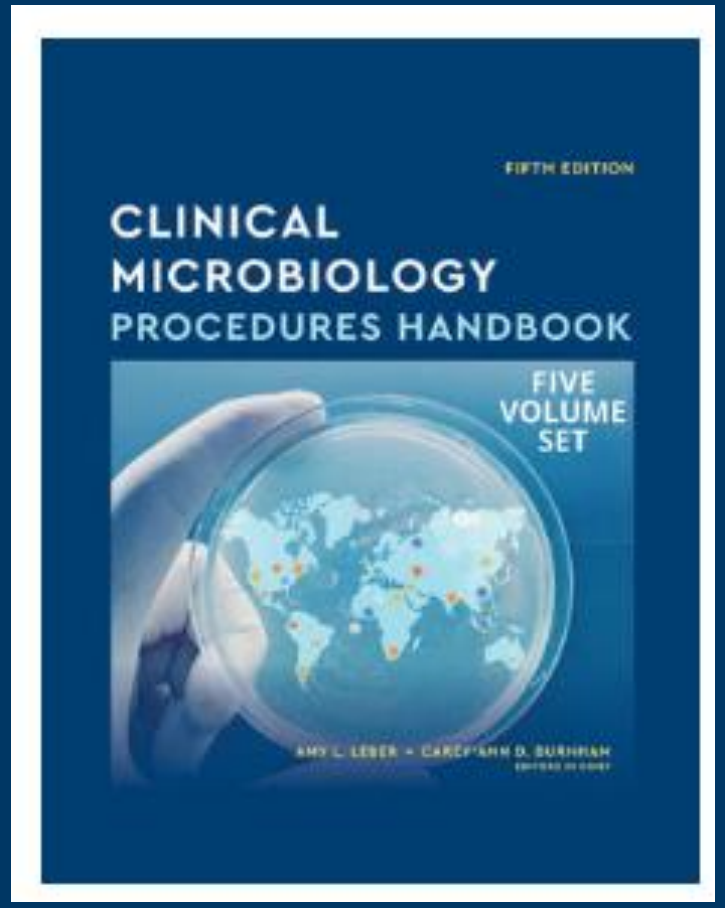
Summary of Treatment

- Outcomes in general appear to be poor. ~40% failure rate
- Surgical debridement improves outcome
- Inverse relationship between the microbiology and wound severity
- It appears as though antibiotics do penetrate the diabetic wound environment, but these studies may be flawed
- Empiric treatment *might* be inadequate

What does all this mean for the role of culture?

Culture Work Up

- **4.** From invasively collected specimens from normally sterile sites, identify up to three microorganisms according to the criteria in Table 3.12.1-2. Exception, work-up all organisms listed as "Any quantity" in Table 3.12.1-2.
- **5.** For noninvasively collected, good-quality specimens, with Gram stain evidence of infection (presence of PMNs and/or few epithelial cells), identify up to two microorganisms according to the criteria in Table 3.12.1-2. Exception, work-up all organisms listed as "Any quantity" in Table 3.12.1-2.
- ◩ **NOTE:** Definition: good quality wound specimen - PMNs in direct smear or a history of diabetes or immunocompromised condition; Poor-quality wound specimen - moderate or numerous squamous epithelial cells on direct smear or no PMNs.



How many organisms to work up?

- Three organisms from sterile sites
- Two organisms from non-sterile sites
- Literature review…

We made it up ☺

Aeromonas hydrophila	Rare, Well-defined pathogen	Any growth
C. perfringens	Common pathogen	Any growth
Pasteurella spp.	Rare, Well-defined pathogen	Any growth
Capnocytophaga spp.	Rare, Well-defined pathogen	Any growth
Aracanobacterium haemolyticum	Rare, Well-defined pathogen	Any growth**
Bacillus anthracis	Rare, Well-defined pathogen	Any growth**
Corynebacterium diphtheriae	Rare, Well-defined pathogen	Any growth**
Nocardia spp.	Rare, Well-defined pathogen	Any growth**
Mycoplasma hominis	Rare, Well-defined pathogen	Any growth**
Vibrio vulnificus	Rare, Well-defined pathogen	Any growth**
Clostridium septicum	Rare, Well-defined pathogen	Any growth**
Clostridium novyi	Rare, Well-defined pathogen	Any growth**
Clostridium sordelli	Rare, Well-defined pathogen	Any growth**
Corynebacterium kroppenstedtii	Rare, Well-defined pathogen	Any growth**

** Although any growth of these organisms would be clinically significant, laboratories should not identify all organisms in a wound culture to ensure that these organisms are not missed. As a practical matter, laboratories can assume that if these organisms are disease causing, they will be cultured in predominant quantities (i.e. - greater than commensal flora).

Doern et al. Journal of Nonsense. How to make things up out of thin air. 2024. 234(5)

Using Relative Abundance to Guide Work Up

From Table 3.12.2 in CMPH

Coagulase negative staphylococci	Commensal, Potential pathogen	Pure	ID only, AST on request or if hardware associated, report as mixed flora if not significant
Enterococci	Commensal, Potential pathogen	Pure or predominant	ID and AST, report as mixed flora if not significant
Enterobacterales	Commensal, Potential pathogen	Pure or predominant	ID and AST, report as mixed flora if not significant
Bacillus spp. (Not Anthrax)	Commensal, Potential pathogen	Pure	ID
Eikenella spp.	Commensal, Potential pathogen	Pure or predominant	ID, AST for deep tissue infections
Glucose non-fermenting Gram negatives	Commensal, Potential pathogen	Pure or predominant	ID and AST
Stenotrophomonas maltophilia	Commensal, Potential pathogen	Pure or predominant	ID and AST

Let's look at some examples.

Microbiology Culture Examples:
Tissue biopsy from Diabetic foot ulcer

Gram Stain Result

Many PMNs
GNRs, GPCs,
Few Squamous Epis

Culture Result

2+ E. coli
2+ Enterococcus faecium
2+ Coagulase Negative Staphylococci
2+ Streptococcus agalactiae (GBS)
2+ Finegoldia magna

Suggested Reporting

Mixed aerobic and anaerobic bacteria resembling intestinal flora including:
2+ E. coli
2+ E. faecium
2+ S. agalactiae

Susceptibility Testing

Performed Upon Request.

Microbiology Culture Examples:
Tissue biopsy from Diabetic foot ulcer

Gram Stain Result

Many PMNs
GNRs, GPCs,
No Squamous Epis

Culture Result

4+ *Enterococcus faecium*
2+ *P. aeruginosa*
2+ *Stenotrophomonas maltophilia*
2+ *Bacteroides fragilis* group
2+ *Parvimonas* spp.

Suggested Reporting

4+ *Enterococcus faecium*
2+ *P. aeruginosa*
Mixed aerobic and anaerobic bacteria including:
2+ *Stenotrophomonas maltophilia*
2+ *Bacteroides fragilis* group

Susceptibility Testing

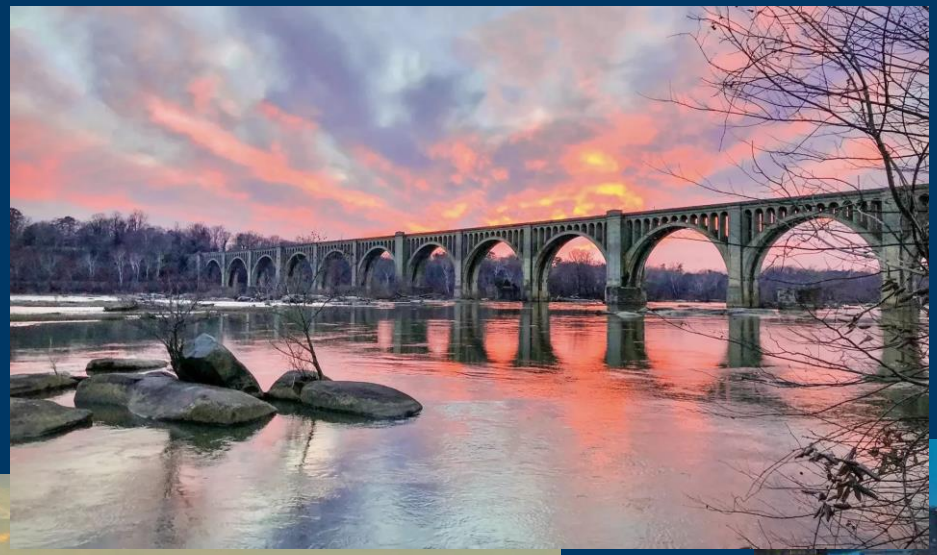
Susceptibility testing performed on the *Enterococcus faecium* and *P. aeruginosa* as the principle pathogens.

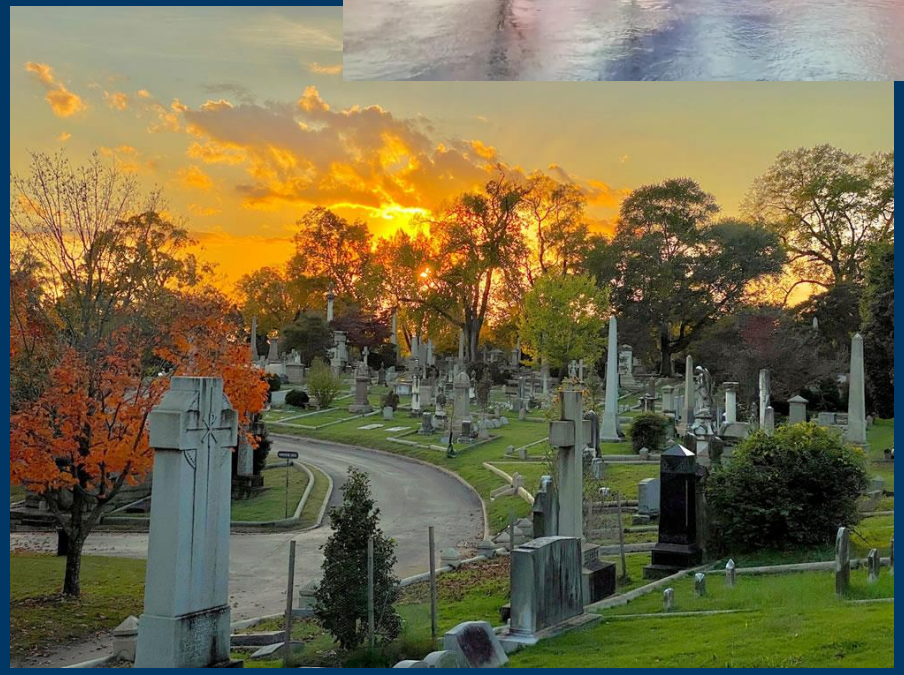
Some last comments

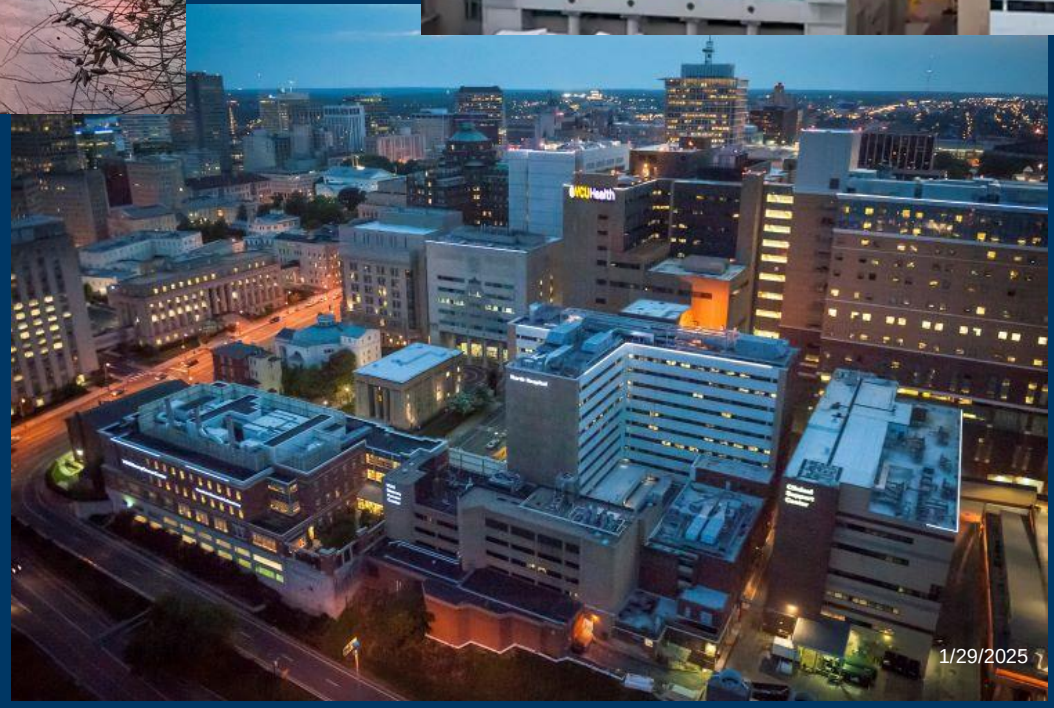
- Diabetic foot infections are often polymicrobic.
- Metagenomic analyses have shown that culture commonly fails to grow all organisms, especially obligate anaerobes.
  - Cultures do not represent a complete picture of a patient's infection.
- Specimen quality can be variable and may be submitted from clinically unifected lesions.
  - Excessive work-up of these cultures can lead to unnecessary antibiotic therapy.
- Per IDSA guidelines, empiric therapy should cover *P. aeruginosa* if present so laboratories should be sure to work-up any amount of this organism.
- Encourage thoughtful culture practice and optimal specimen types.

Thank you for your attention.

Questions – email christopher.doern@vcuhealth.org







Bonus Material

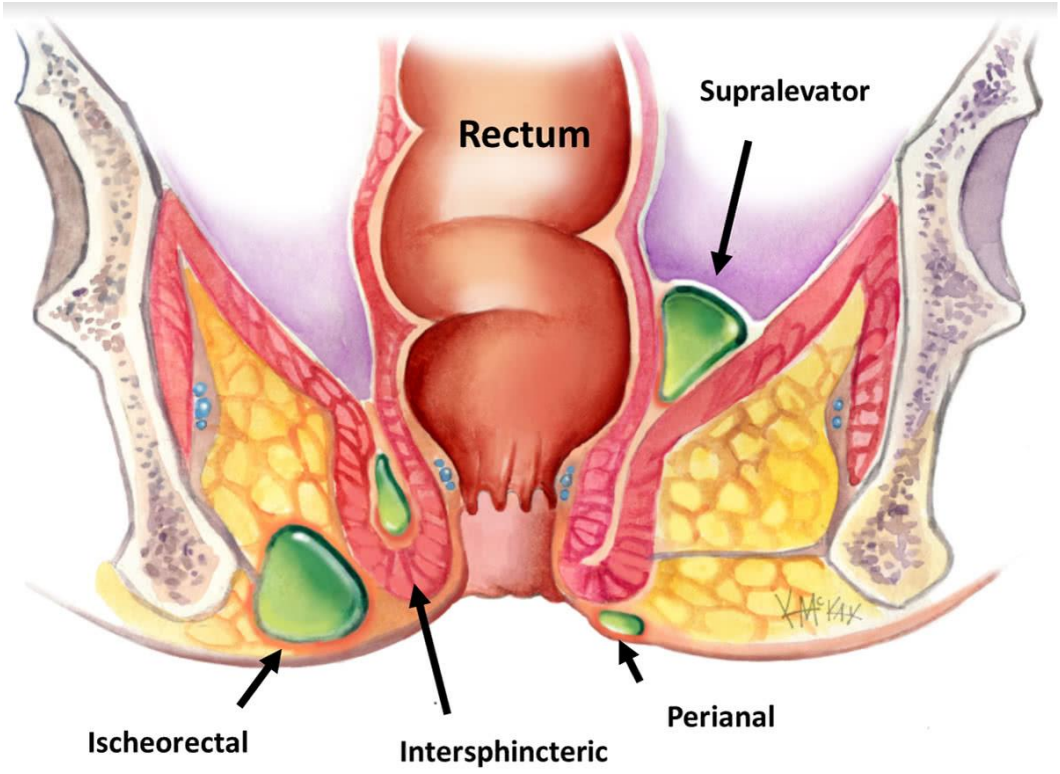
Case

- A 56 year old otherwise healthy male presents with perianal swelling, pain and tendernous.
- Physical exam noted a small, erythematous, well-defined, subcutaneous mass near the anal orifice consistent with an anorectal abscess.
- Patient had a low-grade fever but all other vital signs were normal.
- An incision and drainage was performed and purulent material was collected and sent to the laboratory for aerobic and anaerobic bacterial culture.

Anorectal Abscesses

- The problem
  - Common complaint in the ED
  - Estimated 100,000 cases/year in US
  - Likely underestimate due to misdiagnosis as hemorrhoids.
  - If not diagnosed and treated can progress to “anal sepsis”
  - Can lead to a fistula in ~25% of patients.

- Pathophysiology



Techniques in Coloproctology (2020) 24:127–143
https://doi.org/10.1007/s10151-019-02144-1

ORIGINAL ARTICLE

Evaluation and management of perianal abscess and anal fistula: SICCR position statement

A. Amato[1] · C. Bottini[2] · P. De Nardi[3] · P. Giamundo[4] · A. Lauretta[5] · A. Realis Luc[6] · V. Piloni[7]

Statement: the treatment of anal abscess is surgical incision and drainage

Grade of recommendation: 1B

Statement: antibiotic therapy is unnecessary in uncomplicated anorectal abscess but can prevent fistula-in-ano after incision and drainage of simple anal abscess

Grade of recommendation: 1B

What does this mean for the value of culture?

Antibiotic therapy for prevention of fistula in-ano after incision and drainage of simple perianal abscess: A randomized single blind clinical trial

Table II. Comparison of baseline and surgery related characteristics of patients based on fistula formation

Variable	*Fistula development**			
	Yes	*No*	*Total*	P *value*
Total no. (%)	67 (22.3)	233 (77.7)	300 (100)	
Group*				
1	22 (14.2)	133 (85.8)	155 (100)	<.001
2	45 (31.3)	99 (68.8)	144 (100)	

Cipro and Metronidazole Prophylaxis
Group 1 – Abx
Group 2 – No Abx

Ghahramani et al. Surgery. 2017. 162(5)

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1997, p. 2974–2976 Vol. 35, No. 11
0095-1137/97/$04.00+0

The Aerobic and Anaerobic Bacteriology of Perirectal Abscesses

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Received 23 May 1997/Returned for modification 15 July 1997/Accepted 21 August 1997

TABLE 1. Aerobic and anaerobic organisms recovered in 144 perirectal abscesses

Organism(s)[a]	No. of isolates
Aerobic organisms	
Streptococcus	
α-Hemolytic	6
γ-Hemolytic	2
Group A	9
Group B	2
Group D	9
S. aureus	34
Coagulase-negative staphylococci	6
N. gonorrhoeae	2
Proteus spp.	12
Pseudomonas aeruginosa	4
Other *Pseudomonas* species	3
E. coli	19
Klebsiella pneumoniae	3
Enterobacter species	1
Other gram-negative rods[b]	16
Lactobacillus spp.	3
Total	131
Anaerobic organisms	
P. magnus	11
P. anaerobius	12
P. asaccharolyticus	14
P. prevotii	6
P. saccharolyticus	1
P. micros	15
Other *Peptostreptococcus* spp.	13
Streptococcus intermedius	2
Veillonella parvula	4
Veillonella alcalescens	2
Eubacterium lentum	9
Other *Eubacterium* spp.	2
Propionibacterium acnes	2
Lactobacillus spp.	1
Clostridium perfringens	4
Clostridium butyricum	1
Other *Clostridium* species	10
Fusobacterium nucleatum	6
Fusobacterium mortiferum	2
Other *Fusobacterium* species	13
*Bacteroides fragilis**	58
*Bacteroides distasonis**	4
*Bacteroides ovatus**	9
*Bacteroides vulgatus**	3
*Bacteroides thetaiotaomicron**	11
Prevotella melaninogenica	18
Prevotella intermedia	12
Prevotella oris-buccae	2
Prevotella ureolytica	17
Prevotella oralis	2
Prevotella bivia	14
Prevotella disiens	6
Porphyromonas asaccharolytica	20
Other *Bacteroides* species	19
Total	325

[a] Species marked with an asterisk all belong to the *B. fragilis* group.
[b] Other gram-negative rods include *Klebsiella* spp. other than *K. pneumoniae*, *Citrobacter* spp., *Providencia* spp., *Morganella* spp., *Acinetobacter* spp., and *Aeromonas* spp.

Microbiology Culture Examples:
Perirectal abscess material collected during I&D

Gram Stain Result

Many PMNs
Moderate GNRs, GPCs, and GPRs
Few Squamous Epis

Culture Result

2+ E. coli
2+ Enterococcus faecium
2+ Coagulase Negative Staphylococci
2+ Corynebacterium spp.
2+ Finegoldia magna

Suggested Reporting

Mixed aerobic and anaerobic bacteria resembling mixed intestinal and skin flora.

Comment: The presence of *S. aureus*, beta-hemolytic streptococci, *P. aeruginosa,* and significant growth of other pathogens has been ruled out.

Susceptibility Testing

Not performed.

Microbiology Culture Examples:
Perirectal abscess material collected during I&D

Gram Stain Result	Suggested Reporting
Many PMNs Moderate GNRs, GPCs, and GPRs Few Squamous Epis	1+ *S. aureus* Mixed aerobic bacteria resembling intestinal flora.

Culture Result	Susceptibility Testing
4+ E. coli 3+ Enterococcus faecium 1+ *S. aureus*	Perform on *S. aureus*.

Summary of Anorectal Culture Workup

- Expect intestinal flora – It would be weird if it wasn’t there.
- Don’t overwork these cultures.
  - Look for key pathogens and clear predominance of possible pathogens.