Financial Disclaimers

- None

Personal Disclaimers

- I have no AP training
- I have 2 young children (under 4)
- They did not tolerate the time change well
- Our power was knocked out yesterday
- My basement flooded last night
- I bailed it out last night thru this AM (and just before coming here).

Case report

- 50-year-old woman from northern Mexico with a diagnosis of diabetes presented with a 12-year history of a gradually enlarging right foot demonstrating multiple draining lesions.
- Reported a long course of originally pruritic lesions on the plantar instep of her right foot, which over many years developed into multiple bulbous nodules with central pinpoint ulcerations expressing purulent and granular material.
- Reports having to gradually had to modify her footwear to accommodate the increasing size of her foot, which at the time of presentation was causing her great pain.
- History of repeated / failed treatments, complicated by patient compliance
Case report

- Presented to our ID clinic for further evaluation of (now advanced) disease and treatment recommendations after multiple rounds of failed therapy at outside institutions
- Punch biopsy performed

Patient at “presentation”
### Pathology results
- Punch biopsy
  - Inclusion of filamentous organisms observed on H&E
  - Negative by GMS stain
  - Differential?

### Differential Diagnosis
- Actinomycetoma
- Chronic bacterial osteomyelitis
- Tuberculosis
- Buruli ulcer

### Biopsy submitted for culture
- Specimen submitted to microbiology for identification of organism
- Results:
  - 4+ *Staphylococcus aureus*
  - Unable to hold culture for actinomycetes-like organism
Secondary biopsy submitted for culture

- Results:
- 4+ *Staphylococcus aureus*
- Unable to hold culture for actinomycetes-like organism

Biopsy submitted for molecular diagnosis

- 16S rRNA sequencing
- Results:
  - *S. aureus*
  - 326 bp sequence
  - 100% identity
  - 100% coverage
  - Robust species level classification

Differential Diagnosis

- Actinomycetoma
- Botryomycosis
- Chronic bacterial osteomyelitis
- Tuberculosis
- Buruli ulcer

Microbiology Summary

- All diagnostic specimens returning *S. aureus*
- Filamentous organism more consistent with actinomycetoma
- Next step?
Next-generation Sequencing for Infectious Disease

- High throughput sequencers which can generate gigabase-scale data
- Next-generation sequencing produces information from millions of individual DNA molecules simultaneously
  - Can independently sequence and classify isolated DNA fragments
  - Allows enumeration of individual organisms present
  - Complex mixtures can be deconvoluted
  - Low-prevalence organisms can be detected

Next-generation unbiased shotgun sequencing

Next-generation 16S rRNA sequencing

- In 2013 we developed and clinically validated an NGS application for molecular diagnosis of polymicrobial specimens (NGS16S)
- PCR amplification and sequencing of 16S rRNA
  - Does not amplify human DNA
  - Can function with degraded specimens or trace bacterial DNA
  - Direct testing for non-sterile sites or reflex testing for polymicrobial specimens that are interpretable by Sanger diagnosis.

Next generation sequencing results

- Top hit is *S. aureus*
- *A. madurae* recovered
  - Low [relative] abundance likely reflects organism burden in the tissue section submitted for diagnosis
Primer on Mycetoma

- Chronic subcutaneous infection caused by filamentous bacteria (actinomycetoma) or fungi with thick, septate hyphae (eumycetoma)
- Endemic to Central and South America, Africa, and India.
- Characterized by “grains” or “granules” composed of aggregates of the infecting organism, expelled through skin via draining sinuses.
- More than 30 species of bacteria and fungi have been documented to cause mycetoma (J Glob Infect Dis. 2009 Jan-Jun; 1(1): 64–67).

What is the causative organism?

- In Central America, most common bacterial agents are:
  - Nocardia brasiliensis
  - Streptomyces madurae
  - Actinomadura madurae
- Actinomadura madurae is most common bacterial cause worldwide.
- Most common fungal agents are:
  - Madurella mycetomatis
  - Scedosporium boydii

Characteristics of grains help to ID organism

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the grains.</th>
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A *little* help from stains

- **Gram stain**
  - *Nocardia* are classically Gram-variable
  - Can appear uniformly gram negative if organism is stressed
  - *Actinomadura madurae* is Gram-negative
  - *Streptomyces* species are Gram-positive

- **Other stains**
  - *Nocardia* are weakly positive by Fite-Faraco modified acid-fast stain
  - “Unique” characteristic in differentiating actinomycetomas
  - HOWEVER: reduced mycolic acid expression if organism is stressed
  - Not reliable for speciation

- Definitive diagnosis for all organisms (fungal or bacterial) is achieved by microbiology with **extended culture**
  - Alert the lab!

Outcome

- Patient disease considered too advanced for further antibiotic treatment, agreed to below-the-knee amputation

- Culture on intraoperative specimens grew *A. madurae*, confirming next-generation sequencing diagnosis

- NGS can be used for focused identification of organisms of interest in a complex, polymicrobial specimen
- Can overcome biological “contamination” to reveal organisms of interest