



Microbiology Case Studies

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Objectives

1. Correlate patient's clinical signs and symptoms with clinical laboratory results.
2. Identify organisms based on microbiology culture results.
3. Discuss the diseases associated with each pathogen identified.

Case # 1

66 yo male presents to ED with back pain, fever, chills on 8/12/20

Previous medical history of hepatocellular carcinoma, HCV, COPD (chronic obstructive pulmonary disorder), and hypertension

MRI showed abnormalities – evidence of metastatic disease, but cannot rule out infection

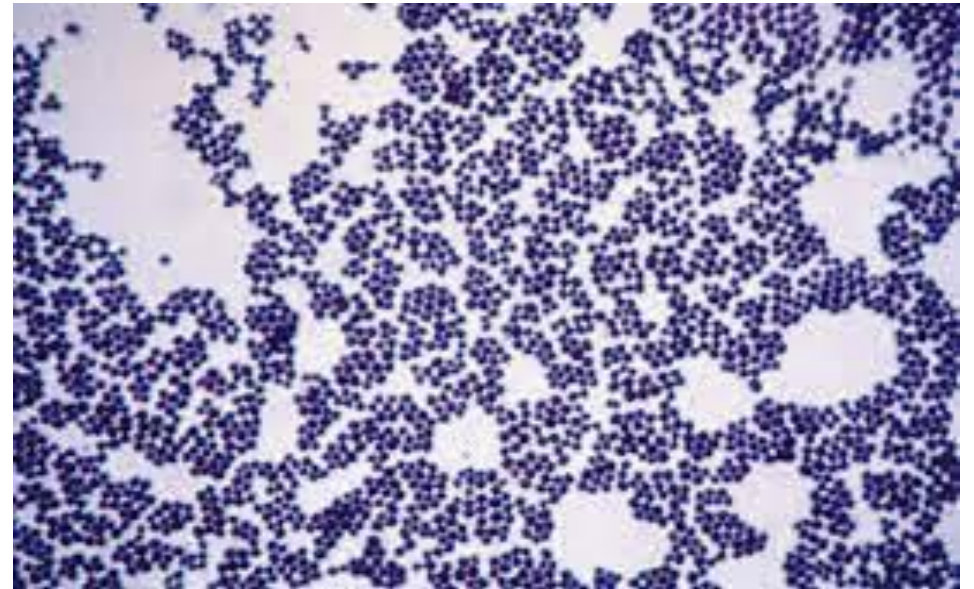
Patient initially started on Ceftriaxone due to history of *Streptococcus pyogenes* bacteremia

Case # 1

Blood and urine were collected for cultures.

Blood cultures turned positive with

Gram positive cocci in clusters.



Case # 1

Growth on blood and urine cultures:

Yellow colonies – beta hemolysis

GPCCL

Catalase positive

BactiStaph Latex positive

Set up Vitek MS and AST card



Case # 1

Pathogen is...

Staphylococcus aureus

Case # 1

ID: Staph aureus

Susceptibility:

Blood

Susceptibility

	Staphylococcus aureus LCMC LAB VITEK
Oxacillin	≥ 4 mcg/ml Resistant
Vancomycin	1 mcg/ml Susceptible

Urine

Susceptibility

	Staphylococcus aureus LCMC LAB VITEK
Nitrofurantoin	≤ 16 mcg/ml Susceptible
Oxacillin	≥ 4 mcg/ml Resistant
Tetracycline	≤ 1 mcg/ml Susceptible
Trimethoprim/Sulfamethoxazole	≤ 10 mcg/ml Susceptible
Vancomycin	1 mcg/ml Susceptible

Case # 1

Positive blood cultures for 2 weeks

First negative blood cultures were collected on 9/1/20.

T12/L1 bone biopsy cultures collected on 9/2/20 – No growth

Patient is finally discharged on 9/8/20.

Case # 1

Patient returns to ED on 11/4/20 with abdominal swelling.

Abdominal paracentesis is performed, and fluid is sent for cultures.

***Klebsiella pneumoniae* – ESBL positive grows out.**

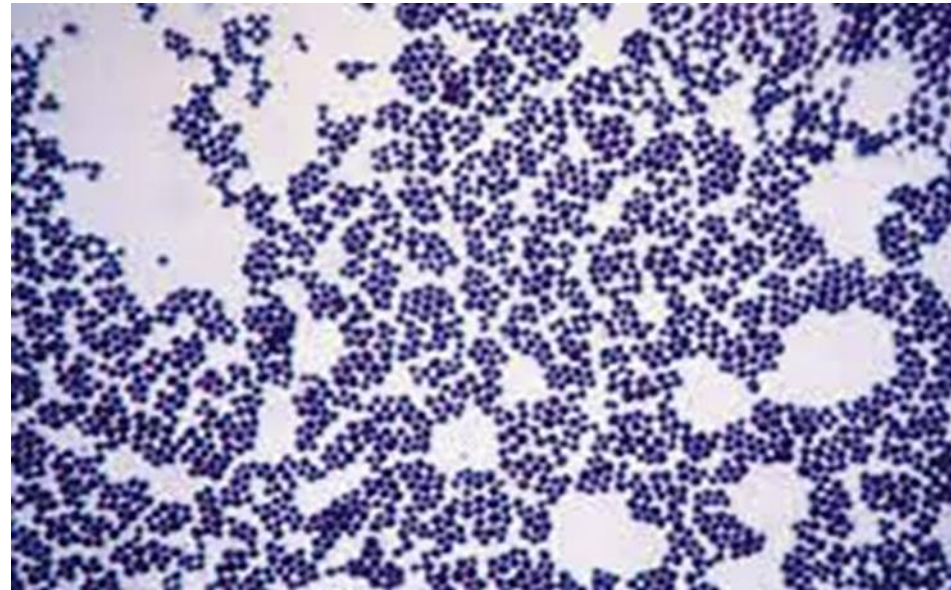
Blood cultures also collected.

Case # 1

Blood cultures turn positive. What do we see on Gram stain?

Do we see Gram negative bacilli?

GPCCL



Case # 1

Growth on blood cultures:

Yellow colonies – beta hemolysis

GPCCL

Catalase positive

BactiStaph Latex positive

Set up Vitek MS and AST card



Case # 1

ID: Staph aureus

Susceptibility:

Blood

Susceptibility

	Staphylococcus aureus LCMC LAB VITEK	
Oxacillin	>=4 mcg/ml	Resistant
Vancomycin	4 mcg/ml	Intermediate

Vancomycin confirmed by Etest and Microscan.

Case # 1

From CLSI M100-30th edition:

Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Staphylococcus spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
				S	SDD	I	R	S	SDD	I	R	
GLYCOPEPTIDES												
(20) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of <i>S. aureus</i> from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of <i>Staphylococcus</i> spp. other than <i>S. aureus</i> , all of which give similar size zones of inhibition.												
B	Vancomycin	<i>S. aureus</i>	—	—	—	—	—	≤2	—	4–8	≥16	(21) For <i>S. aureus</i> , vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. (22) Send any <i>S. aureus</i> for which the vancomycin is ≥8 µg/mL to a referral laboratory. See Appendix A.

Case # 1

Due to elevated MIC to Vancomycin, Daptomycin and Ceftaroline were added to antibiotic therapy.

Daptomycin was the first approved antibiotic in the new class of lipopeptides and exhibits broad spectrum activity against Gram-positive bacteria, including MRSA and extensively-resistant strains, such as Vancomycin-intermediate *S. aureus* (VISA) and Vancomycin-resistant *S. aureus* (VRSA), and Vancomycin-resistant *Enterococcus* (VRE).

Ceftaroline is a new fifth-generation cephalosporin, which exhibits broad-spectrum activity against Gram-positive bacteria, including MRSA and extensively-resistant strains, such as VISA and VRSA.

Case # 1

Patient was discharged on 12/2/20.

Remained on antibiotics:

- Vancomycin – 6 weeks
- Daptomycin – 8 weeks
- Gentamicin – 10 days
- Ceftaroline

Concern for osteomyelitis – etiology of bacteremia was unclear

Vancomycin levels (and kidney function) were closely monitored by Antimicrobial Stewardship PharmD

Follow-up visits: Improvement

Case # 2

45 yo male presents to ER with arm pain and rash

- Gardener
- Symptoms started 2 weeks ago after landscaping
- Denies spider/insect bite
- Lesions slowly tracking up right forearm, moving up above elbow
- Some of the lesions are beginning to rupture

Case # 2

Patient is diagnosed with _____ infection, but ER physician also wants to cover cellulitis.

- Discharged with prescriptions for Itraconazole and Clindamycin.

Case # 2

10 days later.....Patient returns to ER

Lesions are now “necrotic, raised, tender, ulcerated, located on the palmar aspect of right forearm and dorsal aspect of right forearm”.

Case # 2



Case # 2

Patient is admitted.

It is discovered that patient only filled prescription for Clindamycin. He said he could not afford the Itraconazole prescription.

Itraconazole:

Walmart: \$152.60

Target, Winn Dixie, CVS, Walgreens: ~\$200

Clindamycin:

Walmart, Target, Winn Dixie, CVS, Walgreens: \$35 - 50

Case # 2

Physician collected fluid from pustules and performed punch biopsies.

Both were sent to lab for aerobic, anaerobic, fungal, and AFB cultures.

CBC

Fungal antibodies

Case # 2 – Lab Results

CBC:

- WBC 11.8 H
- RBC 5.04
- Hgb 15.9
- HCT 45.8

Normal Ranges

4.0-10.0 K/uL
4.10-5.80 M/uL
13.0-17.5 g/dL
39.0-52.0%

Case # 2 – Lab Results

Differential:

Neutrophils 71 H

Lymphocytes 14 L

Monocytes 6

Eosinophils 8

Basophils 1

Normal Ranges

32-64%

25-48%

4-6%

2-3%

0-1%

Case # 2 – Lab Results

Fungal Antibodies:

◦ Aspergillus spp. Ab	None detected
◦ Blastomyces dermatitidis Ab	None detected
◦ Coccidioides immitis Ab	None detected
◦ Histoplasma spp.	None detected

Case # 2

Which Microbiology cultures will be positive?

Clues:

Gardener

Didn't fill Itraconazole

Case # 2 – Lab Results

Microbiology:

Aerobic cultures

No growth

Anaerobic cultures

No growth

AFB cultures

No growth

But.....

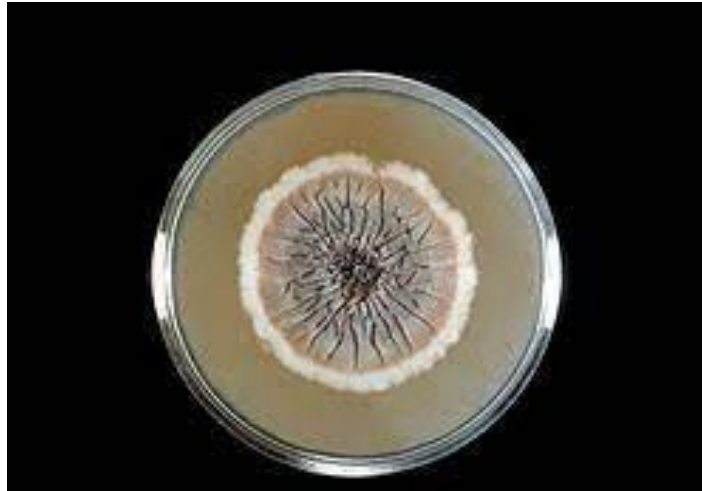
Fungal cultures

Positive

Case # 2

Fungal cultures:

Mold growing after 5 days of incubation

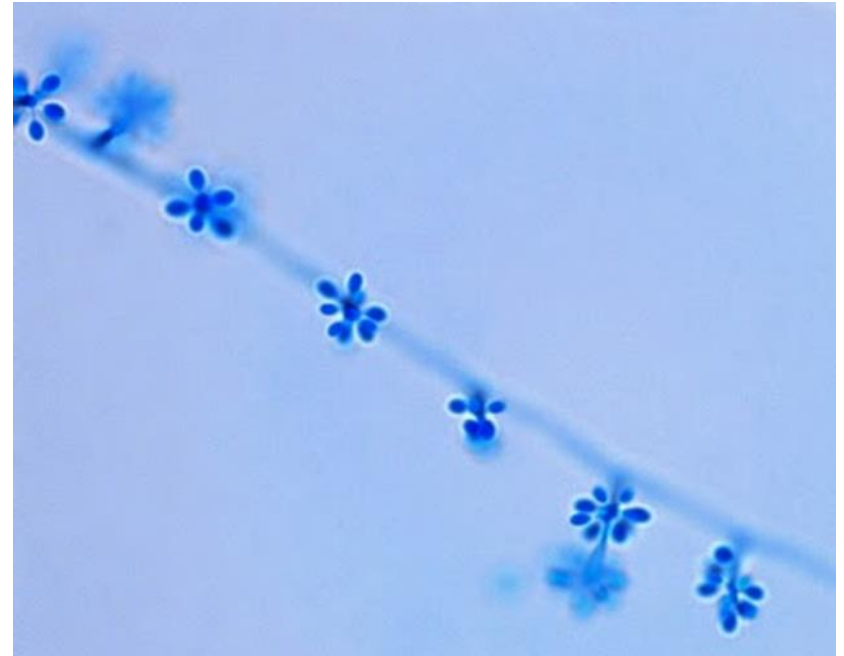
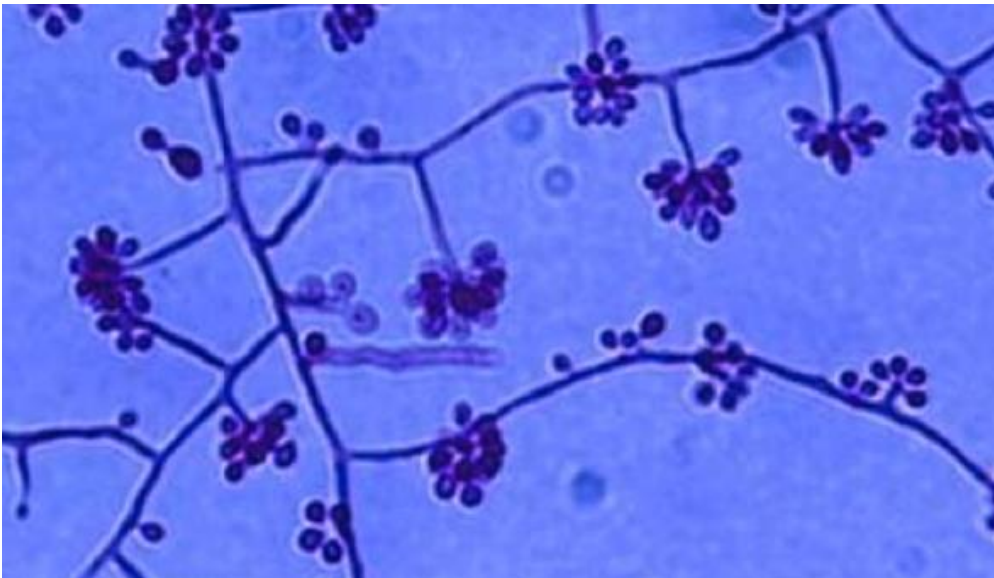


Surface: White periphery with black center, wrinkled

Case # 2

LPCB - 30°C incubation

LPCB: Rosette clusters



Case # 2

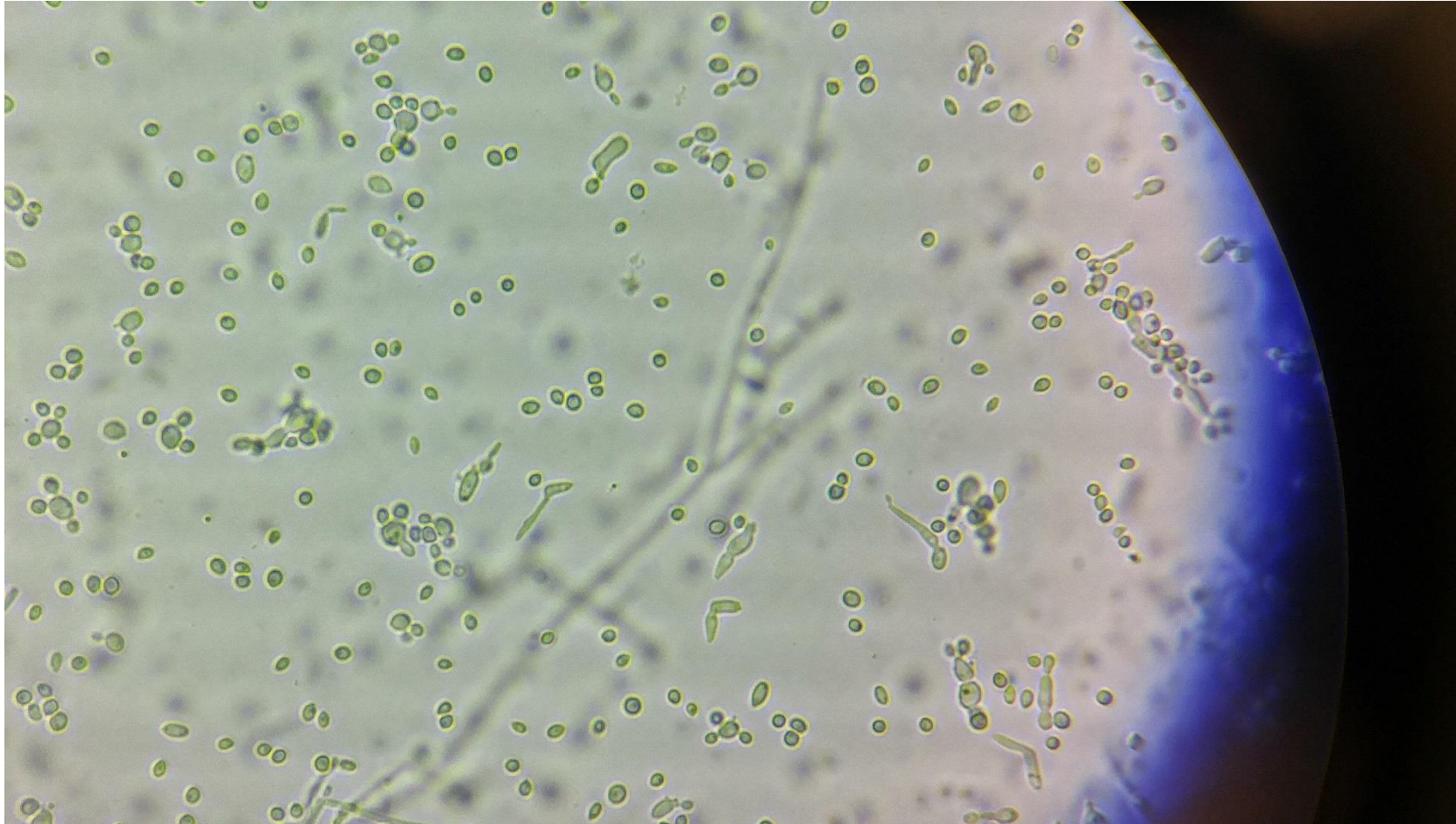
Fungal cultures:

Dimorphic mold:

- Converted to yeast phase at 35°C
- Fusiform budding cells, “cigar bodies”

Case # 2

LPCB - 35°C incubation



Case # 2

Pathogen is...

***Sporothrix schenckii* complex**

Case # 2 - *Sporothrix schenckii* complex

- Grows moderately rapid, usually within 7 days
- Thermally dimorphic, mold phase @ 30°C and yeast phase @ 35°C
- Surface is initially white to pale orange, then darkens and wrinkles
- Narrow septate hyphae with branching
- Slender conidiophores, right angles from hyphae
- Small tear-shaped or round conidia that form “rosettes”
- Yeast phase: round, oval, and fusiform budding cells form “cigar bodies”

Case # 2

Sporotrichosis

- Causes a chronic infection that begins as lesions of the skin and subcutaneous tissue
- Lymphatic channels and lymph nodes – draining the infected area
- Found in soil and decaying matter
- Results from skin puncture by contaminated material (thorns, moss, etc.)
- Rose Handler's Disease
- Can also cause pulmonary sporotrichosis after inhalation
- Rare: fatal in immunocompromised patients

Case # 2

Sporotrichosis

Patient improved and was discharged after treatment with Itraconazole.



Case # 3

83 yo male presents to ER with symptoms of nausea, fever, and left hand pain.

Patient's left ring finger was cyanotic with cellulitis.

History of multiple malignancies (lung, colon, prostate) – still undergoing chemotherapy.

Denies exposure to brackish water, but did “clean” fish wearing gloves.

Case # 3

ER physician - diagnosis

- Sepsis with septic shock
- Cellulitis
- Possible necrotizing fasciitis

Begins antibiotic therapy (Cipro and Pip/tazo) and admits patient.

Case # 3 – Lab Results

CBC:

- WBC 62.4 HH
- RBC 3.14 L
- Hgb 9.6 L
- HCT 28.2 L

Normal Ranges

4.0-10.0 K/uL
4.10-5.80 M/uL
13.0-17.5 g/dL
39.0-52.0%

Case # 3 – Lab Results

Differential:

Neutrophils 96 H

Lymphocytes 1 L

Monocytes 1 L

Eosinophils 0 L

Basophils 0

Normal Ranges

32-64%

25-48%

4-6%

2-3%

0-1%

Case # 3

Lactic acid:

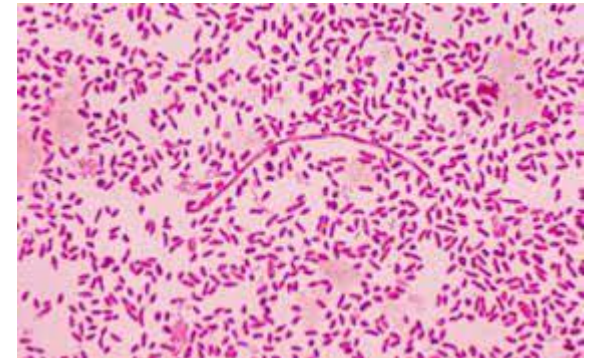
- I-Stat **2.8 H**

Normal range

0.5 – 2.2 mmol/L

Positive blood cultures – 2 sets drawn in ED:

- **Gram negative bacilli**



Case # 3

- On day 3 of admission, patient has I&D (incision and drainage) surgery on left ring finger.
 - Could not perform surgery upon admission due to heparin therapy
 - Found “purulent fluid around the flexor tendon sheath and copious cloudy fluid in dorsal hand”
 - Added Ceftriaxone and Doxycycline to antibiotic therapy
 - Sheath was flushed with saline
 - Penrose drain was inserted

Case # 3

- Infection of Flexor Tendon Sheath
 - Body has limited ability to fight off infection
 - No blood supply to the lubricating synovial fluid within the tendon sheath
 - Immune system – ineffective
 - Must be diagnosed quickly and treated aggressively

Case # 3

- Kanavel's Cardinal Signs of Infected Flexor Tendon Sheath:
 - Swelling of finger
 - Tenderness along the tendon sheath
 - Limited movement with the finger held slightly bent
 - Pain when attempting finger movement

Case # 3

Fluid collected during surgery was sent to Microbiology for cultures.

- Gram stain showed **many Gram negative bacilli**

Meanwhile, positive blood cultures have been worked up, and organism was identified.

Case # 3

Positive blood culture bottles were subbed to BAP, Choc, Mac.

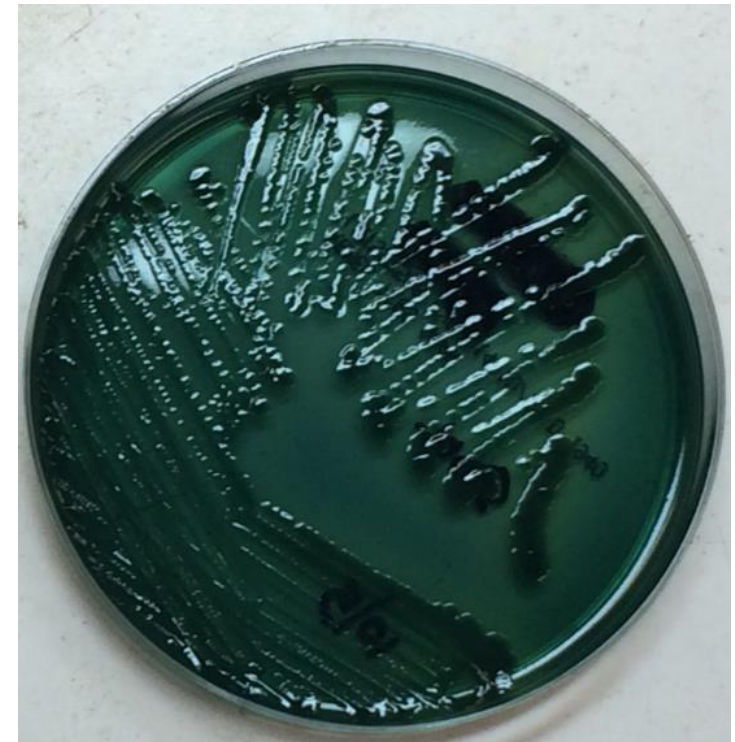
Oxidase = Positive

Set up Vitek MS and AST card.

Tech sees ID results, and it'swait – WHAT?

Isolates organism to TCBS to confirm.

Growth on TCBS: green colonies



Case # 3

Pathogen is...

Vibrio vulnificus

Case # 3

According to the CDC, there are approximately 80,000 illnesses and 100 deaths caused by *Vibrio* species in the United States.

Infection occurs by consuming raw or undercooked shellfish, especially oysters, or by exposing an open wound to brackish or saltwater.

Months with “R” rule

Case # 3

Recovery from mild cases of vibriosis can occur within 3 days with no complications.

Vibrio vulnificus can cause serious illness and may lead to limb amputation.

1 out of 4 patients with *V. vulnificus* infections die - in as little as 2 days of showing symptoms.

Case # 3

Update on patient:

Patient recovered from sepsis and septic shock; however, his finger had to be amputated.

Case # 4

50 yo male sees PCP with complaints of joint pain in right index finger.

- Treated with anti-inflammatory medication
- Returns to PCP – not resolved
- Referred to orthopedic surgeon
- Steroid injection to joint
- Surgery – sent specimens for culture (aerobic, anaerobic, fungal, and AFB)

Case # 4

Microbiology culture results:

- | | |
|---------------------|-----------|
| ◦ Aerobic culture | No growth |
| ◦ Anaerobic culture | No growth |
| ◦ Fungal culture | No growth |

But...

- | | |
|---------------|--|
| ◦ AFB culture | Positive after 2 weeks of incubation in liquid broth, then on LJ slant @30°C after 3 weeks |
|---------------|--|

Case # 4

Infectious disease visits the lab after we call her with the + AFB culture result.

Patient cleaned his mother's aquarium.

Yes, this is an easy one.....

Case # 4

Pathogen is....

Mycobacterium marinum

Case # 4

Patient was treated aggressively by ID physician.

- Infection was in the joint.
- Amputation was considered because he was at risk for losing his hand.
- Patient was very fortunate – did not have to amputate, but did lose mobility in finger

Case # 4

Mycobacterium marinum

Diagnosis is made from tissue biopsies

- Only 70-80% of cultures are positive.

Acid Fast Bacillus

- Takes 2-3 weeks to grow
- Grows well at 30°C on LJ slant
- Photochromogen – yellow pigment when exposed to light
- Produces urease
- Weak catalase positive
- Niacin negative and Nitrate negative

Case # 4

Mycobacterium marinum

Infection usually presents as a localized granuloma but can evolve into an ascending lymphangitis that resembles sporotrichosis or can spread to deeper tissues.



Case # 5

43 yo male presents to ED with abdominal pain, diarrhea (>10 episodes in 24 hours), and fever

Previous medical history includes pancreatitis, ESRD (end-stage renal disease), HIV/AIDS with long standing non-compliance, anemia, hypertension, and intra-abdominal abscess

“Frequent Flyer”

Case # 5

Received stool specimen for the following:

Giardia/Cryptosporidium Antigens

Stool Culture with Shiga Toxin and Campylobacter Antigen

Clostridium difficile Panel

Patient had previously been tested for Clostridium difficile one month ago - Negative

Case # 5

Results:

Giardia Antigen: Negative

Cryptosporidium Antigen: Negative

Stool Culture: Negative

Shiga Toxin: Negative

Campylobacter Antigen: Negative

Clostridium difficile Panel: **Positive**

Case # 5

Patient was treated with oral Vancomycin and Flagyl, then discharged with prescription to complete treatment at home.

Patient then returns to ED a month later – still **positive for C diff.**

Patient then returns to ED a month later – still **positive for C diff.**

Case # 5

Patient was non-compliant with outpatient antibiotic therapy and continued to test positive.

After four months of frequent ED visits and disruptive treatment, patient finally tested negative for C. difficile.

Case # 5

Clostridioides difficile infections:

- causes severe diarrhea and colitis (inflammation of the colon)
- almost 500,000 infections in the US each year
- 1 in 6 patients who get C. diff will get it again in the subsequent 2-8 weeks
- 1 in 11 patients over 65 yo diagnosed with healthcare-associated C. diff infection die within one month

Case # 5

Clostridioides difficile Risk Factors:

Anyone can be infected, but most cases occur after taking antibiotics (cephalosporins, fluoroquinolones, ampicillin, amoxicillin, and clindamycin). Other risk factors include:

- 65 or older
- Recent hospital or nursing home admission
- Immunocompromised (HIV/AIDS, cancer, immunosuppressants)
- Previous infection with C. diff

Case # 5

Clostridioides difficile Symptoms:

- Severe diarrhea
- Fever
- Stomach tenderness or pain
- Loss of appetite
- Nausea

Case # 5

From CAP Microbiology Checklist:

MIC.22440 Stool Specimen Number/Timing

Phase I

There are written policies for the number and/or timing of collection of stool specimens submitted for routine bacterial testing.

NOTE: The laboratory should consider developing policies with its clinicians for the number and/or timing of collection of stool specimens submitted for routine bacterial testing. Suggestions made by the authors of a 1996 CAP Q-Probes study (Valenstein et al) include:

- 1. Accept no more than two specimens/patient without prior consultation with an individual who can explain the limited yield provided by additional specimens*
- 2. Do not accept specimens from inpatients after the third hospital day, without prior consultation*
- 3. Test stool for Clostridium difficile toxin for all patients with clinically significant diarrhea and a history of antibiotic exposure. Consider C. difficile testing as an alternative to routine microbiologic studies for inpatients who have test requests for routine enteric pathogens*
- 4. Positive test results for Clostridium difficile do not correlate well with disease in young children. Follow manufacturer's guidelines for guidance on the testing of pediatric patients.*

These recommendations are for diagnostic testing. Different policies may apply to tests ordered for follow-up.

Case # 5

Our policy (Lab and Infection Control) for C. difficile specimen collection includes:



- Must be loose/liquid stool (takes the shape of the container)
- No repeat testing for 7 days after a negative result
- No repeat testing for 30 days after a positive result
- At least 3 loose stools per 24 hours
- No laxatives for 48 hours prior to specimen collection
- Specimen collected at least 6 hours after new antibiotic initiation

Case # 5

Bristol Stool Chart:

- Tool developed to evaluate stool specimens based on shape and consistency of the stool.
- Developed in 1997 by physicians from British Royal Infirmary in Bristol, England.
- Still useful today

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. Entirely Liquid

Case # 5

Daily Huddle with Infection Control and Unit Directors:

- Review each order for C. diff
- Review patient's symptoms – does patient meet testing criteria?
- Review positive cases (Infection Control and ASP)
- Review rejected specimens
- Reduced the # of formed stools sent for testing
- Decreased # of C diff orders

Questions ???

