

Case Studies in Microbiology



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Objectives

1. Correlate patient's clinical signs and symptoms with organism identifications.
2. Identify organisms based on clinical laboratory results.
3. Discuss the diagnostic and therapeutic implications of identifying organisms correctly.

Touro Infirmary – New Orleans

- 360 beds
- Garden District
- Founded in 1852
- Lab – 64 FTE's
- Microbiology – 7 FTE's, 2 flex
- Annual volume lab testing – 726,000



Case # 1

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 # 1

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

- Discharged with prescriptions for Itraconazole and Clindamycin.

Case # 1

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 # 1



Case # 1

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 # 1

Physician collected fluid from pustules and also performed punch biopsies.

- Both were sent to lab for aerobic, anaerobic, fungal, and AFB cultures.
- CBC
- Fungal antibodies

Case # 1 – Lab Results

CBC:		Normal Ranges
◦ WBC	11.8 H	4.0-10.0 K/uL
◦ RBC	5.04	4.10-5.80 M/uL
◦ Hgb	15.9	13.0-17.5 g/dL
◦ HCT	45.8	39.0-52.0%

Case # 1 – Lab Results

Differential:		Normal Ranges
Neutrophils	71 H	32-64%
Lymphocytes	14 L	25-48%
Monocytes	6	4-6%
Eosinophils	8	2-3%
Basophils	1	0-1%

Case # 1 – Lab Results

Fungal Antibodies:

◦ <i>Aspergillus spp.</i> Ab	None detected
◦ <i>Blastomyces dermatitidis</i> Ab	None detected
◦ <i>Coccidioides immitis</i> Ab	None detected
◦ <i>Histoplasma spp.</i> Ab	None detected

Case # 1

Which Microbiology cultures will be positive?

Clues:
Gardener
Didn't fill Itraconazole

Case # 1 – Lab Results

Microbiology:

Aerobic cultures	No growth
Anaerobic cultures	No growth
AFB cultures	No growth
But.....	
Fungal cultures	Positive

Case # 1

Fungal cultures:

Mold growing after 5 days of incubation

Surface: White periphery with black center, wrinkled

LPCB: Rosette clusters

Case # 1 LPCB - 30°C incubation

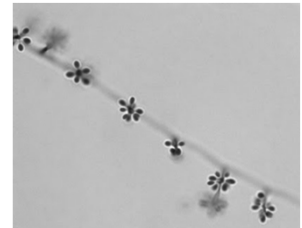
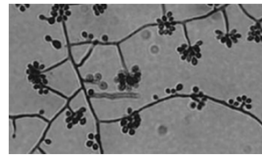


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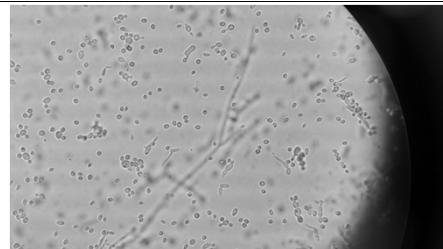
Case # 1

Fungal cultures:

Dimorphic mold:

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

Case # 1 LPCB - 35°C incubation



Case # 1

Final answer....

***Sporothrix schenckii* complex**

Case # 1 - *Sporothrix schenckii* complex

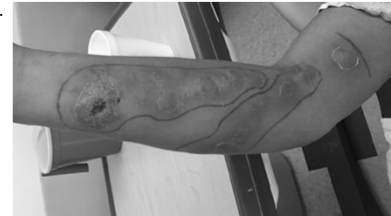
- Grows moderately rapid, usually within 7 days
- Thermally dimorphic, mold phase @ 30°C and yeast phase @ 35°C
- Surface is initially white to pale orange, then darkens and wrinkles
- Narrow septate hyphae with branching
- Slender conidiophores, right angles from hyphae
- Small tear-shaped or round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

Case # 1 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 # 1 Sporotrichosis

Patient improved and was discharged after treatment with Itraconazole.



Case # 2

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 # 2

ER physician - diagnosis

- Sepsis with septic shock
- Cellulitis
- Possible necrotizing fasciitis

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

Case # 2 – Lab Results

CBC:		Normal Ranges
◦ WBC	62.4 HH	4.0-10.0 K/uL
◦ RBC	3.14 L	4.10-5.80 M/uL
◦ Hgb	9.6 L	13.0-17.5 g/dL
◦ HCT	28.2 L	39.0-52.0%

Case # 2 – Lab Results

Differential:		Normal Ranges
Neutrophils	96 H	32-64%
Lymphocytes	1 L	25-48%
Monocytes	1 L	4-6%
Eosinophils	0 L	2-3%
Basophils	0	0-1%

Case # 2

Lactic acid:		Normal range
◦ I-Stat	2.8 H	>=1.7

Positive blood cultures – 2 sets drawn in ED:

- Gram negative bacilli

Case # 2

- On day 3 of admission, patient has surgery (I&D) 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 # 2

- 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 # 2

- 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 # 2

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 # 2

Positive bottles were subbed to BAP, Choc, Mac.

Oxidase = Positive

Set up Vitek GN card and AST card.

Tech sees results, and it'swait – WHAT?

Isolates organism to TCBS to confirm.

Growth on TCBS: green colonies



Case # 2

Final answer....

Vibrio vulnificus

Case # 2

Vibrio vulnificus

Update on patient:

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

Case # 2 Vibriosis

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 # 2 Vibriosis

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

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 # 3

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 # 3

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 # 3

Final answer....

Mycobacterium marinum

Case # 3

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 # 3 *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

While reviewing positive culture results, I came across a blood culture for a NICU baby that grew out *Group B Streptococcus*.

Patient's prenatal screening results were in her medical record.

GBS Strep Screen performed at a reference lab: Negative for GBS

Case # 4

Quality Improvement Project:

Mother is GBS negative, but yet baby still develops GBS sepsis.

Mother later revealed that she had been to an emergency room for urinary tract infection 10 days prior to delivery.

OB/GYN was not aware of the ER visit or diagnosis. Patient did not give her OB/GYN's name to the ER (not listed in her EMR), so he never received results. He retrieved lab results. Urine culture grew out.....

Streptococcus agalactiae

Case # 4

Quality Improvement Project:

- Analyzed how this process works in our ER
(MD and nurse review culture results)
- Currently doing culture by LIM broth for GBS
- Decided to implement GBS assay on Cepheid GeneXpert

Case # 4 Ask the Audience

1. Who is performing culture method for GBS?
2. Who is performing PCR? 24/7?
3. What do you do, if anything, when GBS grows out from genitourinary sources on females of child-bearing age?

Case # 5

49 yo female arrives to ER via EMS and presents with agitation, confusion, and abdominal pain.

Walks without assistance.

Speech is normal.

Unable to obtain H&P due to altered mental status.

However, she was a "frequent flyer".

Past history: Hepatitis B, drug abuse, hepatic encephalopathy, uses street drugs and cocaine

Case # 5

ER observations:

- Fever
- Altered mental status
- Tachycardic
- Respiration rate: 28 (elevated)

Over the next few hours,

BP, pulse, and resp rate – all over the place

Case # 5

ER diagnoses:

- Hepatic encephalopathy
- Fever
- Cocaine abuse
- Pancytopenia
- Metabolic Acidosis
- Sepsis

Patient is admitted to ICU.

Case # 5 – Lab Results

CBC:		Normal Ranges
◦ WBC	0.5 CL	4.0-10.0 K/uL
◦ RBC	2.35 L	4.10-5.80 M/uL
◦ Hgb	7.4 L	13.0-17.5 g/dL
◦ HCT	22.4 L	39.0-52.0%
◦ Platelets	11 L	140-410 K/uL

Case # 5 – Lab Results

Differential:		Normal Ranges
Neutrophils	56	32-64%
Bands	8	
Lymphocytes	36	25-48%
Monocytes	0 L	4-6%
Eosinophils	0 L	2-3%
Basophils	0	0-1%

* Absolute counts for Neutrophils, Lymphocytes, and Monocytes were below normal range.

Case # 5 – Lab Results

Chemistry

BUN/Creatinine	5.0	▽ L	12.0-20.0	
Calcium	8.2	▽ L	8.4-10.2	mg/dL
Total Protein	6.5		5.9-8.4	g/dL
Albumin	1.7	▽ L	3.4-5.0	g/dL
A/G Ratio	0.4	▽ L	0.8-1.7	
Anion Gap	7.0	▽ L	8.0-16.0	
AST	113	△ H	10-40	U/L
ALT	65	△ H	10-36	U/L
Alkaline Phosphatase	115		45-122	U/L
Bilirubin Total	3.8	△ H	0.2-1.0	mg/dL

Case # 5 – Lab Results

		Normal Ranges
Ammonia	216 H	16-60 ug/dL
Lactic Acid	54.4 H	4.5-19.0 mg/dL

Urine Drug Screen: Cocaine Detected

Blood cultures were drawn, urine was collected for UA and culture, and stool was collected for culture.

Case # 5 – Lab Results

Coagulation		Normal Ranges
PT	40.5 H	9.4-12.5 sec
PTT	91.4 H	25.1-36.5 sec
Fibrinogen	48 L	200-393 mg/dL
D-Dimer	12541.0 H	0.00-243.00 ng/ml D-DU

Case # 5 – Lab Results

Positive Blood cultures:

Gram stain = Gram negative bacilli

Urine culture:

>100,000 CFU/ml of *E. coli*

Case # 5 – Lab Results

What is the organism growing from our blood culture?

E. coli?

No, that would be too easy.

Case # 5 – Lab Results

Hint:

Stool culture is positive.....

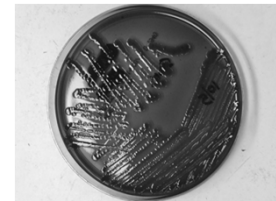
TCBS plate →



Case # 5 – Lab Results

Stool pathogen
is identified as....

Vibrio parahaemolyticus



Case # 5 – Lab Results

So now....what is the organism growing from our blood culture?

Vibrio parahaemolyticus?

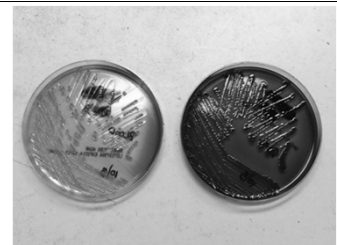
No, that would be too easy.

Case # 5 – Lab Results

Blood culture =
TCBS on the left

Stool culture =
TCBS on the right

Really, I'm not making this up.



Case # 5

So again....what is the organism growing from our blood culture?

Vibrio cholerae

Case # 5

TCBS – Thiosulfate Citrate Bile Salts Sucrose Agar

Selective and differential medium for *Vibrio*

Yellow: *V. cholerae*, *V. alginolyticus*

Green: *V. parahaemolyticus*, *V. vulnificus*

Case # 5

Our patient went into cardiac arrest and died within 48 hours of arriving in ER.

ID physician: “actively dying upon arrival”

Final diagnoses:

Respiratory failure, cirrhosis of liver, hepatic encephalopathy,
Gram negative septicemia, anemia, leukopenia, disseminated
intravascular coagulation, bacterial endocarditis, hypertension,
thrombocytopenia, diabetes mellitus, hypokalemia, hypocalcemia,
Hepatitis B

SEPSIS

Sepsis Facts

1.6 million adults develop sepsis each year in U.S.
◦ Every 20 seconds a new case is diagnosed

~270,000 deaths resulting from sepsis each year
◦ One death every 2 minutes

1 in 3 patients dying in hospitals linked to sepsis which makes it
the leading cause of death in U.S. hospitals (~35%).

Sepsis Facts

It is also the leading cause of readmissions.

- Almost 20% hospitalized for sepsis are readmitted w/i 30 days.
- #1 cause of readmissions.

Mortality rate:

- Increases approximately 8% by every hour of delayed treatment.
- 80% of deaths can be prevented with diagnosing sepsis rapidly and beginning treatment.

Sepsis Facts

Over \$25 billion is spent each year on hospitalizations due to sepsis.

- Ranks #1

\$18,400 – average cost of stay for sepsis hospitalization

- 2x all other diagnoses

Sepsis Facts

Symptoms include:

- Fever
- Elevated heart rate
- Elevated respiration rate
- Dizziness, faintness
- Confusion, altered mental status

Sepsis Facts

Additional symptoms include:

- Diarrhea, nausea, or vomiting
- Severe muscle pain
- Shortness of breath
- Low urine output
- Cool, clammy skin
- Loss of consciousness

Sepsis - Process Improvements in ER

Sepsis Lean Committee was formed.

- consists of ER physician, ER triage nurse, ICU nurse, lab, Infectious Disease physician, Quality Improvement representative, and Sepsis Coordinator

Purpose: Expedite identification of sepsis patients so treatment can be administered asap (antibiotics within one hour of presentation).

Sepsis - Process Improvements in ER

qSOFA – Quick Sequential Organ Failure Assessment Score

Criteria for qSOFA:

1. Altered mental status
2. Respiratory rate ≥ 22
3. Systolic BP ≤ 100

Positive qSOFA = meets at least 2 of the criteria

Sepsis - Process Improvements in ER

qSOFA – analyzing value of implementation in triage; if positive, initiate sepsis protocol.

SOFA score – also defines specific criteria, but also includes lab testing

- Platelet count
- Bilirubin
- Creatinine

References

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Questions

