

MICROBIOLOGY CASE STUDIES: THIS IS JEOPARDY!



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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 diagnostic and therapeutic implications of identifying organisms correctly.



•MOLD IS IN
•THE AIR

•\$100

MOLD IS IN THE AIR \$100

58 yo female presents to ED with shortness of breath on exertion and productive cough with pus-like sputum.

History of COPD, asthma, and emphysema with several recent admissions

Treated for LUL pneumothorax requiring chest tube placement 30 days ago. Completed course of Doxycycline.

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- CT negative for pulmonary embolism but significant for interstitial lung disease, cavitory lesions, and severe COPD.
- ILD = large group of disorders that cause scarring (fibrosis) of the lungs. Fibrosis causes stiffness in the lungs which makes it difficult to breathe.
- Started treatment with Vancomycin and Cefepime although concern for bacterial infection was low

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- Pulmonologist is consulted and orders bronchoscopy for further evaluation; however, procedure cannot be performed due to increasing O2 requirements.
- Sputum was sent for aerobic, fungal, and AFB cultures

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Sputum:

- Aerobic culture = Normal flora
- AFB culture = No growth

Urine: No growth

Blood cultures: No growth

Influenza PCR: Negative

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Sendouts:

- Positive Fungitell - assay that detects β -D Glucan which is presumptive diagnosis of invasive fungal disease
- Positive Galactomannan - assay that detects component of *Aspergillus* cell wall released during growth; indicates invasive aspergillosis infections

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Sputum:

- Fungus culture

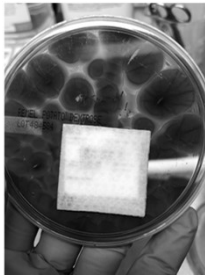
Mold growing after 10 days of incubation

Potato Dextrose:

Surface: yellow – pale orange, velvety

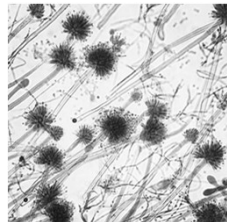
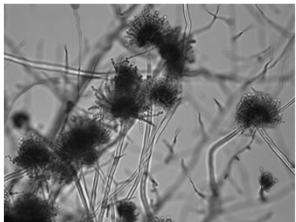
Reverse: dark orange, reddish-brown

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MOLD IS IN THE AIR \$100

Lactophenol Cotton Blue Prep:



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- Alex,
- What is.....

•*Aspergillus versicolor*

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Aspergillus versicolor

- Slow growing, filamentous fungus
- Commonly found in soil and decaying organic matter
- Found in damp indoor environments, such as water-damaged buildings or materials such as wallpaper, carpet, and insulation
- Found on food, such as cereal grains, seeds, nuts, dried meats, and dairy products

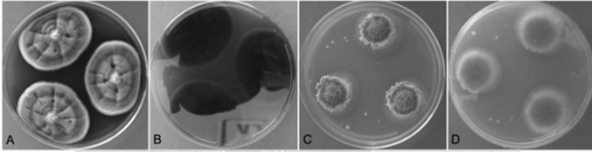
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Aspergillus versicolor

- Unique – can appear in a wide range of colors.
- Initially appears white but turns yellow, orange, tan, pale green, or pink over time.
- Can often be observed in an emerald green color with a powdery texture

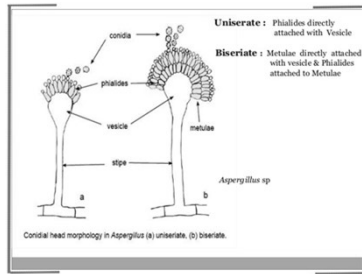
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Aspergillus versicolor



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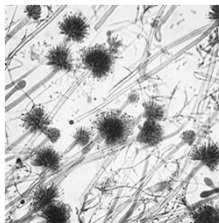
Aspergillus Anatomy:



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Lactophenol Cotton Blue Prep:

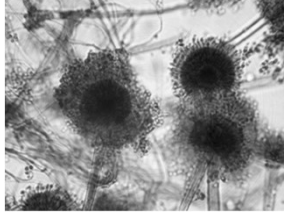
- Hyphae are septate and hyaline.
- Conidial heads are biseriata and loosely radiate.
- Conidiophores measure 120-700 μm in length, are hyaline to pale brown, smooth-walled and brittle (can see where many have “snapped off”).



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Lactophenol Cotton Blue Prep:

- Vesicles are small and variably shaped, with metulae and phialides covering most of the vesicle.
- Conidia measure 2.5-3 μm in diameter, are globose, and may be fine to distinctly roughened.



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Lactophenol Cotton Blue:

- Reduced conidial structures resembling those seen in *Penicillium* species are common.
- Hülle cells may also be present.



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Aspergillus versicolor

- Major producer of mycotoxins (sterigmatocystin and cyclopiaxonic acid) that can cause diarrhea and an upset stomach if ingested in moderate quantities
- Sterigmatocystin- kidney and liver carcinogen

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Aspergillus versicolor

- Highly resilient fungus
- Capable of growing in many extreme conditions, including those of very high salinity
- Found all over the world in various climatic conditions

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Aspergillus versicolor

- Found in the regions of the Arctic, Mir space station, the Dead Sea, and Uranium mines



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Aspergillus versicolor

- Patient was treated with Voriconazole and Anidulafungin
- Lung transplant candidate ??

CRAZY CAT LADY •\$200

CRAZY CAT LADY \$200

42 yo female presents to ED with infected wound to her right hand.

- Warm to the touch
- Purulent
- Possible puncture wounds

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CBC:		Normal range
WBC	18.6 H	4.5 – 11.0 K/uL
RBC	4.38 L	4.50 – 5.90 M/uL
Hemoglobin	15.8	13.5 – 17.5 g/dL
Hematocrit.	39.2 L	40.0 – 51.0%

CRAZY CAT LADY \$200

Differential:

Normal range

Neutrophils	96.5% H	32-64%
Lymphocytes	1.2% L	25-48%
Monocytes	1.8% L	4-6%
Eosinophils	0.5% L	2-3%
Basophils	0%	0-1%

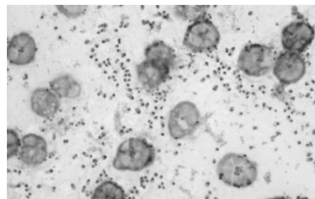
CRAZY CAT LADY \$200

- Cultures (aerobic and anaerobic) were collected from the purulent drainage from the puncture wounds on the right hand.
- Patient describes wound as a cat bite.

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Gram Stain Results:

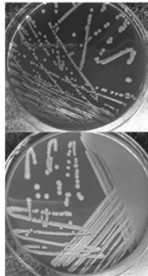
Gram negative coccobacilli



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Culture Results:

- Nonhemolytic on SBA
- No growth on MAC
- Oxidase +
- Indole +



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- Alex,
- What is.....

•*Pasteurella multocida*

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Pasteurella multocida

- commonly associated with infected cat and dog bite wounds.
- carried by almost all feline species in their oropharynx as part of their normal flora
- 400,000 people are bitten or severely scratched by cats annually in the U.S.

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Pasteurella multocida

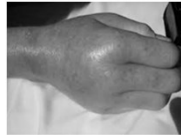
- Cat bites become infected more frequently than dog bites.
- Cat's teeth are small and sharp which can easily penetrate the bones, joints, and tendons.



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Pasteurella multocida

- causes cellulitis, often with gray malodorous discharge
- can lead to septic arthritis, tenosynovitis, and osteomyelitis



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Pasteurella multocida

- Likely to be the pathogen if cellulitis develops within a few hours after the bite.
- Swelling, reddening, and intense pain
- If incubation period is longer, infection with *Staphylococcus* or *Streptococcus* should be suspected.

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Pasteurella multocida Study

- Women accounted for 72% of cat bite victims, compared to 38% of dog bite victims.



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Pasteurella multocida Study

- Cat bites:
 - 19% abscesses
 - 39% purulent wounds
 - 42% nonpurulent cellulitis



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Pasteurella multocida

- Antibiotic therapy:
 - Amoxicillin/CA
 - Trimethoprim/Sulfamethoxazole
 - Doxycycline
 - Fluoroquinolones (Cipro, Levo)
- Duration of therapy depends on type of infection.

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Patient was discharged with prescription for Amoxicillin/CA. (ED physician suspected *Pasteurella multocida*.)



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Antibiotic Stewardship:

- Right Drug
 - Right Dose
 - Right Time
- Reduces readmissions and LOS

•MONEY
MAKER
•\$300

MONEY MAKER \$300

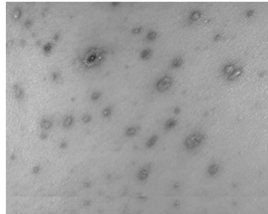
28 yo male presents to ED with rash, fever, chills, headache, nausea, vomiting, abdominal pain, and diarrhea.

- History of HIV and secondary syphilis (not on antiretroviral therapy)
- Recently hospitalized for rash and sepsis

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▪ Diffused papular rash

- Derm consult
- Biopsies collected



Note:

The characteristic rash from secondary syphilis appears as rough, reddish-brown spots that usually appear on the palms of the hands or bottoms of the feet. It may appear in one area of the body or spread to multiple areas.

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▪ Secondary syphilis rash



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▪ CT showed cavitory lesion – concern for TB

- patient placed in isolation
- order AFB cultures x 3



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- Patient is placed on Penicillin G for syphilis
- Also treated with Itraconazole, Vancomycin, and Ceftriaxone
- Leaves AMA
- Outpatient appointment - few weeks later
- Rash is improving on body, but face still has rash

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- Patient declares that his face is his “money maker”
- Postinflammatory hyperpigmentation on neck and face - a condition in which an injury or inflammation to the skin causes increased pigment production. The most common cause is acne, but it also can result from psoriasis, a burn, or an injury.
- Agitated – wants face to return to “original state”
- Leaves without seeing physician



MONEY MAKER \$300

- Postinflammatory hyperpigmentation



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- Lab Results:
 - RPR Titer 1:16 Reactive
- AFB cultures of sputum and bronch:
Positive for *Mycobacterium kansasii*

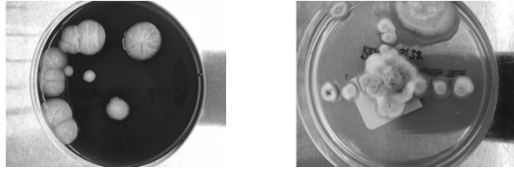
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- Lab Results:

Liver enzymes	Normal Range
AST 130 H	<45 U/L
ALT 53 H	<46 U/L
- Hepatitis C Antibody: Negative
- Possibly related to rash/infection

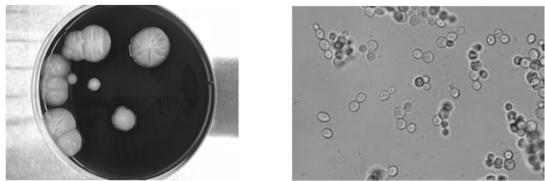
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- Remember the skin biopsies?
- Fungus cultures of right arm biopsies turned positive



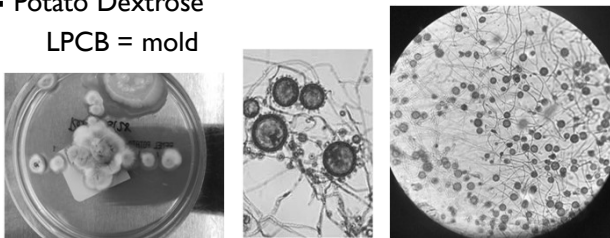
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- Brain Heart Infusion
Wet mount = Yeast



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- Potato Dextrose
LPCB = mold



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- Alex,
- What is.....

•*Histoplasma capsulatum*

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Histoplasma capsulatum

- Lives in the environment, particularly in soil that contains large amounts of bird or bat droppings.
- In the United States, *Histoplasma* mainly lives in the central and eastern states, especially areas around the Ohio and Mississippi River valleys.
- The fungus also lives in parts of Central and South America, Africa, Asia, and Australia.

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Histoplasma capsulatum

- Symptoms of Histoplasmosis (appear after 3 – 17 days):

Fever	Cough
Fatigue	Chills
Headache	Chest pain
Body aches	

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Histoplasma capsulatum

- Only 1% of infections are symptomatic.
- Usually self-limiting
- Patients with history of pulmonary disease can develop chronic pulmonary histoplasmosis.
- Some patients are at risk for developing disseminated histoplasmosis.

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Histoplasma capsulatum

Patients that have a higher risk of infection:

- Weakened immune systems
HIV, organ transplants, steroids
- Infants
- Adults aged 55 or older

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Histoplasma capsulatum

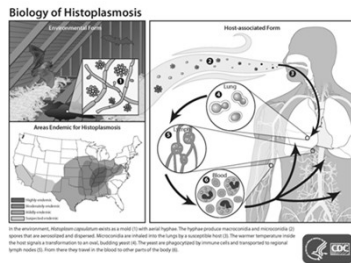
- Not contagious – can't spread from the lungs between people or between people and animals
- Take caution when cleaning chicken coops, exploring caves, remodeling old buildings, and disturbing soil where there are bird or bat droppings

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Histoplasma capsulatum

- Spores circulate in the air after contaminated soil is disturbed.
- People breathe in the spores.
- After the spores enter the lungs, the person's body temperature allows the spores to transform into yeast.
- The yeast can then travel to lymph nodes and can spread to other parts of the body through the bloodstream.

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MONEY MAKER \$300

Histoplasma capsulatum – Dimorphic mold

- Organisms grow in a mold form in the soil.
- After the mold spores are inhaled into the lungs, they undergo a morphological change into a pathogenic yeast form.
- The ability to convert to the yeast form is essential for this class of fungal agents to be pathogenic and produce disease.
- Temperature change is one key stimulus that triggers the phase transition from mold (25°) to yeast (37°).
- Genes that are expressed only in the pathogenic yeast form of these fungi have been identified to help explain how and why this phase transition is required for virulence.

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Histoplasma capsulatum

- Lab tests:
 - Culture – process immediately
 - Antigen detection – urine, serum, CSF, BAL
 - Antibody detection – 2-6 weeks to develop antibodies
 - PCR

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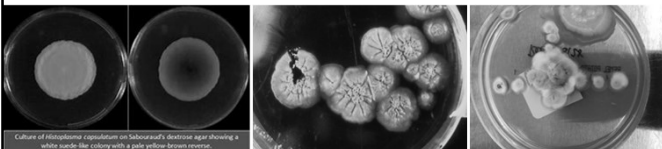
Histoplasma capsulatum

- Slow growing – mycelial forms usually mature within 15-20 days, but may take up to 8 weeks
- Mold phase – 25-30°C, white to brown, or pinkish, with a fine dense cottony texture
- Reverse is white, but sometimes yellow or orange-tan
- Mold phase will grow on most fungal media

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Histoplasma capsulatum

- Mold phase



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Histoplasma capsulatum

- Yeast phase - 37°C, requires brain heart infusion or other enriched agar.
- Colonies are moist, white, and yeast-like.
- Yeast phase is inhibited by cyclohexamide.



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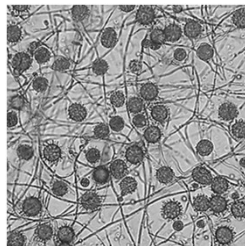
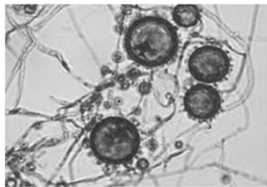
Histoplasma capsulatum

- Young cultures: Septate hyphae bearing round to pear-shaped, smooth or occasionally spiny microconidia on short branches or directly on the sides of the hyphae.
- Mature growth: Large, thick-walled, round macroconidia are formed. Described as tuberculate or knobby with short cylindrical projections.

MONEY MAKER \$300

Histoplasma capsulatum

- LPCB:



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Histoplasma capsulatum

- Patient's rash showed improvement after treatment with Itraconazole.
- Antiretroviral treatment was strongly recommended to patient.

•JUST IN TIME
•\$400

JUST IN TIME \$400

- 70 yo male with history of aortic ulceration presents to ED with tachycardia, abdominal pain, shortness of breath, coughing, and bloody sputum.
- 3 months prior: TEVAR (thoracic endovascular aortic repair) for ruptured abdominal aortic aneurysm

JUST IN TIME \$400

- TEVAR procedure:
 - Minimally invasive surgery
 - Stent graft is inserted through small incision to reinforce the aneurysm, usually through the groin
 - Stent graft – metal tube covered in fabric helps prevent the aneurysm from bursting

JUST IN TIME \$400

- CT reveals serious concerns:
 - Rapid aneurysm expansions
 - vs.
 - Hemorrhage into the wall of the aorta with progressive changes of penetrating ulcer
 - vs.
 - Contained rupture

JUST IN TIME \$400

CBC:		Normal range
WBC	22.3 H	4.5 – 11.0 K/uL
RBC	3.88 L	4.50 – 5.90 M/uL
Hemoglobin	10.3	13.5 – 17.5 g/dL
Hematocrit	32.8 L	40.0 – 51.0%

JUST IN TIME \$400		
Differential:		Normal range
Neutrophils	90.5% H	32-64%
Lymphocytes	5.9% L	25-48%
Monocytes	3.5% L	4-6%
Eosinophils	0.0% L	2-3%
Basophils	0.1%	0-1%

JUST IN TIME \$400		
		Normal range
BNP	368 H	<100 pg/mL
Lactic Acid	2.2 H	0.3 – 2.0 mmol/L
Blood cultures x2 collected.		
Patient started on antibiotics: Ceftriaxone, Pip/Tazo, Vancomycin		

- | | |
|--|--|
| JUST IN TIME \$400 | |
| <ul style="list-style-type: none"> ▪ Patient is declining the following day ▪ Rushed into surgery <ul style="list-style-type: none"> ▪ Decortication of left chest ▪ Ruptured thoracoabdominal aorta repair ▪ Left heart bypass ▪ Left groin cutdown ▪ Removal of the infected TEVAR - purulence and hematoma sent for culture | |

JUST IN TIME \$400

- Blood cultures turn positive

Gram negative bacilli

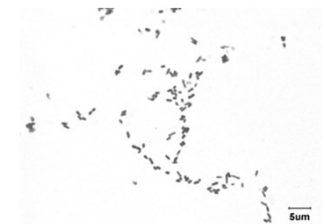


A black and white microscopic image showing numerous small, rod-shaped bacteria (bacilli) scattered across the field of view. The bacteria appear to be Gram-negative, as they are not stained purple.

JUST IN TIME \$400

- Gram stains on aorta and graft

Gram negative bacilli




A black and white microscopic image showing Gram-negative bacilli. The bacteria are arranged in several distinct chains and some are in pairs. A small scale bar labeled '5um' is visible in the bottom right corner of the image.

JUST IN TIME \$400

- Routine plates set up for tissue and graft
- Positive bloods subbed to BAP, Choc, and Mac

Mac BAP



Two petri dishes are shown side-by-side. The left dish is labeled 'Mac' and contains a dark, uniform agar surface. The right dish is labeled 'BAP' and shows a grid of bacterial colonies growing on the agar surface.

JUST IN TIME \$400

Does this help?

XLD	HE
	

JUST IN TIME \$400

•Alex,
•What is.....

•*Salmonella enterica* serotype Enteritidis

JUST IN TIME \$400

- *Salmonella* is a typical cause of aortitis, implicated in 33% to 50% of all cases.
- Rare, but associated with high morbidity and mortality.
- Thoracic endovascular aortic repair is favored over open surgery – less invasive
- Treat with antibiotics for up to 12 months.
Ceftriaxone and/or Ciprofloxacin

JUST IN TIME \$400

- Possible mechanisms are septic emboli to the vasa vasorum, continuous infection from a focus extending to the arterial wall, direct bacterial inoculation, and bacterial seeding in an existing intimal injury or an atherosclerotic plaque.
- Typical risk factors are male gender, age over 50 years, diabetes, and pathological alterations of the aortic wall (most commonly atherosclerosis).

JUST IN TIME \$400

- Symptoms are nonspecific and a high index of clinical suspicion is thus necessary to establish a diagnosis.
- The most common symptoms are fever, back pain, abdominal pain, and chills. Our patient only had 1 out 4.
- Early diagnosis and prompt initiation of empirical antibiotic therapy are crucial.

JUST IN TIME \$400

- Our patient continued antibiotic treatment with Ceftriaxone by IV.
- Discontinued Pip/Tazo.
- Continued on Vancomycin until blood cultures and operative cultures were finalized. There was concern for presence of *Staph* also.
- Patient was improving.

FINAL JEOPARDY

PATIENT SAFETY

FINAL JEOPARDY

Guidelines or a coordinated program that promotes the appropriate use of antimicrobials, improves patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms.

FINAL JEOPARDY

- Alex,
- What is.....

- Antibiotic Stewardship

FINAL JEOPARDY

Questions?