The Clinical Role of Diagnostic Microbiology

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Presentation Objectives

- Describe the role of diagnostic microbiology in patient healthcare
- Understand how proper specimen collection impacts patient results
  - Blood cultures
  - Urine cultures
- Overview of antimicrobial susceptibility testing methods and reporting guidelines

Purpose of Diagnostic Microbiology

- Assist in the diagnosis & treatment of infectious diseases
  - Determine the etiology of infections
  - Determine the susceptibility of the etiologic agent to antibiotics – guide antimicrobial therapy

CDC PHIL

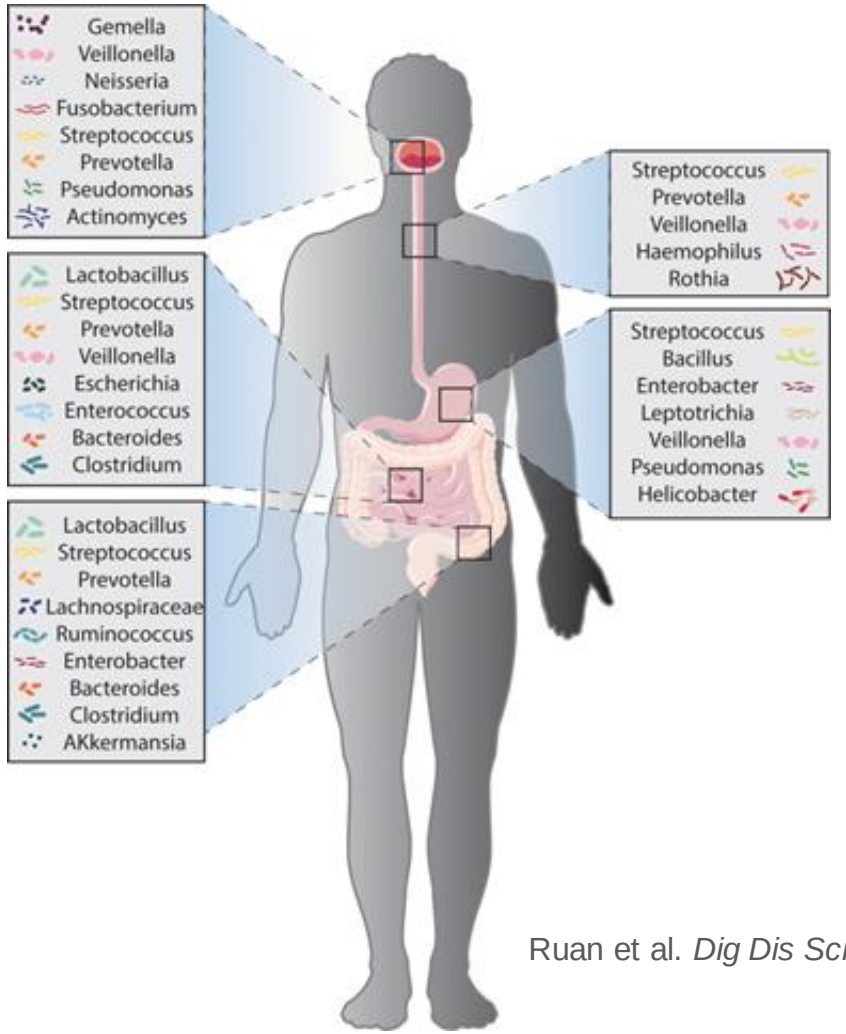
Laboratory Methods

- What is the etiology (cause) of infection?
  - Direct detection
  - Culture
  - Serology
- What is the antimicrobial susceptibility of the causative agent?
  - Culture-based methods (phenotypic)
  - Molecular-based methods (genotypic)

CDC PHIL

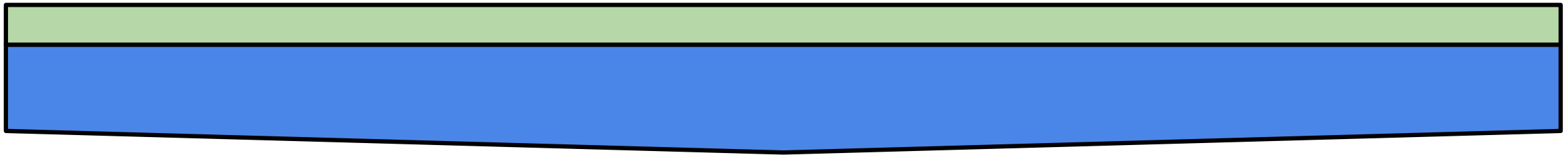
Importance of Specimen Collection

- Quality of specimen submitted for analysis is of ***critical*** importance
- If specimen quality is poor
  - Results may be inaccurate
  - Worse, results may be misleading
  - Specimen should be rejected & recollected
  - If not, workup limited



Ruan et al. *Dig Dis Sci.* 2020.

Overview of Blood Cultures

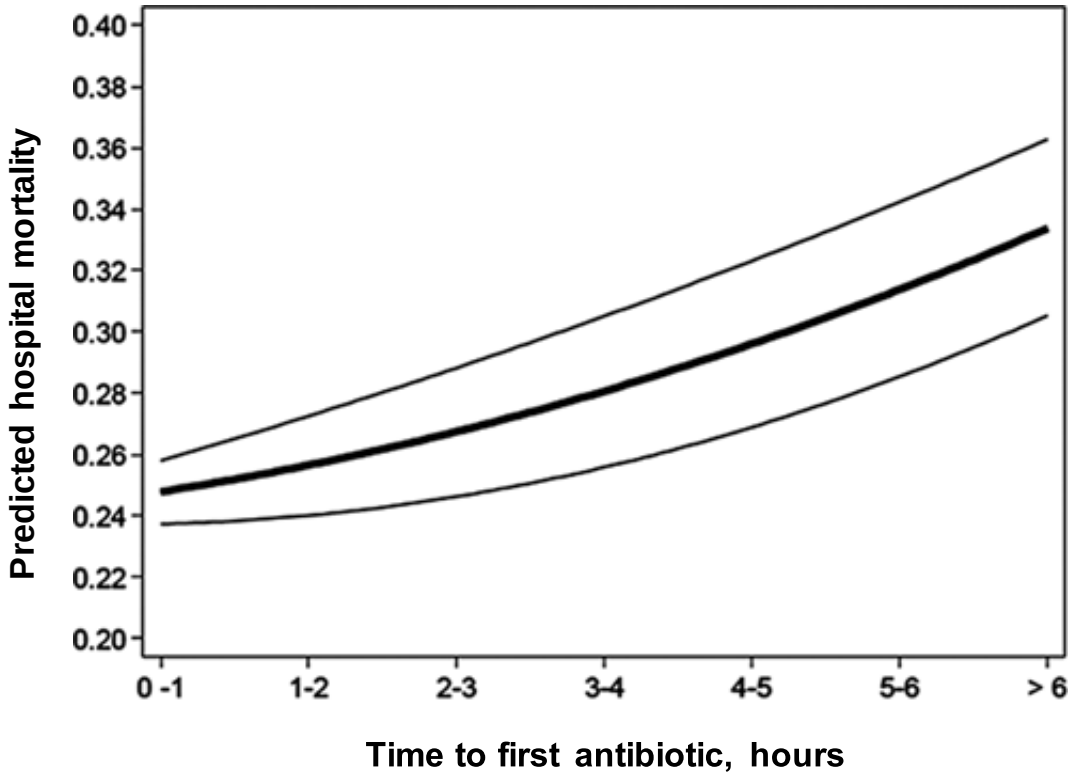


Purpose of Blood Cultures

- Determine if a bloodstream infection is present
- Identify the organism present
- Provide information about the source of infection
- Guide antimicrobial therapy

Blood Cultures

- Mortality rates increase when appropriate antimicrobial therapy is delayed
- Recovery of infectious agents is dependent on prompt delivery of appropriately collected blood cultures to the laboratory

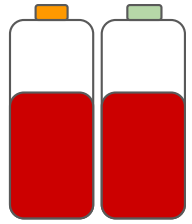


Ferrer et al. *Critical Care Medicine.* 2014.

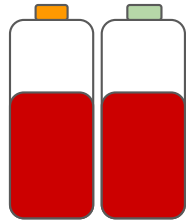
Optimal Blood Culture Collection

- Optimal collection volume: 10 mLs per bottle
  - the number of infection-causing organisms in the blood can be very low
- Standard collection: 20 total mLs collected into 1 aerobic and 1 anaerobic bottle
  - suboptimal volume could cause infections to be missed
- 2 separate draws are performed
  - helps identify pathogens and possible contaminants
  - common skin commensals identified in only one set have limited workup

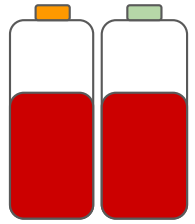
1 set = 1 needlestick = 2 bottles



1 order = 2 sets = 4 bottles



Site 1



Site 2

Blood Culture Detection

- Continuous monitoring instruments
- colorimetric sensor detects pH changes caused by carbon dioxide production
- Positive culture bottles alert technologist for immediate action

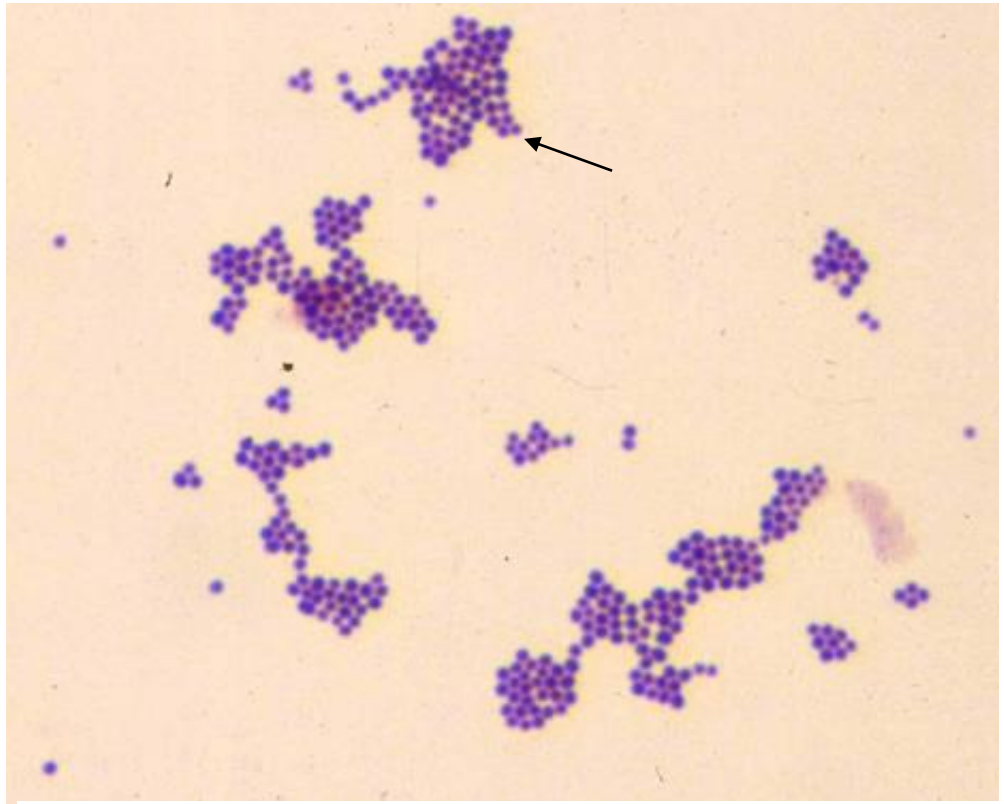
biomerieux.com

Microscopic Morphology

- Performed on patient specimens and culture growth
- initial indication of organisms present and specimen quality
- Gram reaction - positive (blue/purple), negative (red/pink)
- Shape - cocci (spheres), bacilli (rods), spirochetes (spiral shaped)
- Arrangement - clusters (staphylo-), chains (strepto-)

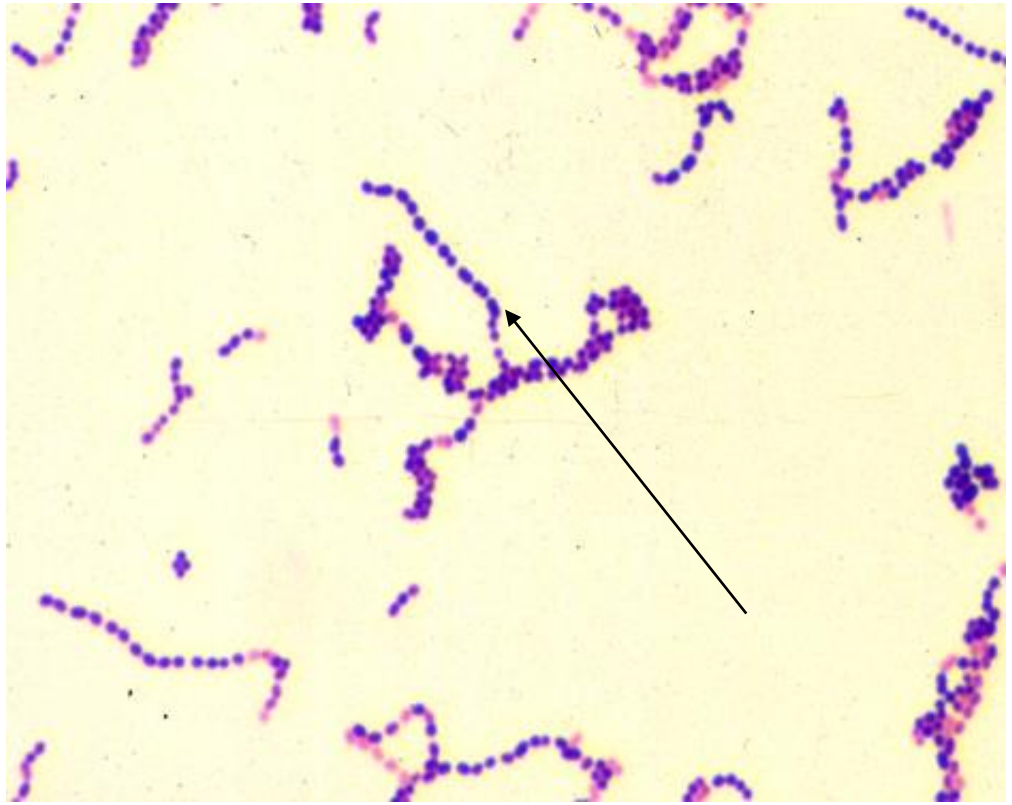
Microscopic Morphology

Staphylococci



Gram positive cocci in clusters

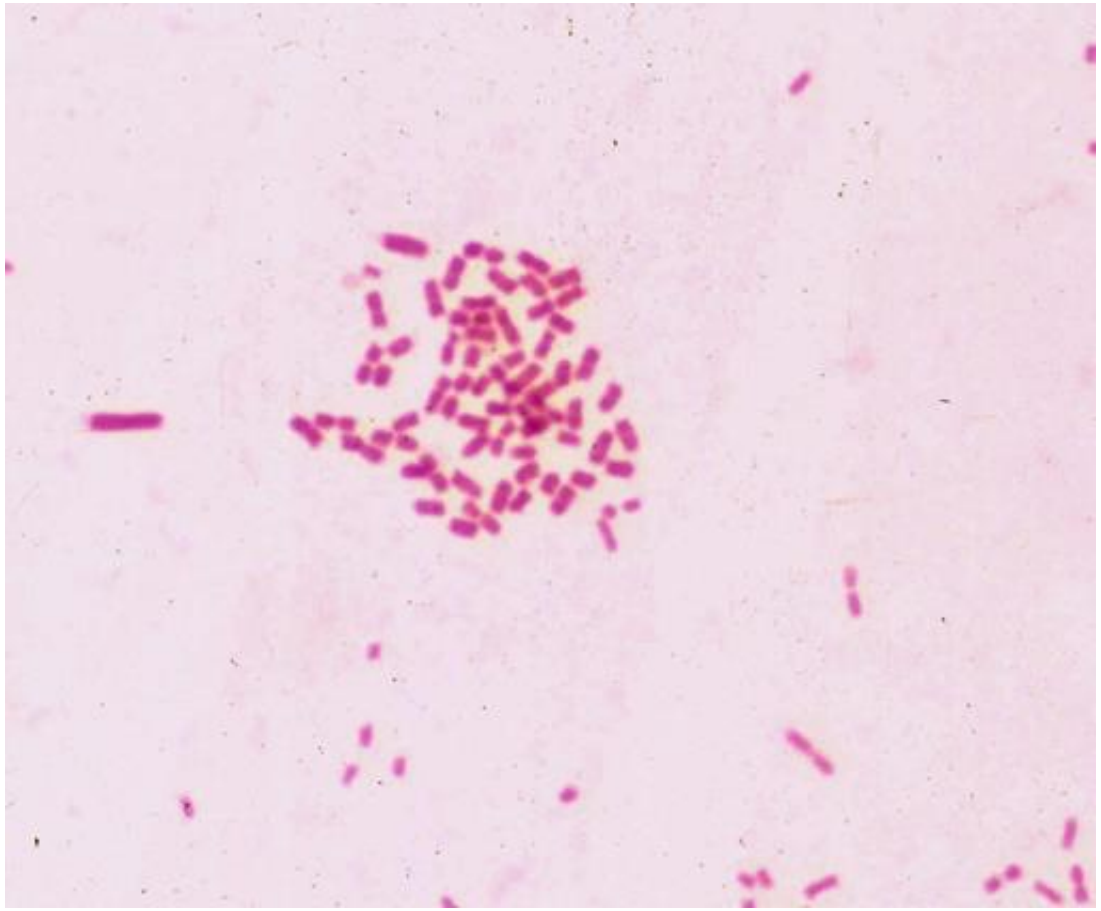
Streptococci



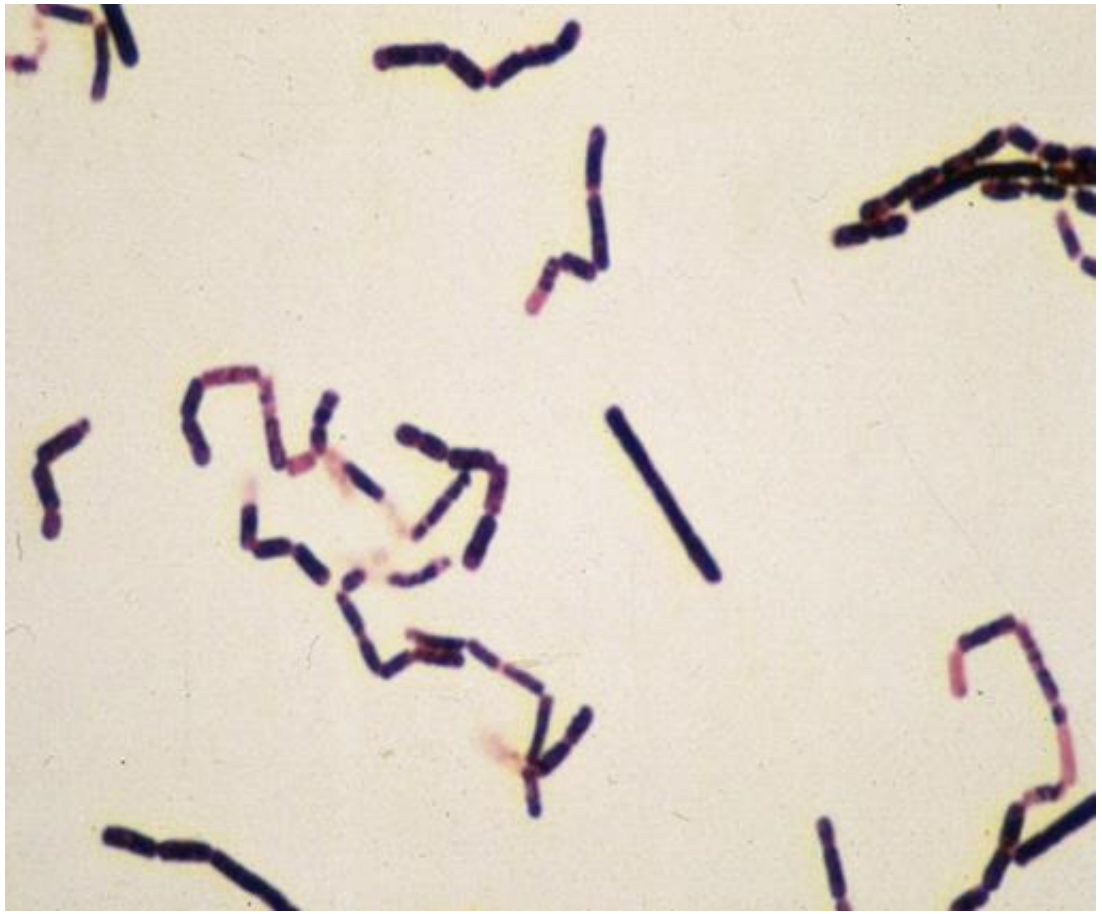
Gram positive cocci in chains

Microscopic Morphology

Gram Negative Bacilli

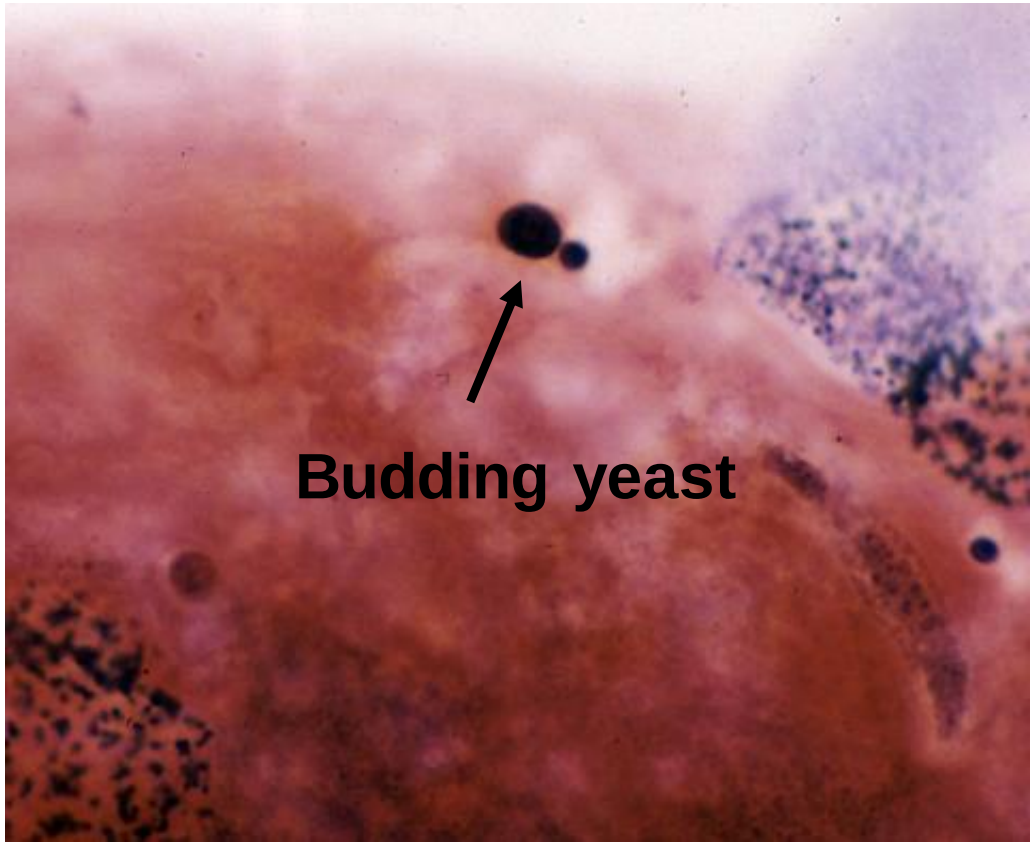


Gram Positive Bacilli



Microscopic Morphology

Yeast



Sporulating Structure - Mould

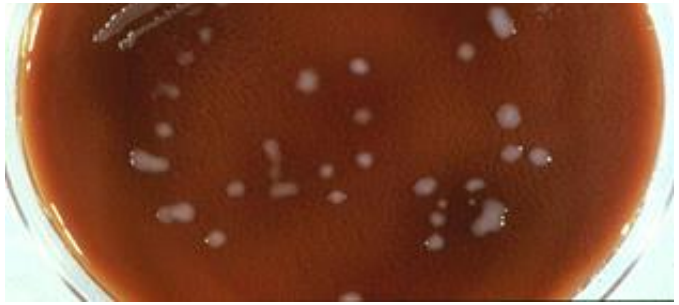
Rapid Molecular Detection

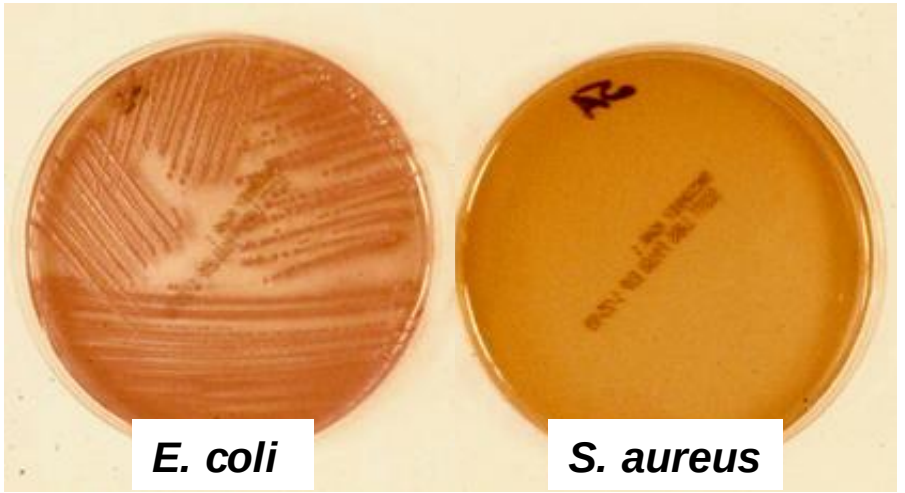
- Multiplex PCR directly from positive blood culture bottle
- Detects the 33 bacteria and yeast commonly associated with bloodstream infections and
- 10 resistance genes
  - mecA/C
  - vanA/B
  - carbapenemases
- Run time approximately 1 hour

Culture

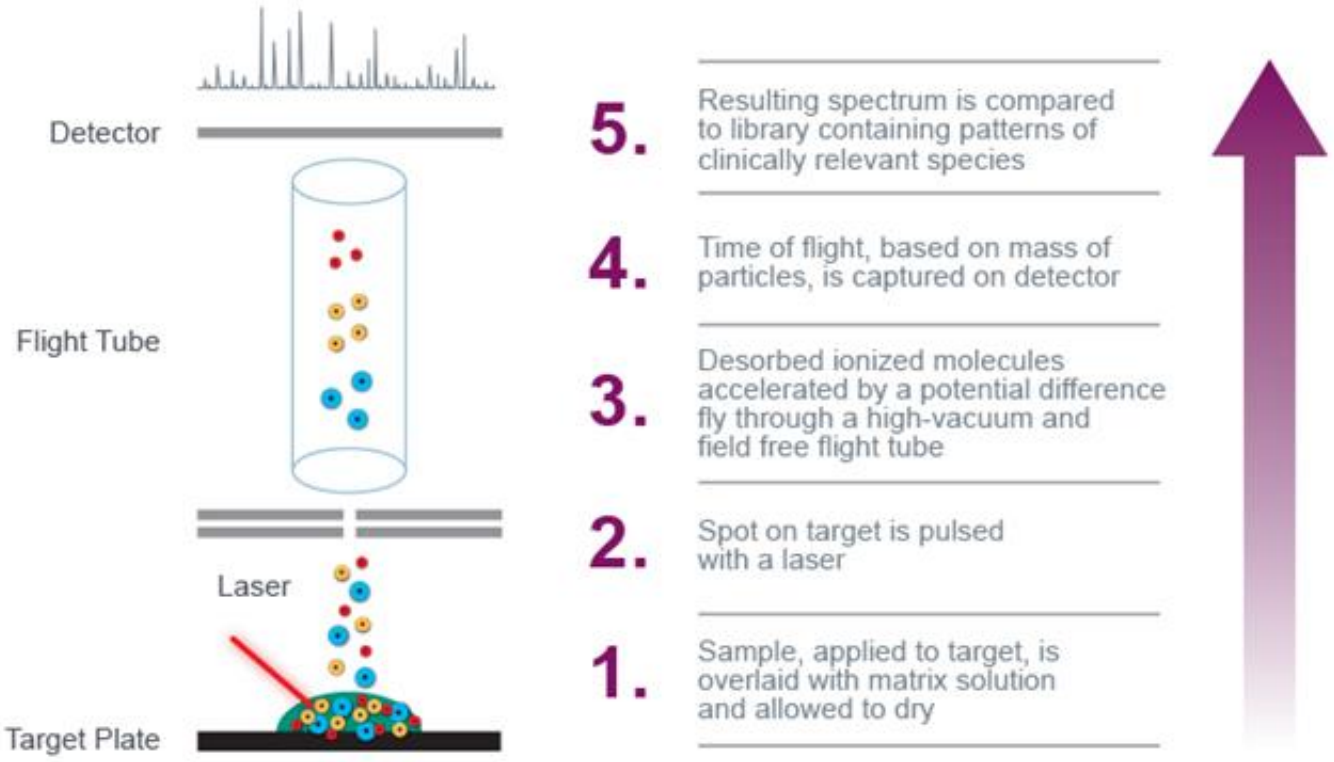
- Grow pathogenic microorganism(s) from appropriately collected specimens
  - Isolate and identify causative agent
  - Requires 18-48 hours; sometimes longer
  - Agar media types
    - Enriched
    - Selective
    - Differential

Chocolate agar with *Haemophilus*





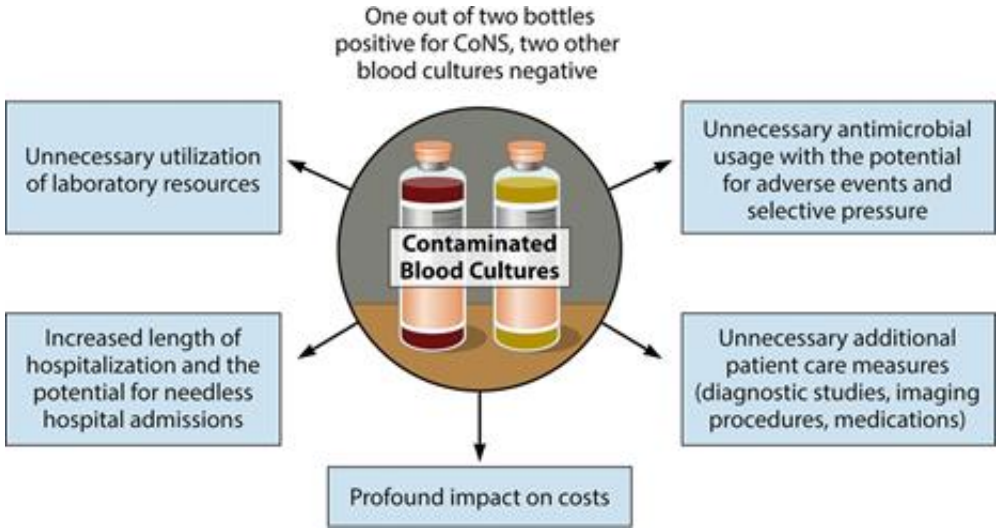
Molecular Identification



https://www.beckmancoulter.com/products/microbiology/maldi-tof-mass-spectrometry

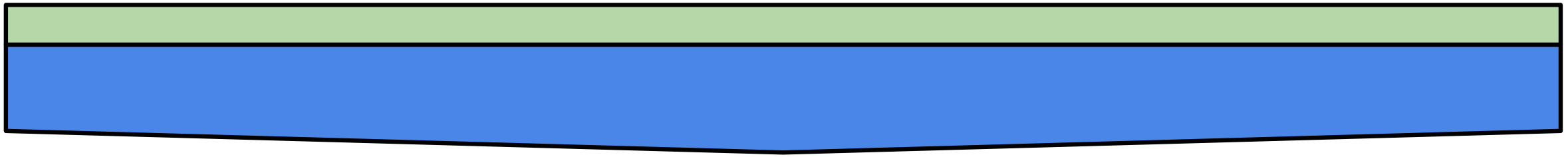
Blood Culture Contamination

- Common skin commensals isolated from single blood culture set
- Negative impact to patient care and healthcare costs
- Laboratory requirement to monitor blood culture contamination rates AND provide feedback
- Continuous quality improvement measure
  - education and feedback
  - skin and bottle disinfection
  - collection site
  - ordering practices



Doern et al. *Clin Microbiol Rev.* 2019.

Overview of Urine Cultures

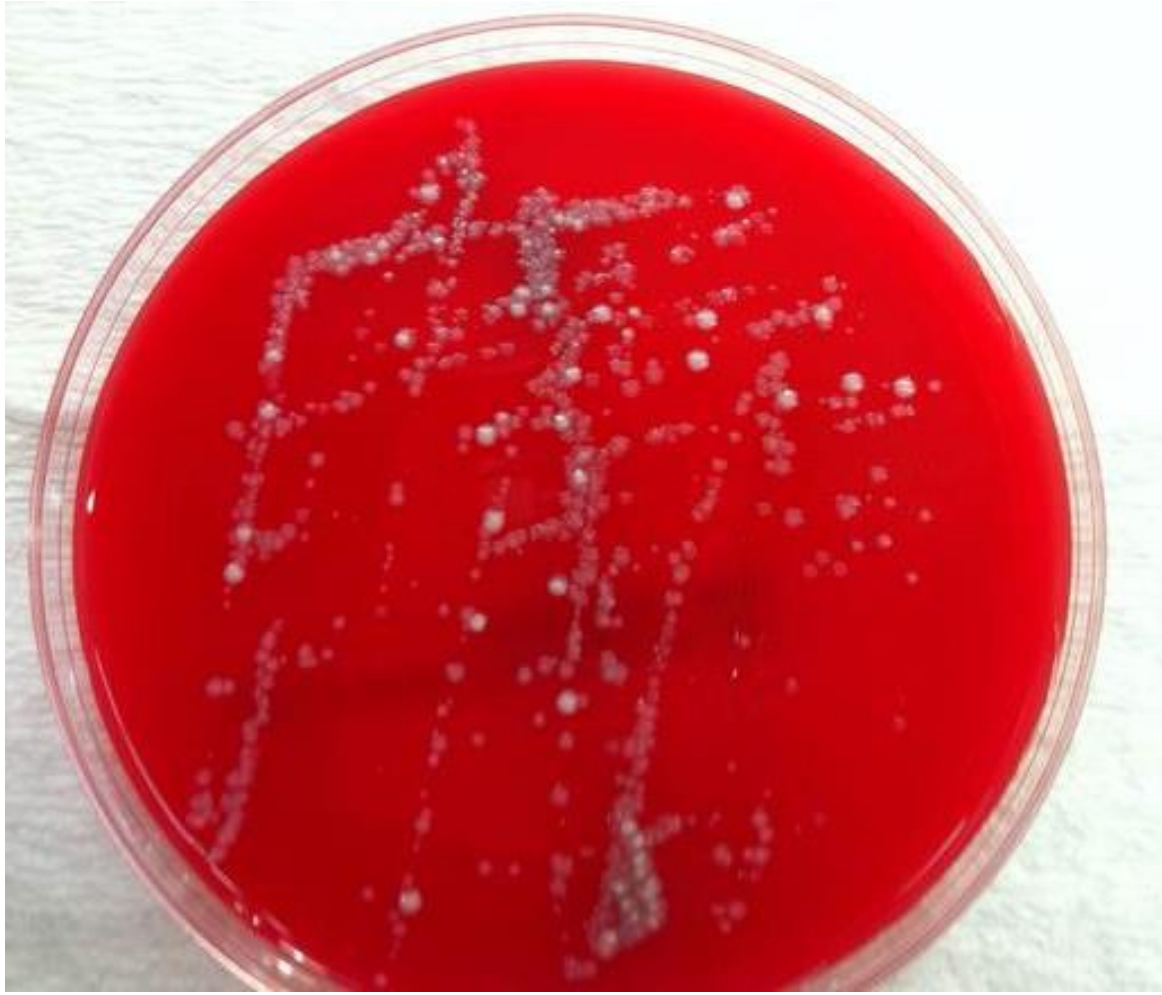


Urine Culture

- Aids in diagnosis of urinary tract infection and guides antimicrobial therapy
  - cystitis, pyelonephritis
- Noninvasive specimens
  - clean catch midstream collection
  - foley catheter
  - pediatric collection bag
- Invasive specimens
  - suprapubic aspirate
  - straight catheter

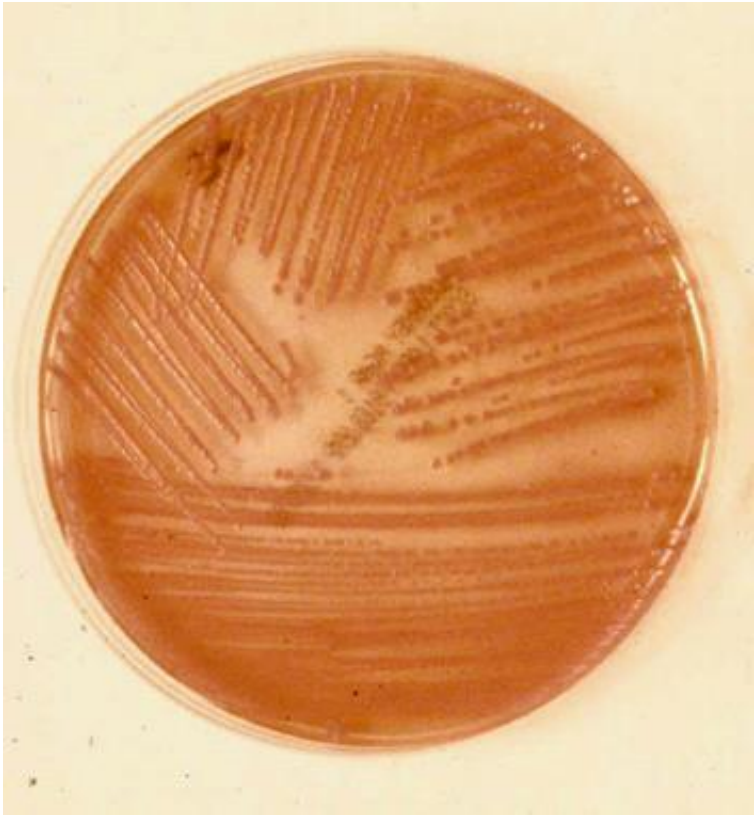
Urine Culture

- Interpretation of cultures is complicated by improper collection and transport conditions
- Specimen can easily be contaminated by skin and rectal microbiota
  - adherence to proper collection instructions
- Transport stability
- importance of collection in symptomatic patients



Urine Culture

- Quantitative culture - separate true UTI from contamination
- Traditional interpretation
  - ≥10^5 CFU/mL - UTI
  - < 10^5 CFU/mL contamination
- Culture volume
  - noninvasive: 1µL
  - invasive: 10µL



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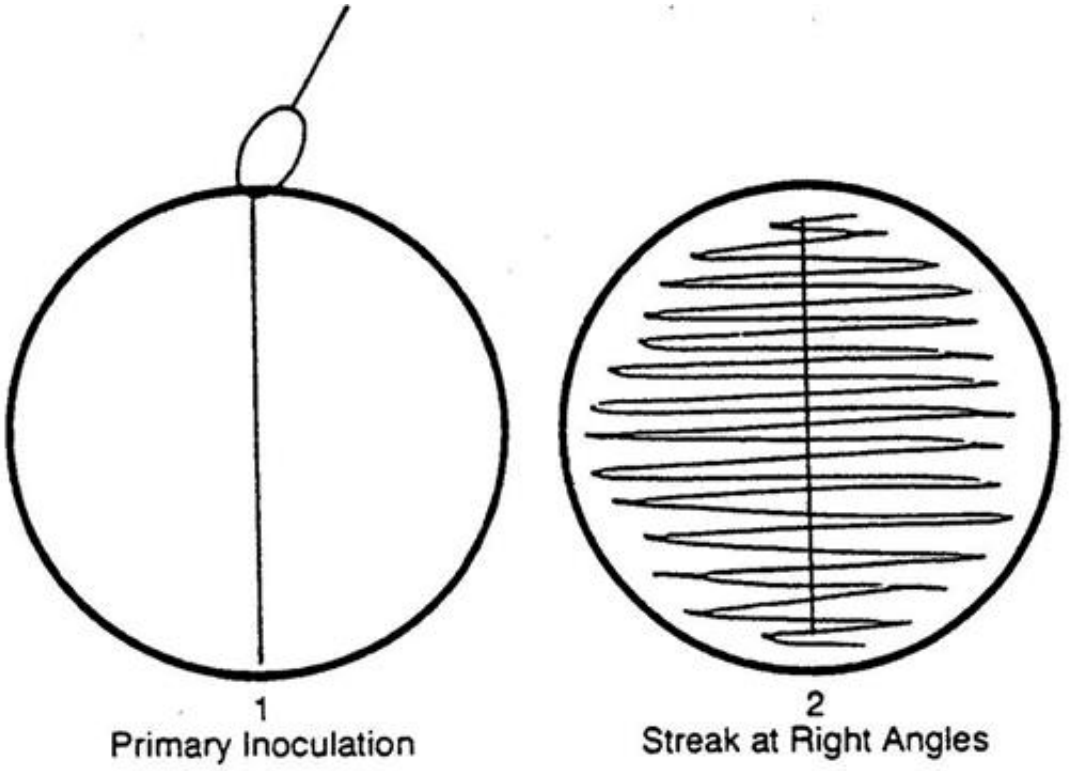
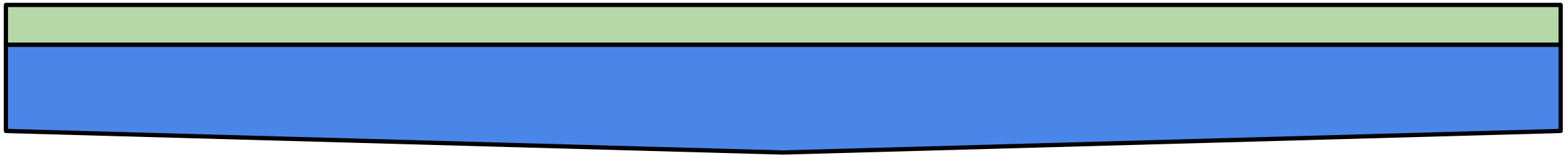


Urine Culture

- Reporting criteria for identification and susceptibility testing of uropathogens
  - noninvasive specimens: ≥ **10,000** cfu/mL
  - invasive specimens: ≥ **1,000** cfu/mL

Susceptibility Test Methods



Antimicrobial Susceptibility Testing (AST)

- Determine if one or more antibiotics may be effective as treatment for infection
  - Guideline for therapy
- Methods
  - Culture based - phenotypic
  - Molecular based - genotypic

When to Perform AST?

Three overarching principles

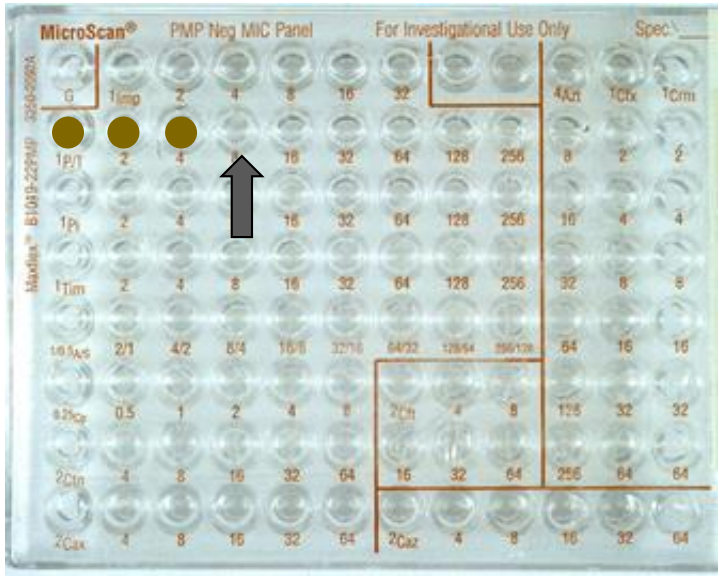
1) Isolate must be clinically significant,
   - i.e., a (potential) pathogen
2) Antimicrobial susceptibility pattern of isolate is unpredictable
3) A standardized method is available for AST performance and interpretation on the isolate

Phenotypic AST

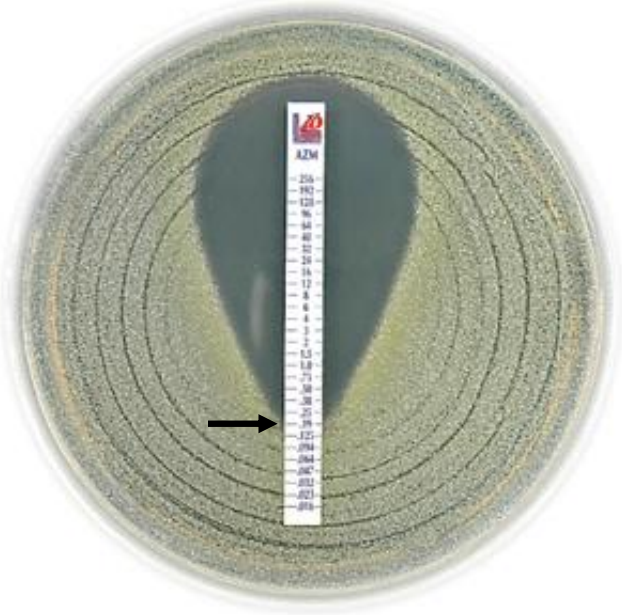
- Requires isolation of pathogen – pure culture
- Generally covers all resistance mechanisms
- Determine relative susceptibility to relevant antibiotics
  - MICs of drugs against the pathogen; TAT – 6-24 hours
  - Using PK/PD parameters, determine if MIC is low enough for drug to be useful for therapy (susceptible)
    - Susceptible – may be useful for therapy
    - Resistant – not useful for therapy
    - Intermediate – “gray” area; avoid use or use maximal safe dose

Antimicrobial Agent	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	
PENICILLINS										
Ampicillin	10 µg	≥ 17	–	14–16^	≤ 13	≤ 8	–	16^	≥ 32	**(5)** Results of ampicillin testing can be used to predict results for amoxicillin. **(6)** Breakpoints when oral ampicillin is used are only for therapy of uncomplicated UTIs due to *E. coli* and *Proteus mirabilis*.
Piperacillin*		–	–	–	–	≤ 8	16	–	≥ 32	**(7)** Disk diffusion breakpoints have been removed because no disk correlate data are available for the revised piperacillin MIC breakpoints. Disk diffusion breakpoints will be reassessed if data become available.

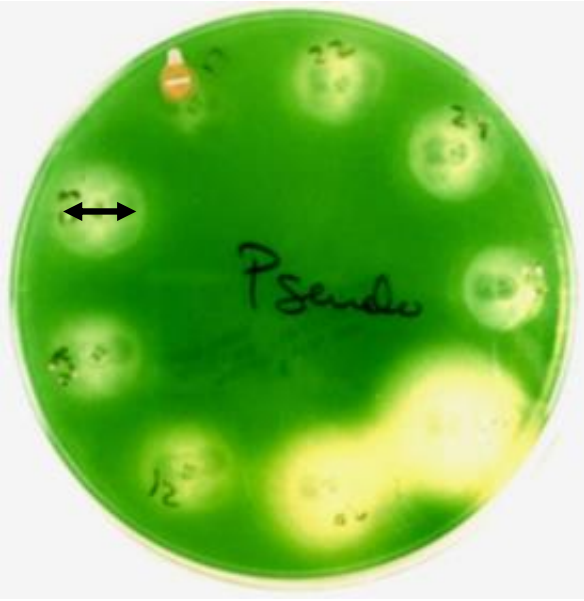
Microdilution



Gradient Diffusion



Disk Diffusion



Vitek 2

Genotypic AST

- On isolate or directly on specimen
- Detects the common genetic determinant of resistance to the particular antibiotic; generally not comprehensive
- Detect resistance gene by molecular method, e.g., PCR; faster (TAT 1-4 hrs) but more expensive
  - If present – assume resistance; drug not effective
    - Caveat: Gene may be present but not expressed
  - If absent – assume susceptibility; drug may be effective
    - Caveat: Other mechanisms of resistance may be present

Summary

- Timely and accurate laboratory diagnosis of infectious diseases is important for patient management
- Specimen quality is a critical determining factor in value of results
- Rapid molecular methods for identification and susceptibility testing are becoming more common
- Antimicrobial susceptibility testing provides a useful guide for selecting therapy

Questions?