Introduction to the Clinical Microbiology Laboratory

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At the conclusion of this program, you will be able to:

- Describe the **primary role** of a clinical microbiology laboratory with a focus on bacteriology.
- Explain how **improperly collected** specimens can contribute to misleading results.
- List examples where **bacteria reported** may **NOT** be contributing to an infection.
- Discuss tests used to determine if a bacterium is **susceptible or resistant to an antimicrobial agent.**
- Describe a **cumulative antibiogram** and how this report can be used to guide empiric therapy and monitor % of bacteria susceptible (%S) to specific antimicrobial agents.

Scenario: Sick patient in the hospital

Physician

- Physician sends a specimen to the clinical microbiology lab
- What does he/she want to know?
  - Does the specimen contain pathogens?
    - What type?
    - How many?
  - What antimicrobials can I use to treat this patient?

Infection Prevention

- Reviews microbiology laboratory reports
- What does he/she want to know?
- Could the pathogens isolated have been acquired while the patient was in this facility?
- What can be done to prevent the spread of the pathogens?

What is Clinical Microbiology?

- **Function** of the clinical microbiology laboratory:
- **Clinical**: diagnosis and management of infections
- **Epidemiological**: understand infectious microbes in patients and populations, and to find sources and routes of transmission necessary for prevention efforts
- **General rules in clinical microbiology:**
  - **#1:** Positive cultures do not make an infection
  - **#2:** No lab test is 100% accurate

Who are clinical microbiologists?

www.wikipedia.com

- Bachelor's degree that includes 2 years of specialized training in clinical laboratory sciences
- **CLS:** clinical laboratory scientist
- **MLT:** medical laboratory technician
- **Ph.D.**: perform research and development

Where are Clinical Microbiologists?

- Huntington Memorial Hospital
- **Pasadena**, CA
- Tertiary care, non-academic
- 619 beds
- 6 outpatient clinics
- 1 urgent care

What is Clinical Microbiology?

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- **Epidemiological**: understand infectious microbes in patients and populations, and to find sources and routes of transmission necessary for prevention efforts

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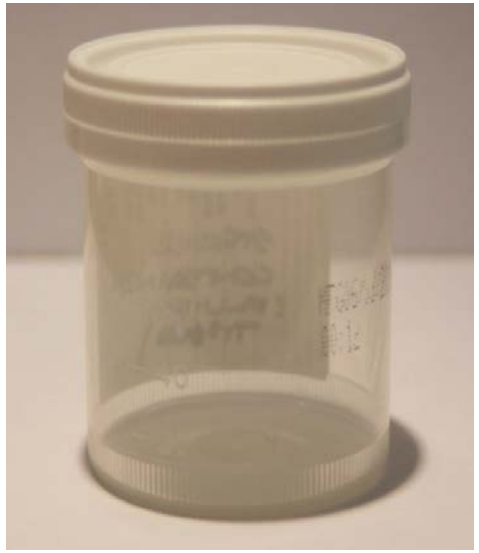
Specimen collection

- Proper specimen collection is one of the **most important factors** in diagnosing infection
- Follow instructions for collecting and transporting specimens for microbiology tests
- The **best** specimens are tissue, aspirate, pus, body fluid
- Swabs are low yield and prone to contamination

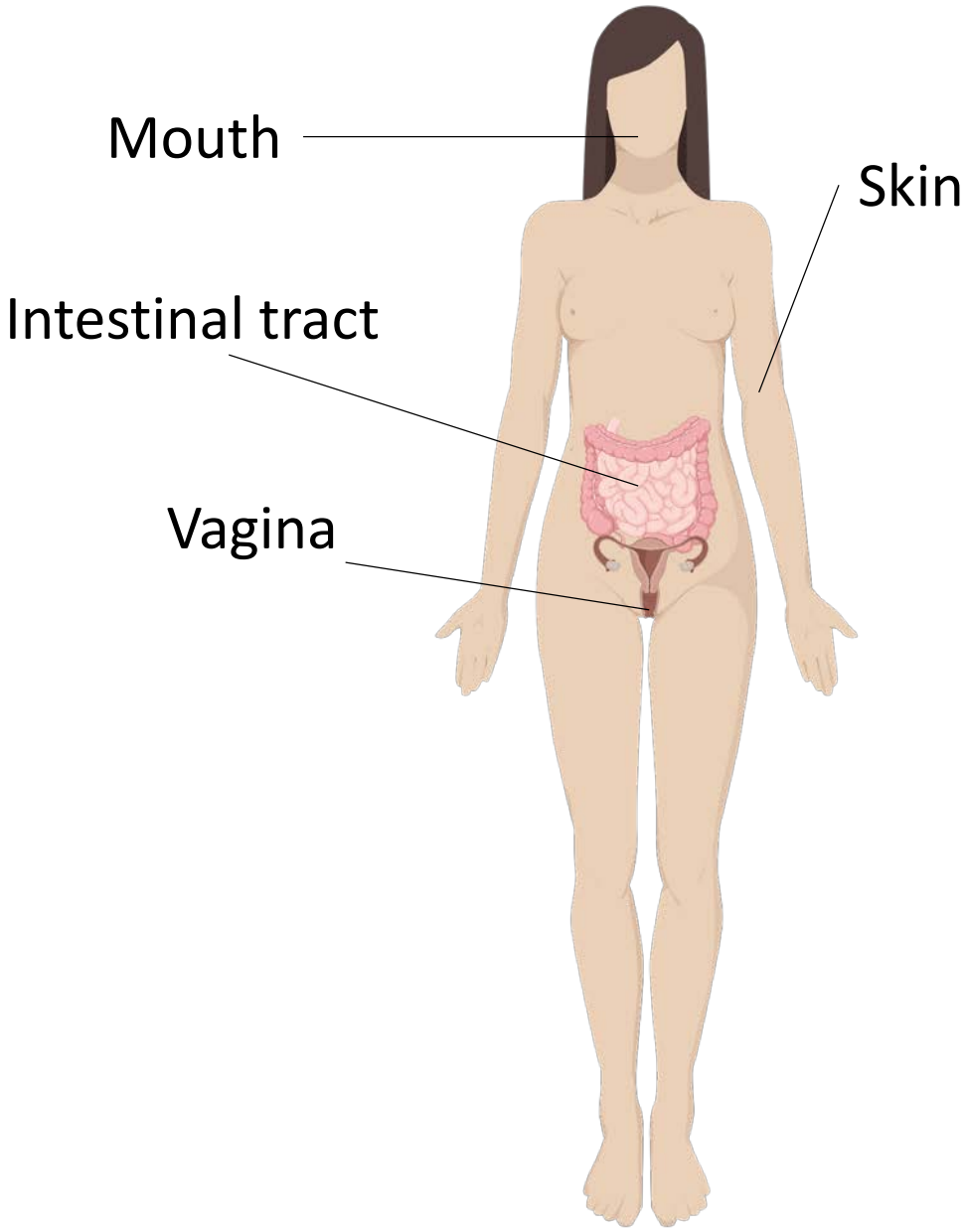


E-swab

Viral transport

Challenge: Microbiome

- Microorganisms that live on and within our bodies
- AKA "**normal flora**"
- Trillions of cells!
- 1000s of species!
- Both beneficial and potentially harmful
- **Challenge:** differentiating normal flora from pathogens



Specimen processing

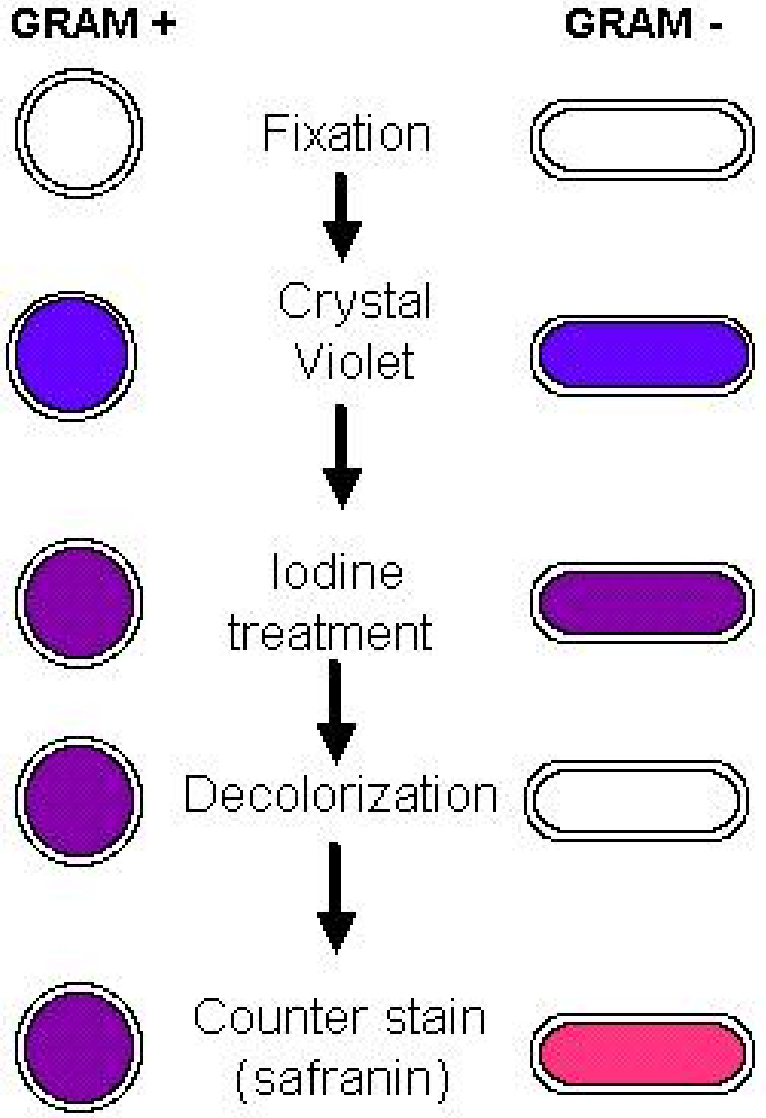
Biosafety Cabinet (BSC)

Direct Gram stain

Culture

Gram stain

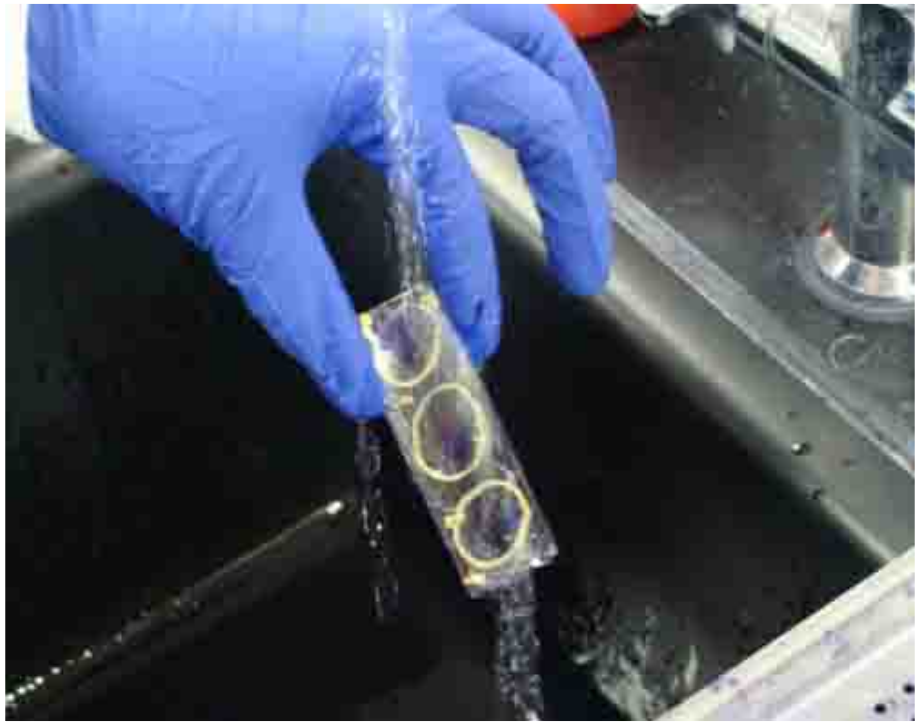
- Han Christian Gram: 1884
- Method of classifying bacteria into 2 large groups: Gram positive (+) and Gram negative (-)
- Differentiates bacteria by the chemical and physical properties of their cell walls
- Helpful in guiding initial empiric therapy
- Reported to physicians ASAP



Gram positive: thick cell wall

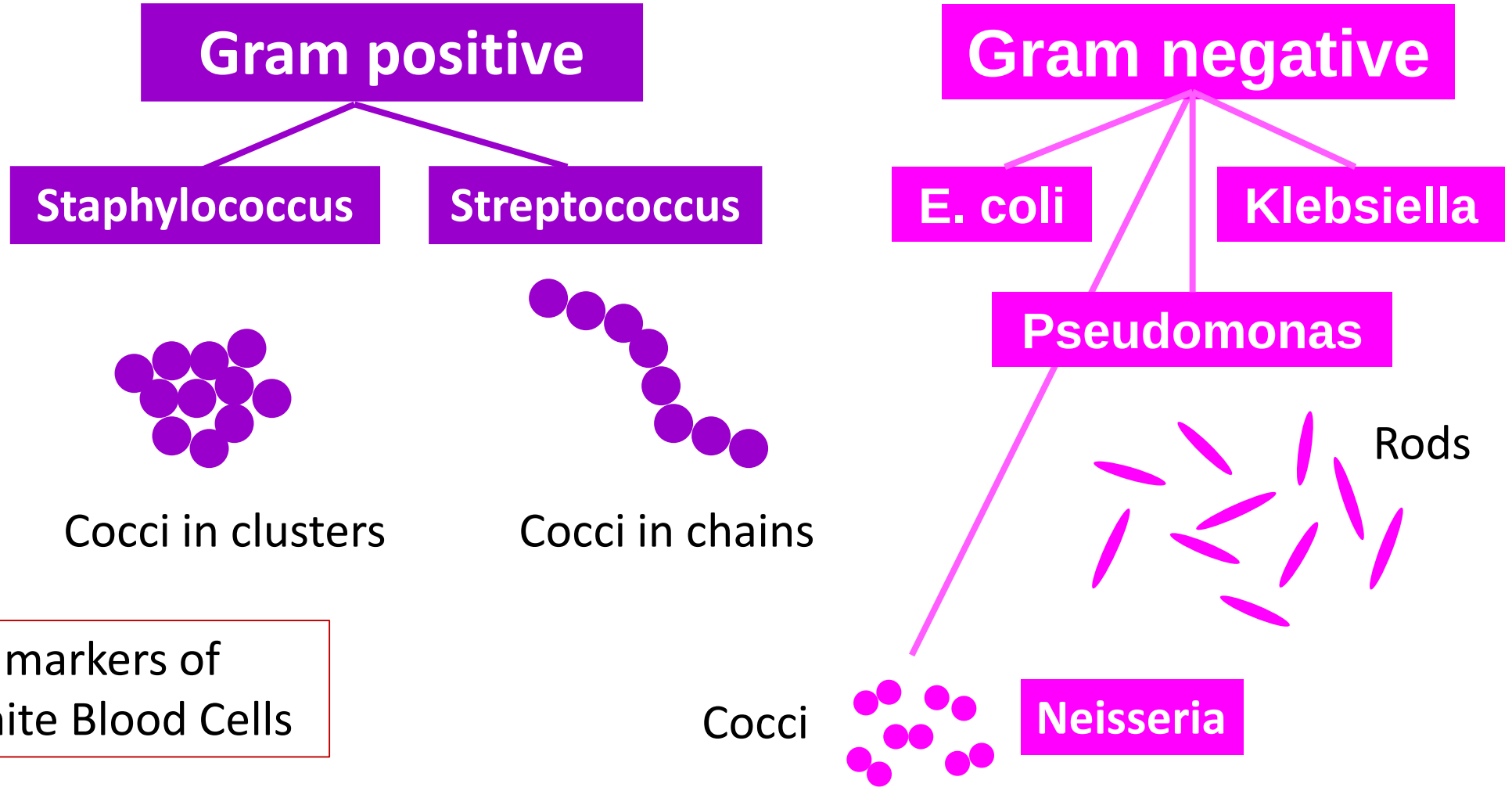
Gram negative: thin cell wall

Perform Direct Gram stain for bacteria



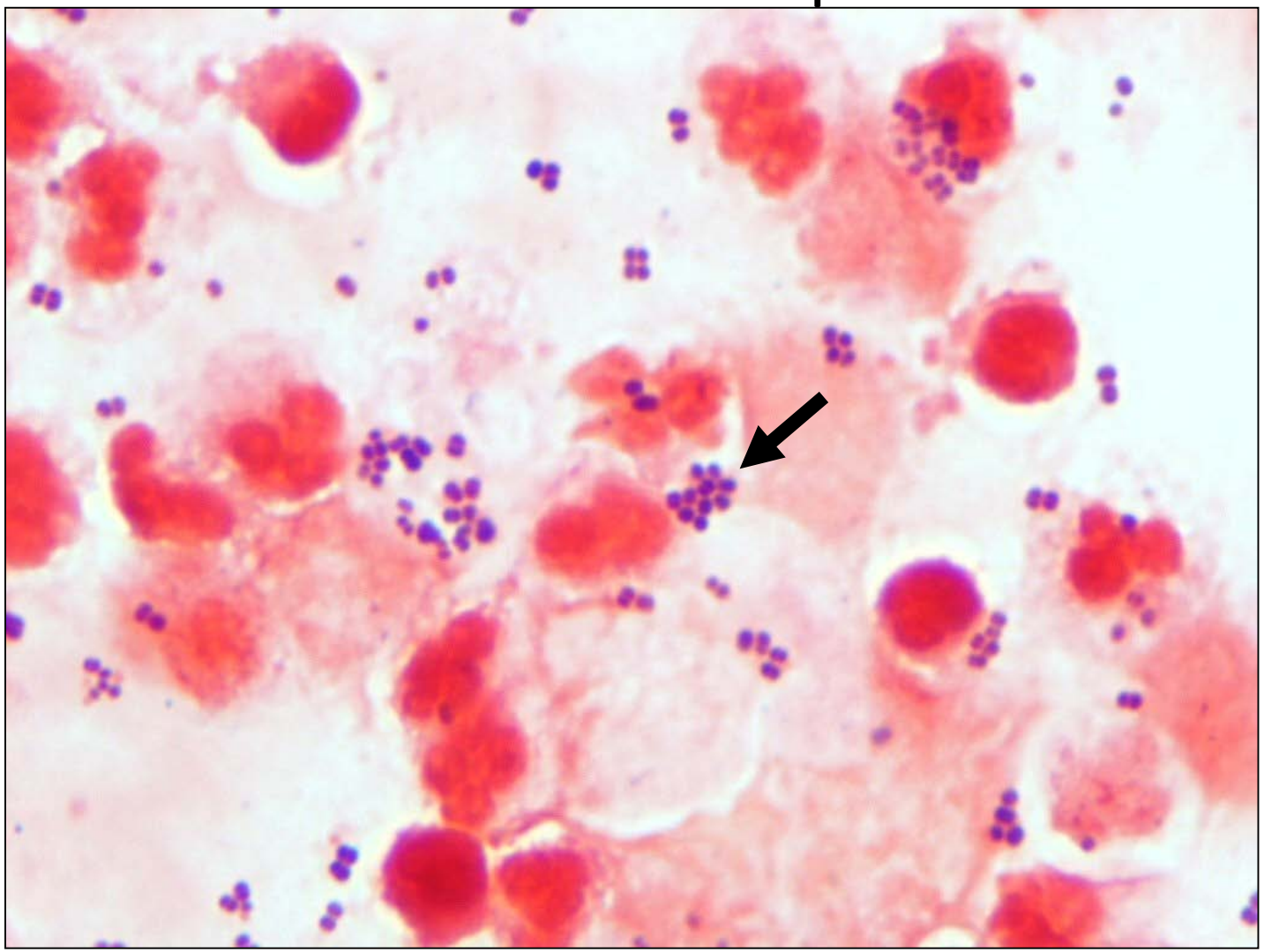
Report results within a few hours
Quick insight into possible causes of an infection

Gram stain reactions for select bacteria



Also detects markers of infection White Blood Cells

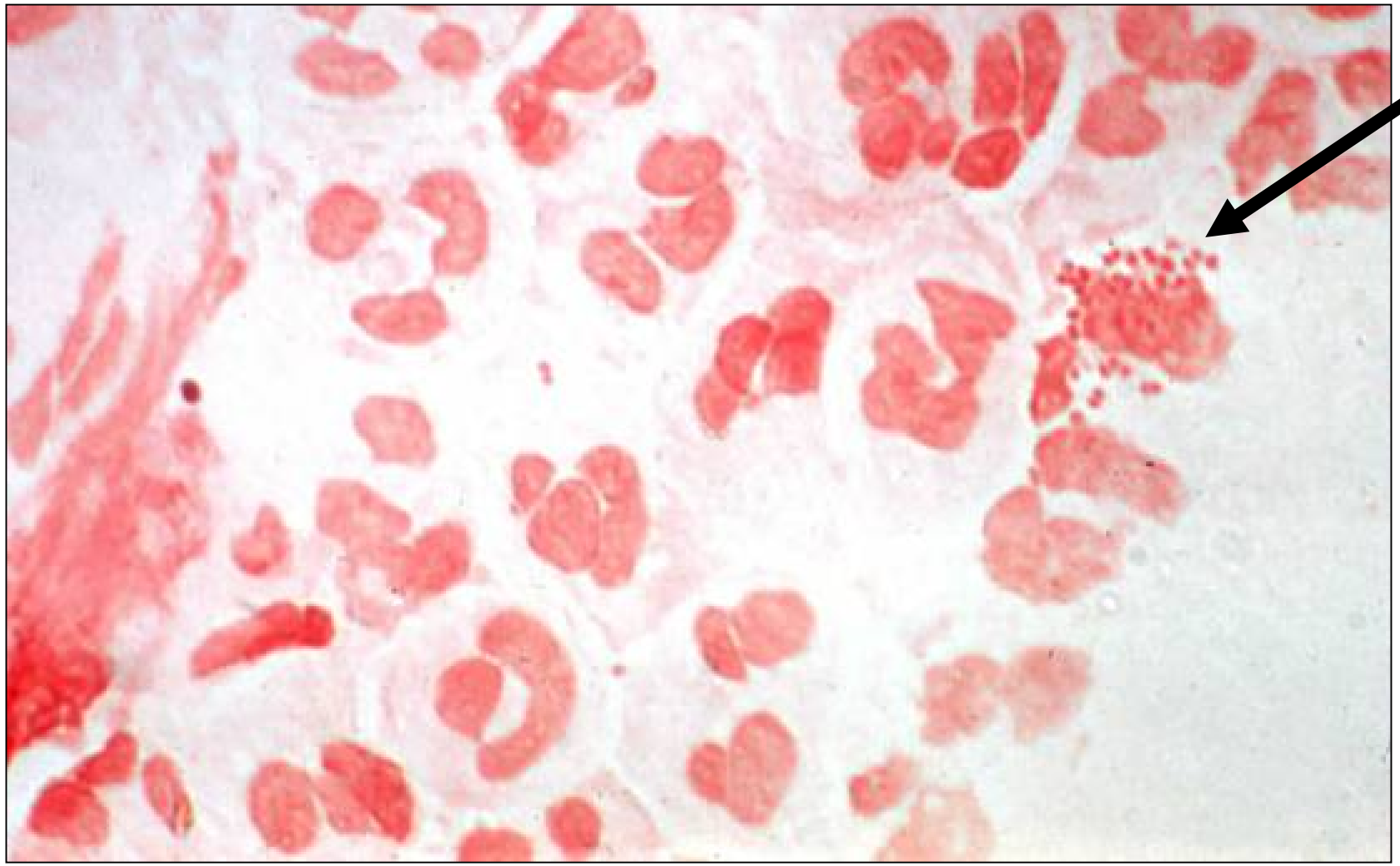
Direct Gram stain of pus from a wound



Gram-positive cocci in clusters
White blood cells

Staphylococci

Direct Gram stain of urethral discharge



Gram-negative cocci within white blood cells

Gonorrhoeae

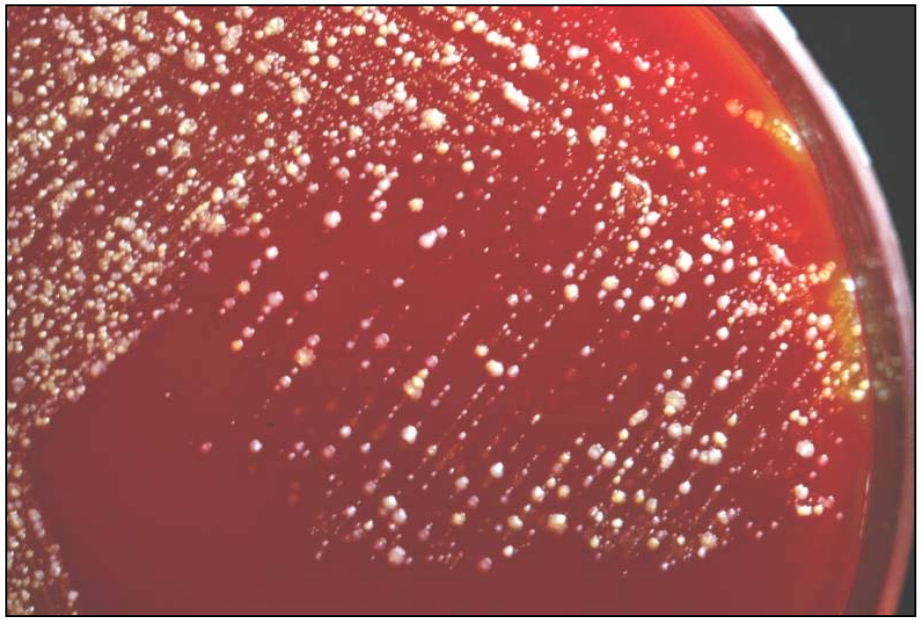
Culturing bacteria

Incubator:
human body temperature

Should I identify these bacteria and perform antimicrobial susceptibility tests?

Criteria for identification

- Should I identify these bacteria and perform antimicrobial susceptibility tests?
- Body site
- Pure versus mixed culture
- Pathogen versus normal flora



Methods Used to Identify Bacteria

Traditional methods:

- Gram stain and microscopic exam
- Growth rate and colony appearance on various types of agar media
- Reactivity with various chemicals/reagents

Modern methods:

- DNA/RNA content of microorganisms
- Protein profile (MALDI-TOF MS) of microorganisms

Case 1

- 85 year old male
- He has been sick for 3 days
- Getting progressively worse
  - Shortness of breath
  - Fever, chills, sweats, productive cough
- Temperature of 102°F
- Pneumonia
  - Sputum cultures
  - Blood cultures

10 ml sputum in Luken's trap
Avoid collecting saliva

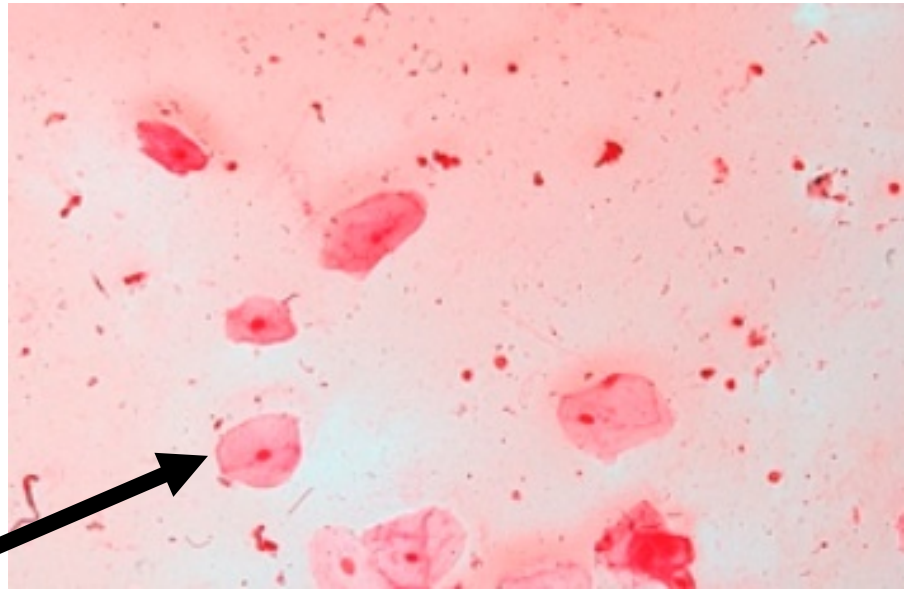
Blood culture

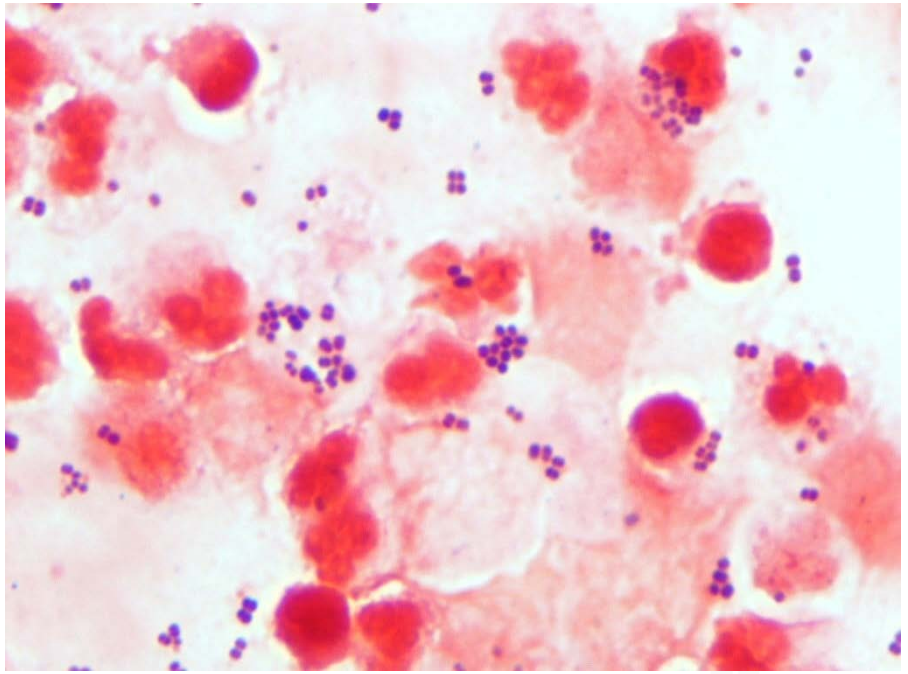
Common Lower Respiratory Tract Pathogens

- Community-acquired pneumonia (**CAP**)
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Moraxella catarrhalis*
  - "Atypicals" – *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila*
    - Often more difficult to recover / identify
- Hospital-acquired pneumonia (**HAP**): Most often ICU or ventilator-associated
  - *Klebsiella pneumoniae*
  - *Pseudomonas aeruginosa*
- Either CAP or HAP
  - *Staphylococcus aureus*
    - MRSA or MSSA

Assessing Sputum Specimen Quality

- If saliva instead of sputum is collected, we may not recover the “true pathogens”
- Prepare a direct Gram stain
- Count the number of squamous epithelial cells (SEC)

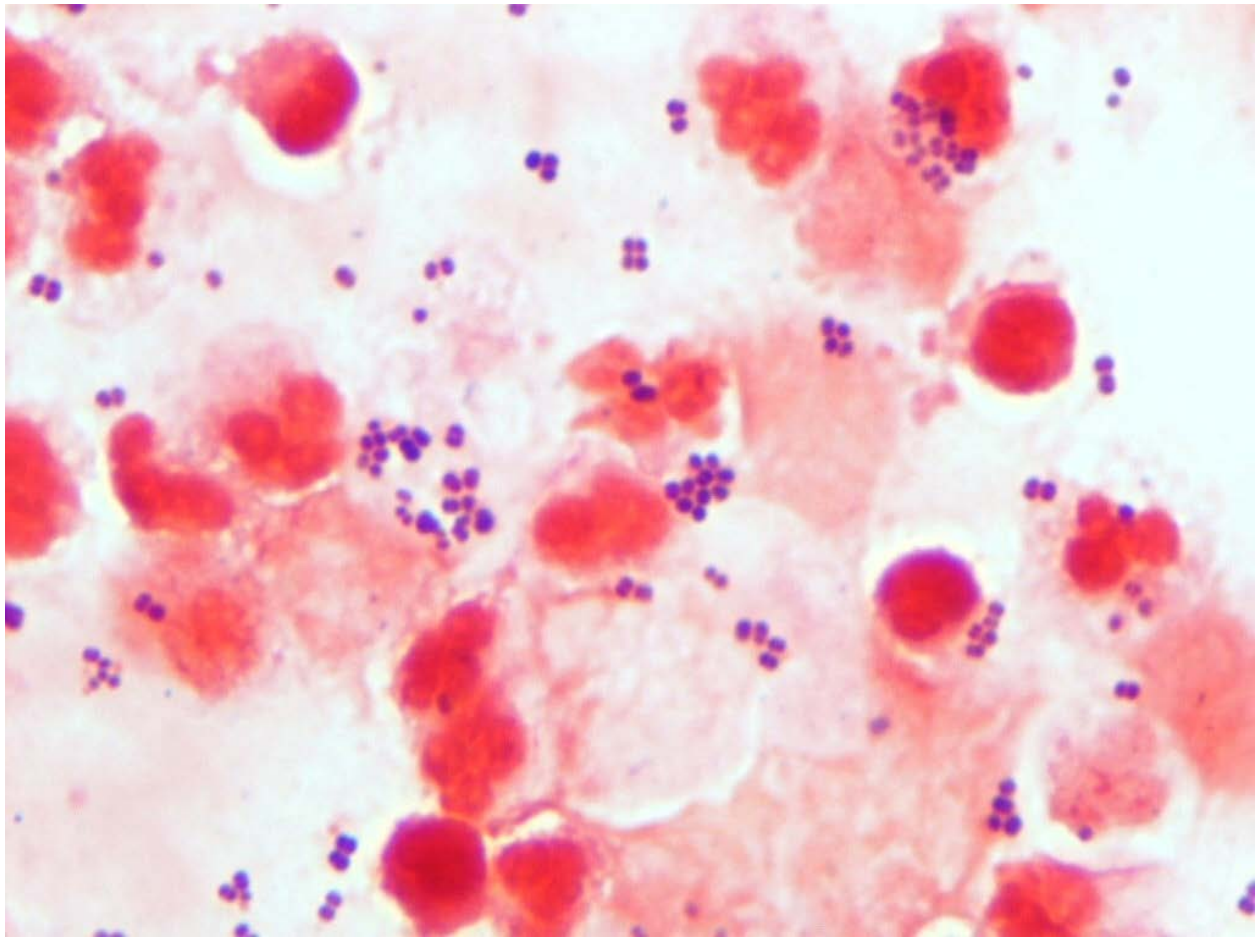




# SEC/low power field	Interpretation
< 10	No significant oral contamination
≥ 10	Indicates poorly collected specimen

Direct Gram Stain Results

- Report:
  - Many WBCs
  - Many **Gram-positive** cocci in clusters
  - Moderate normal oral flora
- Physicians:
  - Staphylococcus!



When *Staphylococcus* is suspected…

- Many different species of Staphylococci
  - Pathogenicity
  - Antimicrobial susceptibility profiles
- Questions:
  - Is this ***Staphylococcus aureus***?
    - If yes, is this methicillin-resistant *S. aureus* (MRSA) or methicillin-susceptible *S. aureus* (MSSA)?
  - Is this another species of *Staphylococcus*?
    - Typically lumped into "**coagulase-negative Staphylococci**" (CoNS)
    - Often **contaminant**; less clinically significant than MRSA or MSSA

Treatment of *S. aureus* infections

MSSA

Oxacillin* or Nafcillin*

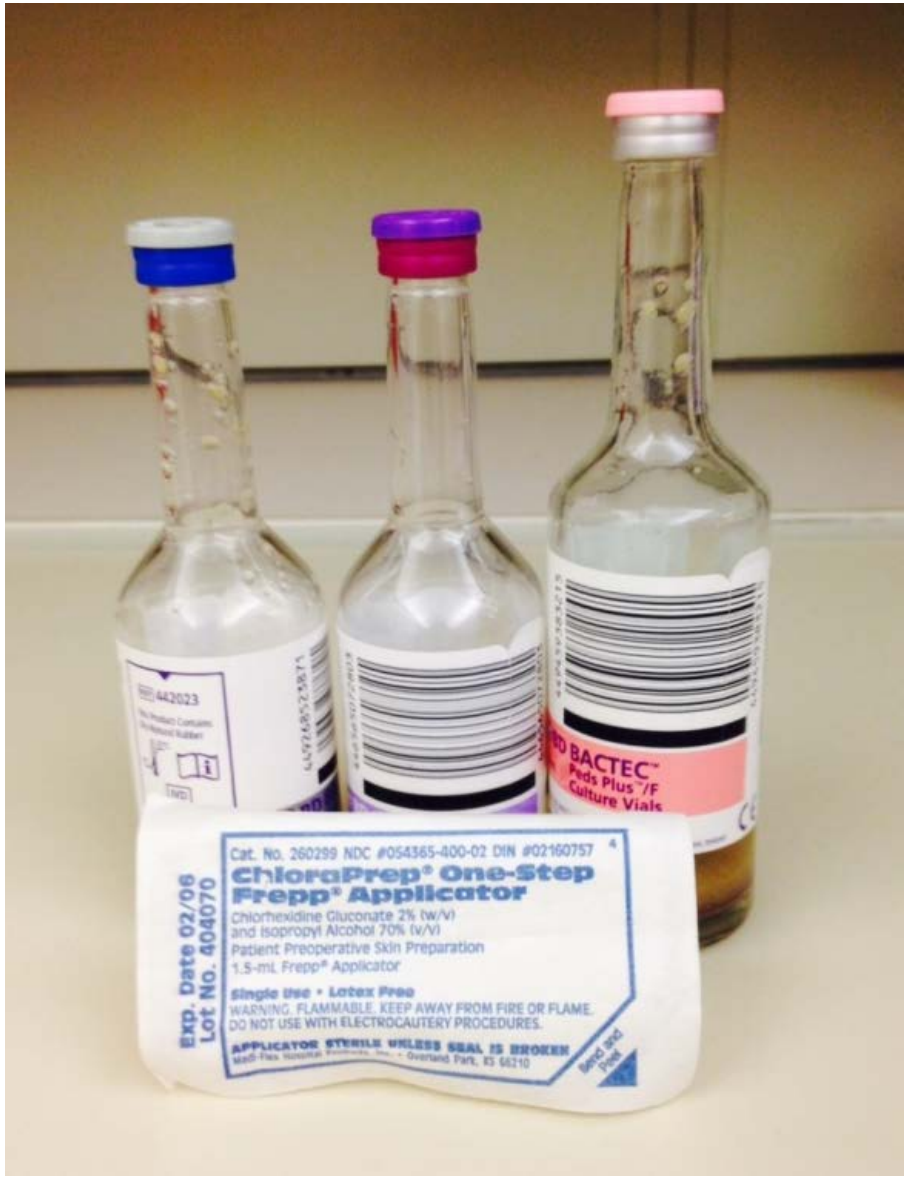
MRSA

Vancomycin

*Methicillin very similar but no longer available

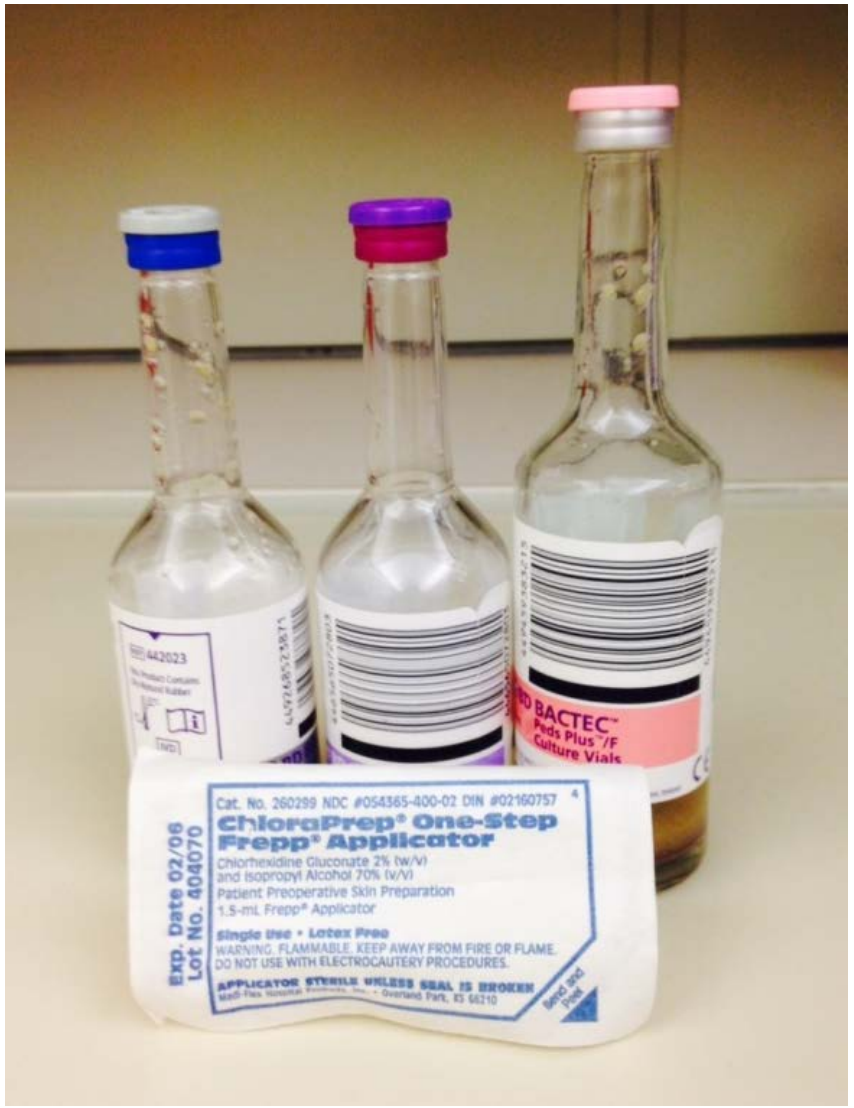
Blood Culture

- Blood culture bottles
  - **Blue**: Aerobic bottle
  - **Purple**: Anaerobic bottle
  - **Pink**: Pediatric bottle
- Contain:
  - SPS
    - Anticoagulant
    - Antiphagocytic
  - Media
  - Resin



Blood Culture: Collection

- Proper collection site preparation is important for avoiding contamination by normal flora
- Collection sites:
  - Peripheral or IV catheter
  - If drawn from IV catheter, also draw from peripheral site
- Disinfect bottle tops with an alcohol wipe
- Disinfect puncture site using ChloroPrep
  - Scrub for 30 seconds

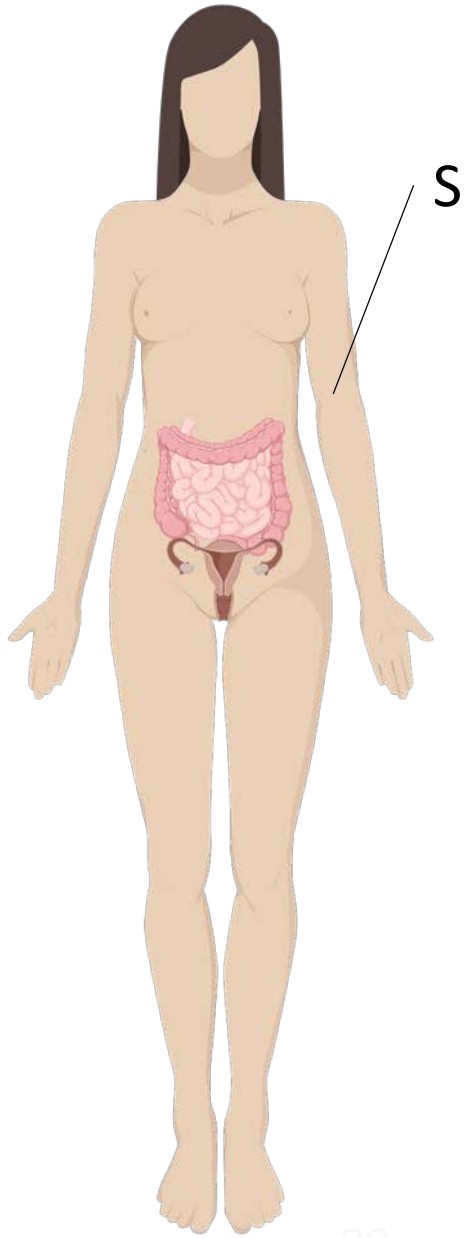


Blood Culture: Volume

- **Volume** is the most critical factor in pathogen recovery
- Adults:
  - Collect 2-3 blood culture sets per episode prior to antibiotic treatment
  - Order a minimum of 2 sets
  - Aerobic and anaerobic bottles should be drawn as a set
  - Optimum: 16-20 ml/set, 8-10 ml/bottle
  - Inoculate aerobic bottle first
  - Do not order more than 3 sets in a 24-hour period

Bloodstream infections

- Any organism present in blood is significant except **blood culture contaminants**
- Typically found in 1/2 blood culture bottles
  - Coagulase-negative staphylococci (CoNS)
  - Diphtheroids (Corynebacteria)
  - *Bacillus* spp.
  - *Propionibacterium* spp.
  - Viridans streptococcus
  - *Micrococcus* spp.
- True infection:
  - 2 sets of blood cultures must be positive
  - Patient shows signs and symptoms of a bloodstream infection

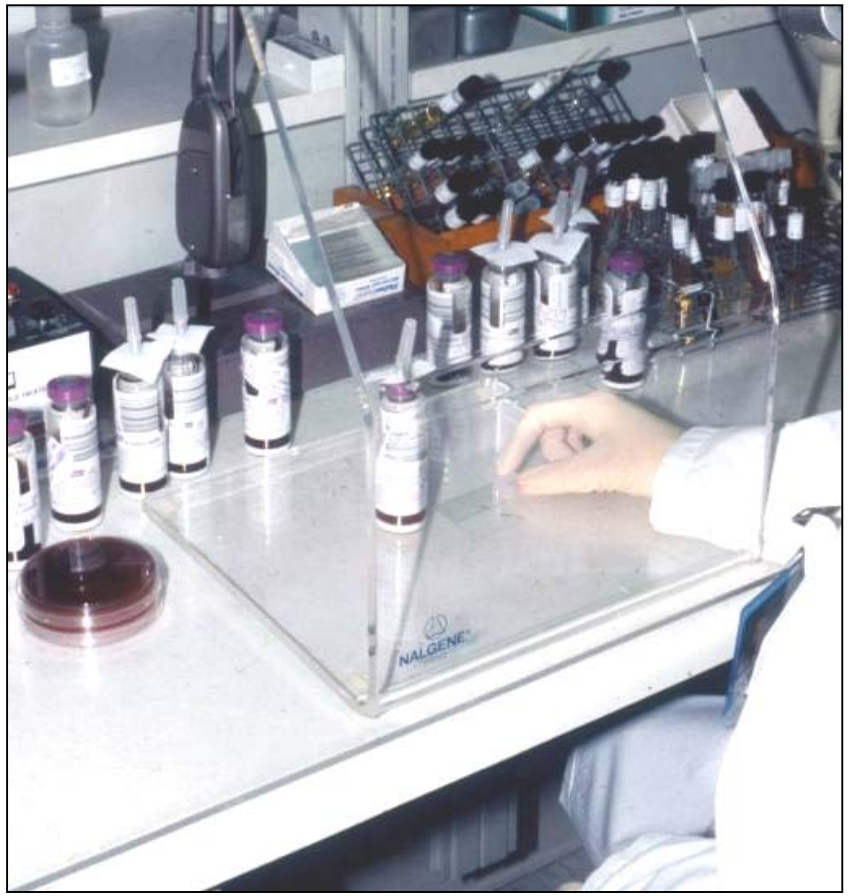


Automated blood culture

- Blood culture bottles are placed in automated blood culture instruments
- If bacteria are present, they multiply and produce **CO2**
- The machine detects **CO2** and sounds an **alarm** when it reaches a certain threshold

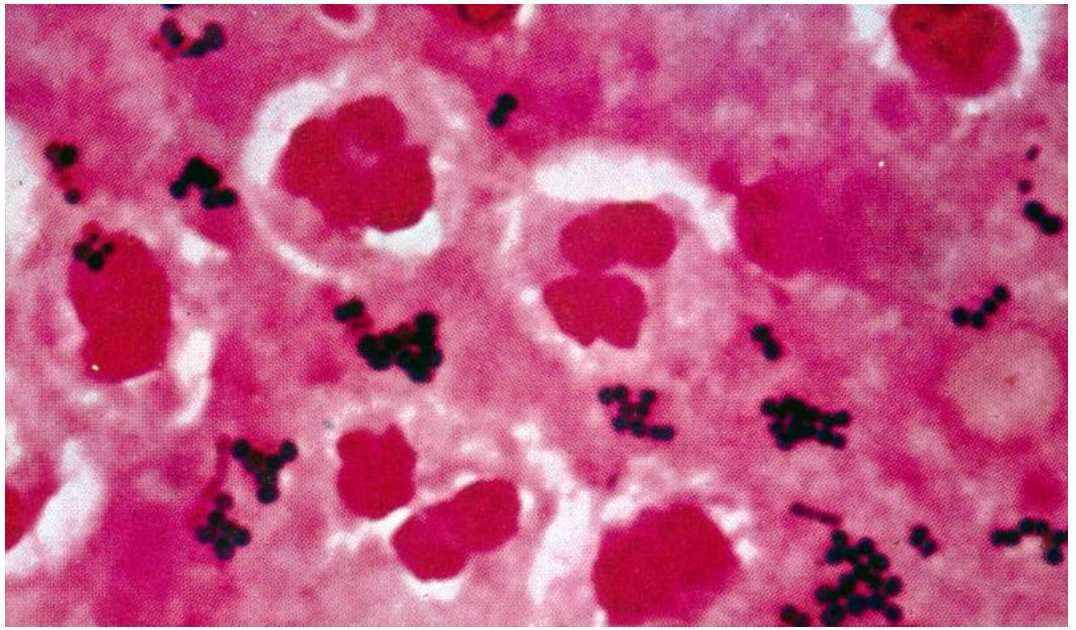
Positive blood culture bottle work-up

Gram stain and culture



Gram stain:

Gram-positive cocci in clusters

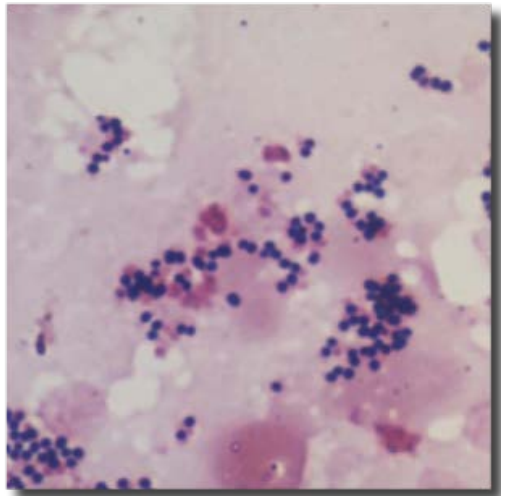


Blood “Traditional” Culture Workup (1)

Pos Blood Culture

15 m

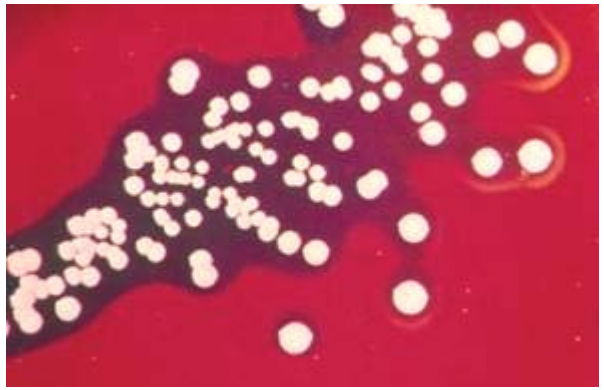
Gram Stain



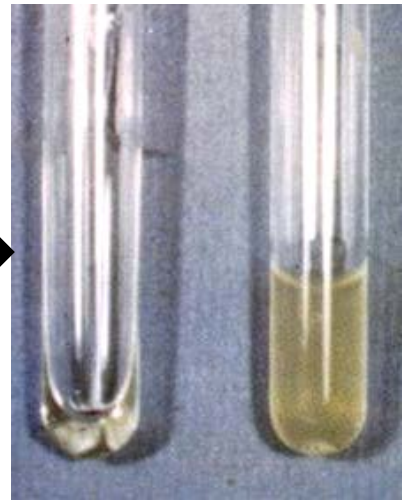
GPCC+

16-20 h

Sheep’s Blood Agar Medium
Staphylococcus spp.



Coagulase



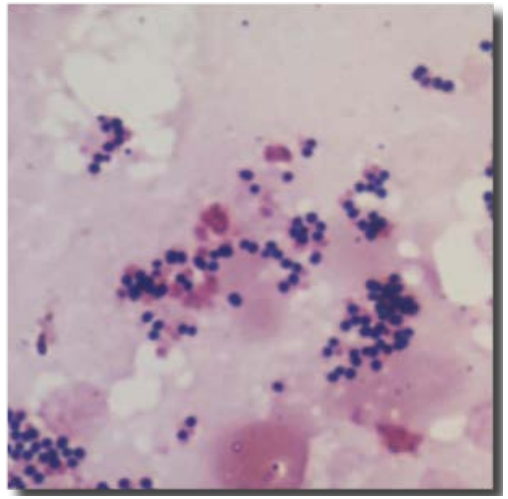
neg pos

Blood “Molecular” Culture Workup (2)

Pos Blood Culture

15 m

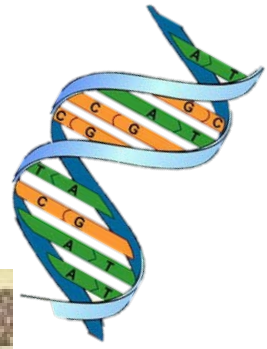
Gram Stain



GPCC+

1-2 h

Molecular Assay
Results: MSSA or MRSA or CoNS



www.bd.com/geneohm

Final report

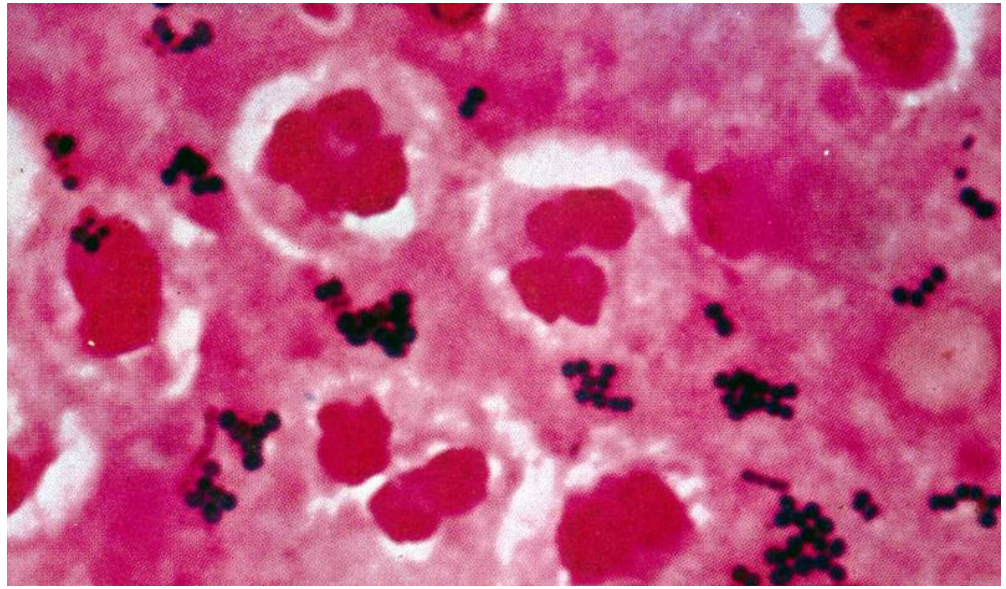
Gram Stain:

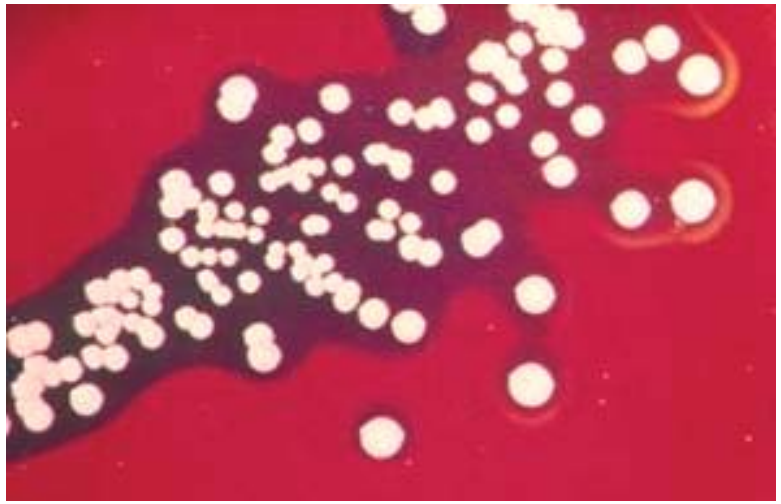
Gram-positive cocci in clusters

Culture:

Staphylococcus aureus (MRSA)

Clindamycin	R
Daptomycin	S
Linezolid	S
Oxacillin	R
Vancomycin	S

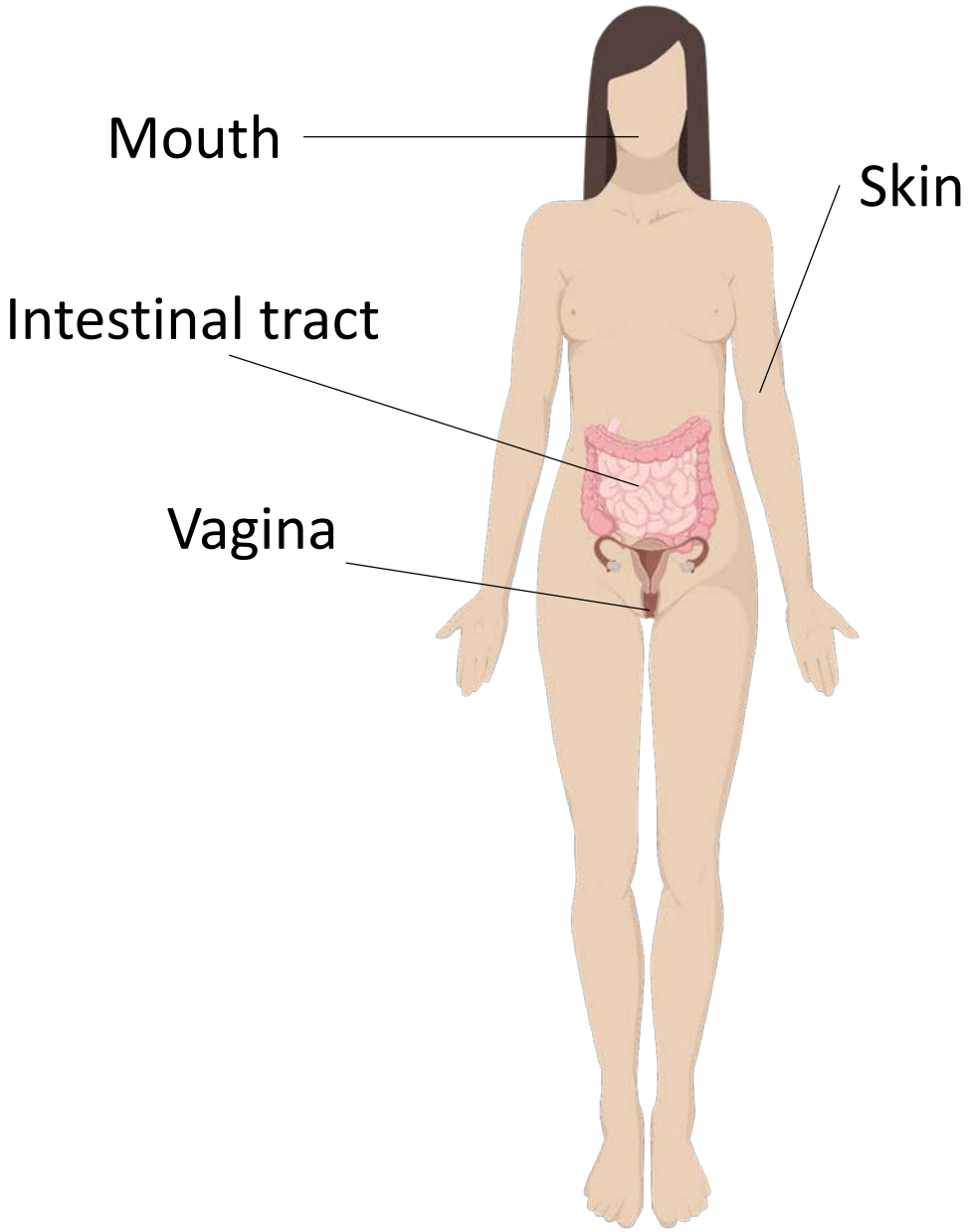




"MRSA isolated. Please check infection control policies."

Case #2

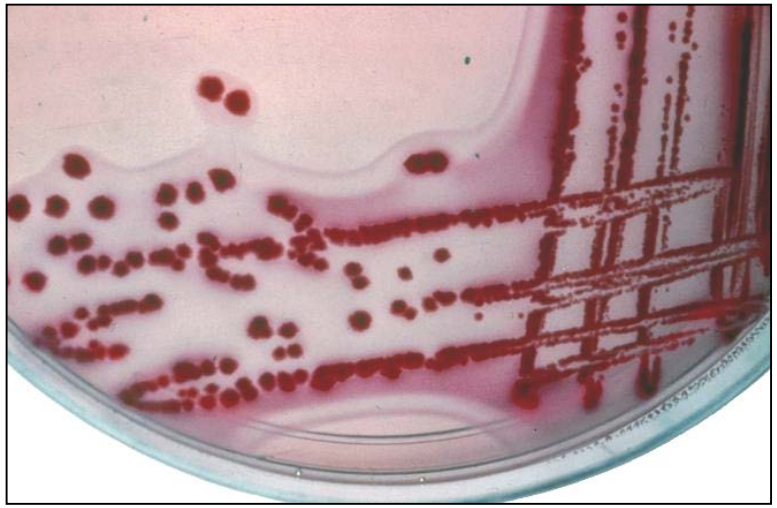
- 25 year old female
- Presents with dysuria (painful urination)
- Frequency of urination
- Urinary tract infection (UTI)
- Challenge: pathogens from normal flora



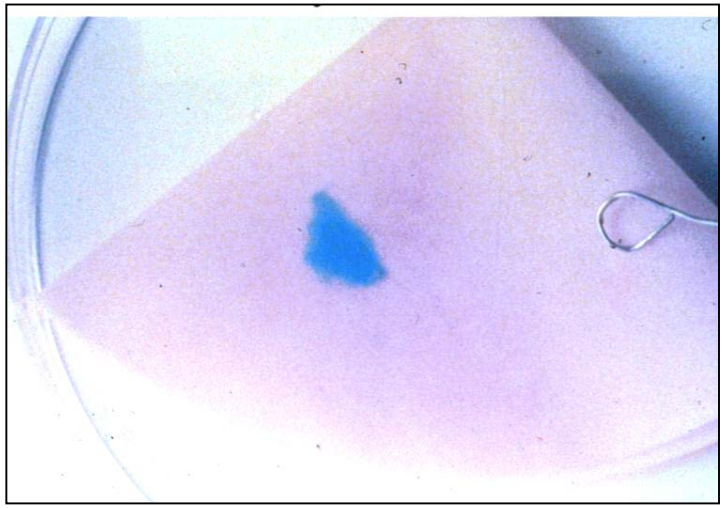
Most common UTI pathogens

- **Community acquired**
  - Most common: *E. coli*
  - *Klebsiella,* other Enterobacteriaceae
  - *Staphylococcus saprophyticus*
- **Hospital acquired**
  - *E. coli, Klebsiella,* other *Enterobacteriaceae*
  - *Pseudomonas aeruginosa*
  - Enterococci
  - Staphylococci

Spot indole test



Positive



E. coli

Urine collection and transport

- Must test within 2 hours of collection if stored at room temperature
- Must test with 24 hours if refrigerated
- Must test within 2 days if in boric acid preservative (“Grey top”)

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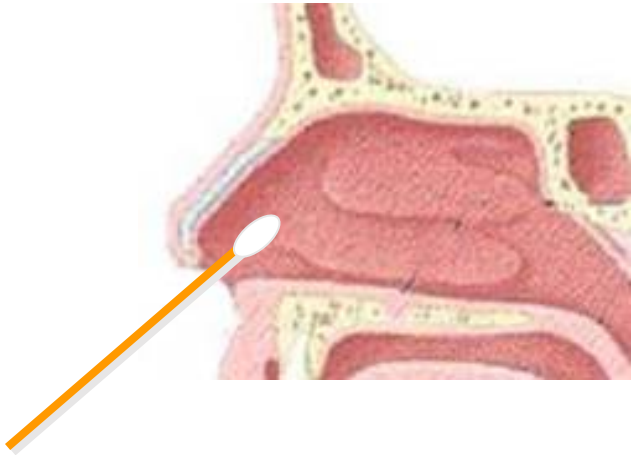
Infection Prevention: Surveillance Cultures

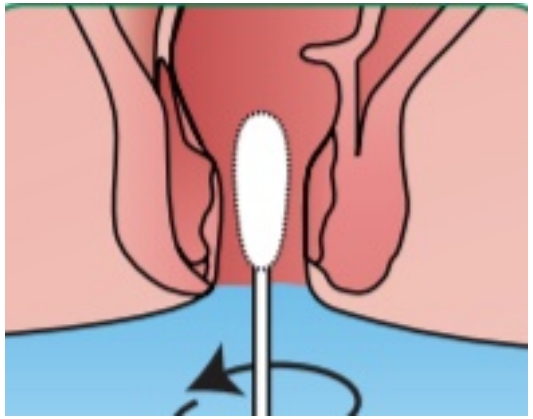
- Different than diagnostic cultures
- Must order as “surveillance culture”
- Must send appropriate specimen
- Only tested for “targeted” pathogen
  - **MRSA**: nares swab
  - **Rectal swab**: carbapenem resistant *Enterobacteriaceae* (CRE)

Nares Swab



MRSA

Rectal Swab

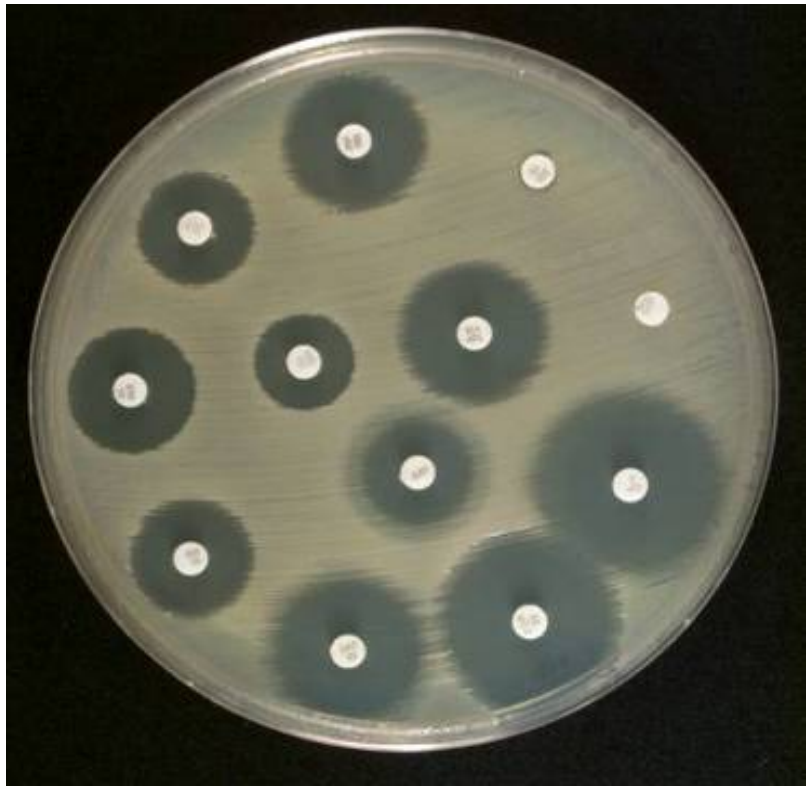


CRE

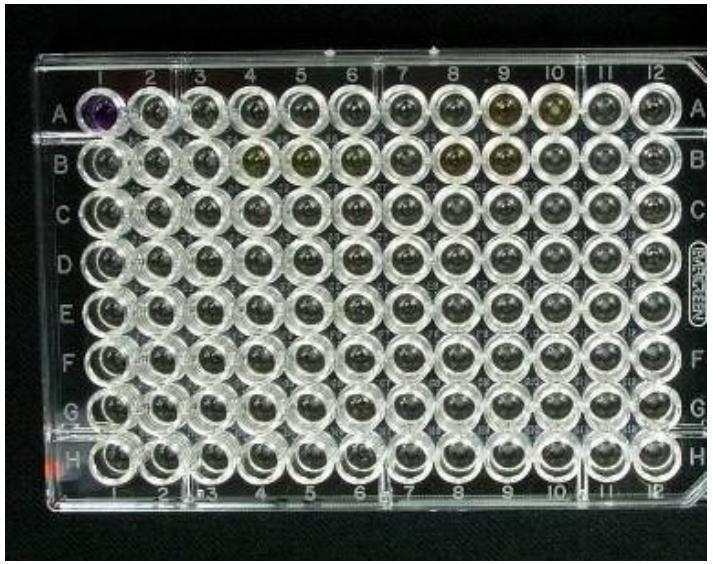
Tests to Detect Antimicrobial Susceptibility

Antimicrobial Susceptibility Testing (AST)

Disk diffusion (Kirby Bauer)



Broth Microdilution MIC



MIC = minimal inhibitory concentration (lowest concentration of drug that inhibits growth of the test bacteria)

Reported results:

- **Susceptible (S)** – drug likely to work providing it can get to the infection site
- **Resistant (R)** – drug won't work
- **Intermediate (I)** – drug may or may not work depending on site of infection and patient's status

Disk Diffusion (Kirby Bauer)

Pick colonies

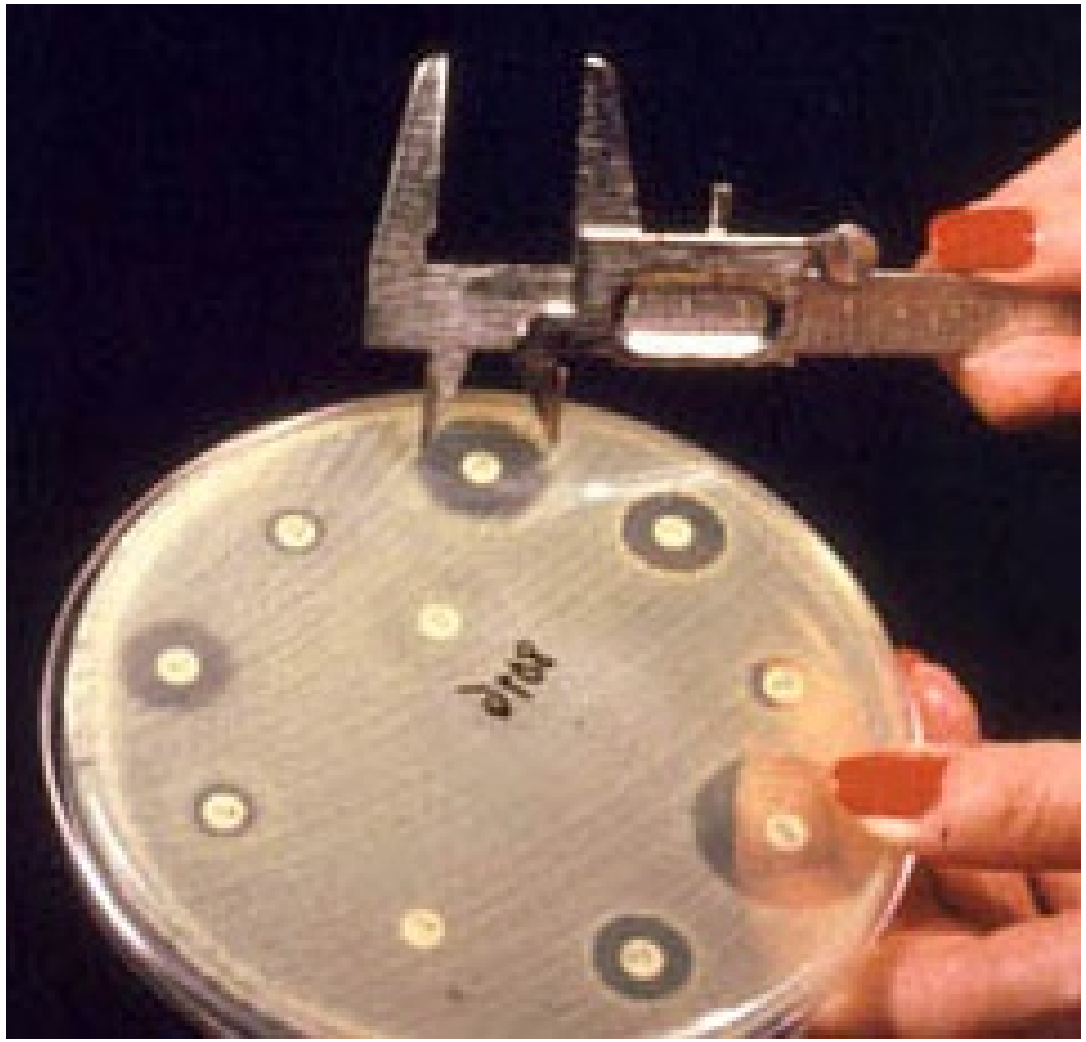
Prepare inoculum suspension

Swab plate

Add disks

Incubate overnight

Measure zone sizes



Larger zone of clearing = more susceptible

Zone Diameter “Breakpoints” (mm)

CLSI, Clinical and Laboratory Standards Institute

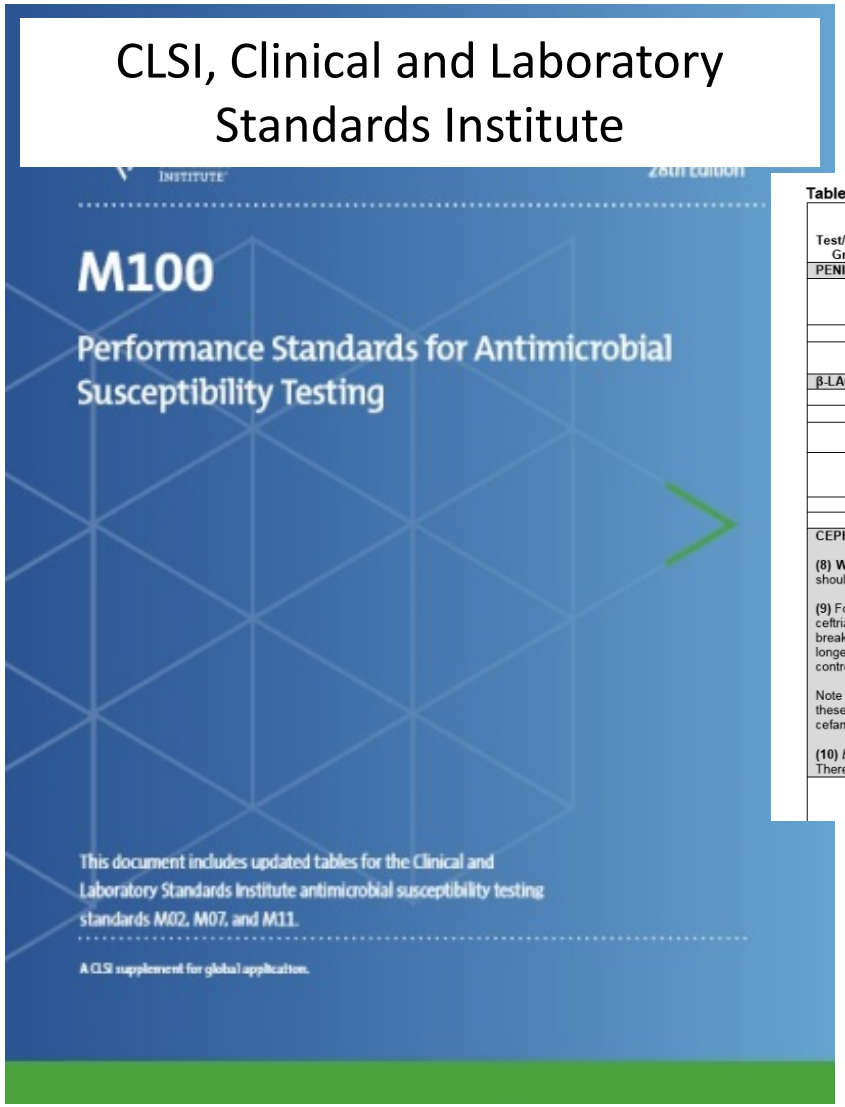
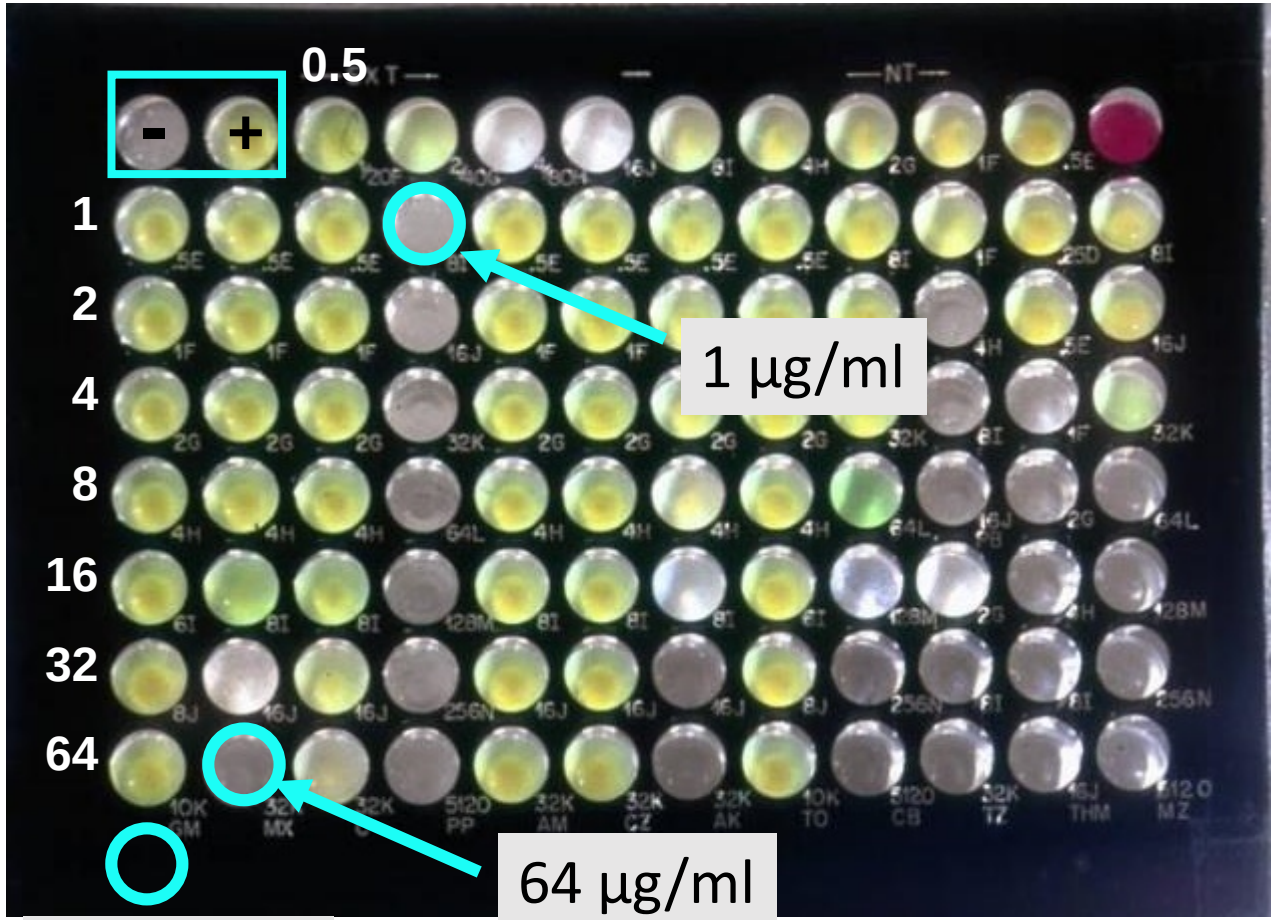


Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	SDD	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	SDD	I	R	Comments
PENICILLINS											
A	Ampicillin	10 µg	≥17	–	14–16	≤13	≤8	–	16	≥32	**(4)** Results of ampicillin testing can be used to predict results for amoxicillin. See general comment (2).
O	Piperacillin	100 µg	≥21	–	18–20	≤17	≤16	–	32–64	≥128	
O	Mecillinam	10 µg	≥15	–	12–14	≤11	≤8	–	16	≥32	**(5)** For testing and reporting of *E. coli* urinary tract isolates only.
β-LACTAM COMBINATION AGENTS											
B	Amoxicillin-clavulanate	20/10 µg	≥18	–	14–17	≤13	≤8/4	–	16/8	≥32/16	
B	Ampicillin-sulbactam	10/10 µg	≥15	–	12–14	≤11	≤8/4	–	16/8	≥32/16	
B	Ceftolozane-tazobactam	**30/10 µg**	**≥21**	–	**18–20**	**≤17**	≤2/4	–	4/4	≥8/4	**(6)** Breakpoints are based on a dosage regimen of 1.5 g every 8 h.
B	**Ceftazidime-avibactam**	**30/20 µg**	**≥21**	–	–	**≤20**	**≤8/4**	–	–	**≥16/4**	**(7) Breakpoints are based on a dosage regimen of 2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h over 2 days.**
B	Piperacillin-tazobactam	100/10 µg	≥21	–	18–20	≤17	≤16/4	–	32/4–64/4	≥128/4	
O	Ticarcillin-clavulanate	75/10 µg	≥20	–	15–19	≤14	≤16/2	–	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)											
			(8) WARNING: For *Salmonella* spp. and *Shigella* spp., 1st- and 2nd-generation cephalosporins and cephamycins may appear active *in vitro*, but are not effective clinically and should not be reported as susceptible.								
			(9) Following evaluation of PK-PD prop ceftriaxone) and aztreonam were first breakpoints was necessary for the dos longer necessary to edit results for ceph control purposes. For laboratories that h								
			Note that breakpoints for drugs with lin these drugs for *E. coli*, *Klebsiella* spp., cefamandole, and cefoperazone should								
			(10) *Enterobacter*, *Citrobacter*, and *Ser* Therefore, isolates that are initially susc								
A	Cefazolin										

Drug	S	I	R
Ciprofloxacin	≥21	16-20	≤15
Gentamicin	≥15	13-14	≤12

MIC Testing

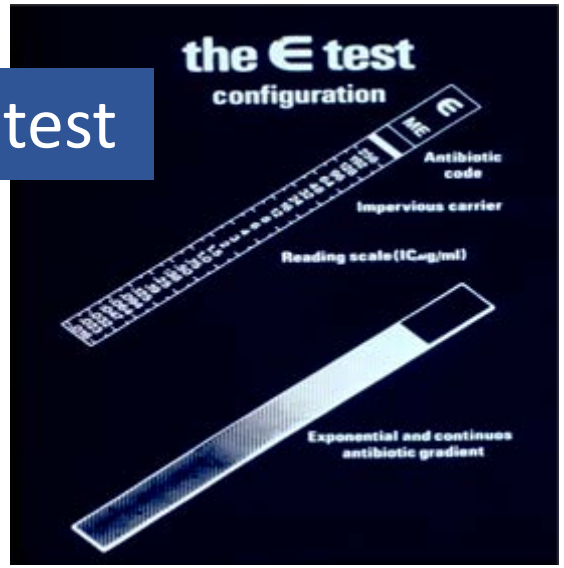


MIC "Breakpoints" (µg/ml)
Enterobacteriaceae

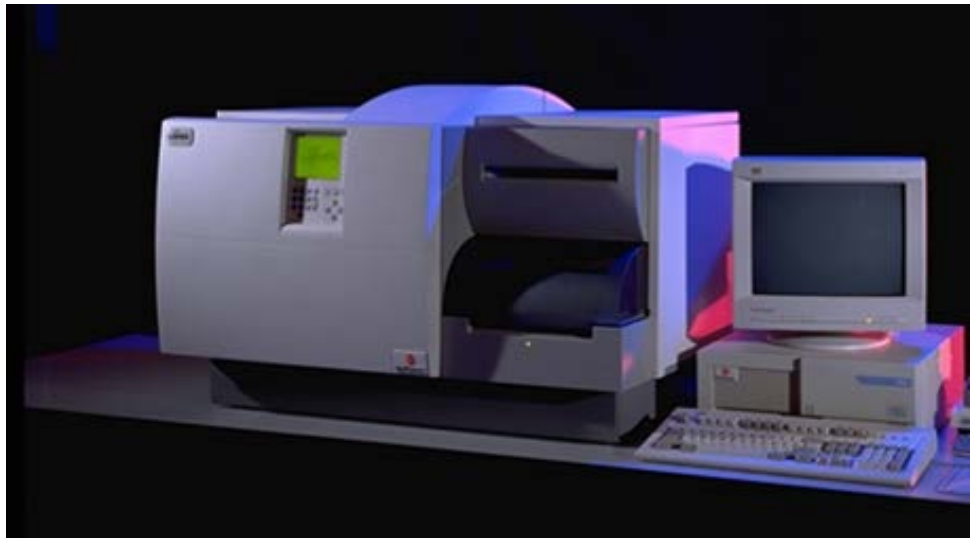
Drug	S	I	R
Ciprofloxacin	≤1	2	≥4
Gentamicin	≤4	8	≥16

Commercial Antimicrobial Susceptibility Test Systems

Etest



Vitek 2



MicroScan

Phoenix

Sensititre

Antimicrobial susceptibility testing (AST)

- Criteria on when to perform:
  - If **1 or 2 potential pathogens** is isolated form culture
  - If it is likely that the bacteria are **causing an infection**
  - If bacteria have a susceptibility pattern that is **unpredictable**

Urine culture

- **Report 1**
  - > 10^5 CFU/ml *E. coli*

Perform AST

Significant quantity of potential pathogen

E. coli is a common UTI pathogen

No contaminants

- **Report 2**
  - > 10^5 CFU/ml *Corynebacterium*
  - 40,000 CFU/ml *E. coli*
  - 10,000 CFU/ml yeast
  - 40,000 CFU/ml *Lactobacillus*

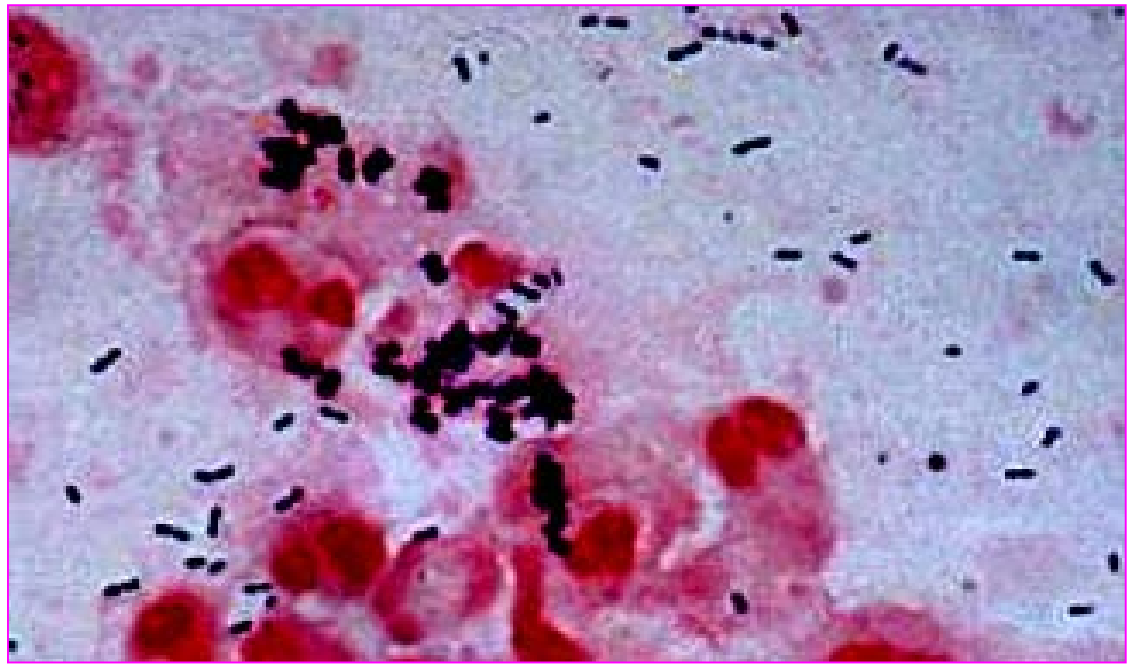
Do not perform AST

Contamination likely: mixed

Encourage **re-collection** if UTI is still suspected

Sputum culture

- Gram stain:
  - Many oral flora
  - Many Gram positive diplococci
  - Many WBCs
- Culture:
  - Many normal flora
  - Many *Streptococcus pneumoniae*



Perform AST

Good correlation of Gram stain with culture

Significant quantitate of a potential pathogen

48

Throat culture

- Culture
  - Many **Group A Streptococcus**
- **Do not perform AST routinely**
  - Group A Streptococcus is **always susceptible** to penicillin
  - Penicillin allergy
- Not necessary to perform AST on bacteria that are always (**predictably**) susceptible to the antimicrobial agents typically prescribed

AST on all organisms

- Why do we **NOT** perform AST on every potential pathogen isolated?
  - AST results on a report suggest that bacteria are causing an infection
  - Report results when NOT needed may lead to:
    - Unnecessary or inappropriate therapy
    - Selection of resistant bacteria
    - Patients on antibiotics are at higher risk for *Clostridium difficile* infections
    - Failure to look further to identify the true cause of the patient's symptoms

AST: Infection Prevention

Review of **S, I, R** most important for IP

For MIC tests, must report S, I, R with or without MIC value.

Susceptibility

	Morganella morganii [iso1]	
	MIC (MCG/ML)	
Amikacin		
Ampicillin	R	R
Azithromycin		
Cefepime	<=1	S
Ceftazidime		
Ceftazidime/Avibactam		
Ceftolozane/Tazobactam		
Ceftriaxone		
Ciprofloxacin	>=4	R
Colistin		
Ertapenem	<=0.5	S
Fosfomycin		I [2]
Gentamicin	<=1	S
Imipenem		
Levofloxacin		
Meropenem		
Minocycline		
Moxifloxacin		
Nitrofurantoin	64	I
Oral Cephalosporins		
Piperacillin + Tazobactam	<=4	S
Tobramycin		
Trimethoprim/Sulfamethoxazole	>=320	R

Two *E. coli* urine isolates

Agent	#1	#2
Ampicillin	S	R
Cefazolin	S	R
Cefepime		R
Ceftriaxone		R
Ciprofloxacin	S	R
Ertapenem		S
Gentamicin	S	S
Meropenem		
Nitrofurantoin	S	R
Pip-tazo		S
Trimeth-sulfa	S	R

Note: Broad Spectrum drug results suppressed when "S" to narrow spectrum drugs

Isolate 1: "Typical" *E. coli* - NO "R"!

Isolate 2: Acquired "R" to all PO agents. Request fosfomycin – usually not tested routinely!

3 more *E. coli* isolates

Potential outbreak?

Agent	#1	#2		#3	#4	#5
Ampicillin	S	R		R	R	R
Cefazolin	S	R		R	R	R
Cefepime		R		R	R	R
Ceftriaxone		R		R	R	R
Ciprofloxacin	S	R		R	R	R
Ertapenem				R	R	R
Gentamicin	S	S		S	S	S
Meropenem				R	R	R
Nitrofurantoin	S	R		R	R	R
Piper-tazo		S		R	R	R
Trimeth-sulfa	S	R		R	R	R

CRE = carbapenem-resistant Enterobacteriaceae

CRE = R to doripenem, ertapenem, imipenem OR meropenem

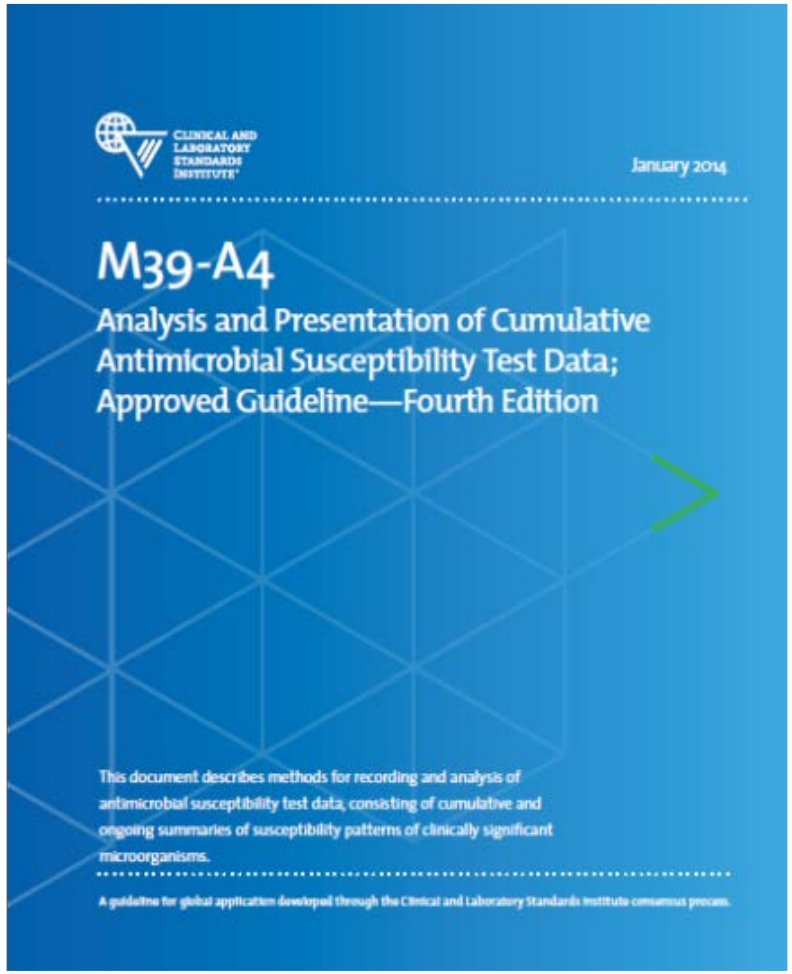
The Cumulative Antibiogram Report

- Analyzes data from routine antimicrobial susceptibility tests performed in the clinical laboratory
- Separate report prepared for each healthcare facility
- Primarily used to guide empiric therapy
- Sometimes used to monitor resistance
  - Changes in %S from year to year

Recommendations: Preparation of Cumulative Antibiogram

- Analyze/present data at least annually
- Include only species with ≥ 30 isolates of each species
- Include diagnostic isolates
- Include the 1st isolate/patient; no duplicate patient isolates

Note: Often difficult to get 30 isolates in LTCFs



GRAM NEGATIVE ORGANISMS ADULT (≥18 yr) INPATIENT	# total isolates tested	Aminoglycoside			βL	βL/βLi		Cephalosporin				FQ	Carbapenem		
		Amikacin	Gentamicin	Tobramycin	Ampicillin	Ampicillin/sulbactam	Piperacillin/tazobactam	Cefazolin	Ceftazidime	Ceftriaxone	Cefepime	Ciprofloxacin	Ertapenem	Meropenem	Trimethoprim-Sulfamethoxazole
ORGANISMS	No.	% Susceptible													
Acinetobacter baumanii	42	48	31	33	0	33	73	-	-	-	37	29	0	36	50
Citrobacter freundii	62	100	85	87	0	0	73	0	-	71	94	84	90	-	81
Enterobacter aerogenes	63	100	98	94	0	0	63	0	-	60	86	95	90	-	92
Enterobacter cloacae	131	100	98	98	0	0	74	0	-	68	87	99	80	98	94
Escherichia coli (all)	1366	100	91	90	52	57	96	97	-	97	99	74	99	99	71
(Non-urine)	373	100	89	90	47	55	94	70	-	96	98	78	97	98	69
Urine isolates	993	100	91	90	53	53	97	92	-	97	99	73	99	99	71
Klebsiella oxytoca	67	100	100	100	0	36	91	43	-	91	96	100	100	100	100
Klebsiella pneomoniae	484	99	98	98	0	79	92	91	-	98	99	94	99	100	91
Morganella morganii	73	100	78	88	0	8	96	0	-	85	97	55	99	100	58
Proteus mirabilis	310	99	82	83	42	77	98	54	-	89	94	52	99	100	67
Pseudomonas aeruginosa (all)	598	99	92	97	0	0	72	0	80	0	73	72	0	76	0
Urine	226	100	95	98	0	0	77	0	88	0	82	77	0	78	0
Blood	23	100	100	100	0	0	78	0	88	0	67	83	0	83	0
Respiratory	180	97	85	94	0	0	61	0	69	0	59	61	0	65	0
Body Fl/Wound/Tissue	137	100	95	99	0	0	76	0	85	0	79	76	0	86	0
Serratia marcescens	59	98	98	81	0	0	93	0	-	72	98	95	97	100	97
Stenotrophomonas maltophilia	42	0	0	0	0	0	0	0	45	0	0	0	0	0	98
E.coli, ESBL (all)	354	100	60	45	0	18	78	0	0	0	0	13	95	99	37
Urine	264	100	58	45	0	18	78	0	0	0	0	14	95	98	34
K. pneumo, ESBL (all)	60	100	58	47	0	3	67	0	0	0	0	28	91	98	22
Urine	38	100	53	42	0	5	24	0	0	0	0	34	86	97	24

Summary

- Assessment of patient's clinical symptoms together with reliable clinical microbiology laboratory results are essential for accurate diagnosis of infections.
- Reliable clinical microbiology laboratory results are dependent on:
  - Appropriate collection and transport of specimens.
  - Accurate identification and antimicrobial susceptibility testing.
  - Good communication between healthcare providers and lab.
- Review of clinical microbiology laboratory results is key to identification of potential hospital transmission of microbes.
- Additional clinical microbiology laboratory tests may be needed for epidemiological investigations.
- A local cumulative antibiogram can help guide empiric therapy decisions and monitor "%S" for antimicrobial agents appropriate for common pathogens.

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