

CLINICAL MICROBIOLOGY AND *INFECTION PREVENTION & CONTROL*

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Expert care with a personal touch

Objectives

- Describe role of the clinical laboratory in infection prevention
- Describe factors that can adversely affect reliable lab results
- Discuss the importance of the gram stain
- Discuss common pathogens that may contribute to HAIs
- Discuss the interpretation, use and importance of the antibiogram

Microbiology and Infection Prevention

Microbiology has two important functions related to infections

Clinical: diagnosis and management of infections

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



Clinical Microbiology

Physician's perspective:

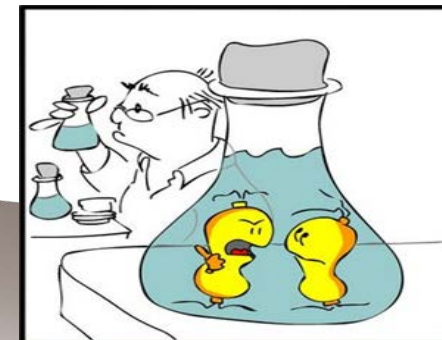
- What's growing?
- What antibiotic can be used?
 - Determined either by predictive value of the organism type (e.g. gram negative bacillus) or by complete result with sensitivities

IP or Epidemiologist's perspective:

- Surveillance for determining clusters/outbreaks and assessing trends
- Need to know organism so IP can implement proper transmission-based precautions as needed in a timely fashion

Assessing Accuracy of Lab Results

- No Lab Test is 100% Accurate 100% of the time
- Many factors can affect accuracy of laboratory tests
 1. Pre-testing: specimen collection, handling, transportation, and preservation prior to arrival in the lab
 2. During testing: specimen processing, skill of the laboratory technician, accuracy of biochemicals and instrument system
 3. Post-testing: Accuracy of result transcription, results communicated accurately



I'M FED UP WITH THIS GUY -
LET'S BECOME PATHOGENIC

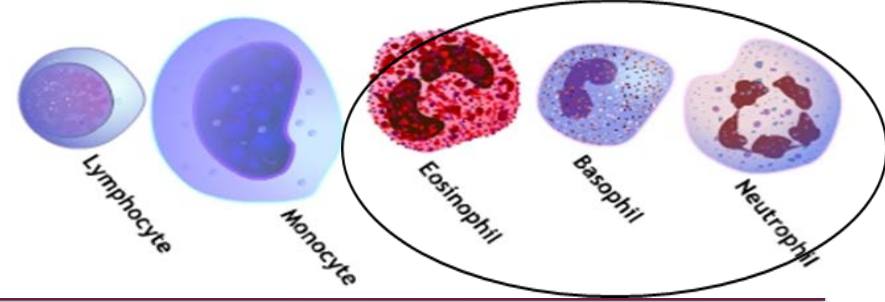
Interpreting Microbiology Test Results

- Presence of an organism does not mean it is causing disease
 - For sterile body sites, bacterial growth may confirm an infection
- Interpret all cultures in the context of what pathogens are normally found in that body site
- Contamination of samples can result in inaccurate results and pseudo-outbreaks
- To interpret microbiology test results, use in conjunction with complete blood cell counts (CBC)

What might indicate invasion into Tissue???



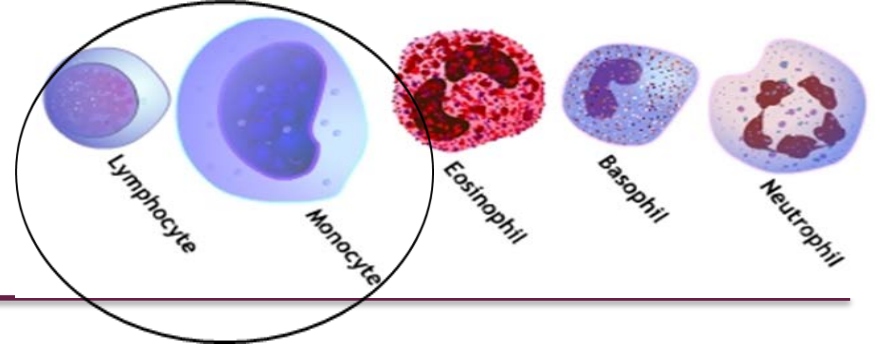
White Blood Cell (WBC)



- PMNs (polymorphonuclear leukocytes) made in bone marrow; provide general response to threat
- Neutrophils (~50-60% wbc) are first line of response to infection; may also be called **'segs'**
- Eosinophils (1-7% wbc); allergic reactions and parasites)
- Basophils (<1%); allergic reactions, help mediate strength of immune response)
- Left shift: presence of immature neutrophils (called **'bands'** or **'stabs'**) in blood count; are indicative of acute infection or inflammatory process

www.rnceus.com/cbc/cbcdiff.htm

Lymphocytes & Monocytes



- Lymphocytes (lymphs) mature in the lymphatic portion of the immune system
 - Include pathogen-specific immune response (B cells, T cells)
 - Increase may be indicative of viral infection
- Monocytes (or macrophages) phagocyte function (or eat) cellular debris and foreign pathogens from the immune system

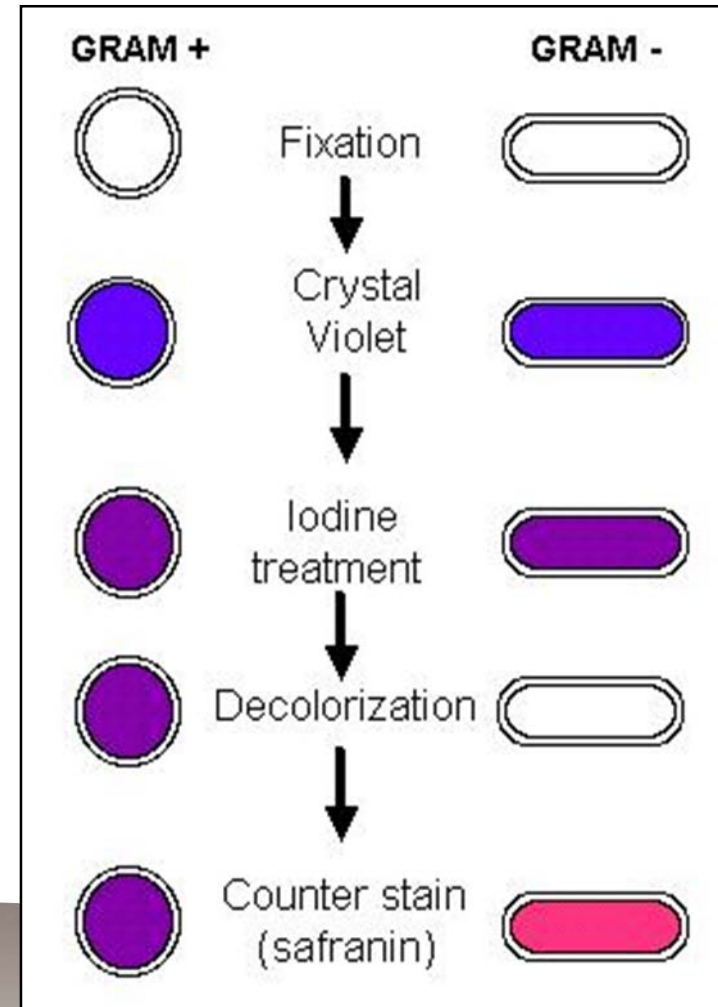
www.rnceus.com/cbc/cbcdiff.htm

Serology

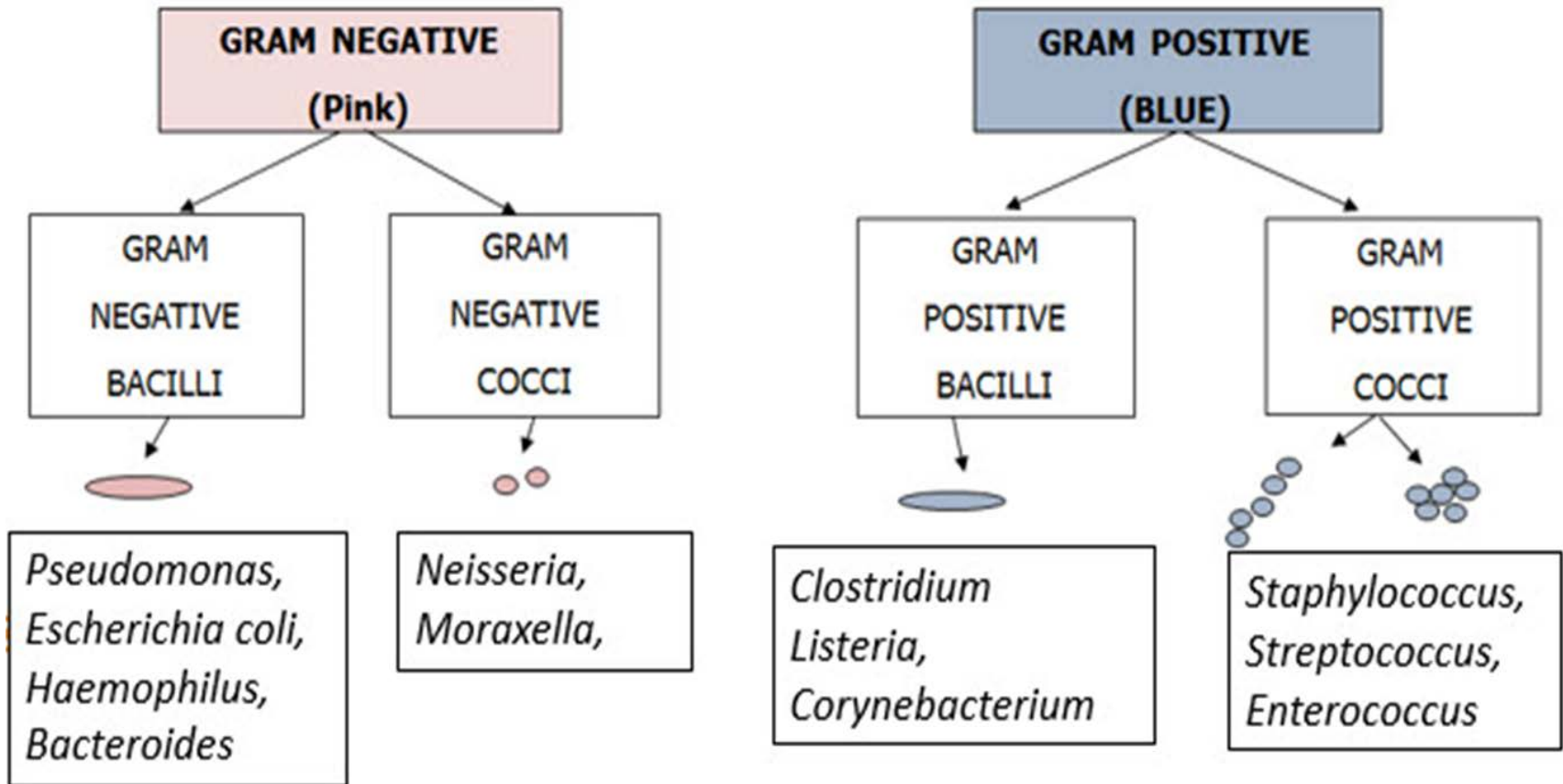
- Diagnostic test that identifies immunoglobulins (antibodies) in blood serum
- Immunoglobulins (Ig) are proteins that bind to viruses and bacteria
- Types
 - IgM: produced immediately after exposure (acute phase of disease)
 - IgG: most abundant; long term response to disease (chronic disease)
 - IgA: secretory, present in mucosal linings
 - IgE: plays a role in hypersensitivity reactions

What is Gram Stain?

- Method of classifying bacteria into 2 large groups: positive (+) and negative (-)
- Differentiates bacteria by the chemical and physical properties of their cell walls
- Helpful in guiding initial empiric therapy
 - results should get to physician ASAP
 - Helps physician with treatment plan

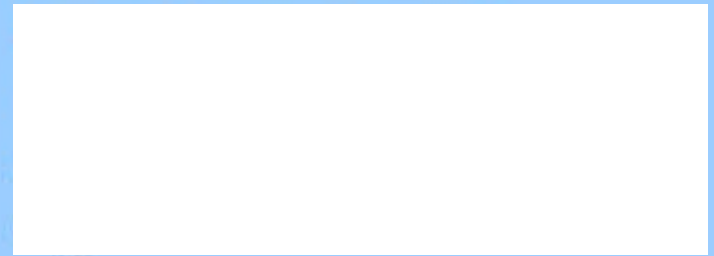


Gram Stain Identifies Four Basic Bacteria Groups

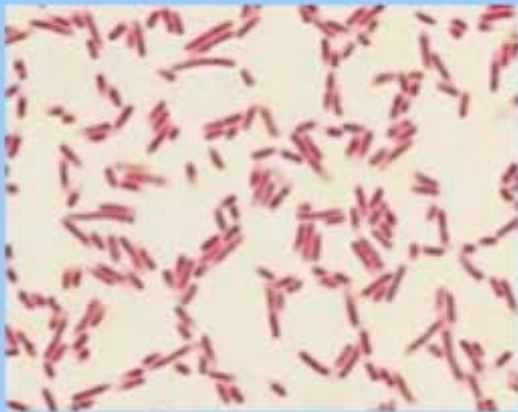




Gram positive cocci
in pairs and chains
Streptococcus



Gram negative rod – Fusiform
Shaped – Fusobacterium
species

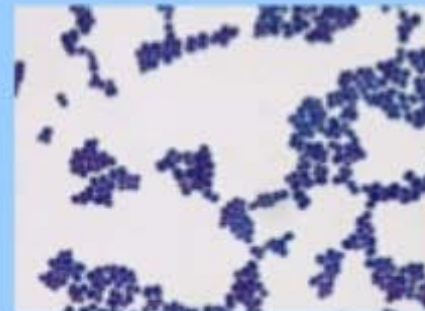


Gram negative
bacillus
Resembling an
Enteric

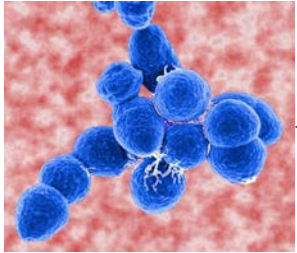


Gram positive rod
most likely a
Bacillus species

Gram positive cocci in clusters,
Staphylococcus



Common Lower Respiratory Tract Pathogens



- Community-acquired pneumonia (CAP)

- *S. pneumoniae*

- *H. influenzae*

- *Mycoplasma*

- Hospital-acquired, most often ICU or ventilator-associated

- *Pseudomonas aeruginosa*

- *Stenotrophomonas maltophilia*

- Either CAP or hospital-acquired pneumonia

- *Staphylococcus aureus* (MRSA or MSSA)

- ↑ mortality; must be recognized quickly

- *Moraxella catarrhalis* (most often CAP)



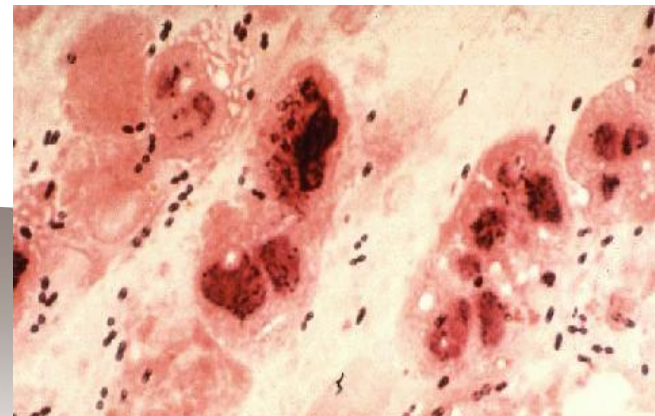
Note: Yeast is NOT usually an infecting organism for pneumonia or other lower respiratory tract infections unless it constitutes >90% of organisms in a specimen and specimen is not contaminated with oral flora

Testing for Lower Respiratory Bacterial Pathogens

- Sputum and bronchial washing are often contaminated with oral flora
- Tracheal aspirates and protected brush specimens not contaminated with oral flora

Interpreting Results from Sputum Specimens

- Results are affected by **quality of sputum** specimen
 - Squamous epithelial cells (SEC) shed from the lining of the mouth and pharynx; presence indicates saliva and oral flora
 - <10 - excellent specimen, no appreciable contamination
 - 10-25 - equivocal but acceptable
 - >25 - reject due to unacceptable levels of oral contamination
- Assess number of WBC
 - < 10 - no infection or poor immune response
 - 10-25 - equivocal
 - >25 - purulence indicates presence of infection

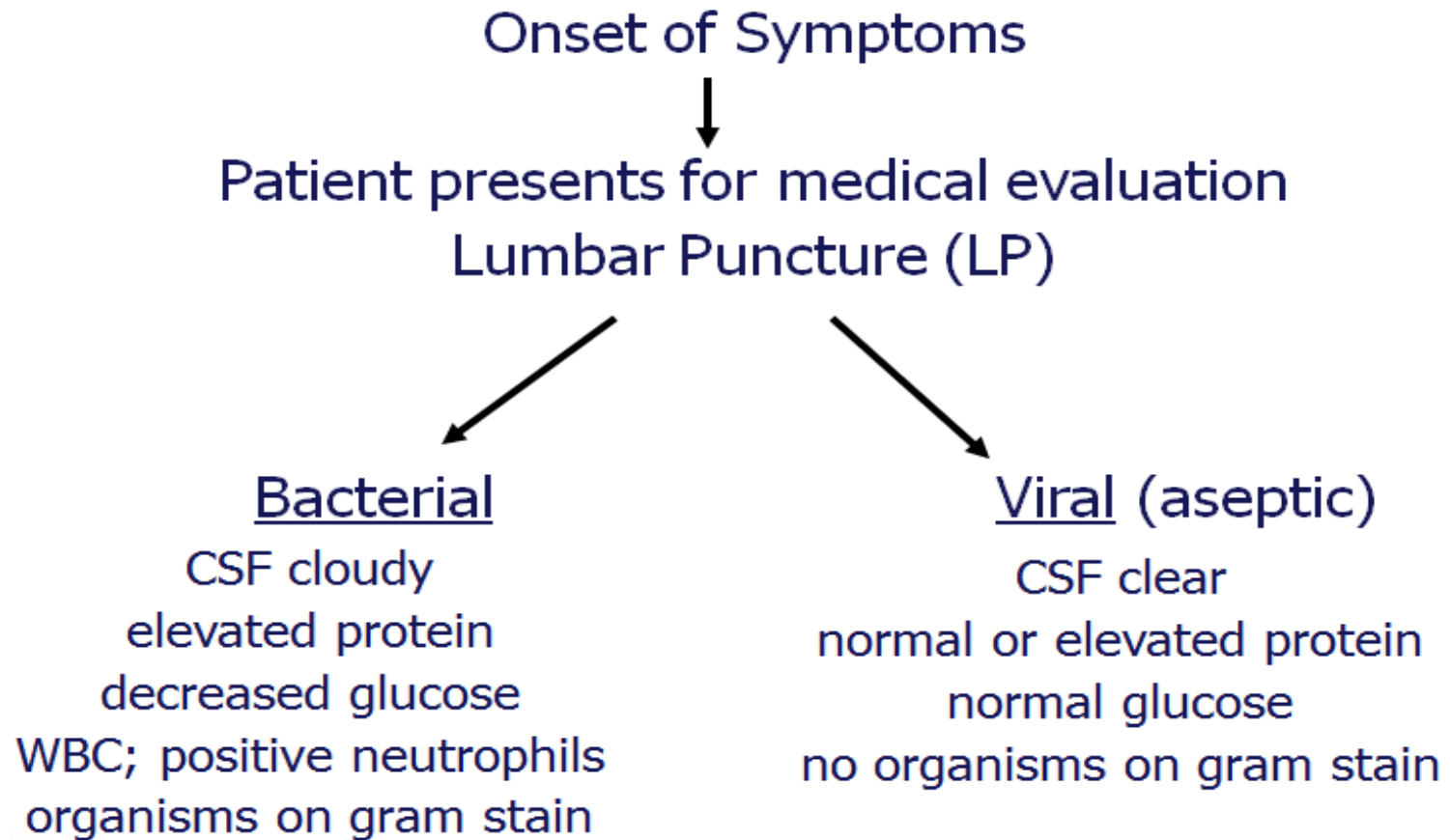


Cerebrospinal Fluid (CSF) Bacteria

- Meningitis often from viruses or upper respiratory flora
- Meningitis due to gram negative rods or *Staphylococcus* usually associated with predisposing factors such as trauma
- Most common meningitis in an adult: *Streptococcus pneumoniae* (gram positive cocci in pairs)
 - generates increased WBC response
- Meningococemia: gram stain showing **gram-negative diplococci** is diagnostic
 - a single case is a true infection emergency



Meningitis



Blood Cultures



- A single blood culture consists of two bottles
 - Bottles designed to recover aerobes and anaerobes
 - Growth may occur in one or both bottles
- Adults: low numbers of bacteria in blood ($\leq 30/\text{mL}$)
 - Can lead to negative gram stain and false negative
 - Volume is important; usual 4 bottles/40cc blood
 - Less blood needed for children
 - Poor specimen collection technique can introduce contaminants to the specimen which are often common skin commensal flora

Common UTI Pathogens

- Gram negatives
 - *E. coli*: Causes 80% of all UTI
 - Proteus, Klebsiella, Enterobacter, Pseudomonas, Gardnerella cause 5-10%
- Gram positives
 - Staph, Enterococcus, *Staph saprophyticus*, 10-20%
- Positive leukocyte esterase and/or nitrite found on a UA can be helpful in determining infection status.
- Increased WBC in urine w/ negative cultures may indicate infection w/ chlamydia or gonorrhea.

Presence of yeast are not part of the NHSN definition for a urinary tract infection

Common Pathogens of Deep and Organ Space SSI

- Anaerobic (does not require O₂ for growth)
 - *B. fragilis*
 - Clostridium
 - *Peptostreptococcus*
 - *Propionibacterium* (septic arthritis, endocarditis, suture sites for craniotomy)
- Aerobic examples
 - Staphylococcus
 - Streptococcus
 - Gram negative rods (GNR)

Common Bowel Flora

- Normal mix of bacterial flora keeps numbers of yeast, *C. difficile*, and other potential pathogens in the gut in check
- With altered flora, yeast, *C. difficile*, pseudomonas species, VRE, and others can proliferate and dominate the flora

Of note: Stool samples contain digestive enzymes; enzymes continue to work after collection, necessitating addition of a preservative and/or prompt processing of specimens

Antibiotics Resistance

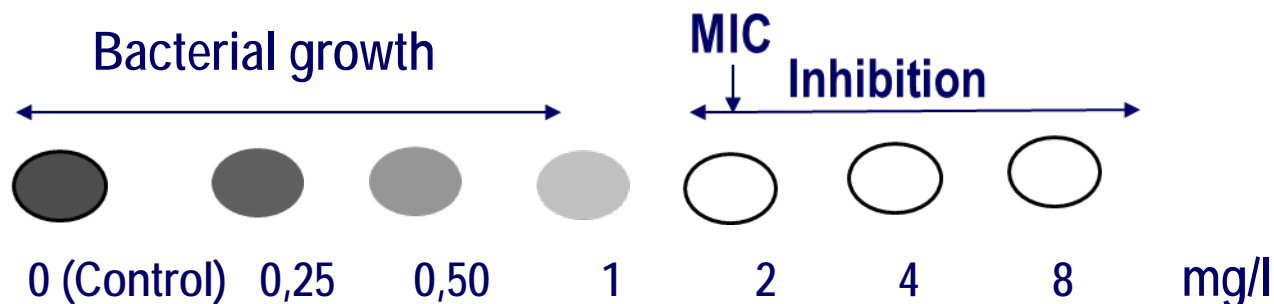
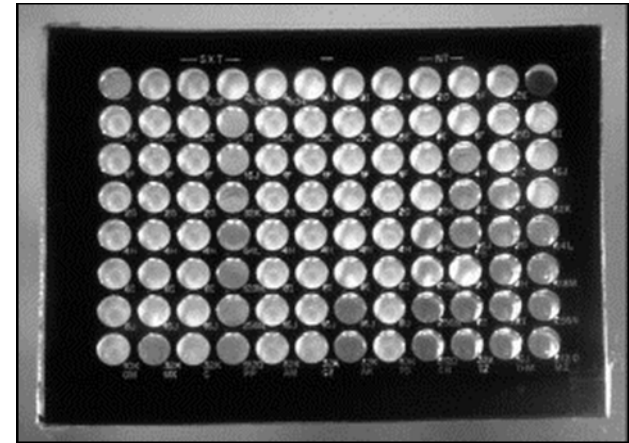
- Emerges when some or all of a species/subspecies of bacteria survive exposure to an antibiotic
- Can be intrinsic or transferred
- Multi-drug resistance organisms (MDRO) - resistant to multiple antibiotic agents; defined by organism type/specific agents



Kirby-Bauer Disk
Diffusion Susceptibility
Plate

Sensitivity Testing: Dilution in liquid broth

- Tubes containing increasing antibiotic concentrations
- Incubation during 18 hr at 37°C



Resistance: Extended Spectrum Beta-Lactamase (ESBL) Producing Gram-Negative Rods (GNR)

- Each new generation of Cephalosporins have greater activity on GNR through new forms of beta-lactam
 - Resistance develops to new beta-lactams by new forms of beta-lactamases
- GNR are now resistant to 3rd generation Cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone) and Monobactams (e.g., aztreonam) by ESBLs
- ESBL producing GNR remain susceptible to cephamycins (e.g., cefoxitin, cefotetan, cefmetazole) and carbapenems (e.g., meropenem, imipenem)

Resistance: Carbapenem Resistant Enterobacteriaceae (CRE)

- Carbapenems are becoming the last β -Lactam antibiotic class for treatment of ESBL infections
- New Delhi metallo-beta-lactamase 1 (NDM-1) carbapenemase-resistant Enterobacteriaceae (CRE) was detected in 2008; susceptible only to polymyxins and tigecycline.
- Few treatment options are available

CDC guidance for management of CRE infected patients, 2015

<https://www.cdc.gov/hai/organisms/cre>



CRE – Reportable to LA PHD



**LAC DPH Health Information:
New Health Officer Order – Health Facilities
to Report Carbapenem-Resistant
Enterobacteriaceae and Antimicrobial
Resistance**



January 19, 2017

This notification is to inform Los Angeles health care providers of a new Health Officer order. All affected health care facilities will receive detailed communications and guidance regarding this mandate and its implementation.

The Los Angeles County Department of Public Health (LACDPH) Interim Health Officer has mandated that all Los Angeles County acute care hospitals and skilled nursing facilities report all clinical laboratory carbapenem-resistant *Enterobacteriaceae* (CRE) positive tests from any specimen. The order also requires that acute care hospitals, and skilled nursing facilities that generate an annual antibiogram, submit it to LACDPH each year.

CRE, defined as *Enterobacteriaceae* (*Klebsiella sp.*, *E. coli*, and *Enterobacter sp.*) resistant to carbapenem antibiotics or that produce carbapenemases, are a persistent and growing public health threat. These healthcare-associated pathogens are difficult to treat, have a high mortality rate, and are easily spread between patients in health care facilities. The mandate is a necessary step to control the spread of this healthcare-associated infection.

What is Antibiogram ?

- An **antibiogram** is an overall profile of antimicrobial susceptibility testing results of a specific microorganism to a battery of antimicrobial drugs. ... Only results for antimicrobial drugs that are routinely tested and clinically useful should be presented to clinician
- Used for Clinical decision making

ANTIBIOTIC

Results as Percent Susceptible
(ID Restriction and Relative Cost noted
below)

Acinetobacter baumannii
Enterobacter cloacae
E. coli (All Isolates)
E. coli (Urine only)
Klebsiella pneumoniae
Klebsiella oxytoca
Citrobacter freundii
Proteus mirabilis
Providentia stuartii
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Enterococcus faecalis
Enterococcus faecium
Streptococcus pneumoniae
Haemophilus influenzae

Total-1st isolate only reported 84 126 2589 2035 648 79 60 443 74 582 61 934 169 213 62 39 34

Penicillins

Nafcillin	*\$\$												51	30				
Penicillin	\$\$												11	4	97	21	100-NONMEN 77-MENINGITIS	76
Ampicillin	\$												NT	NT	97	21	NT	76
Ampicillin/sulbactam	\$	27	NR	50	52	73	58	NR	80	NI	IR	NR						
Piperacillin/Tazobactam	*\$	NT	79	93	93	85	91	75	100	68	77	NT						

Cephalosporins

Cefazolin	\$	IR	NI	74	80	74	48	0	71	NI	IR	NI						
Cefoxitin	\$	NI	NI	86	86	88	97	0	88	82	IR	34						
Ceftriaxone	\$	NI	84	84	84	84	88	72	93	53	NI	79					100-NONMEN 95-MENINGITIS	
Ceftazidime [ID restricted]	\$	NI	75	89	89	85	96	72	98	53	83	72						
Cefepime [ID restricted]	\$	23	94	92	92	91	100	98	95	66	86	75						

Carbapenems

Meropenem [ID restricted]	\$\$	30	95	100	100	93	99	93	38	64	75	100						
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Fluoroquinolones

Ciprofloxacin	\$	18	96	66	66	86	95	93	53	23	67	69	NI	NI				
Levofloxacin	\$	18	98	66	65	87	95	93	58	21	65	69	NI	NI			91	

Aminoglycosides

Gentamicin	\$	16	96	86	86	93	91	100	79	54	88	97	89	68				
Tobramycin	\$	17	93	83	83	86	91	100	80	32	88	66						
Amikacin	\$	NR	100	97	94	84	97	100	95	100	98	100						

Miscellaneous Antibiotics

Nitrofurantoin	\$	NI	34	96	95	24	86	93	NI	NI	NI	NI	100	97	99	15		
Clindamycin	*\$												67	57			76	
Erythromycin	*\$												42	22			67	

HOSPITAL ANTIBIOGRAM FROM 01/01/2007 TO 01/12/2007
 RESULT SHOWS PERCENT ORGANISMS SUSCEPTIBLE TO ANTIBIOTIC TESTED
 GRAM POSITIVE COCCI



Organism	Number of Isolates Tested	Ampicillin	Penicillin	Oxacillin	Erythromycin	Clindamycin	Ciprofloxacin	Gentamicin	Vancomycin	Trimethoprim/Sulfamethoxazole	Tetracycline	Rifampin	Nitrofurantoin (Only Urines Tested)	Percent of isolates from urine
Staph. aureus, methicillin susceptible (OP)	8	0	14.29	50	87.5	87.5	100	100	100	50	100	100	100	0
Staph. aureus, methicillin susceptible (IP+ICU)	3	0	0	50	75	100	75	100	100	75	100	100	100	0
Staph. aureus, methicillin resistant (OP)	2	0	0	0	50	50	--	--	100	100	--	--	--	0
Staph. aureus, methicillin resistant (IP+ICU)	3	0	0	0	100	50	100	100	100	100	100	100	100	0
Staph epidermidis (IP+ICU)	0	--	--	--	--	--	--	--	--	--	--	--	--	--
Staph. epidermidis OP	0	--	--	--	--	--	--	--	--	--	--	--	--	--
Enterococcus faecalis (TOTAL)	1	100							100					
Enterococcus faecium (TOTAL)	0	--							--					
Enterococcus faecalis (IP+ICU)	0	--							--					
Enterococcus faecium (IP+ICU)	0	--							--					
Enterococcus faecalis (OP)	1	100							100					
Enterococcus faecium (OP)	0	--							--					

Medical Center Adult Empiric Infection Therapy Pocket Guide

Antibiotic doses may require adjustment for renal dysfunction. For further information, see Antimicrobial Stewardship website on PVHMC Intranet

Pneumonia	Community-Acquired (CAP)	Mild-Moderate without risk for Pseudomonas	Ceftriaxone 1 g IV q24H PLUS Azithromycin 500 mg IV q24H <i>Severe β-lactam allergy: Levofloxacin</i> 750 mg IV q24H <i>If known/suspected MRSA: ADD Vancomycin</i> per pharmacy and obtain culture	Procalcitonin on admit and every 2-3 days BNP if concomitant heart failure or fluid overload Suction for sputum or BAL for gram stain & culture if Pseudomonas risk Influenza PCR during influenza season or with clinical suspicion
	Pseudomonal risk factors: bronchiectasis, severe COPD, chronic oral steroids, frequent recent antibiotics	Severe (ICU) without risk for Pseudomonas	Ceftriaxone 1 g IV q24H PLUS Levofloxacin 750 mg IV q24H <i>Severe β-lactam allergy: Aztreonam</i> 2 g IV q8H PLUS Levofloxacin 750 mg IV q24H <i>If known/suspected MRSA: ADD Vancomycin</i> per pharmacy	
		Any severity with risk for Pseudomonas (Obtain culture for all patients)	Piperacillin-Tazobactam per pharmacy PLUS Levofloxacin 750 mg IV q24H <i>Severe β-lactam allergy: Aztreonam</i> 2 g IV q8H PLUS Levofloxacin 750 mg IV q24H <i>If known/suspected MRSA: ADD Vancomycin</i> per pharmacy	
	Healthcare-Associated/Hospital-Acquired (HCAP/HAP) (Obtain culture for all patients)	Standard risk (Concern for Pseudomonas, MRSA)	Piperacillin-Tazobactam per pharmacy PLUS Tobramycin per pharmacy PLUS EITHER Vancomycin per pharmacy OR Linezolid* 600 mg IV q12H <i>Severe β-lactam allergy: Aztreonam</i> 2 g IV q8H PLUS Tobramycin per pharmacy PLUS EITHER Vancomycin per pharmacy OR Linezolid* 600 mg IV q12H	Procalcitonin on admit and every 2-3 days. BNP if concomitant heart failure or fluid overload
	Expanded risk (Concern for multi-drug resistant orgs, strongly consider ID consult)	Vancomycin per pharmacy OR Linezolid* 600 mg IV q12H PLUS AGENTS BELOW: <i>For ESBL/AmpC: Meropenem*</i> 1 g IV q8H <i>For CRE/KPC: Cefazidime-Avibactam*</i> 2.5 g IV q8H <i>MDR Acinetobacter: Ampicillin-Sulbactam*</i> 3 g IV q6H PLUS Minocycline* 200 mg IV x 1 dose then 100 mg IV q12H PLUS EITHER Colistin* or Polymixin B*	Suction for sputum or BAL for gram stain & culture required Influenza PCR during influenza season or with clinical suspicion	
Urinary Tract Infection	Asymptomatic bacteriuria		Antibiotic contraindicated unless pregnant or GU surgery in next 4 days	
	Cystitis (symptomatic)		Ceftriaxone 1 g IV q24H or Fosfomycin* 3 g PO x1 dose	
	Pyelonephritis	Uncomplicated	Ceftriaxone 1 g IV q24H (<i>if Severe β-lactam allergy: Aztreonam</i> 1 g IV q8H) <i>Uncomplicated = No recent antibiotics, instrumentation, healthcare-association, obstruction, immunosuppression, prolonged symptoms, pregnancy</i>	Confirm UA & urine culture collected before antibiotics given
		Complicated	Piperacillin-Tazobactam per pharmacy (<i>if Severe β-lactam allergy: Aztreonam</i> 1 g IV q8H) <i>Suspected ESBL/AmpC/Pseudo: Meropenem*</i> 1 g IV q8H +/- Tobramycin per pharmacy <i>Suspected VRE: ADD Daptomycin*</i> 6 mg/kg IV q24H OR Linezolid* 600 mg IV q12H	If foley >2 wks, collect UA & urine culture after changing foley
SSTI	Nonpurulent Cellulitis	Mild (no SIRS)	Cephalexin 500 mg PO q6H (<i>if β-lactam allergy: Clindamycin</i> 300 mg PO QID)	Gram stain/culture of purulent drainage or abscess
		Moderate (SIRS)	Cefazolin 1 g IV q8H	
		Severe/Complicated	Piperacillin-Tazobactam per pharmacy PLUS Vancomycin per pharmacy	
	Purulent cellulitis/abscess	Abscess Only (no SIRS)	I&D and consider TMP/SMX 1-2 DS tab PO BID (dose adjusted for renal function) OR Doxycycline 100 mg PO q12H	
		Abscess with cellulitis	<i>Outpatient, ED discharge: TMP/SMX</i> 1-2 DS tab PO BID (dose adjusted for renal function) OR Doxycycline 100 mg PO q12H <i>Inpatient, Severe: Vancomycin</i> per pharmacy OR Linezolid* 600 mg IV q12H	

Hepatitis **A** Viral Test Results

- Hepatitis A Virus (HAV)
 - HAV, total – current or past HAV
 - HAV, IgM – definitive diagnosis of active HAV infection

All Hepatitis (acute and chronic) are reportable communicable diseases via local public health

Acute hepatitis A requires immediate notification

Hep A – Outbreak CA



KAREN L. SMITH, MD, MPH
Director and State Public Health Officer

State of California—Health and Human
Services Agency
**California Department of
Public Health**



EDMUND G. BROWN JR.
Governor

October 17, 2017

AFL 17-21

TO: Hospital Emergency Departments, Hospital Infection Preventionists, and Hospital Administrators

SUBJECT: California Hepatitis A Outbreak and Use of Hepatitis A Vaccine for At-risk Health Care Personnel including Health Care-based Environmental Services Staff

All Facilities Letter (AFL) Summary

The purpose of this AFL is to provide updated California Department of Public Health (CDPH) vaccination recommendations to health care facilities in light of constrained hepatitis A virus (HAV) vaccine supplies and review infection control recommendations for preventing HAV transmission. Other recommendations related to hepatitis A that are provided in AFL 17-13 remain in effect.

Currently, there is an ongoing HAV outbreak in California. Homeless populations and persons using injection or non-injection illicit drugs are considered at risk of exposure to HAV, particularly those in settings of limited sanitation. Use of adult hepatitis A vaccine to help control recent outbreaks has resulted in concerns that the supplies for adult immunization for the last quarter of 2017 could become limited. Therefore, CDPH recommends:

Interpretation of the Hepatitis B Panel

Tests	Results	Interpretation
HBsAg	negative	Susceptible
anti-HBc	negative	
anti-HBs	negative	
HBsAg	negative	Immune due to natural infection
anti-HBc	positive	
anti-HBs	positive	
HBsAg	negative	Immune due to hepatitis B vaccination**
anti-HBc	negative	
anti-HBs	positive	
HBsAg	positive	Acutely infected
anti-HBc	positive	
IgM anti-HBc	positive	
anti-HBs	negative	
HBsAg	positive	Chronically infected
anti-HBc	positive	
IgM anti-HBc	negative	
anti-HBs	negative	
HbeAG	positive	Highly infectious

Ag = antigen c = core
 Ab = antibody s = surface

Hepatitis C Viral Test Results

- Hepatitis C Virus (HCV)
- Anti-HCV
 - Presence of antibodies to the virus, indicating exposure to HCV
 - Active, chronic or resolved
- HCV RIBA (recombinant immunoblot assay)
 - Confirmatory test of antibodies to the virus
 - Demonstrates if HCV was true positive (present or past is unanswered)

All Hepatitis (acute and chronic) are reportable communicable diseases via local public health

Laboratory Tests for Tuberculosis (TB)

- ***Acid Fast Bacillus (AFB)***
 - Distinguishes bacteria that retain stain in the presence of an acid decolorizer.
 - Present with Mycobacterium species (tuberculosis, avium and others)
 - Very few structures are acid-fast; which makes acid-fastness particularly useful in diagnosis
 - First morning specimen or bronch lavage are best
 - Specimens must be at least 8hrs apart from each other

Rapid Diagnostic Laboratory Tests

- Rapid human immunodeficiency virus (HIV) test detects antibodies with high sensitivity and specificity
 - Use confirmatory testing to verify false positives
- Fast antigen detection for influenza but 44-60% false positives
 - Use confirmatory testing to verify
- Rapid Group A Streptococci antigen detection with 95% specificity
 - Will also detect carriers

Rapid Diagnostic Laboratory Tests - 2

- Polymerase chain reaction (PCR) assays
 - Makes thousands of copies of a DNA segment specific to an organism so it can be detected by identifying tests
 - Available for a number of bacterial and viral pathogens
 - Highly sensitive; may not indicate viability of organism
 - Expensive, but getting less so

Role of Microbiology in HAI Prevention

Microbiology support is critical to

- Outbreak management
- Performing additional tests for epidemiologic analyses
- Infection surveillance
- Alert to unusual pathogens or changes in antibiotic susceptibility in the population
- Design of antibiotic formulary (antibiogram)
- Interpretation of microbiological results



The Infection Preventionist's Guide to the Lab

Edited by
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*Produced in collaboration
with the American Society
for Microbiology. [ASM™]*





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