CIC Study Group

Week 2

HAI Prevention Program

Agenda

- Housekeeping Items
- Review
  - Chapter 24 Microbiology Basics
  - Chapter 25 Laboratory Testing and Diagnosis
  - Chapter 26 Antimicrobial Resistance
- Next week assignments

Chapter 24
Microbiology Basics

Background

The field of microbiology includes the study of ***bacteria, fungi, protozoa, viruses, and algae.***

- Fundamental knowledge of microorganisms, their identification, significance, and basic laboratory techniques provide the infection preventionist with an understanding of pathogenic organisms.

- Proper specimen collection and transport are integral to the recovery of valid microbiological data.

- The Clinical microbiology laboratory can provide information on microbes determined to be of clinical significance.

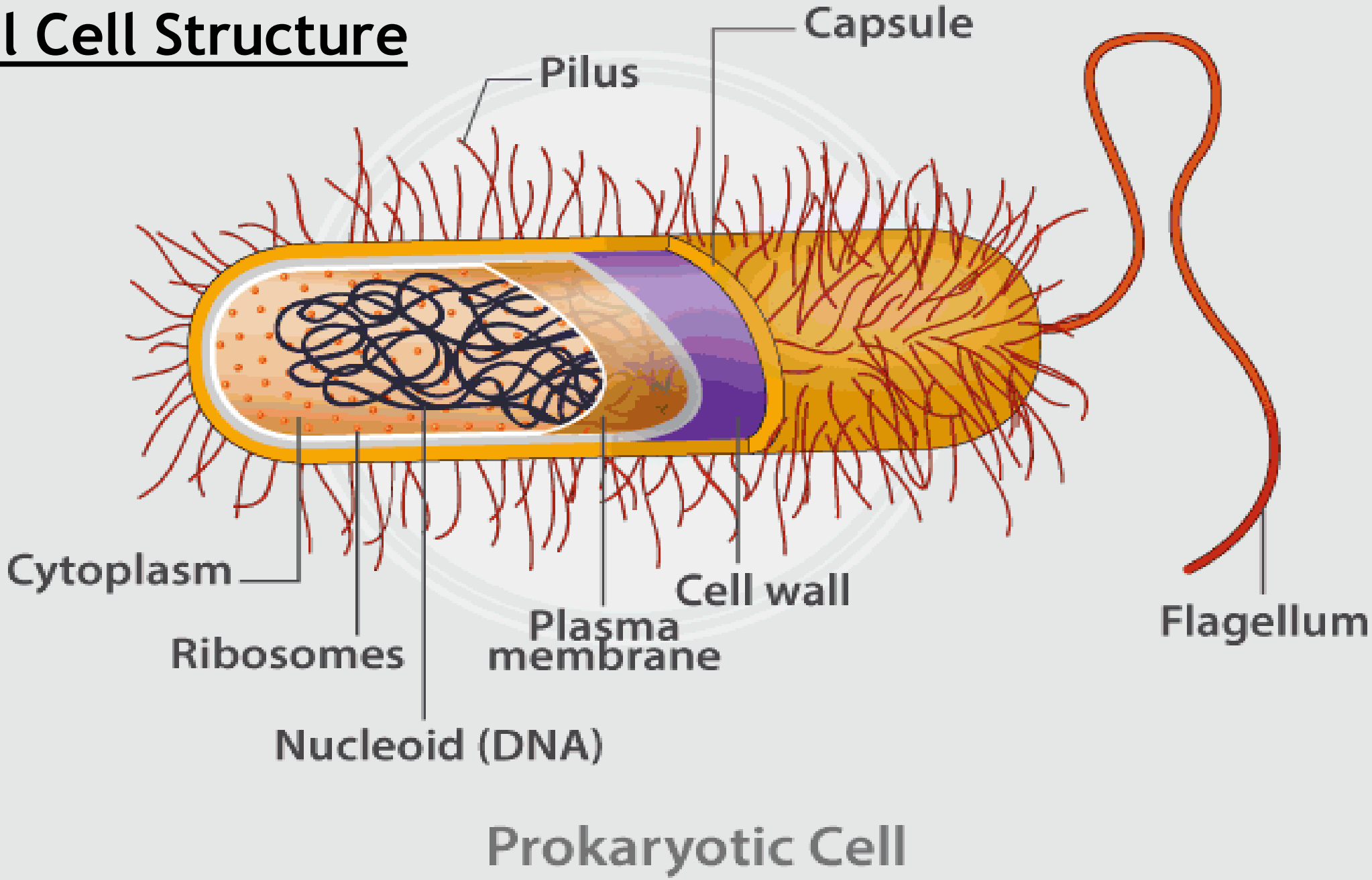
Bacteria

There are at least 17 groups of bacteria; these groups have a similar cell structure that is known as **prokaryotic**.

- Prokaryotic cell structure is microscopically characterized by lack of a visible nucleus, by the lack of a membranous nuclear envelope, and by lack of membrane-bound internal structures such as mitochondria.

- There is no agreed-upon definition of species in bacteriology; bacteria have binomial names containing a genus name, following a specific epithet: *Staphylococcus aureus, S. aureus.*

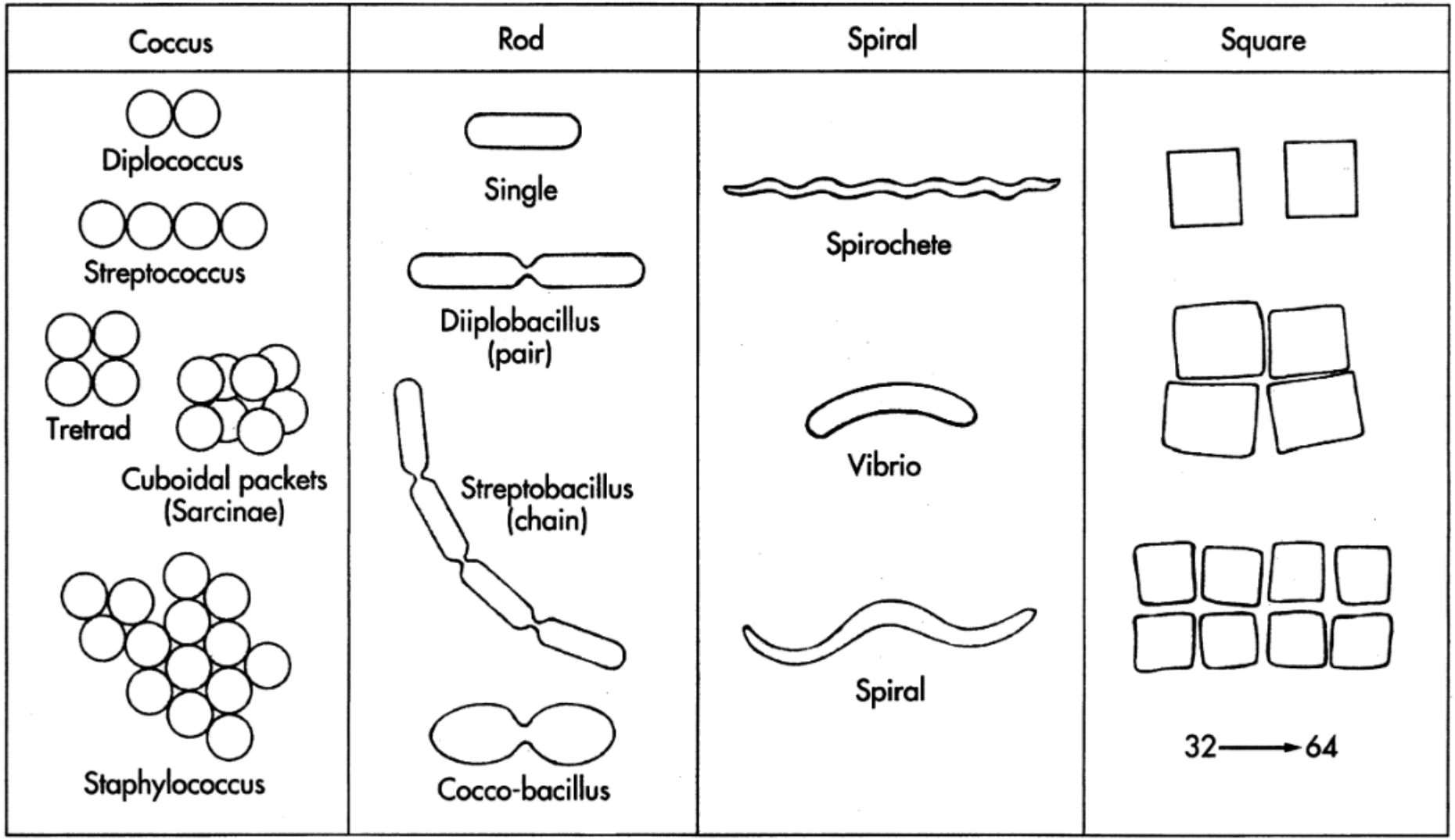
Bacterial Cell Structure



Source: https://byjus.com/biology/prokaryotic-and-eukaryotic-cell

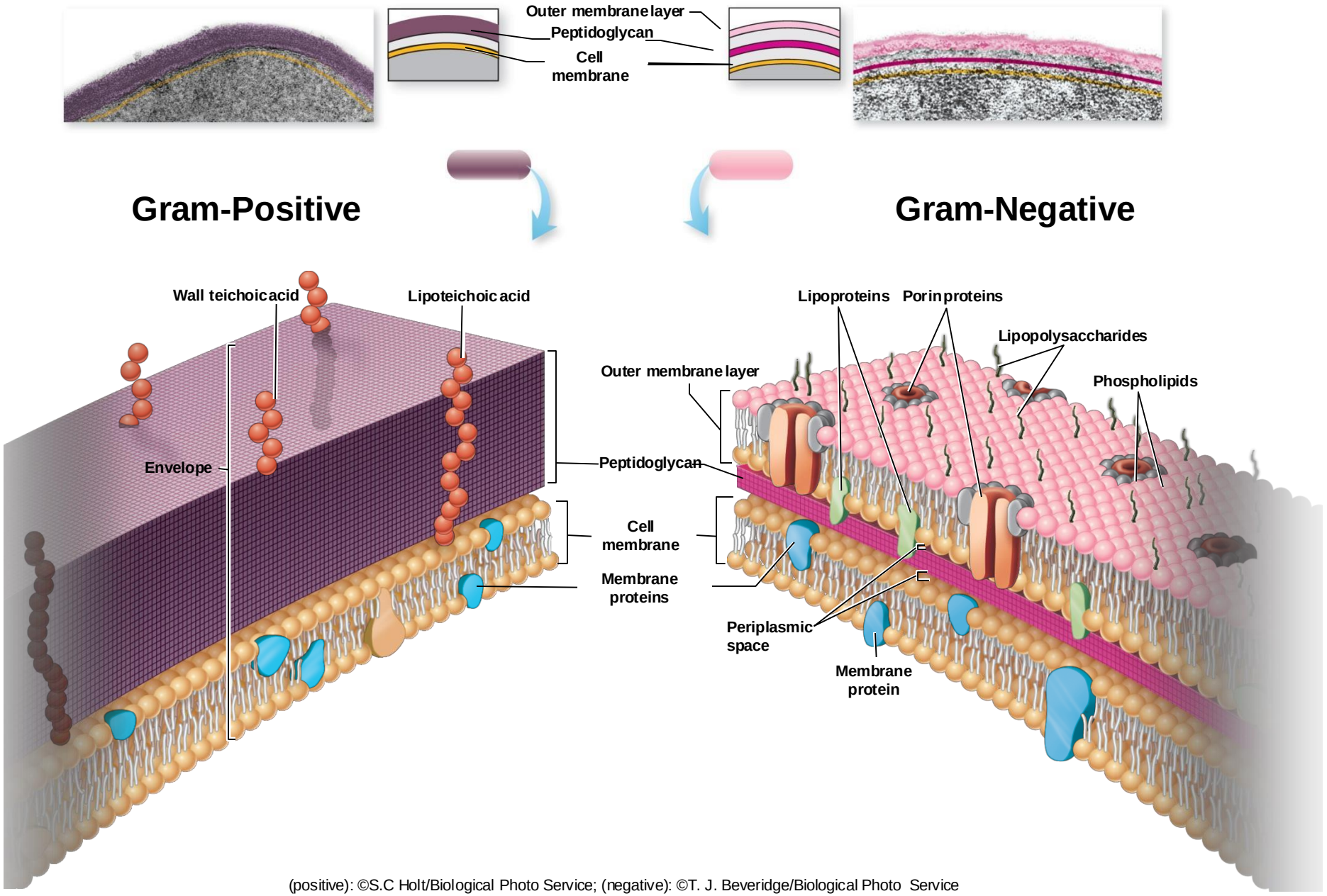
Bacterial Shapes and Arrangements

Figure 24-1. Characteristic bacterial cell shapes and arrangements



Source: APIC Text Chapter 24 Figure 24-1

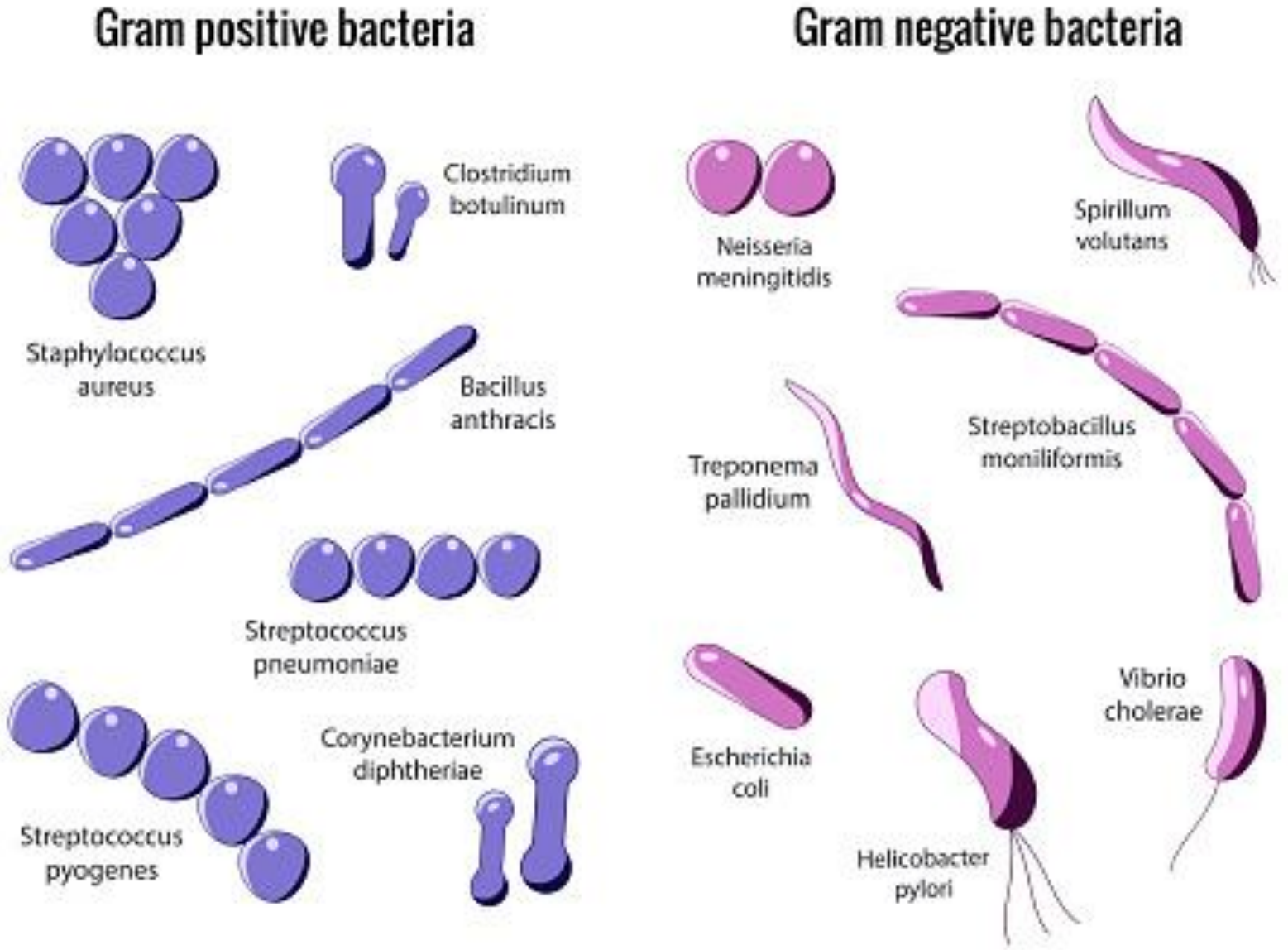
Gram-Positive and Gram-Negative Bacteria



(positive): ©S.C Holt/Biological Photo Service; (negative): ©T. J. Beveridge/Biological Photo Service

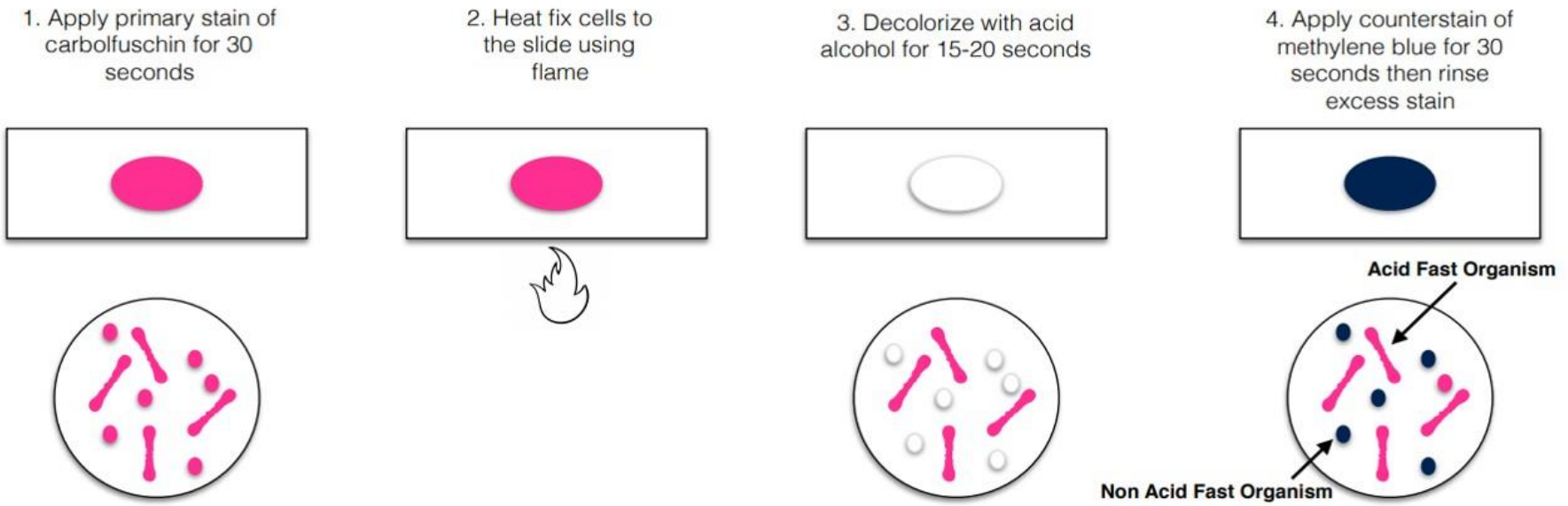
Figure 4.16, 3 of 3

Bacterial Shapes and Arrangements



Source: https://www.istockphoto.com/vector/gram-positive-and-gram-negative-bacteria-gm1214639783-353468560

Acid-Fast Stain



This is a diagram of the basic steps of a Ziehl-Neelsen (Acid Fast) staining procedure.

ACID-FAST STAIN

IMAGE Courtesy of Elizabeth Gray. https://en.wikipedia.org/wiki/File:Acid_Fast_Stain.pdf

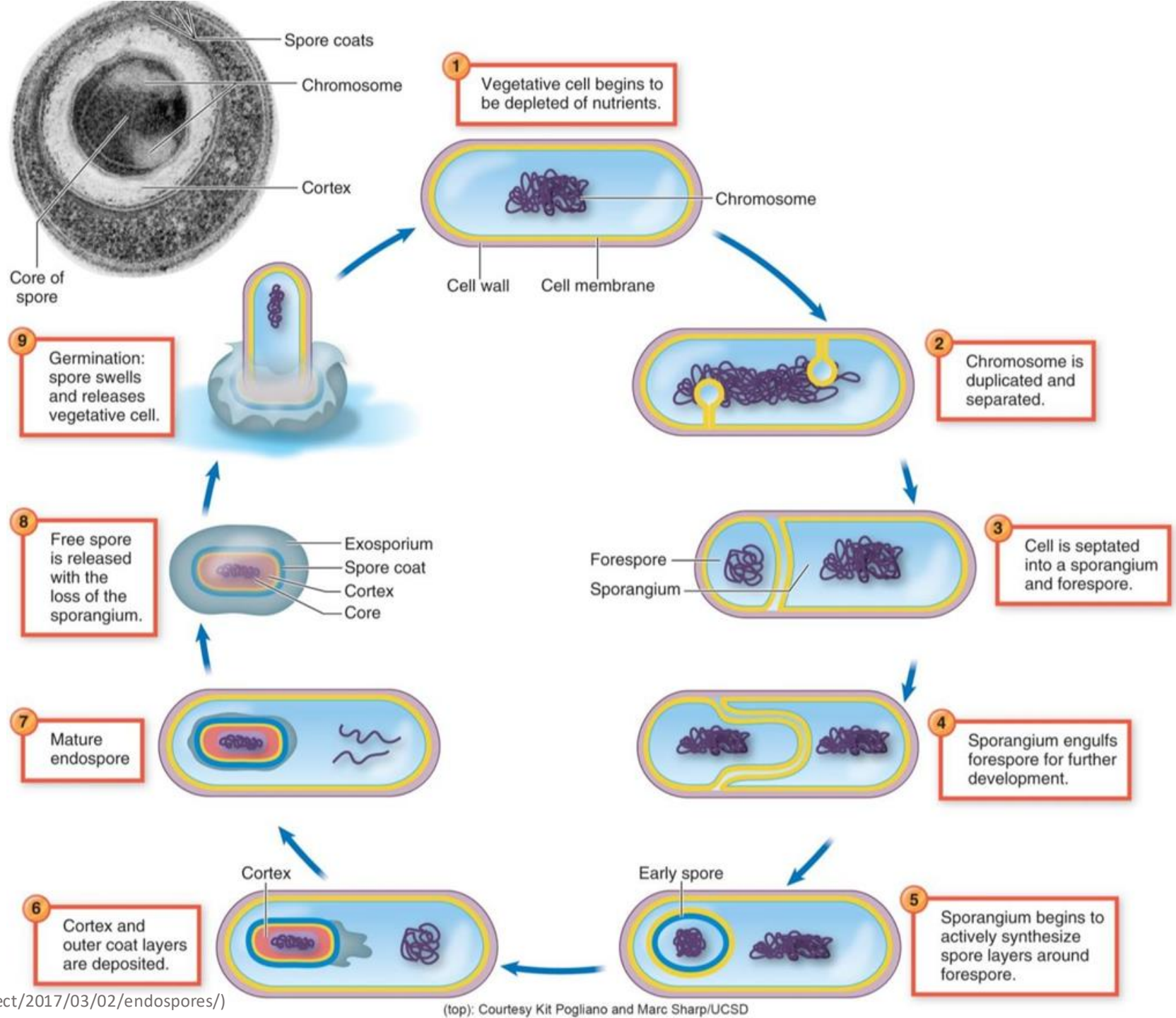
*Useful in identifying *Mycobacterium spp*. as well as *Nocardia* and *Actinomyces* organisms

Endospore Formation

Composed of nuclear material and protein.

Able to survive:

- Water heated to above boiling
- Drying
- Exposure to toxic chemicals
- Antibiotics



(Image: Endospores Source: http://sites.gsu.edu/microbiologyteamproject/2017/03/02/endospores/)

(top): Courtesy Kit Pogliano and Marc Sharp/UCSD

Endospore Formation, Cont.

Bacillus anthracis

- Anthrax

Clostridium tetani

- Tetanus

Clostridioides difficile

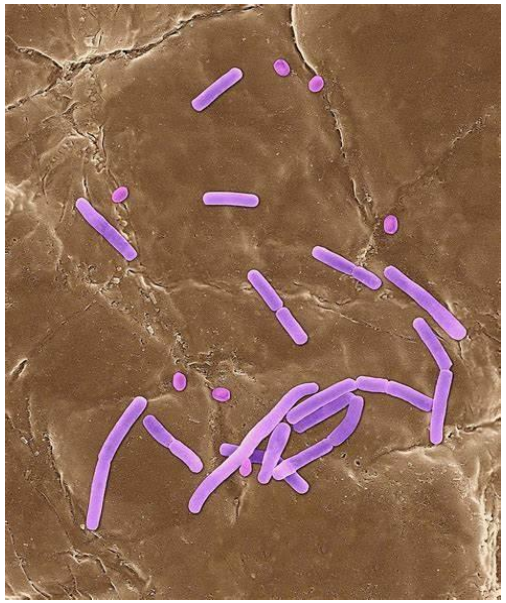
- C. diff

Clostridium perfringens

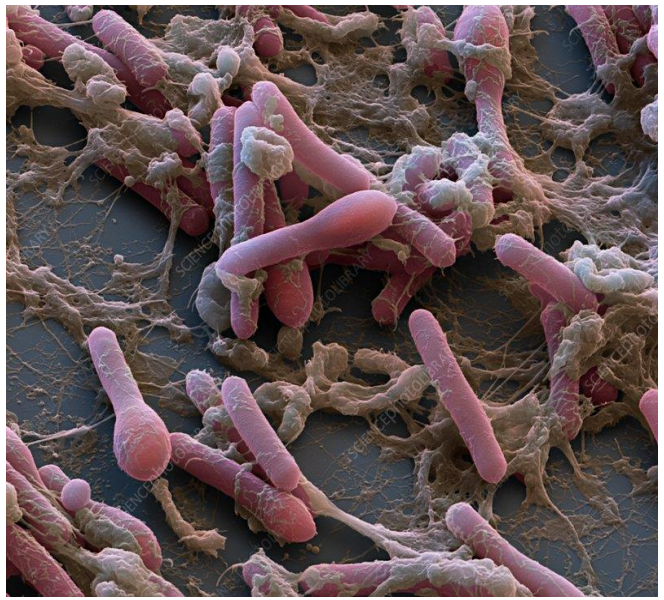
- Gas gangrene

Clostridium botulinum

- Botulism



Source: https://fineartamerica.com/featured/30-bacillus-anthracis-dennis-kunkel-microscopyscience-photo-library.html



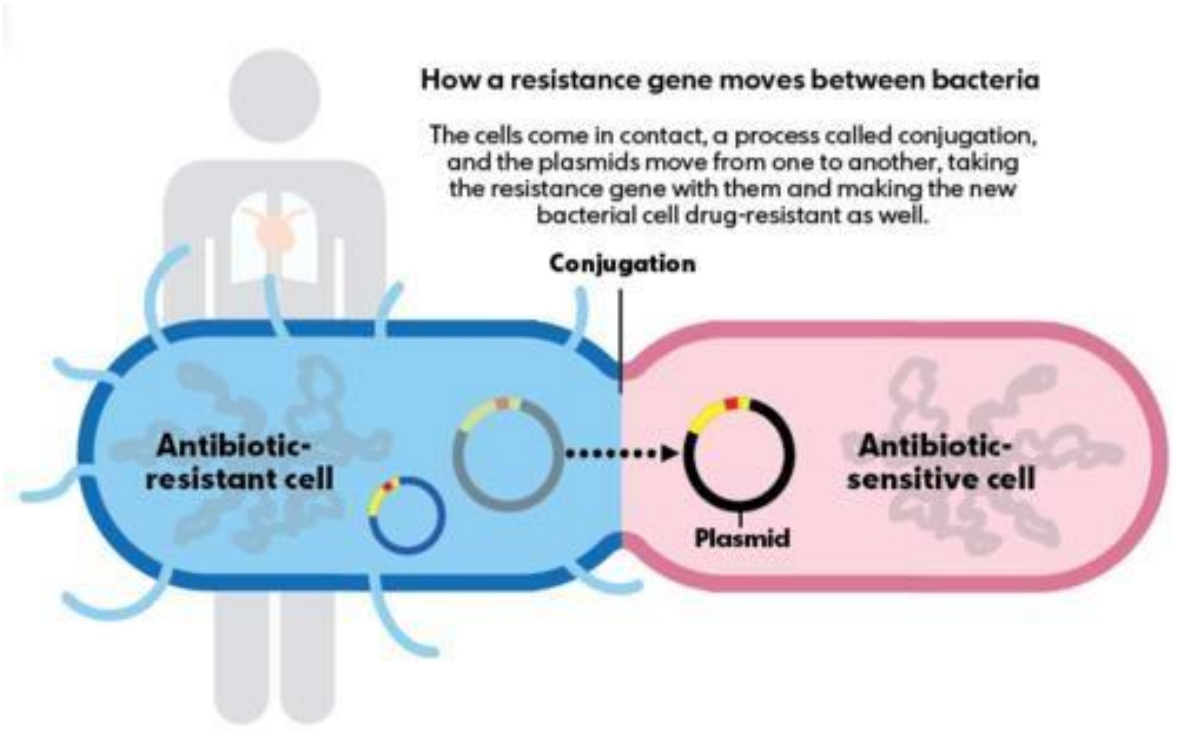
Source: https://www.thermofisher.com/antibody/product/Clostridium-botulinum-Toxin-A-Antibody-clone-H7-Recombinant-Monoclonal/MA5

Bacterial Reproduction and Genetic Diversity

- Genome change can occur not only by mutation but also by transfer of genes from other bacteria.

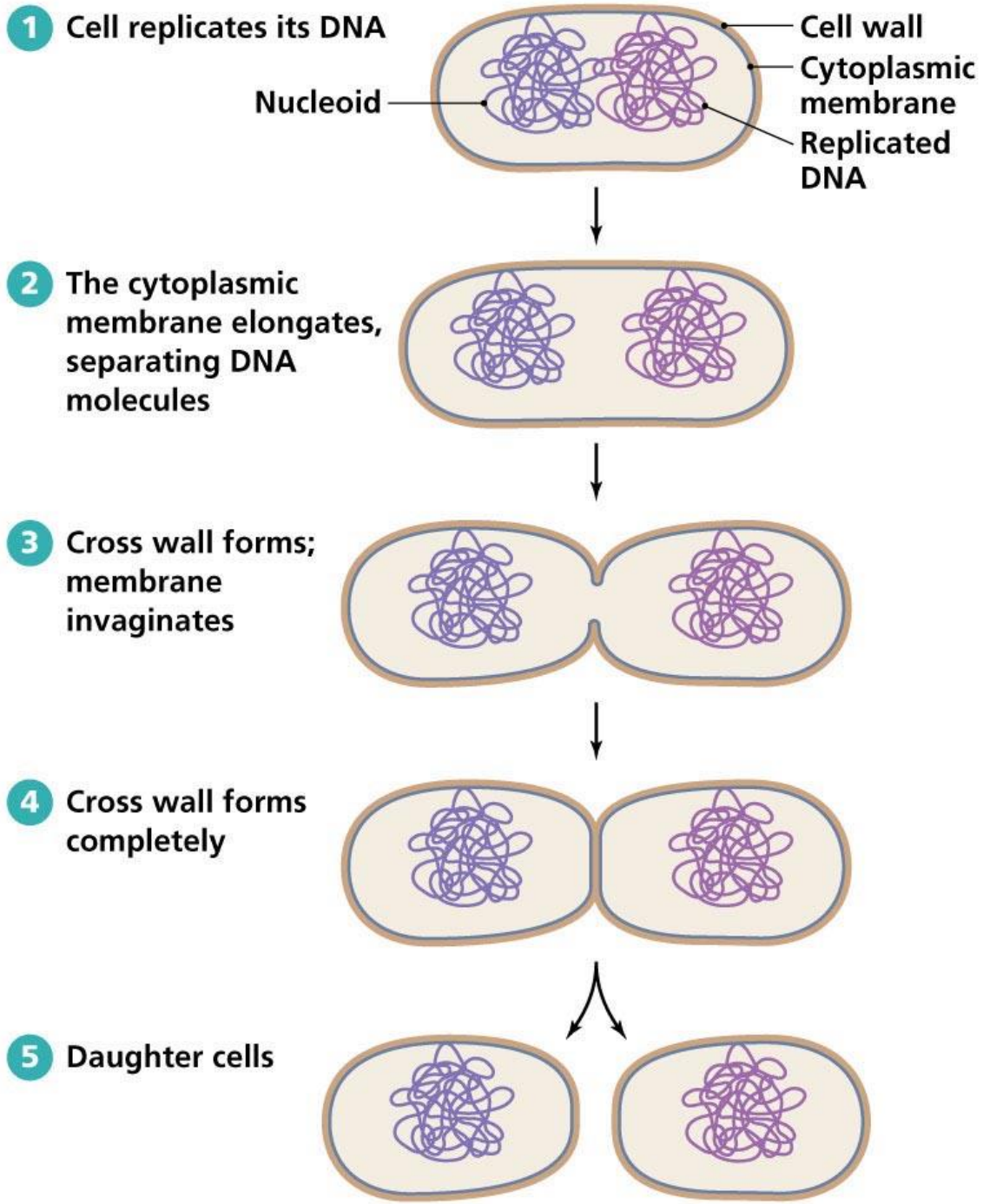
- Plasmids are DNA molecules that are generally circular and not essential to the microbe under some environmental conditions.

  - Acquisition of an R plasmid can render a bacterium and its descendants immediately resistant to antibiotics if resistance genes are encoded on the plasmids.



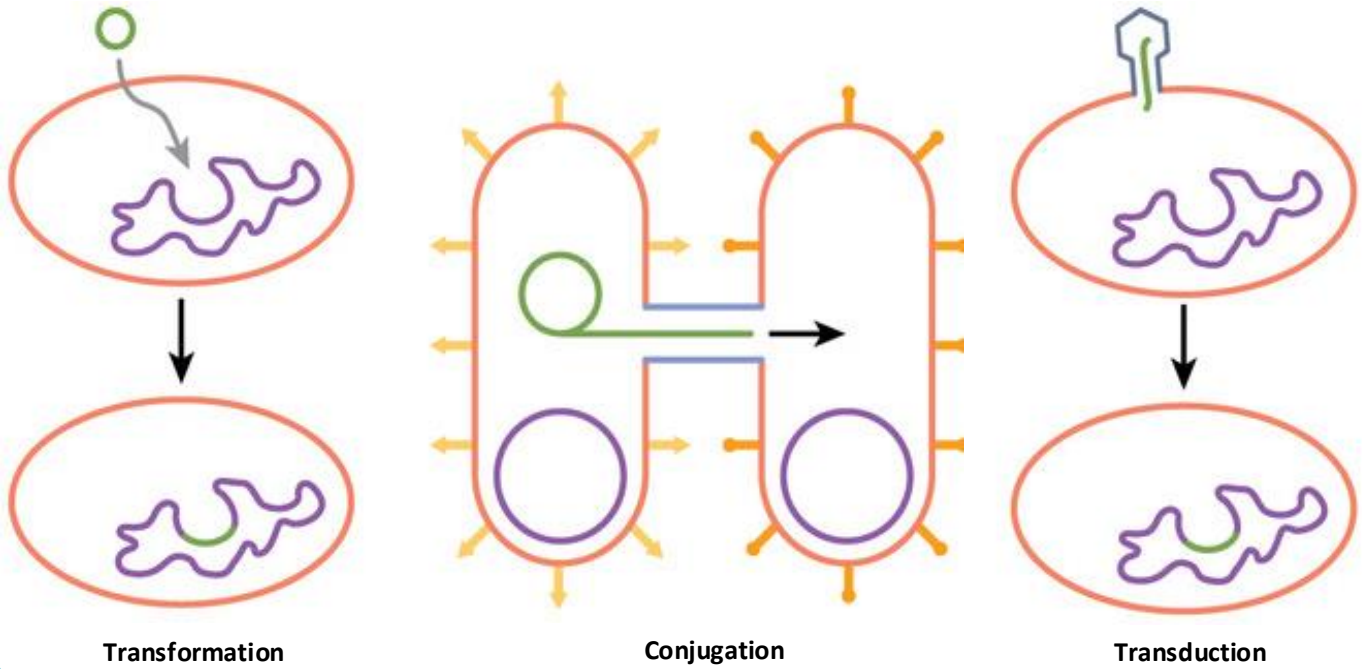
Source: www.moriachambers.com/amanda-riley/

Binary Fission



Source: https://socratic.org/questions/why-is-binary-fission-so-effective-for-bacteria

Transformation, Conjugation and Transduction



Source: https://www.nursinghero.com/study-guides/boundless-microbiology/genetic-transfer-in-prokaryotes

Transformation

- Occurs when naked DNA in the environment, possibly from dead bacteria, enters another bacterium.

Conjugation

- Occurs when all or part of a plasmid is transferred from a donor to a recipient cell; the cell must be in direct contact, and transfer occurs via the sex pilus.
- Can occur between widely separated species, leading to rapid dissemination of genetic information.

Transduction

- Occurs when bacterial DNA is transferred from a donor cell to a recipient cell via a virus capable of infecting bacteria.

Intracellular Parasites

Mycoplasma

- Extremely small pleomorphic bacteria, lack a cell wall and surrounded only by an outer plasma membrane; many are facultative intracellular parasites. Example: *Mycoplasma pneumoniae*

Chlamydiae

- Gram negative coccoid, obligate intracellular parasite; Example: *Chlamydia trachomatis*

Rickettsiae

- Gram negative coccobacilli; obligate intracellular parasite; Example: *Rickettsia typhi*

Viruses

- Viruses are neither eukaryotes or prokaryotes.
- They are obligate intracellular parasites, which means they reproduce within living cells and are dependent on the cell's synthetic and metabolic machinery.
- Virion—outside the host cell, is metabolically inert until it enters a host's cell.

Diagnosing Viral Infections

Direct Detection

- Electron microscopy
- ELISA (enzyme linked immunosorbent assay)
- Latex agglutination
- DNA Probes
- PCR for DNA detection of viruses
- Virus culture

Antibody assay

Viral culture

Clinical Microbiology
Microscopic Slide Preparation

Direct examination or direct wet mount of clinical specimens should be performed **as soon as possible after collection.**

- Examples of specimens examined by direct wet mount: sputum, drainage from lesions, body fluid aspirates, stool, vaginal discharge and urine sediment.
- Examples of pathogens identified in by direct wet mount: *Giardia lamblia* in stool, *Trichomonas vaginalis* in vaginal discharge and *Entamoeba histolytica* from liver abscess aspirate.

Clinical Microbiology
Bacteria Pure Cultures

The choice of growth media depends on:

- The site being cultured (e.g., throat, blood, urine).
- The growth requirements of common or suspected pathogens.
- The likelihood of normal flora.

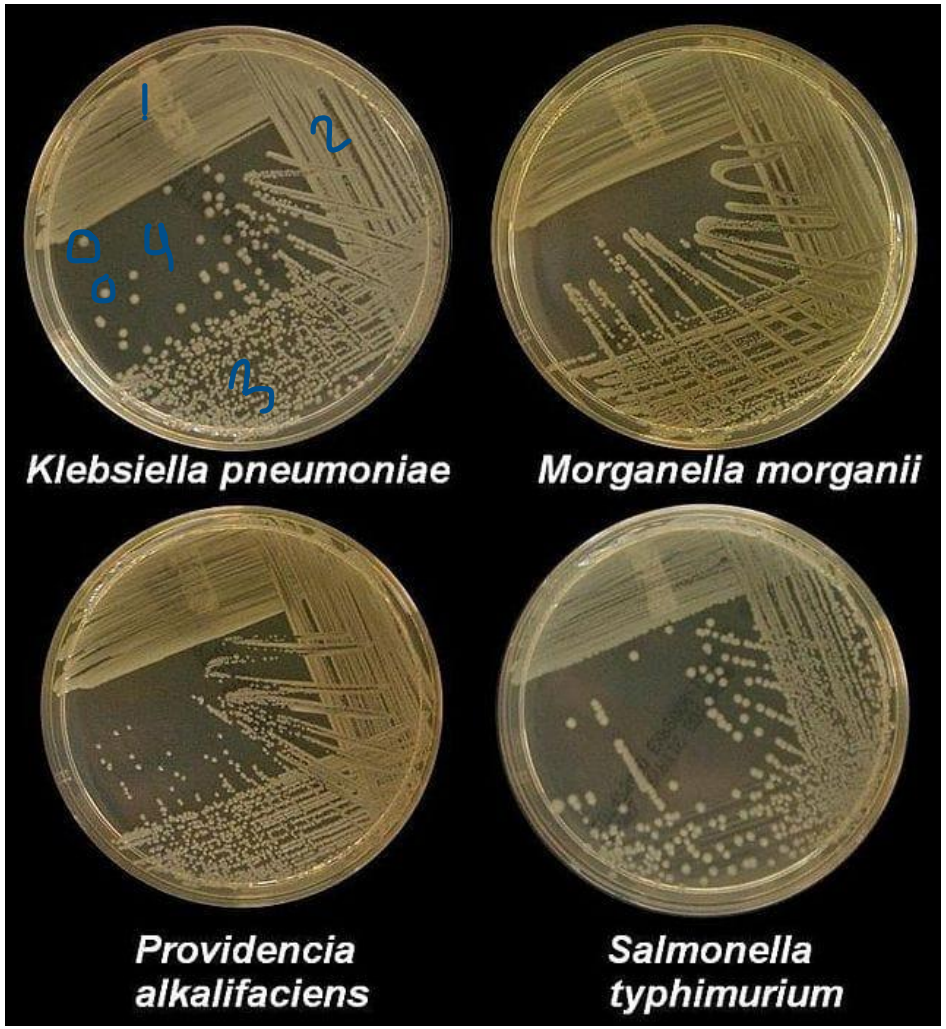
Categories of growth media:

- Nutrient agar
- Enrichment medium
- Selective media
- Differential media

Nutrient Agar

- Supports the growth of a wide variety of bacteria.
- Frequently used for isolation and purification of cultures.

Example: Trypticase soy agar with 5% sheep's blood.



(Image: Four Nutrient agar plates growing colonies of gram negative bacteria
Source: https://microbiologyinfo.com/nutrient-agar-composition-preparation-and-uses/)

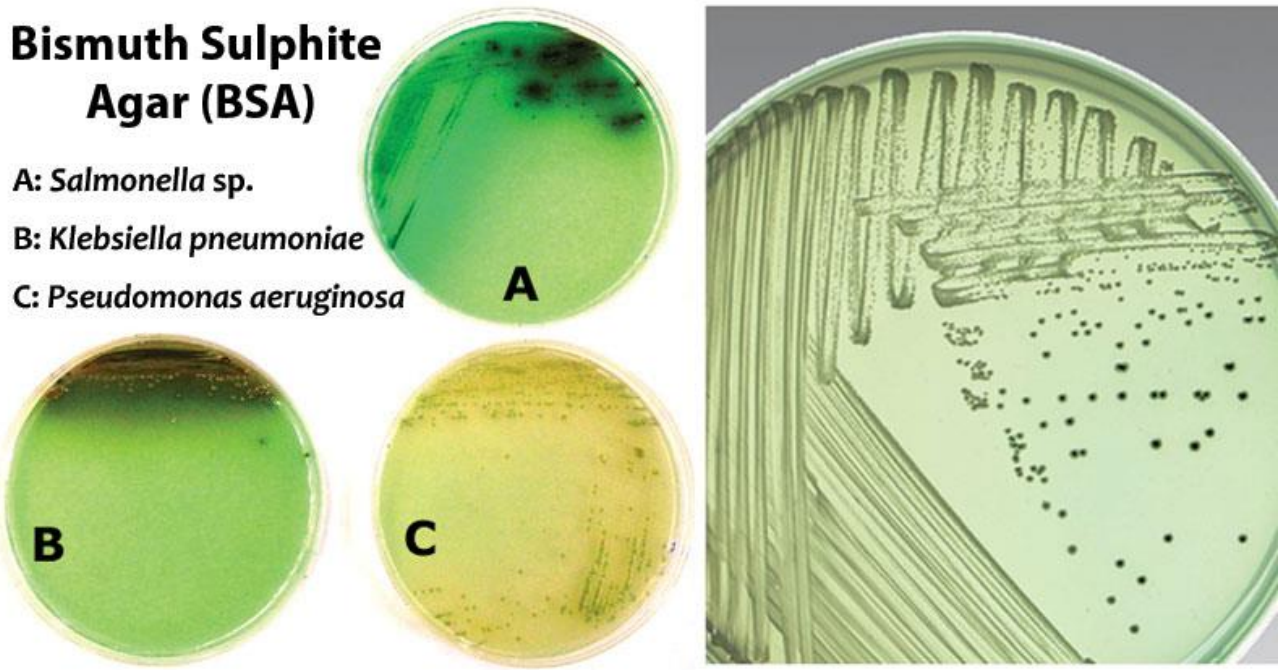
Enrichment Medium

- Contains special nutrients necessary for the growth of fastidious bacteria.

Ex: Chocolate agar utilized for the growth of *Neisseria meningitidis*.

(Image: Enrichment Media Purpose, Principle, and Overview Source: www.labtestsguide.com/enrichment-culture)

Selective Media



(Image: Bismuth Sulphite Agar Source: microbenotes.com/bismuth-sulphite-agar-bsa/)

MacConkey Agar

(Image:https://mltexpo.blogspot.com/2018/04/macconkey-agar-principles-composition.html)

- Contains chemicals or antibiotics designed to inhibit normal commensal bacteria.

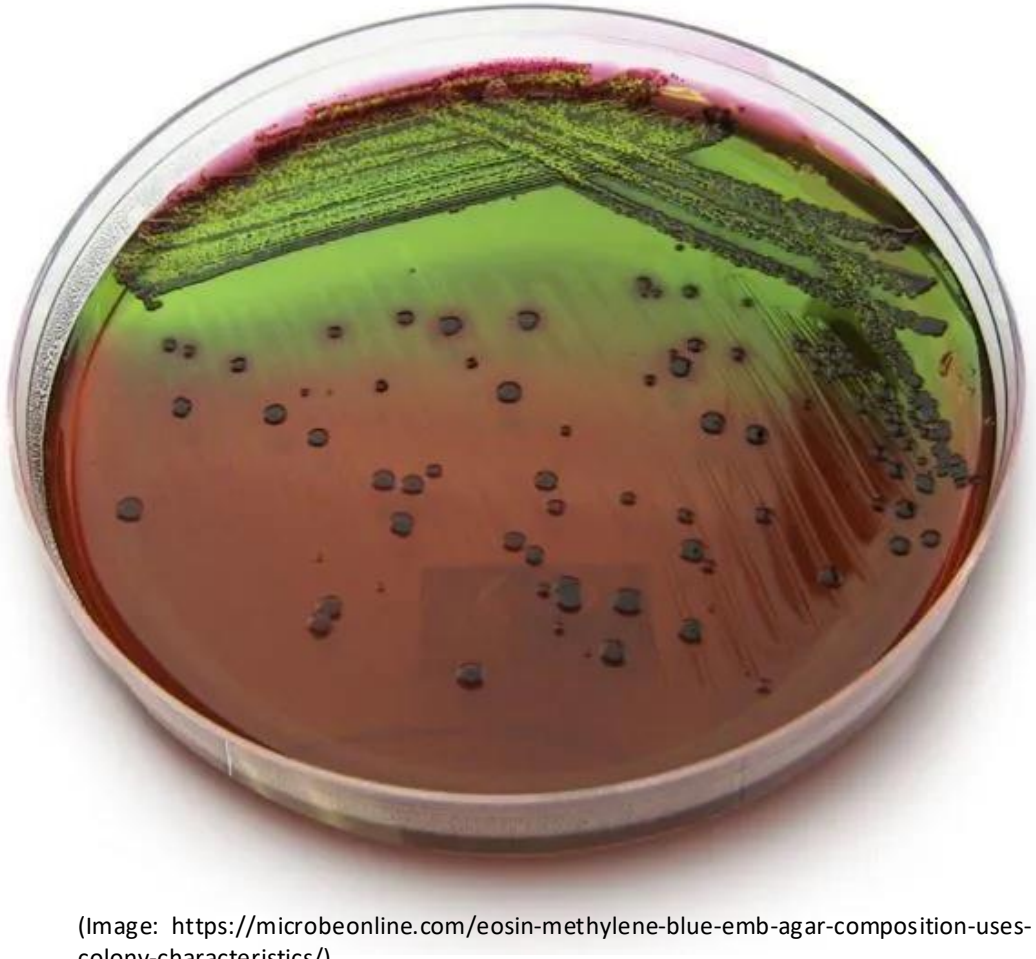
Ex: Bismuth sulfite agar for the isolation of *Salmonella* spp.

Ex: MacConkey agar for the isolation of gram-negative bacteria.

Differential Media

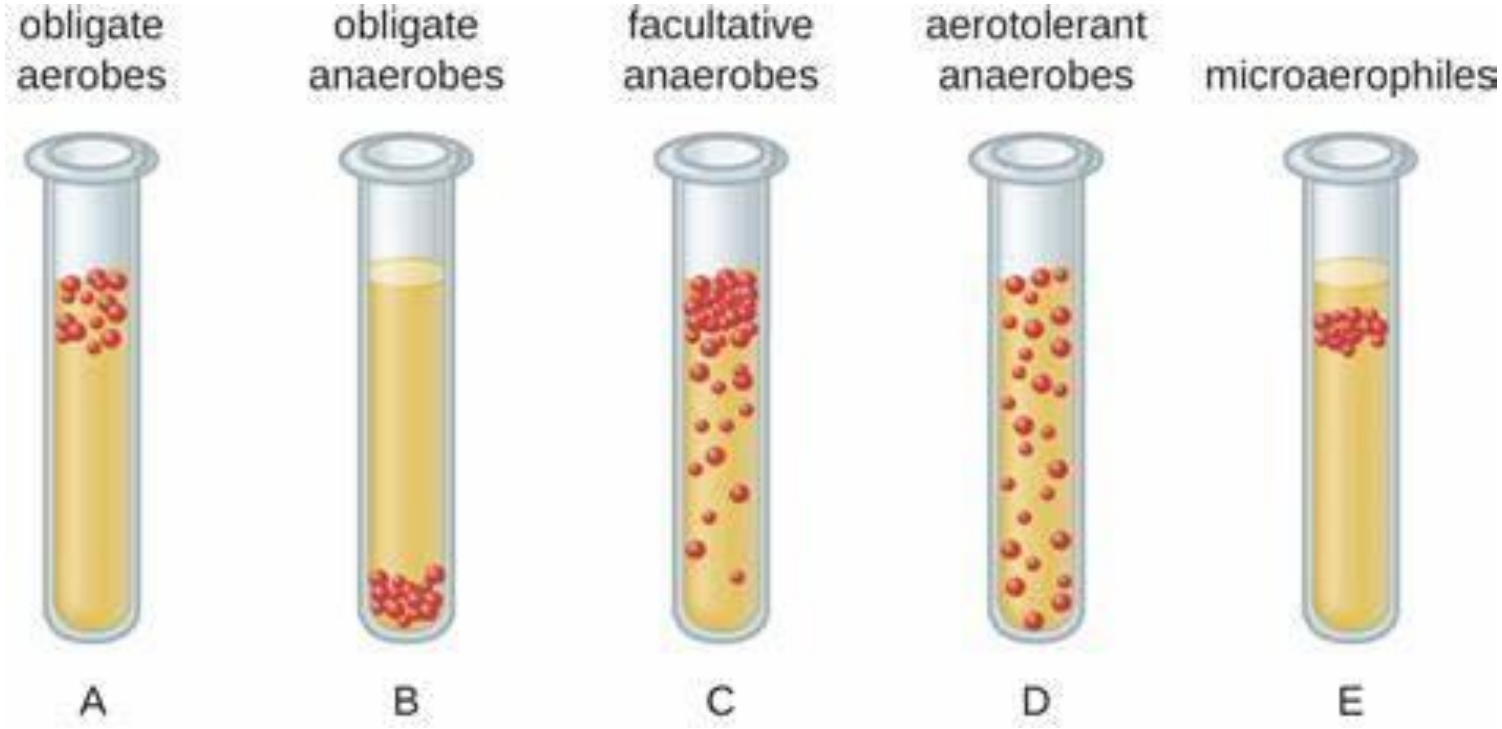
Stains colonies of specific organisms, while inhibiting the growth of others.

Ex: Acetate agar to differentiate between *E. coli* from *Shigella.*



(Image: https://microbeonline.com/eosin-methylene-blue-emb-agar-composition-uses-colony-characteristics/)

The Effect of Oxygen on the Growth of Various Types of Bacteria



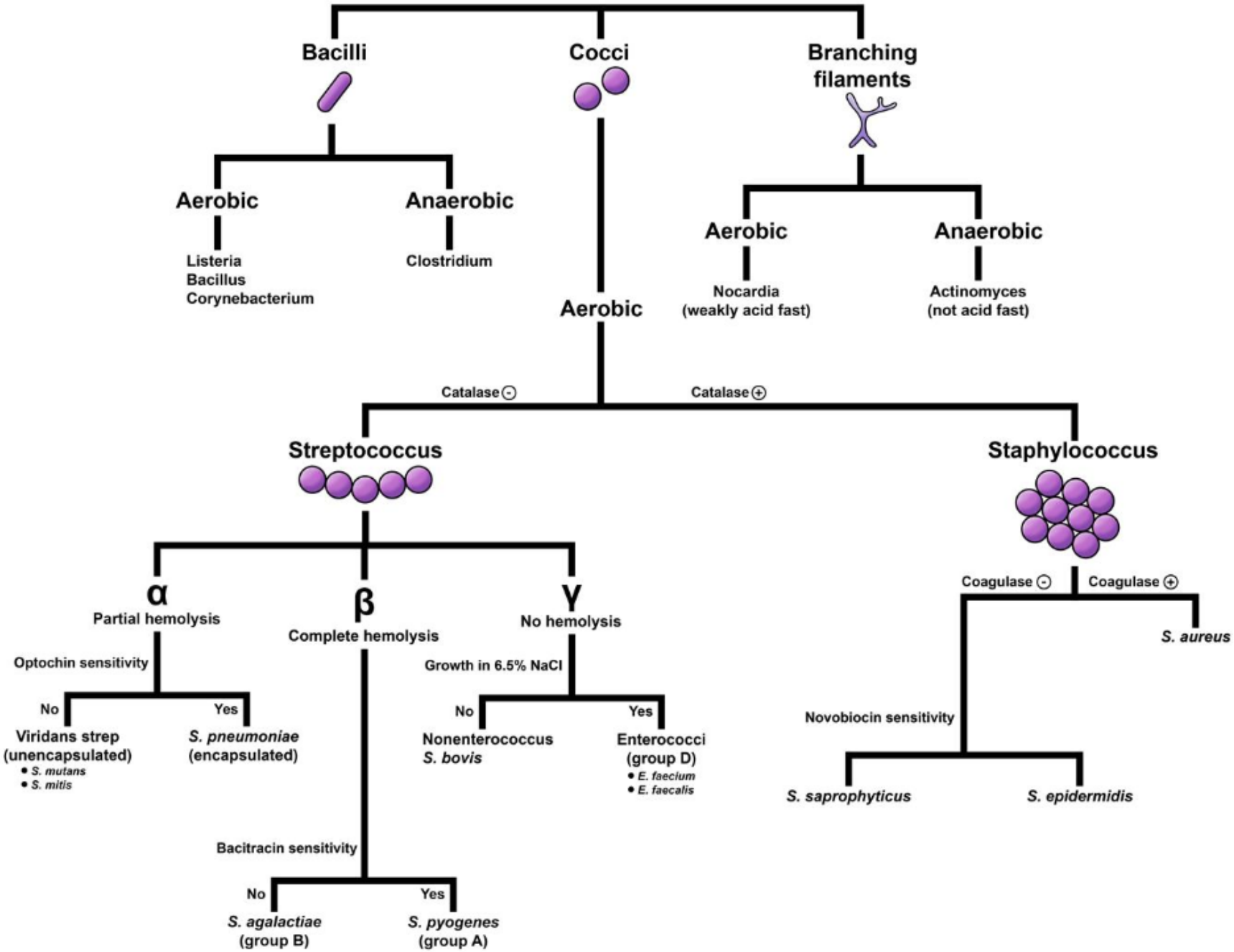
(Image: https://ecampusontario.pressbooks.pub/microbio/chapter/oxygen-requirements-for-microbial-growth/

Catalase and Coagulase Testing

Source: microbeonline.com/diagnostic-tests-biochemical-tests-coagulase-test/

- The catalase test is used to differentiate between streptococci and staphylococci.
- The coagulase test is used to differentiate *S. aureus* from other staphylococci such as *S. epidermidis*
  - CoNS

Gram-Positive Bacteria



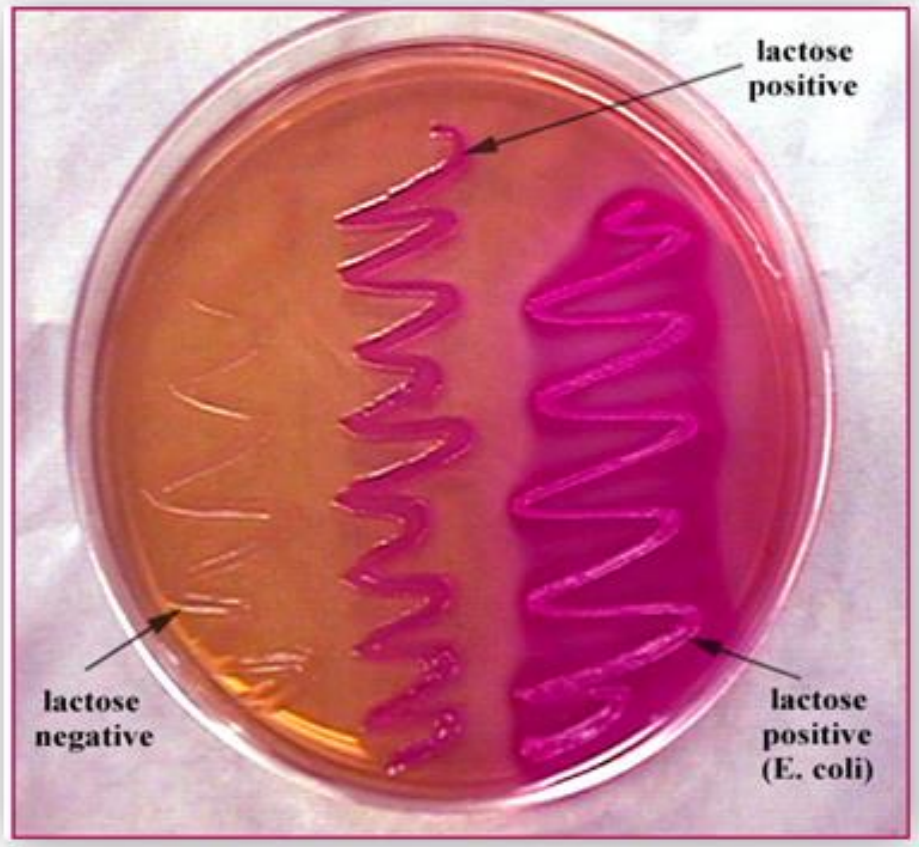
Lactose Fermenting Bacteria

Lactose-fermenting Gram-negative bacilli are included in a biological group known as Enterobacteriaceae.

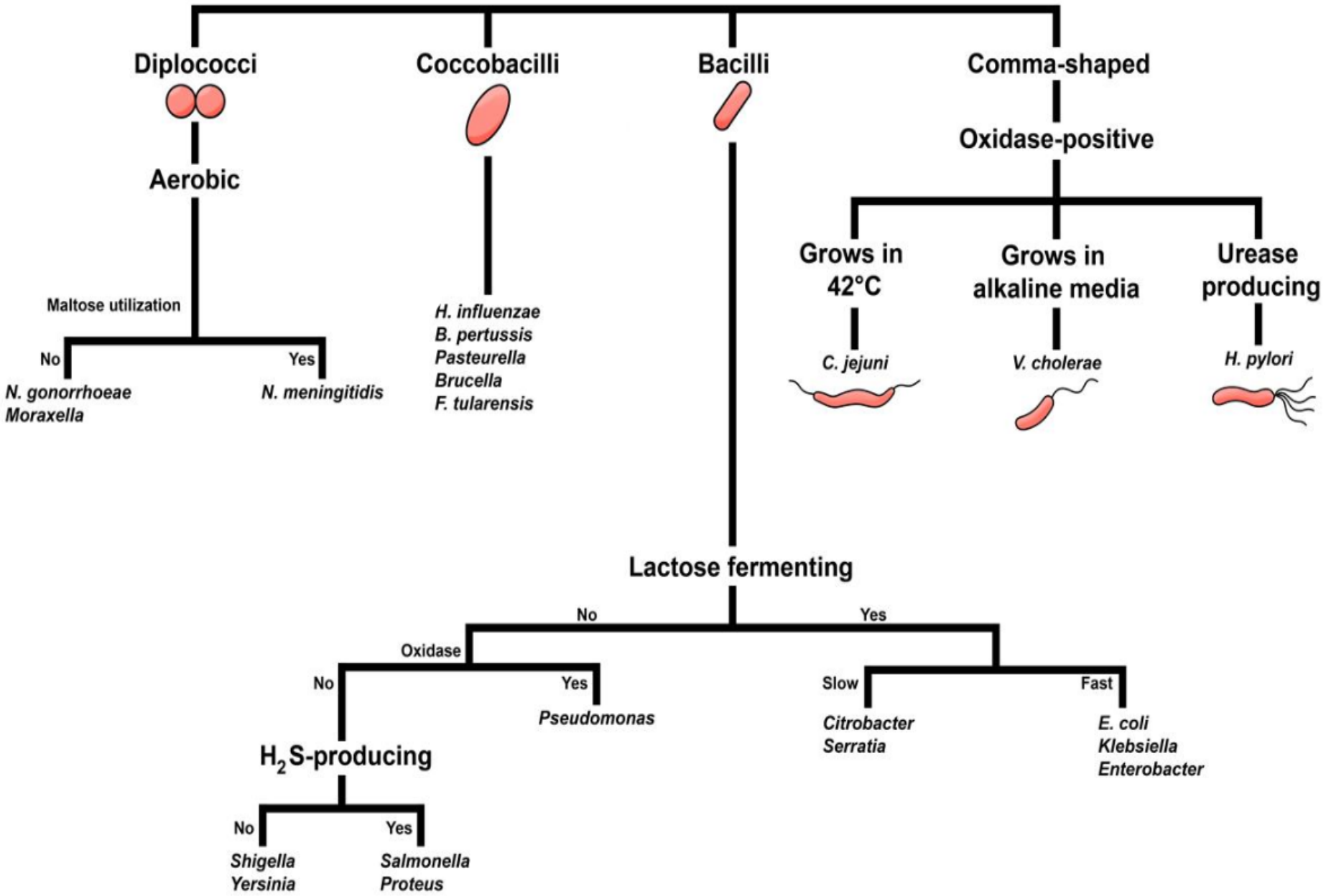
- Escherichia coli
- Klebsiella spp.
- Enterobacter spp.
- Citrobacter spp.
- Serratia spp.

Non-lactose fermenting gram-negative bacilli include:

- Pseudomonas spp.
- Salmonella spp.
- Shigella spp.
- Stenotrophomonas maltophilia
- Acinteobacter baumannii
- Burkholderia cepacia



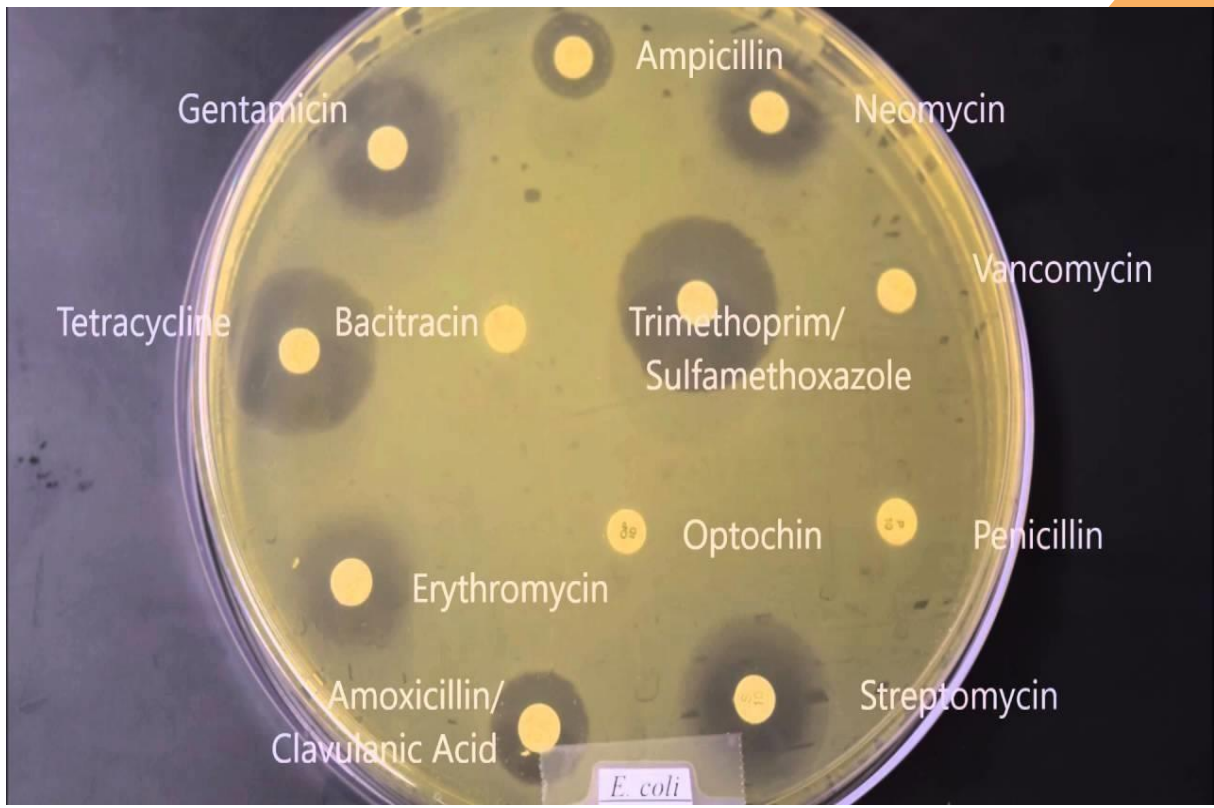
Gram-Negative Bacteria



Antimicrobial Susceptibility Testing

Disk Diffusion (Kirby-Bauer Method)

- Bacteria are spread in a lawn fashion onto Mueller-Hinton agar.
- Paper disks impregnated with a standard amount of antibiotic are placed onto the agar surface.
- Organism growth is either inhibited by the concentration of the antibiotic in the agar or not.
- Zone of inhibition—area in which the concentration of the antibiotic prohibits the growth of the organism.



Concept Check

In a Gram stain procedure, gram-positive bacteria stain purple because:

A. They have lipopolysaccharide layer in their cell wall that is decolorized with alcohol
B. Their cell walls contain long-chain fatty acids that take up crystal violet easily
C. They have a thick peptidoglycan cell wall that retains the primary stain during the alcohol decolorization
D. Gram staining is simple staining, so the only stain used is crystal violet

Concept Check

The microbiology laboratory has isolated a microbe. Chemical analysis shows that it contains proteins, peptidoglycan, lipids, DNA and teichoic acid. What sort of bacteria is this?

A. Gram-positive
B. Gram-negative
C. Cannot tell from this information

Concept Check

A microbe that can grow in the absence of oxygen but is also able to utilize oxygen for growth is a/an:

A. Aerotolerant anaerobe
B. Obligate anaerobe
C. Obligate aerobe
D. Microaerophilic anaerobe
E. Facultative anaerobe

Concept Check

What bacterial structure facilitates bacteria implanting on medical devices?

A. Cell wall
B. Slime Layer
C. Pillus
D. Capsule

Concept Check

Microorganisms are grown on culture media made of an agar base. Additives to media vary according to growth requirements of organism and/or the desire to select out a specific organism. Fastidious organisms require_____ media, and _____ media is used to inhibit normal commensals.

1. Differential
2. Enriched
3. Selective
4. Nutrient broth
5. Synthetic sheep blood agar

A. 1, 3
B. 3, 4
C. 5, 1
D. 2, 3

Concept Check

An example of a selective media that inhibits gram-positive bacteria is:

A. MacConkey's agar
B. Chocolate agar
C. Trypticase Soy agar
D. Sabouraud agar

Chapter 25
Laboratory Testing and Diagnosis

Specimen Collection and Transport

Care should be taken when collecting and transporting specimens.

- Collect specimens aseptically and place in leakproof, sterile container
  - Blood and genital specimens may be placed directly into culture media
    - Golden rule of blood collection: Collect prior to the initiation of antimicrobial therapy and ensure sufficient volume.
  - Special handling may be necessary for specimens collected for anerobic culture
- Deliver specimens promptly to the laboratory
  - If transport is delayed, refrigerate any urine, stool, or sputum specimens
  - Some specimens may remain at room temperature such as genital samples and cerebrospinal fluid (CSF)

Accuracy of Laboratory Testing

Accuracy is the closeness of the result obtained to the true value and is described by two terms: sensitivity and specificity.

Sensitivity

- The ability of a test to detect all true cases of the disease
- The absence of a false-negative result.
- a/a+c

Specificity

- The ability of a test to correctly identify a negative result when the disease is absent.
- The absence of false-positive results.
- d/b+d

a True +	b False +
c False -	d True -

Concept Check

Which of the following statements are true of clinical tests with a higher sensitivity than specificity?

1. The test will be more accurate at predicting who is ill
2. Patients require further testing
3. The test has a high type II error rate
4. There will be a higher rate of false positives

A. 2, 3
B. 2, 4
C. 1, 3
D. 1, 2

Cerebrospinal Fluid Analysis

Table 25-3 Findings in Cerebrospinal Fluid Analysis for Meningitis

Component	Adult	Neonate	Bacterial Infection	Viral Infection	Fungal Infection
Color/clarity	Clear/colorless		Cloudy	Clear/hazy	Clear/hazy
Protein (mg/dL)	15 to 45	115 to 170	↑	Normal to ↑	Normal to ↑
Glucose (mg/dL)	50 to 80	60 percent of plasma value	↓	Normal to ↓	Normal to ↓
WBC count					
Agglutinating capacity	0 to 5	0 to 30	↑	Normal to ↑	↑
WBC differential					
Lymphocytes	62 to 34	20 to 18	↓	Normal to ↑	Normal to ↑
Monocytes	36 to 20	72 to 22	↓	Normal to ↑	Normal to ↑
Neutrophils	2 to 5	3 to 5	↑	Normal to ↓	Normal to ↓
Eosinophils	Rare	Rare	Rare	Rare	Rare

*Remember from Ch. 22 Microbial Pathogenicity and Host Response
Polymorphonuclear leukocytes = neutrophils, eosinophils, basophils, and mast cells.

Concept Check

Important considerations regarding the collection of blood culture specimens should include:

1. Collection prior to the initiation of antimicrobial therapy
2. Collection from a central venous catheter whenever possible
3. Adequate collection of volume of the specimen
4. Refrigeration of specimen after collection

A. 1, 3
B. 2, 4
C. 1, 4
D. 3, 4

Chapter 26
Antimicrobials and Resistance

Key Concepts

To reduce antimicrobial resistance, Infection Preventionists (IPs) need a working knowledge of:

- Available antimicrobials
- Principles for their appropriate use
- The mechanisms by which these drugs inhibit microbial growth.
- Mechanisms by which the organisms develop resistance.

Characteristics of Antimicrobial Agents

Definition:

- Antimicrobial: a substance that inhibits or kills microbes (viruses, bacteria, fungi, parasites)
- Antibiotic: a type of antimicrobial that is synthesized by a living microorganism, usually a fungus.

Administration:

- Common routes of administration include intravenous (IV), Intramuscular (IM) oral, and topical.

Mechanism of Action:

- All antimicrobials are bactericidal/fungicidal or bacteriostatic/fungistatic
  - -cidal agents kill >99.9%, while -static agents only kill 90–99%

Antibacterial Classes

	CLASS	ACTIVITY	EXAMPLE	MECHANISM OF ACTION
β-Lactam	Penicillin	Broad spectrum	Penicillin G, Amoxicillin, Methiclin	Inhibits cell wall synthesis
	Cephalosporin - 1st Gen	Broad spectrum	Cefazolin, Cephalexin	
	Cephalosporin - 2nd Gen	Enteric, gram-negative bacilli Gram-negative diplococci	Cefotaxime, Cefuroxime, Cefoxitin	
	Cephalosporin - 3rd Gen	Gram-negative bacilli	Ceftriaxone	
	Cephalosporin - 4th Gen	Broad spectrum	Cefepime	
	Cephalosporin - 5th Gen	Broad spectrum	Ceftaroline	
	Carbapenem	Broad spectrum	Imipenem, Meropenem, Doripenem	
	Monobactam	Aerobic, gram-negative bacilli	Aztreonam	
	Aminoglycoside	Broad spectrum	Gentamicin, Tobramycin, Amikacin	Inhibits protein synthesis
	Fluoroquinolone	Broad spectrum	Ciprofloxacin, Levofloxacin Moxifloxacin	Inhibits nucleic acid synthesis
	Macrolide	Broad spectrum	Azithromycin, Clarithromycin	Inhibits protein synthesis
	Glycopeptide	Gram-positive	Vancomycin	Inhibits cell wall synthesis
	Oxazolidinone	Gram-positive	Linezolid	Inhibit protein synthesis
	Tetracycline	Broad spectrum	Tetracycline, Doxycycline	Inhibit protein synthesis
	Sulfonamides	Broad spectrum	Trimethoprim/sulfamethoxazole	Interferes with metabolic pathway

*Remember: Please Call Carol Monday Afternoon For Many Great Opportunities To Succeed.

Florida HEALTH

Concept Check

Patients with cell-mediated immunity dysfunction are susceptible to infections attributed to pathogenic intracellular bacteria. Examples of these organisms include:

1. *Salmonella Typhi*
2. *Bacteroides fragilis*
3. *Listeria monocytogenes*
4. *Staphylococcus aureus*

A. 3, 4
B. 1, 2
C. 1, 3
D. 2, 3

Concept Check

What is the highest risk antimicrobial associated with causing *Clostridioides difficile* infection (CDI)?

A. Third-generation cephalosporins

B. Fluoroquinolones

C. Clindamycin and vancomycin

D. Metronidazole

Concept Check

What class of antibiotics do Third-Generation Cephalosporins belong to?

A. Macrolide
B. Aminoglycoside
C. Glycopeptide
D. Beta-lactam

Week 3 Assignments

Action Items

Assigned chapter readings:

- Chapter 97 TB and Other Mycobacteria
- Chapter 72 *C. diff* and Pseudomembranous Colitis
- Chapter 74 Coronavirus Disease
- Chapter 84 Influenza

Week 3 Group Member Spotlight