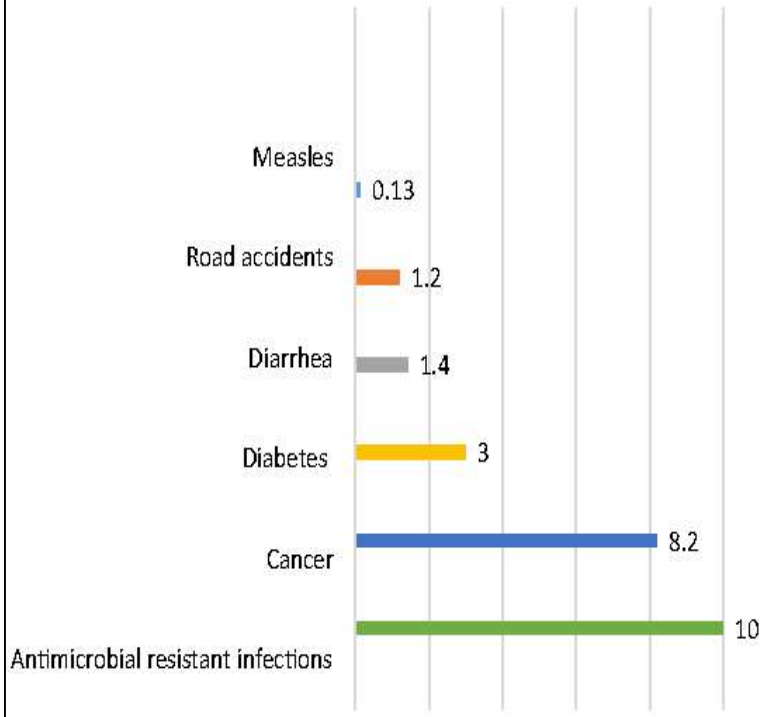
Antibiotherapy and antibioprofilaxy

The Impact of Antimicrobial Resistance in 2050

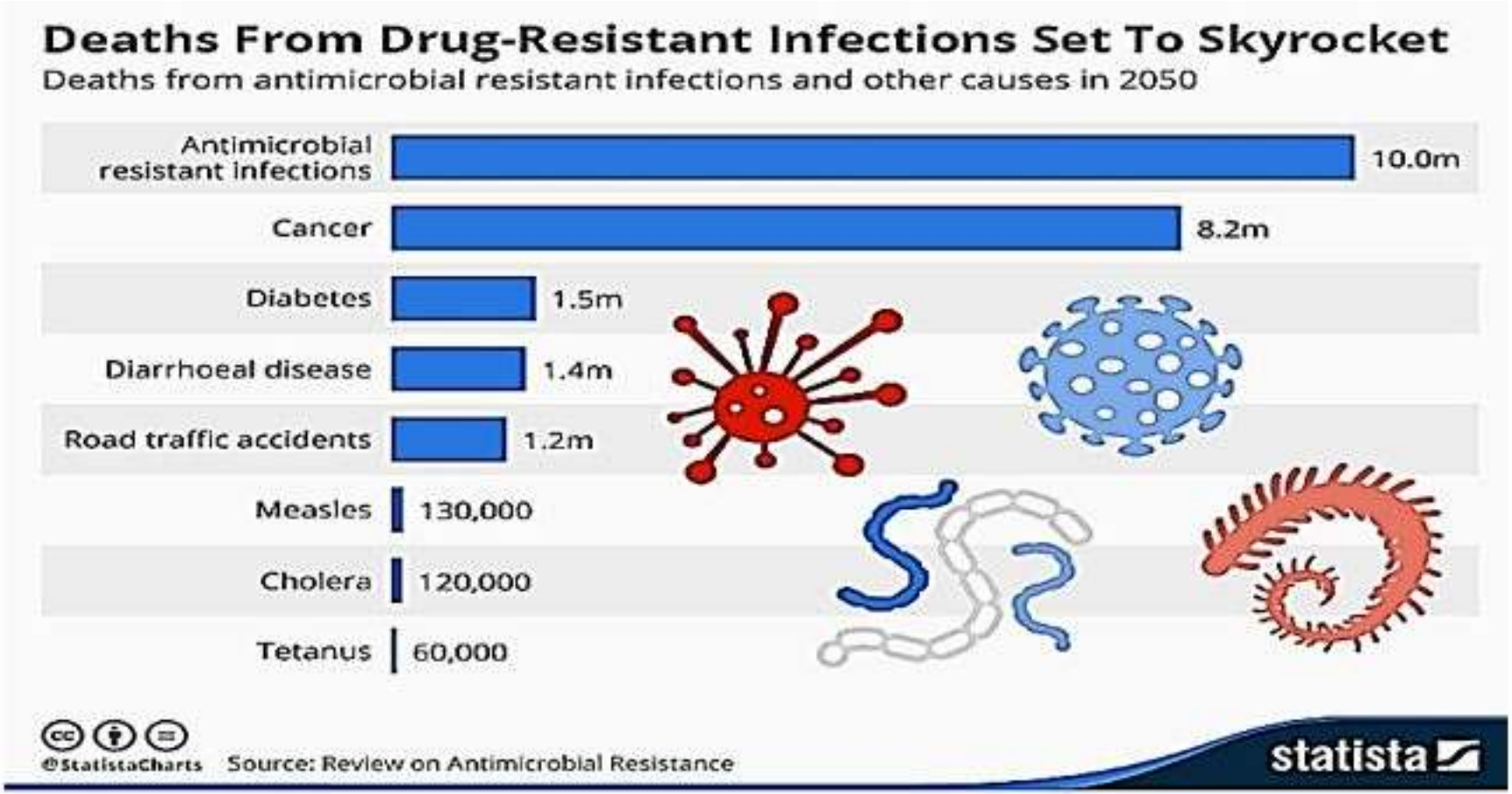
DEATHS PER ANNUM FOR ANTIMICROBIAL RESISTANT INFECTIONS AND OTHER CAUSES BY 2050 IN MILLIONS. [1] AND HTTP:// AMR-REVIEW.ORG/



Death attributable to antimicrobial resistance every year by 2050 in different countries [1]

Bassetti M, Carnelutti A, Peghin M. Patient specific risk stratification for antimicrobial resistance and possible treatment strategies in gram-negative bacterial infections Expert Rev Anti Infect Ther. 2017 Jan;15(1):55-65. Epub 2016 Nov 7 *Fig. 1 The impact of antimicrobial resistance in 2050*

The prognosis of Antimicrobial Resistance in 2050

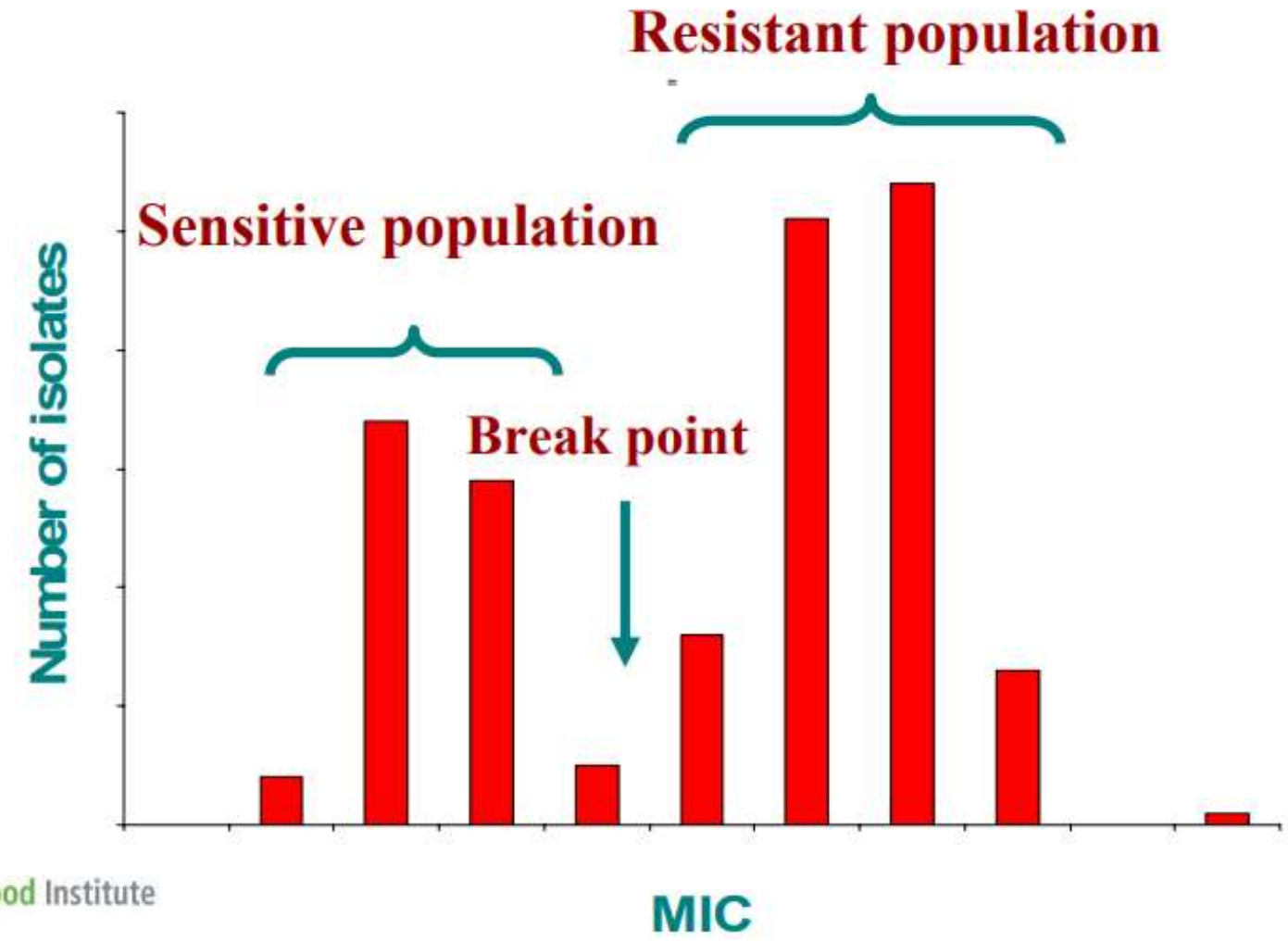


Antimicrobial Resistance

- The ability of a microorganism to survive at a given concentration of an antimicrobial agent at which the normal population of the microorganism would be killed

This is called the “Epidemiological breakpoint”

Population Distribution



National Food Institute

DTU

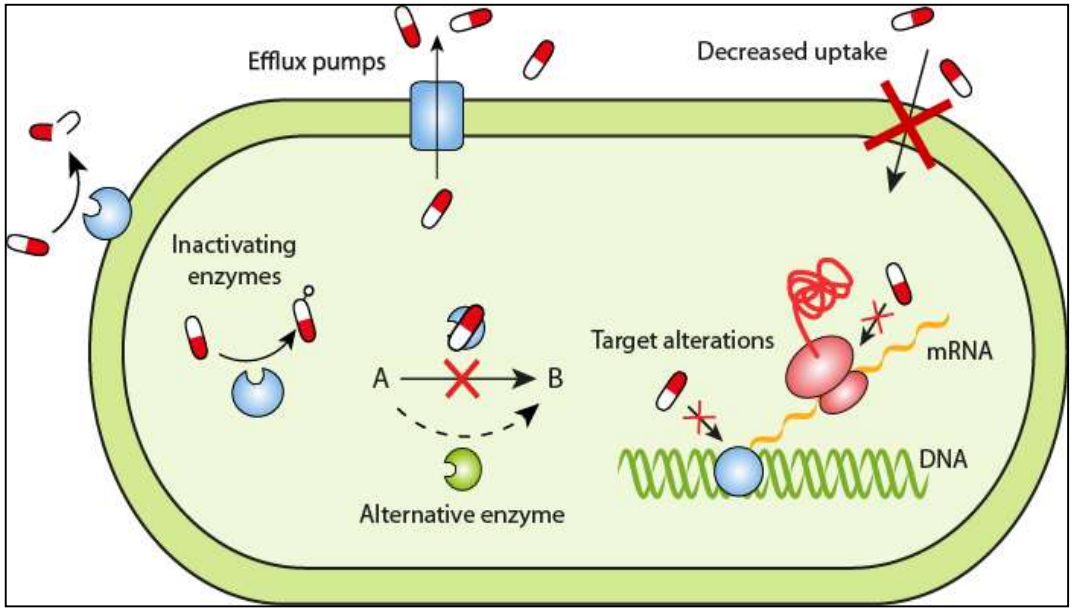
Antimicrobial Resistance

- The ability of a microorganism to survive treatment with a clinical concentration of an antimicrobial agent in the body

This is called the “Clinical breakpoint”

Bacterial Mechanisms of Antibiotic Resistance

1. Prevent antibiotic from reaching its target – impaired influx or increased efflux
   1. Tet(AE) and Tet(K) efflux pumps (tetracyclines)
   2. Altered active transporters (aminoglycosides)
2. Enzymatic inactivation (degradation, alteration)
   1. Bacterial esterases (macrolides)
   2. Acetyl-, phospho-, adenylyltransferases (aminoglycosides)
3. Alter target – "ribosomal protection"
   1. Tet(M) ribosomal protection protein (teracyclines)
   2. "MLSB resistance" vs. macrolides, lincosamides, streptogramin B



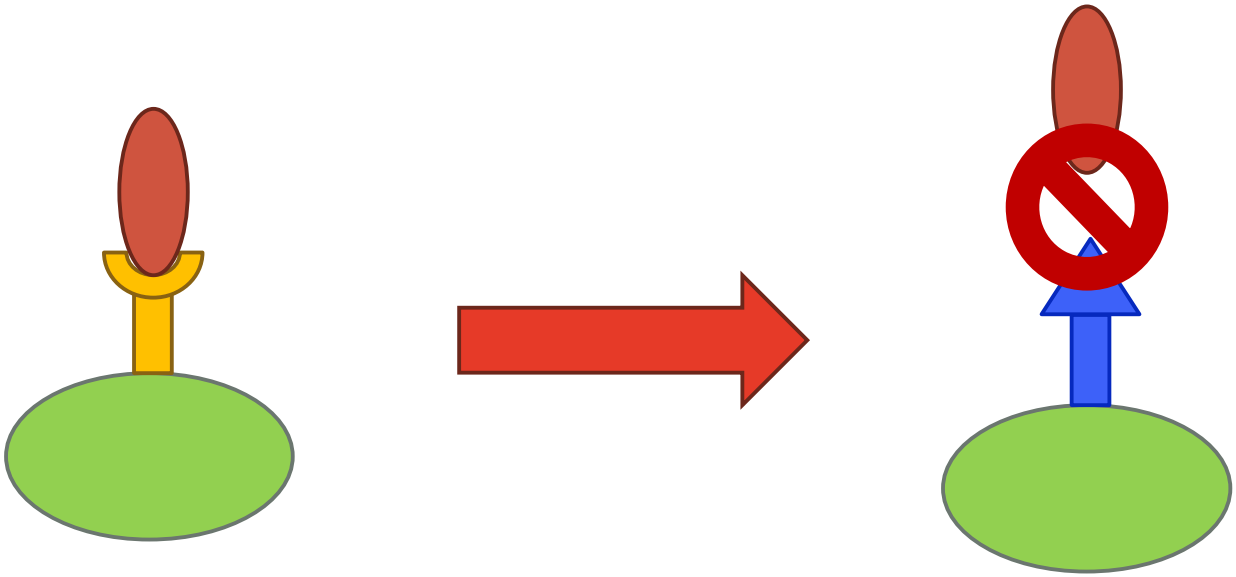
Antibiotic resistance strategies in bacteria. Courtesy of Dr. E. Gullberg

Antibiotic Resistance Mechanisms

Antibiotics	Mechanism of action	Resistance mechanisms
B-lactams: penicillins, cephalosporis	Block cell wall formation	Inactivation, mutation
Glycopeptides: vancomycin	Block cell wall formation	Mutation of binding molecules
Aminoglycosides: gentamycin	Block protein synthesis	Inactivation
Tetracyclines	Block protein synthesis	Inactivation
Macrolides	Block protein synthesis	Ribosome protection
Quinolones	Inhibit DNA replication	Mutation of binding molecules
Rifampin	Inhibits bacterial RNA-polymerase	Mutation in binding molecules
Trimethoprim Sulfonamide	Block formation of nucleic acids and f-met	Mutation in binding molecules

Antibiotic Resistance Mechanisms

- Point mutations in target genes / influx pumps



Genetic Variations / Point Mutations

DNA-gyrase – quinolone resistance

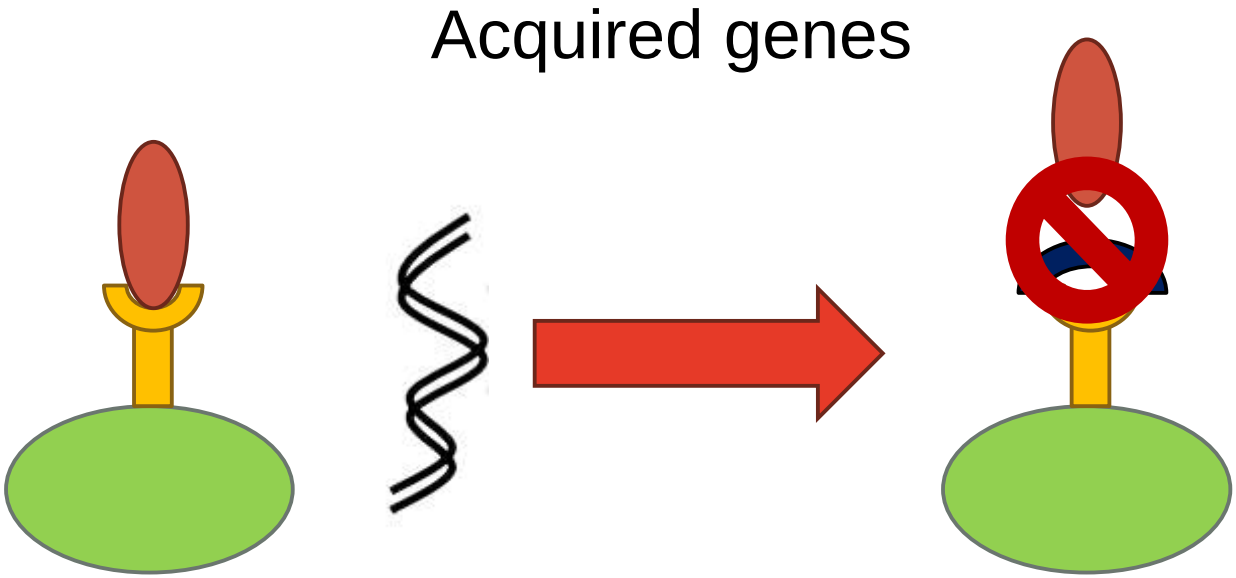
			110	120	130	140	150
Na1S		**101**	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTCCGCA	GTGTATGACA
Na1R	**MUT83A**	**101**	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTACGCA	GTGTATGACA
Na1R	**MUT83T**	**101**	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTTCGCA	GTGTATGACA

Codon 83: TCC → Ser

TAC → Tyr

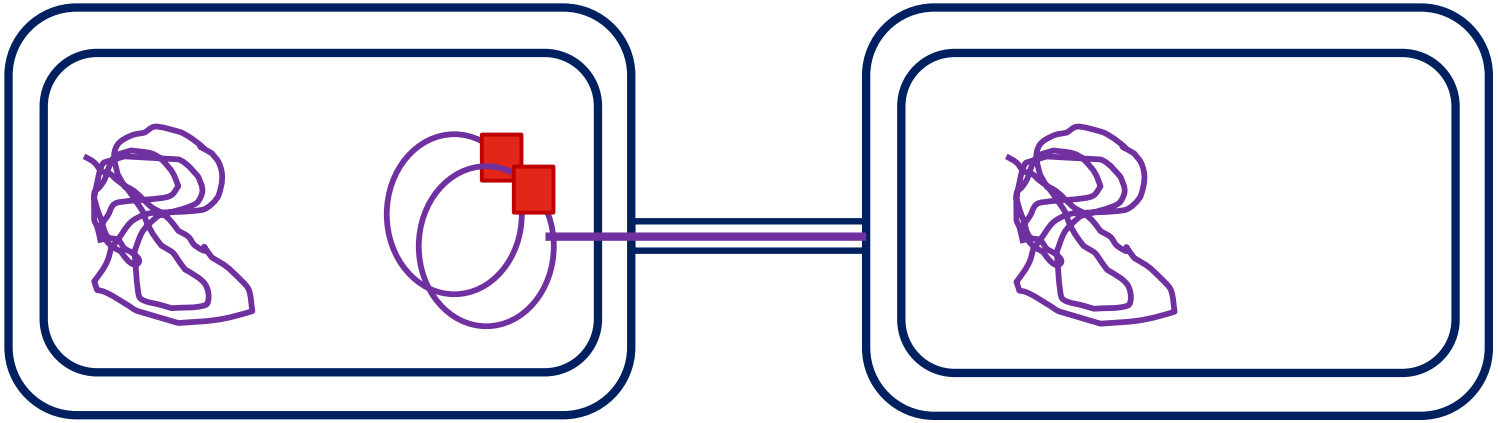
TTC → Phe

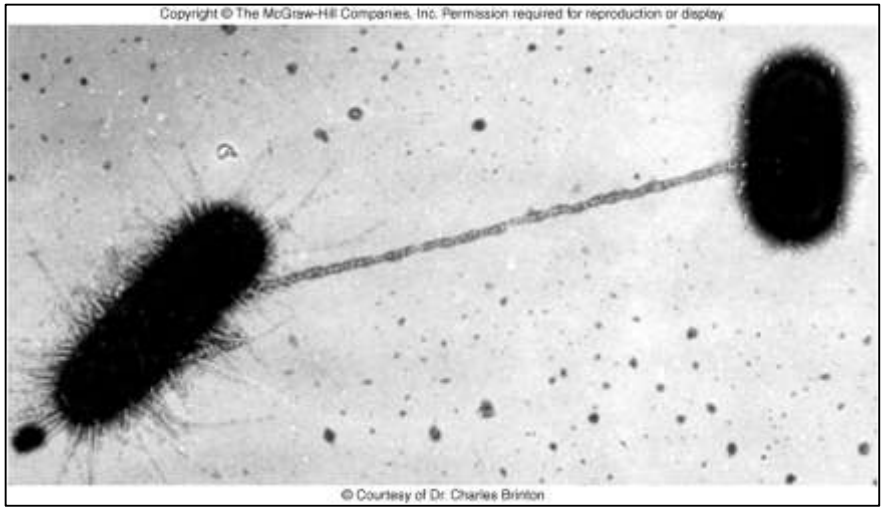
Antibiotic Resistance Mechanisms



Acquisition of Resistance

E. coli / *Salmonella* spp.





Resistance Mechanisms
Decreased Permeability of the Drug

- Prevents the drug reaching the target penicillin binding proteins (PBPs)
- Presence of an Efflux pump also reduces the amount of the intracellular drug

Resistance mechanisms in Carbapenem-resistant *Enterobacteriaceae*

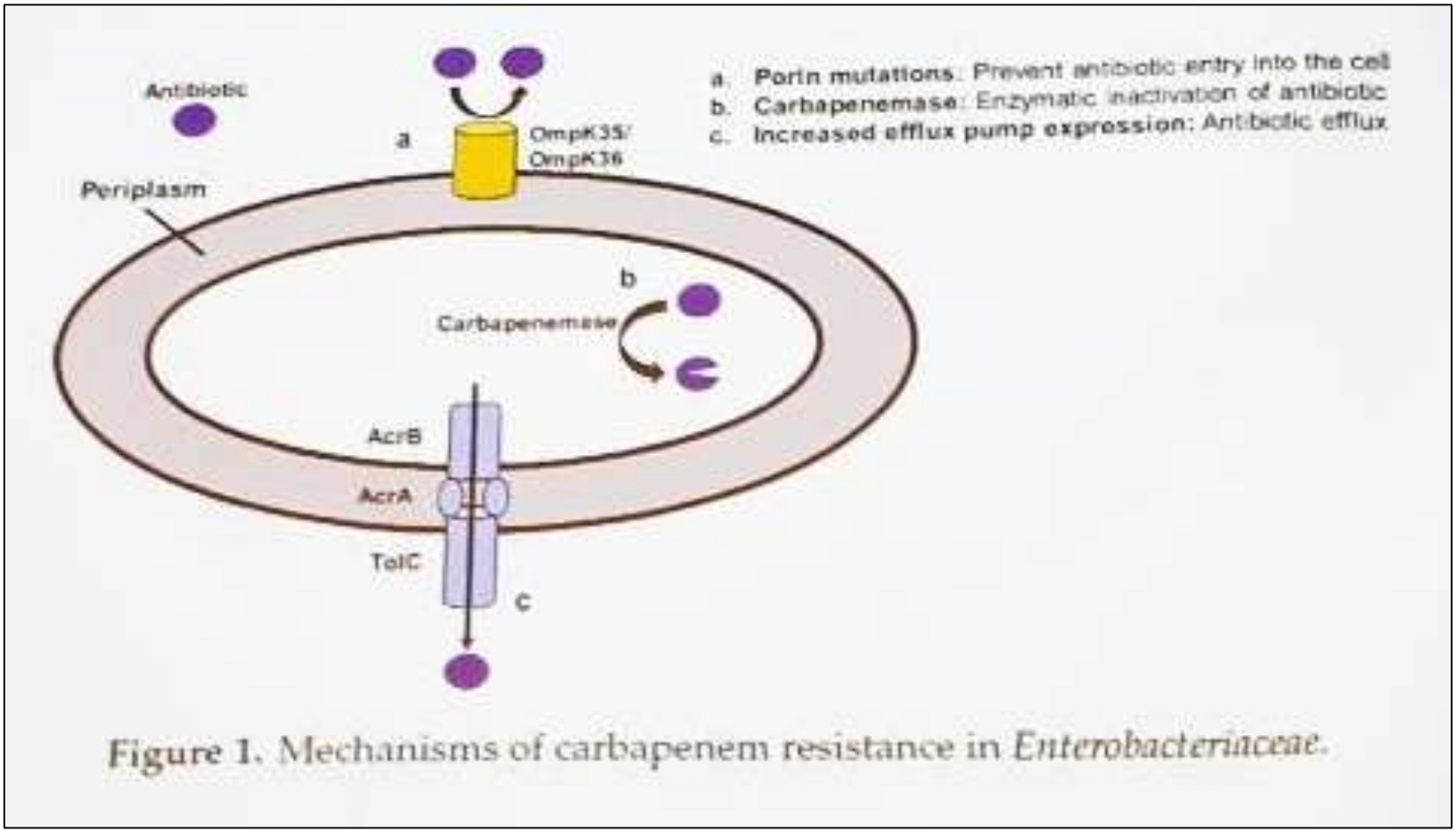


Figure 1. Mechanisms of carbapenem resistance in *Enterobacteriaceae*.

Antibiotics 2019, 8, 37

Resistance mechanisms in *Pseudomonas aeruginosa*

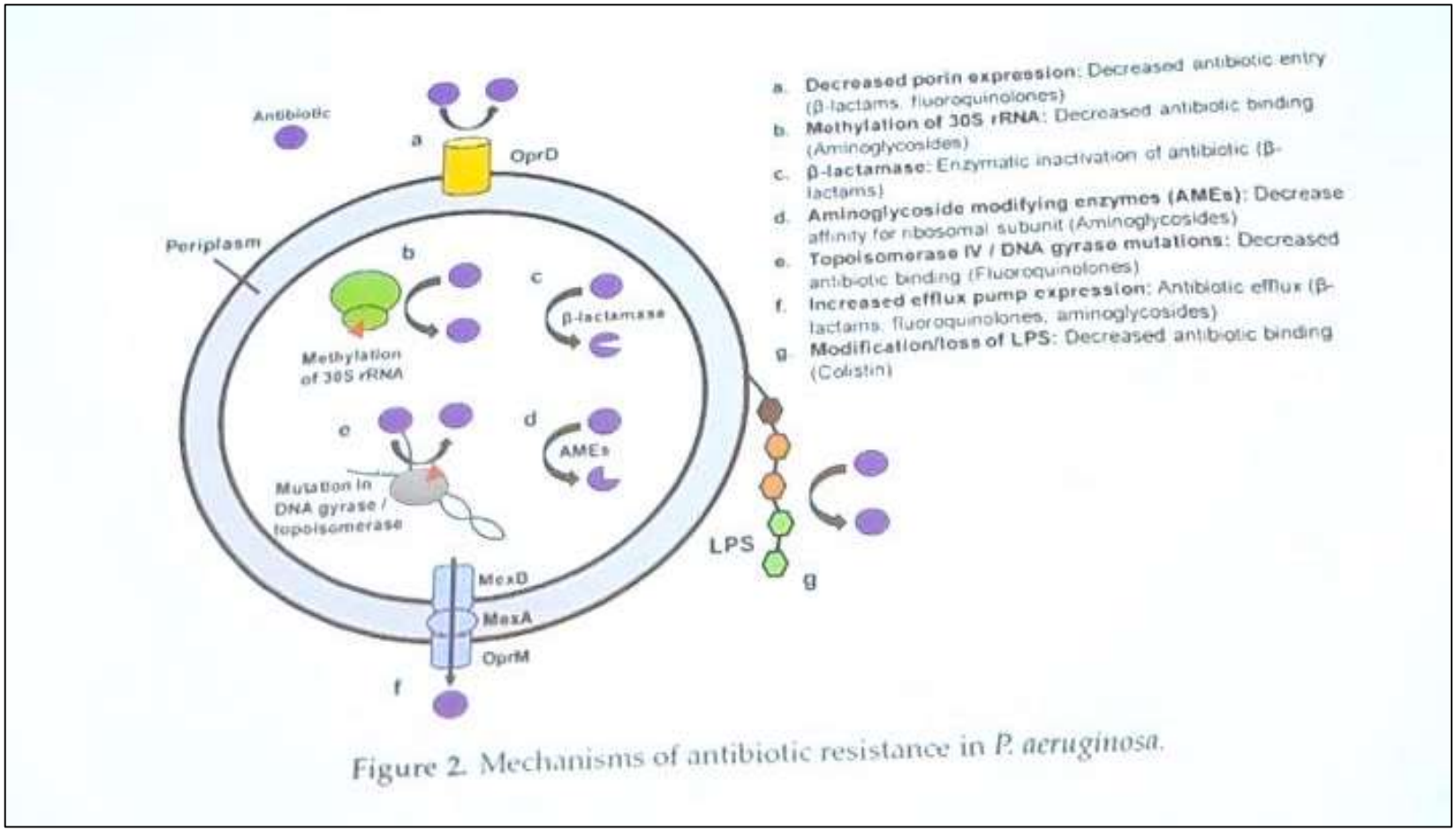


Figure 2. Mechanisms of antibiotic resistance in *P. aeruginosa*.

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Resistance mechanisms in *Acinetobacter baumanii*

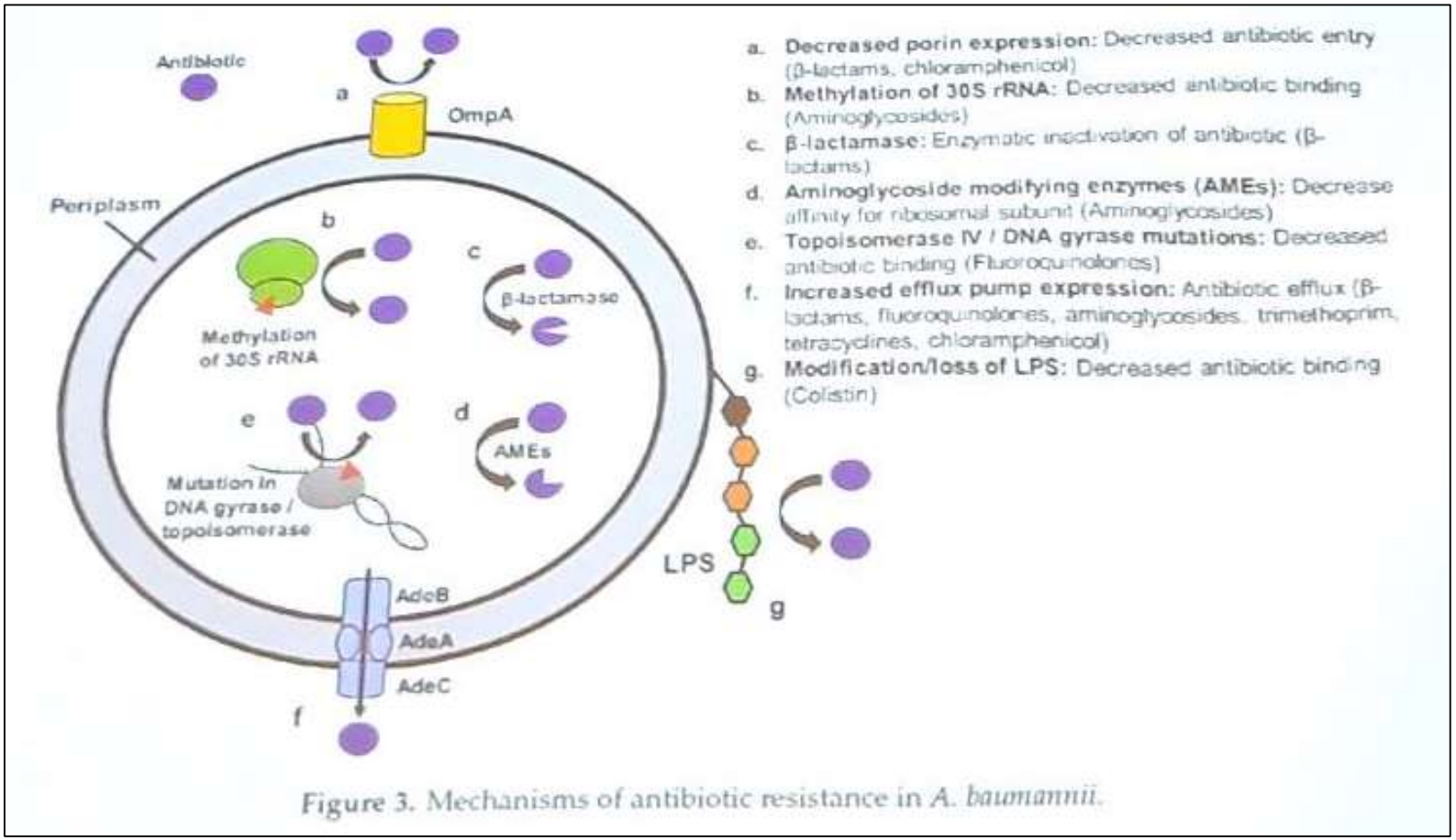
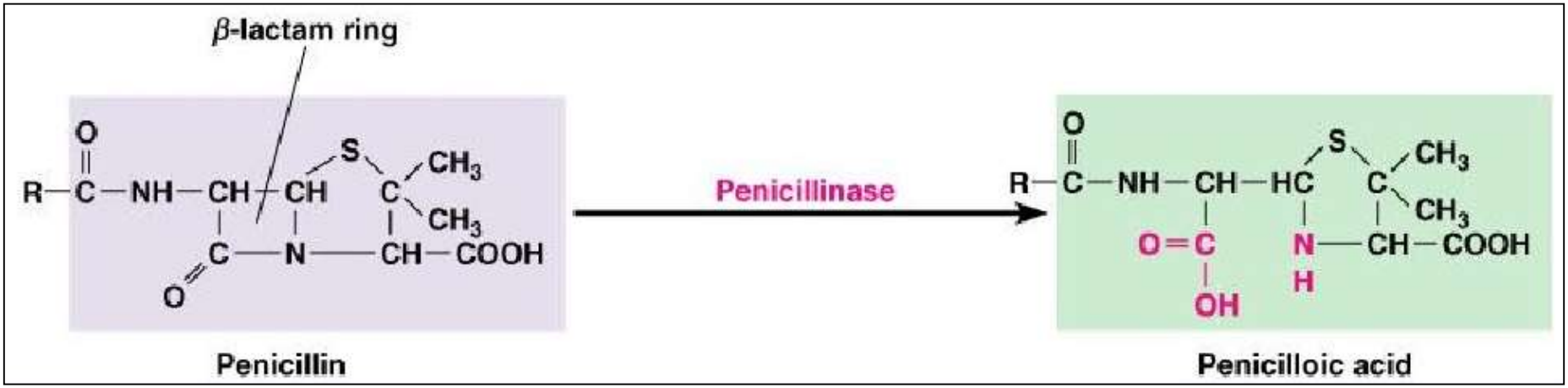


Figure 3. Mechanisms of antibiotic resistance in *A. baumannii*.

Antibiotics 2019, 8, 37

Resistance Mechanisms
β-lactamase

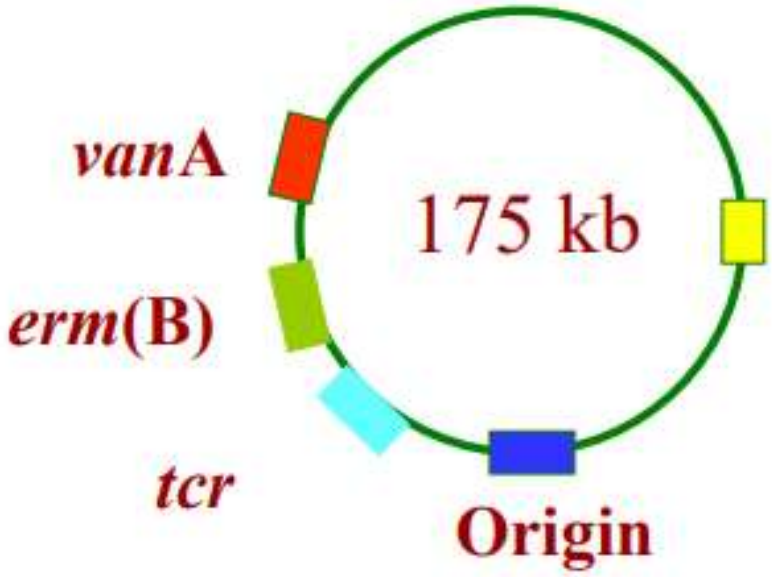
- β -lactamase destroys β-lactam ring, rendering β-lactam antibiotics ineffective
- **Solution**
  - Add clavulanic acid (β-lactamase inhibitor)
    - **e.g. co-amoxiclav (Augmentin)**
    - **Combination of piperacillin and tazobactam (Tazocin)**



Super Bug

A bacterium carrying several antibiotic-resistant genes is called multi-resistant Bacteria or casually, a “super Bacteria” or “super bug.”

Co-selection of Resistance



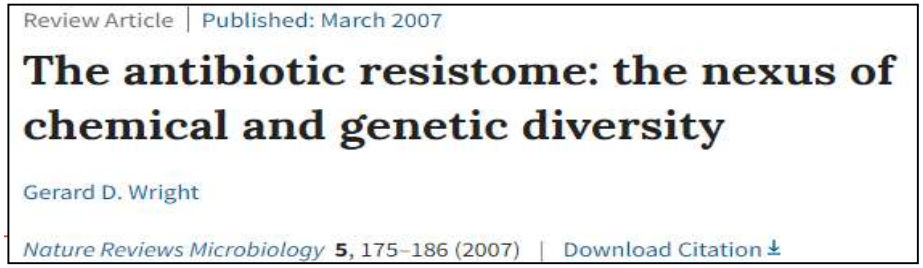
Usage of copper or erythromycin selects for presence of vancomycin resistance

tcr – transferable copper resistance

Genes of resistant

- The vancomycin-resistance gene, *van*A, from enterococci
- The methicillin-resistance gene, *mec*A, from staphylococci
- The β-lactam-resistance gene, *amp*C, specific to some Enterobacteriaceae
  - Class D *OXA* β-lactamases are characterized as penicillinases that can hydrolyze oxacillin and cloxacillin and are poorly inhibited by clavulanic acid and EDTA.
    - OXA-48 is one of the few members of this family to possess notable carbapenem-hydrolyzing activity. First described in 2004 in Turkey, OXA-48 has recently started to spread in Europe and the Middle East
    - carbapenem-hydrolysing β-lactamase, OXA-232, differing from OXA-181 and OXA-48 by 1 and 5 amino acid substitutions, respectively. Compared with OXA-181, OXA-232 had a lower ability to hydrolyse carbapenems but conversely possessed higher hydrolytic activities against penicillins.
- *NDM-1* (New Delhi metallo-ß-lactamase-1). A medical team first isolated the gene in a Swedish patient of Indian origin who traveled to India in 2008
- *KPC* (*Klebsiella pneumoniae* carbapenemase) belongs to the Ambler class A, Bush subgroup 2f, serine-based carbapenemases, which are active against all β-lactams, including the carbapenems.
  - Ten KPC variants (KPC-2 to -11) are currently known.
  - The KPC gene was detected in *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolates, indicating the widespread dissemination of the KPC gene in clinically significant nosocomial isolates.

Resistome


Review Article | Published: March 2007

The antibiotic resistome: the nexus of chemical and genetic diversity

Gerard D. Wright

Nature Reviews Microbiology **5**, 175–186 (2007) | Download Citation

- The **resistome** is a proposed expression by Gerard D. Wright for the collection of all the antibiotic resistance genes and their precursors in both pathogenic and non-pathogenic bacteria.
- This complete set of antibiotic resistance genes is composed of four different types of genes:
  1. Resistance genes found on **pathogenic bacteria**. These are the fewest but also the most problematic ones at present.
  2. Resistance genes found on **antibiotic producers**. The microorganisms such as soil-dwelling bacteria and fungi that naturally produce antibiotics have their own protection mechanisms to avoid the adverse effects of the antibiotics on themselves. The genes which code for these resistances are a strong source[for the pathogenic bacteria.
  3. **Cryptic resistance genes**. These genes are embedded in the bacterial chromosome but do not obviously confer resistance, because their level of expression is usually low or they are not expressed.[
  4. **Precursor genes**. These genes do not confer antibiotic resistance. However they encode proteins that confer to some kind of basal level activity against the antibiotic molecule or have affinity to the molecule. In both cases this interaction may evolve to a full resistance gene given the appropriate selection pressure.
- **Note** that these groups are not independent, and some overlapping is expected between them.

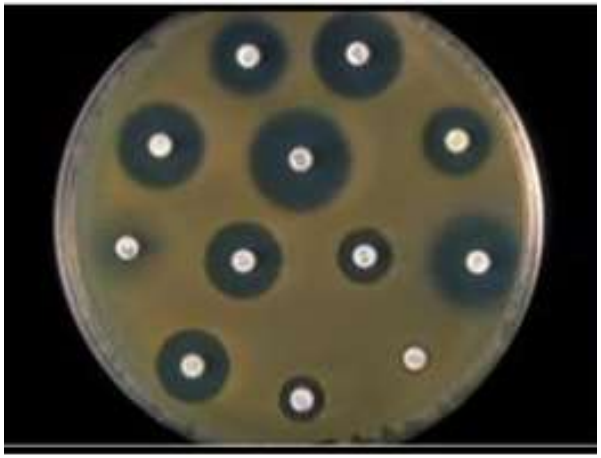
The Most Frequent Multidrug-Resistant Organisms (MDROs) in ICU

Accurate identification of colonized patients is an important strategy for decreasing the spread of MDROs

1. *Methicillin-Resistant Staphylococcus aureus* (MRSA)
   - Hospital-acquired (HA) is the most common type
   - Community-acquired (CA) is becoming more common
   - MRSA in animals (report of high prevalence of high prevalence of MRSA in pigs in the Netherlands – now also found in Danish animals)
2. Vancomycin-Resistant *Enterococci* (VRE)
3. *Acinetobacter baumannii* (natural resistant)
4. *Pseudomonas aeruginosa* (natural resistant)
5. *Klebsiella pneumoniae. E. coli,* 3rd gen. Cephalosporin-resistant Salmonella (Extended spectrum β-lactamases – ESBL)
6. Carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase-producing *Enterobacteriaceae* (CPE)
7. Multi-resistant Gram-negative bacilli (MRGN)
8. Fluoroquinolones-resistant *Salmonella*
9. Fluoroquinolone- and macrolide-resistant *Campylobacter*

Antibiotic Susceptibility and Resistance

- *In vitro* values are guides, not rules
- *In vivo* bacteria are resistant if cidal concentrations are toxic to the host
- Achievable serum concentrations are what determine susceptibility or resistance to drug:
  - Low pH, high protein concentrations, anoxia
  - Pharmacological parameters of drugs (serum *vs*. other bodily fluids)

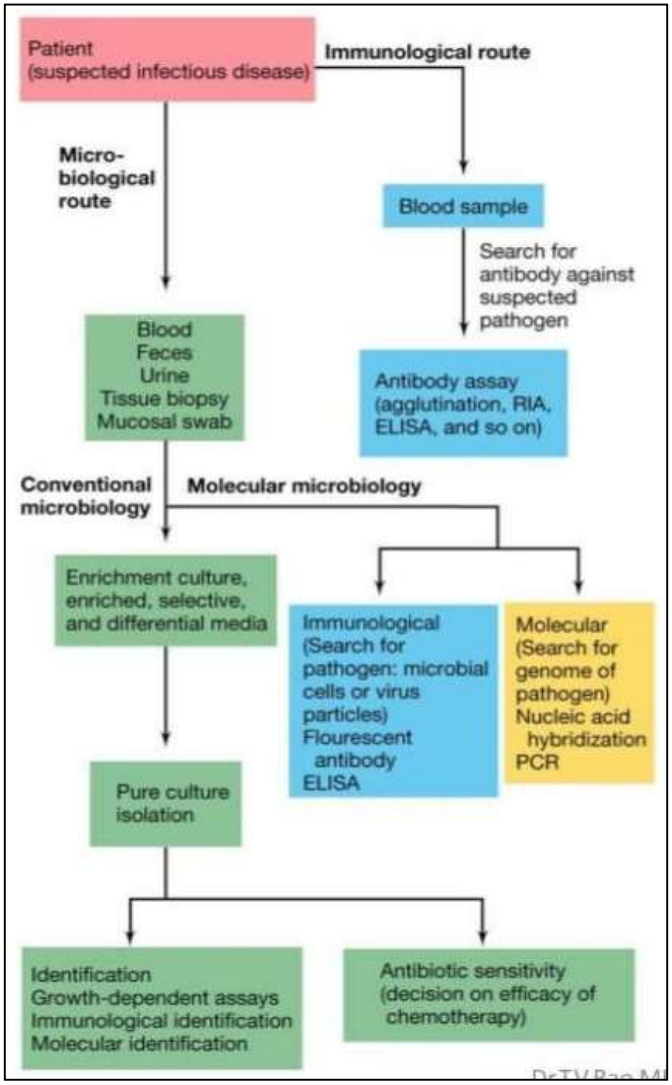


Kirby-Bauer Plate
(Disk diffusion test)

Importance of Identification

- Determining the clinical significance of particular pathogen
- Guiding physician care of the patients
- Determining the laboratory testing for detection of antibacterial resistance is warranted
- Determining the type of antibacterial therapy that is appropriate
- Determining the whether infectious organisms are risk for others patients in the hospital, the publicand other laboratory workers

Microbe Identification



Identification Methods

- Traditional method – phenothypic method
- Immunochemical method – serological methods
- Genotypic method – molecular method

Nutritional Growth Characteristics

Autotrophs/lithotrophs

- Able to utilize simple inorganic compounds
  - CO_2 as carbon source, ammonium salts as nitrogen source
- Include photoptrophs (photosynthesis) and chemo-lithotrophs (oxidation of inorganic compounds)

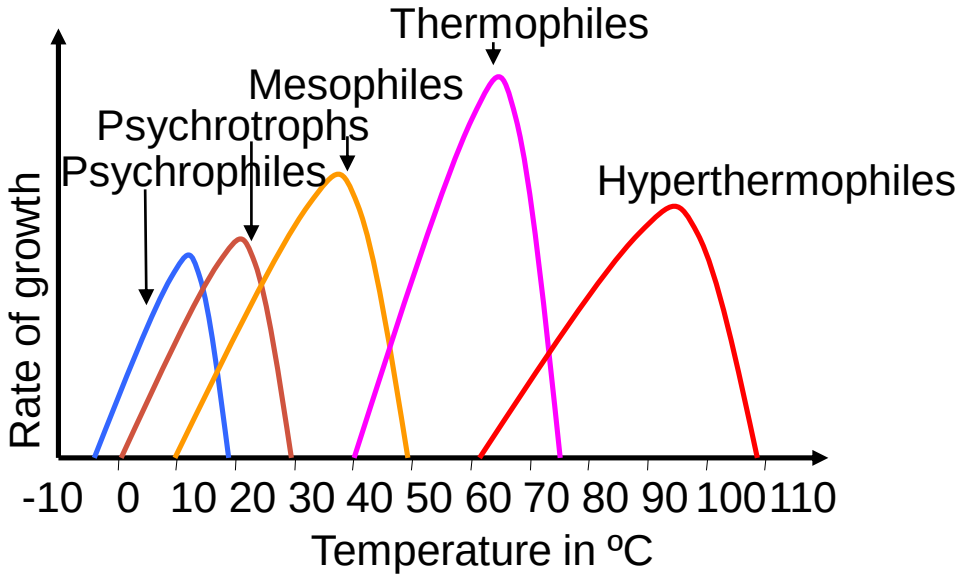
Heterotrophs (bacteria in human body)

- Unable to synthesize own metabolites
- Depend on preformed organic compounds
- Nutritional needs are variable

Growth Requirements

Physical

- Temperature
- pH
- Osmotic pressure
- Moisture & desiccation



Chemical

- Carbon source
- Nitrogen, sulfur phosphorus
- Oxygen

Temperature

Psychrophiles (cold loving)

- True psychrophiles
  (optimum growth at 15 ºC)
- Psychrotophs
  (optimum growth at 20-30 ºC)

Most pathogenic bacteria are mesophiles
And grow optimally at 37 ^{0}C
(human body temperature)

Mesophiles (moderate temperature loving)

Thermophiles (heat loving)

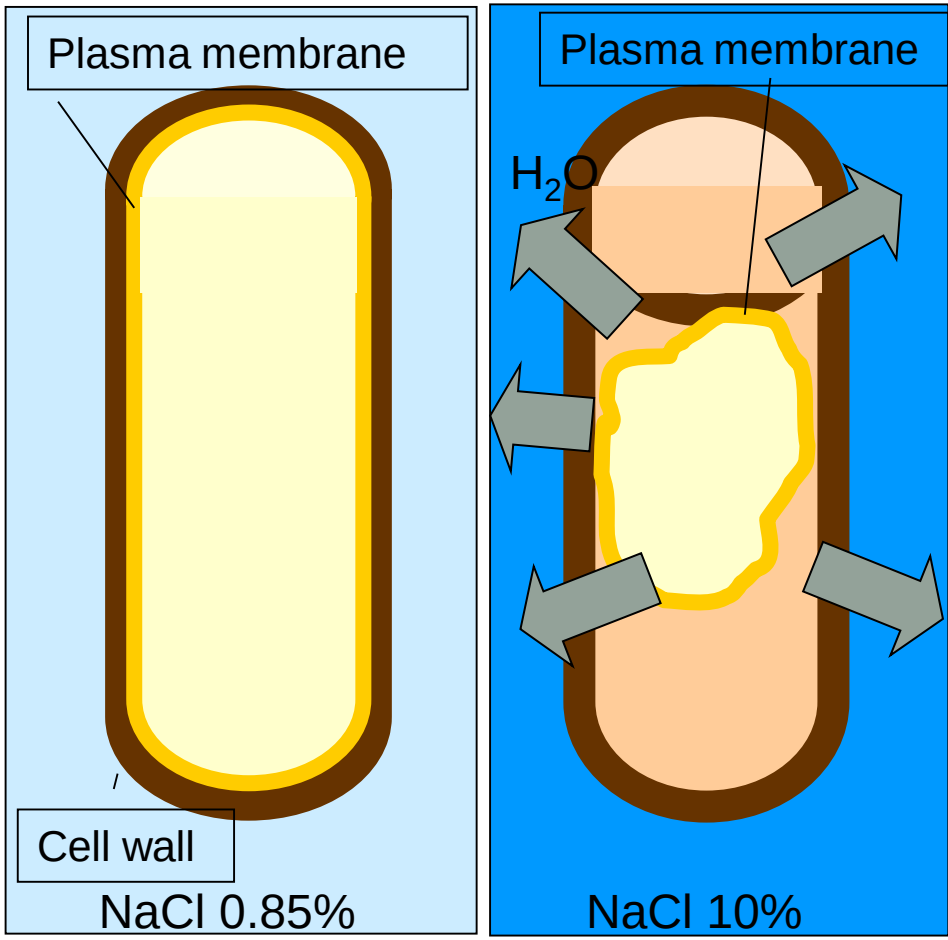
Hyperthermophiles (tolerate extreme temperatures)

pH

- Most medically important bacteria grow at neutral or slightly alkaline pH (7.2 to 7.6)
- Very few bacteria grow below pH 4
- Lactobacilli grow in acidic pH; cholera *vibrio* grow in alkaline pH
- Growth media includes chemical buffers to prevent acid production
- Foods are preserved by acids produced by bacterial fermentation

Osmotic Pressure

- High osmotic pressure (hypertonic) removes water causing plasmolysis – inhibits growth i.e. salt as preservative
- Low osmotic pressures (hypotonic) cause water to enter and can cause lysis
- Bacteria are more tolerant to osmotic variations because of the mechanical strength of the cell wall



Moisture and Desiccation

- Moisture is essential - 80% body weight is water
- Effect of drying varies by organism
  - *T. pallidum*, gonococcus are very susceptible
  - Tubercle bacilli, staphylococci may survive for weeks
  - Bacterial spores survive several years
- Lyophilization
  - Freeze dry process that protects bacteria

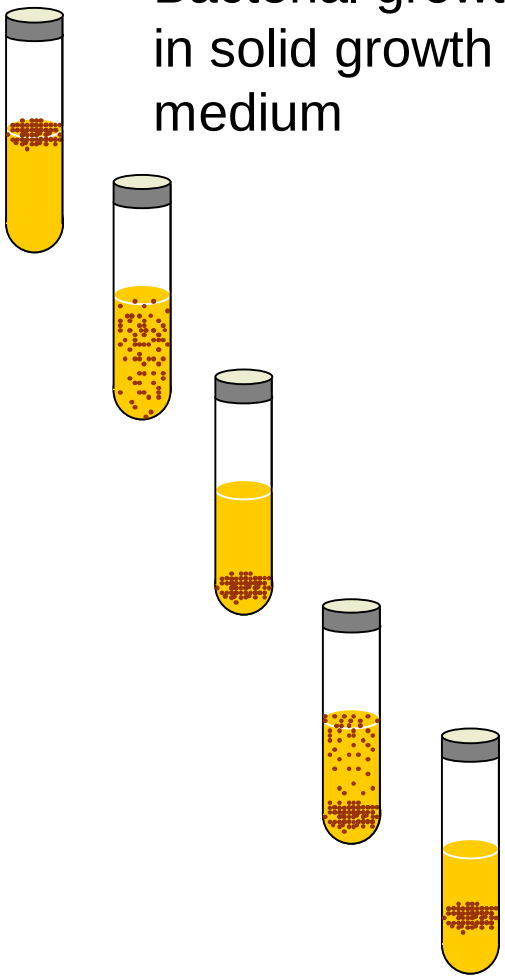
Carbon

- Chemo- and photo-autotrophs fix CO_2
- Chemoheterotrophs obtain energy from organic compounds

Oxygen

- Obligate aerobes
  - Only aerobic growth, oxygen required
- Facultative anaerobes (most human pathogens)
  - Greater growth in presence of oxygen
- Obligate anaerobes
  - Only anaerobic growth, cease with oxygen
- Aerotolerant anaerobes (e.g., *C. perfringens*)
  - Only anaerobic growth, continues with oxygen
- Microaerophiles (e.g., *M. tuberculosis*)
  - Only aerobic growth with little oxygen

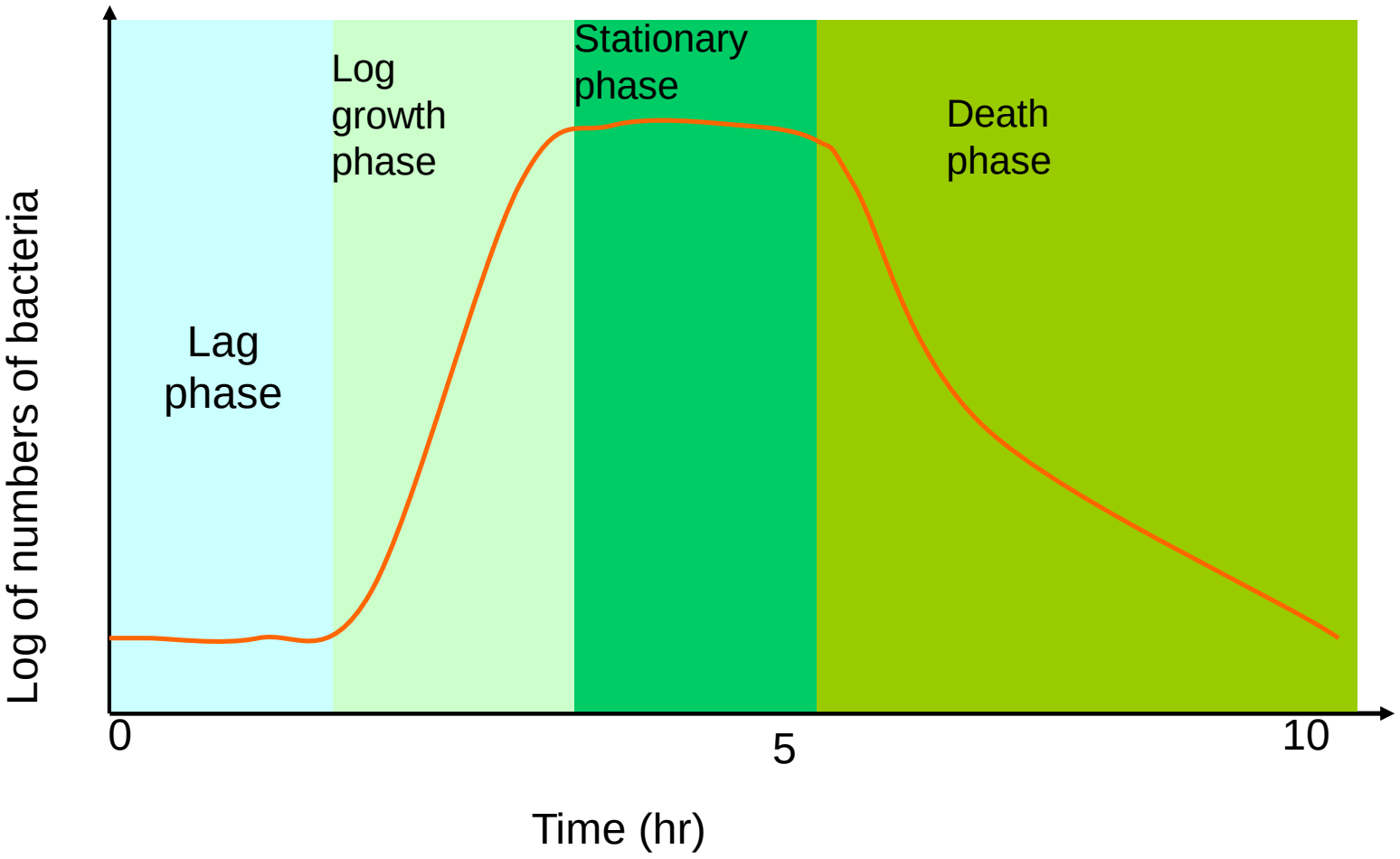
Bacterial growth in solid growth medium



Bacteria Grow by Binary Fission

- In rich broth, the number of bacteria doubles every 30 minutes (generation time)
- If start with 30 people in the room:
  - In 30 minutes we would have 60 people
  - In 60 minutes we would have 120 (very uncomfortable)
  - In 90 minutes we would have 240 (suffocation)

Phases of Bacterial Growth



Inside the Tissue

Interpretation of the Bacterial Growth Curve

- Explosiveness of exponential growth
  - Short generation time: small number of bacteria initiate a dangerous illness (e.g. acute meningococcal meningitis).
  - Long generation time: tuberculosis bacillus causes chronic illness
- Inside body tissues
  - Bacteria are stressed
  - Bacterial populations are rarely fully viable
  - May cease growth but continue synthetic activities to meet adaptive stress
- Non-growing bacteria can also be harmful:
  - Immunogenic
  - Production of toxins starts or accelerates during stationery phase
  - Sporulation can release toxins

Rate of Bacterial Death

- Death is exponential
  - After 1’ – 10% remain alive
  - After 2’ – 1% remain alive
  - After 3’ – 0.1% remain alive

- Effectiveness of antimicrobials
  - Number of organisms – larger number longer to eliminate
  - Environmental factors – organic materials reduce effectiveness
  - Timing of exposure

Measurement of Cell Growth

- Measure total counts
  - Measure both viable and non-viable bacterial cells
  - Direct microscopy using Gram stain; automated cell counter
- Measure viable counts
  - Measure only viable cells
  - Pour plate cultures to give quantitative number of viable bacteria

Measurement of Cell Growth

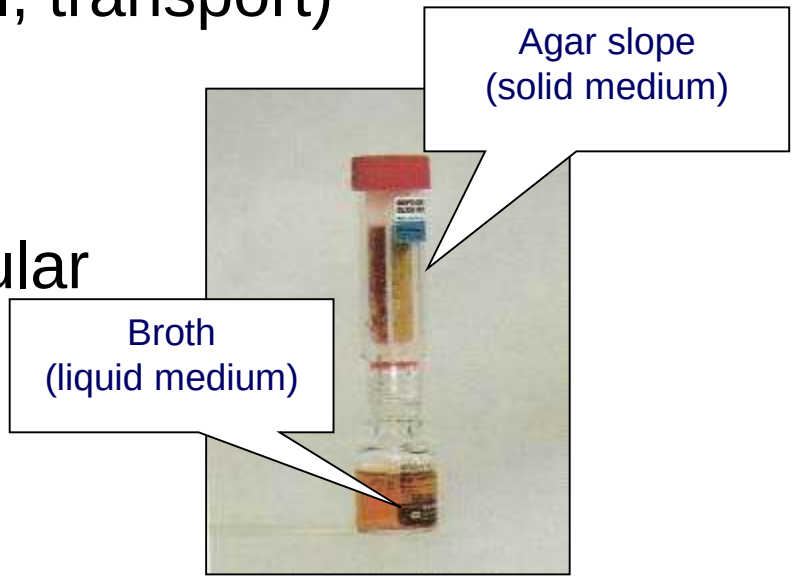
- Semi-quantitative methods
  - Give less accurate but working estimate of bacterial load to aid in decision making
  - Semi-quantitative urine culture; MPN test for water bacteriology
- Quantitative methods
  - Give accurate estimate of bacterial number; more exact applications
  - Vaccine production

Rapid Cultivation and Automation

- Lysis centrifugation system
  - Pre-treatment of blood culture
- Instrument-based systems
  - Periodic and continuous monitoring systems; growth detected by:
    - Colorimetric or fluorescent detection of CO2
    - Consumption of gasses
    - Fluorescent detection of growth
- Bioluminescence assay for viable organisms
- Colorimetric filtration (urine screening)

Types of Bacterial Culture Media

- Solid, semisolid, liquid, biphasic
- Simple media, special media (enriched, selective, enrichment, indicator/ differential, transport) synthetic media
- Aerobic and anaerobic media
- Cell culture for obligate intracellular bacteria (e.g., *Chlamydia spp*)



Biphasic culture medium

Selective and Differential Media

Selective

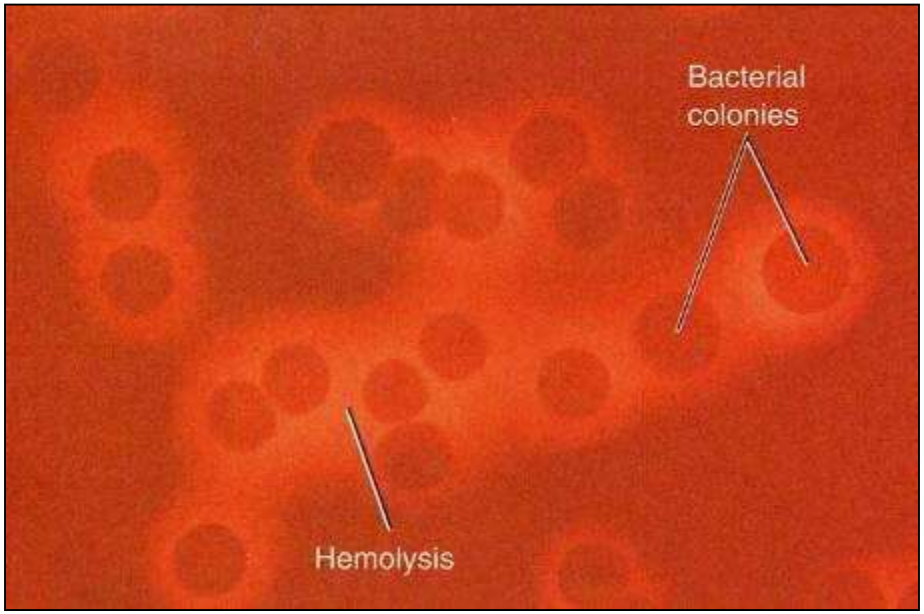
- Bismuth sulfite for *Salmonella typhi* (inhibits gram-positive and most gram-negative intestinal bacteria)

Differential

- Blood agar plates for *Streptococcus pyogenes*

Selective & differential

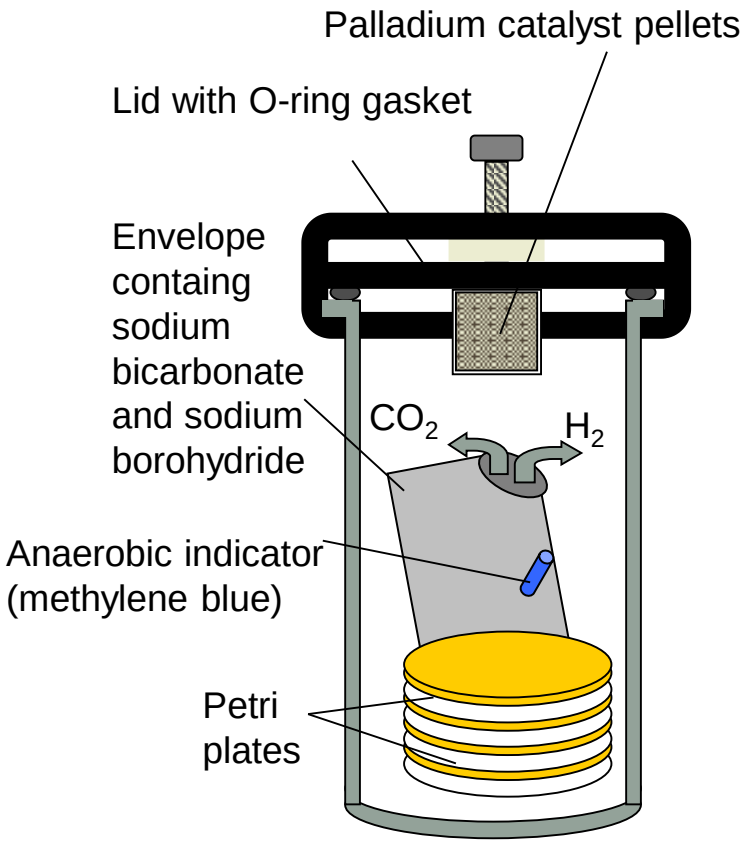
- Mannitol salt agar for *Staphylococcus aureus*



Type of hemolysis reaction aids identification of *S. pyogenes*

Anaerobic Growth

- Reducing media containing thioglycolate to deplete oxygen; cooked meat broth
- Anaerobic jar, anaerobic chamber, anaerobic bags/ pouch

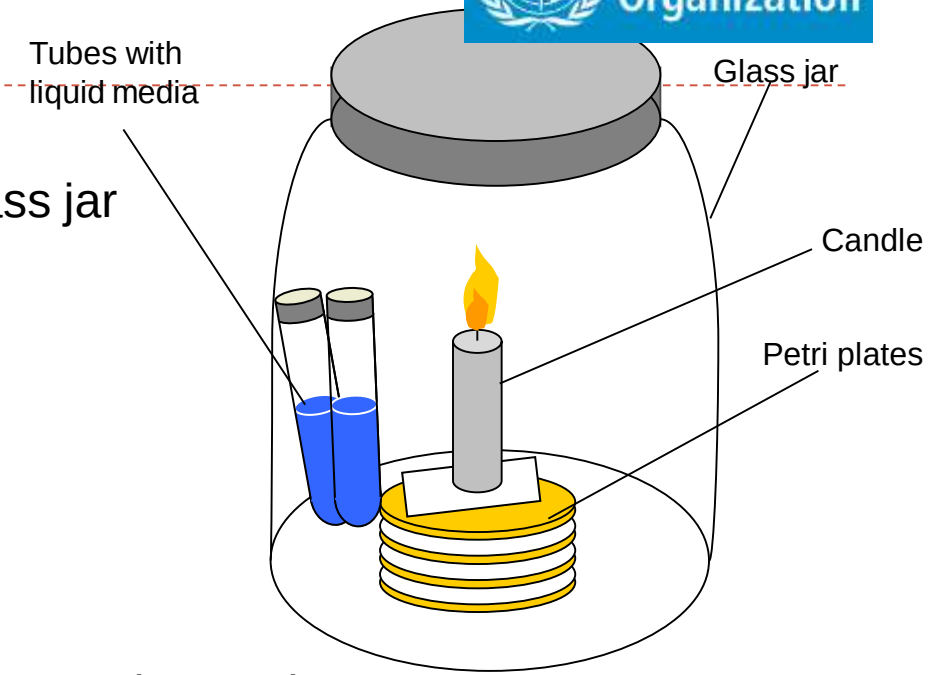


Capnophiles

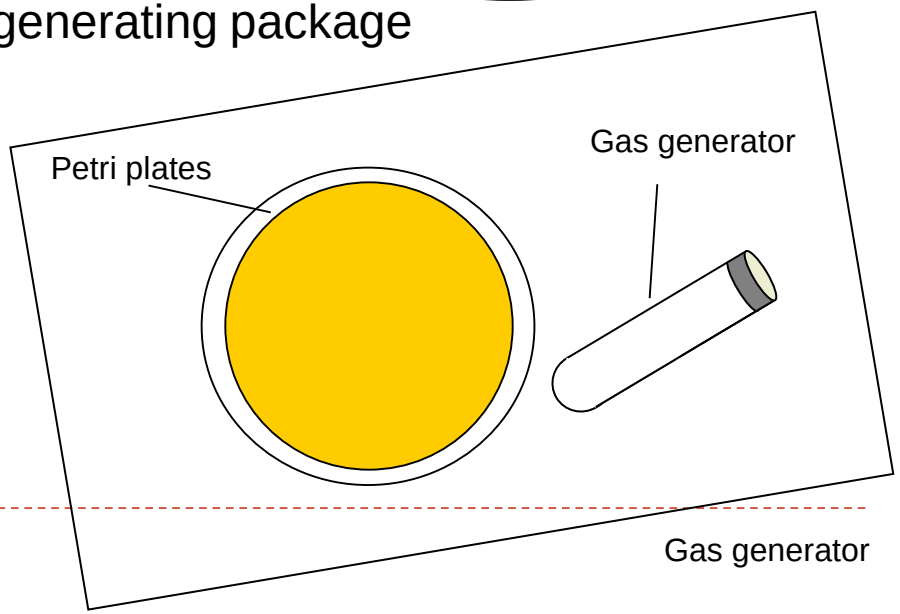
Capnophiles require high concentration of CO

e.g. *Brucella abortus*

A. Glass jar



B. CO_2 generating package



Cultivation of Bacteria

The process of growing microorganisms in culture by:

- Taking bacteria from an infection site by specimen collection - *in vivo*
- Growing bacteria in the artificial environment of the laboratory - *in vitro*

Why Cultivate Bacteria?

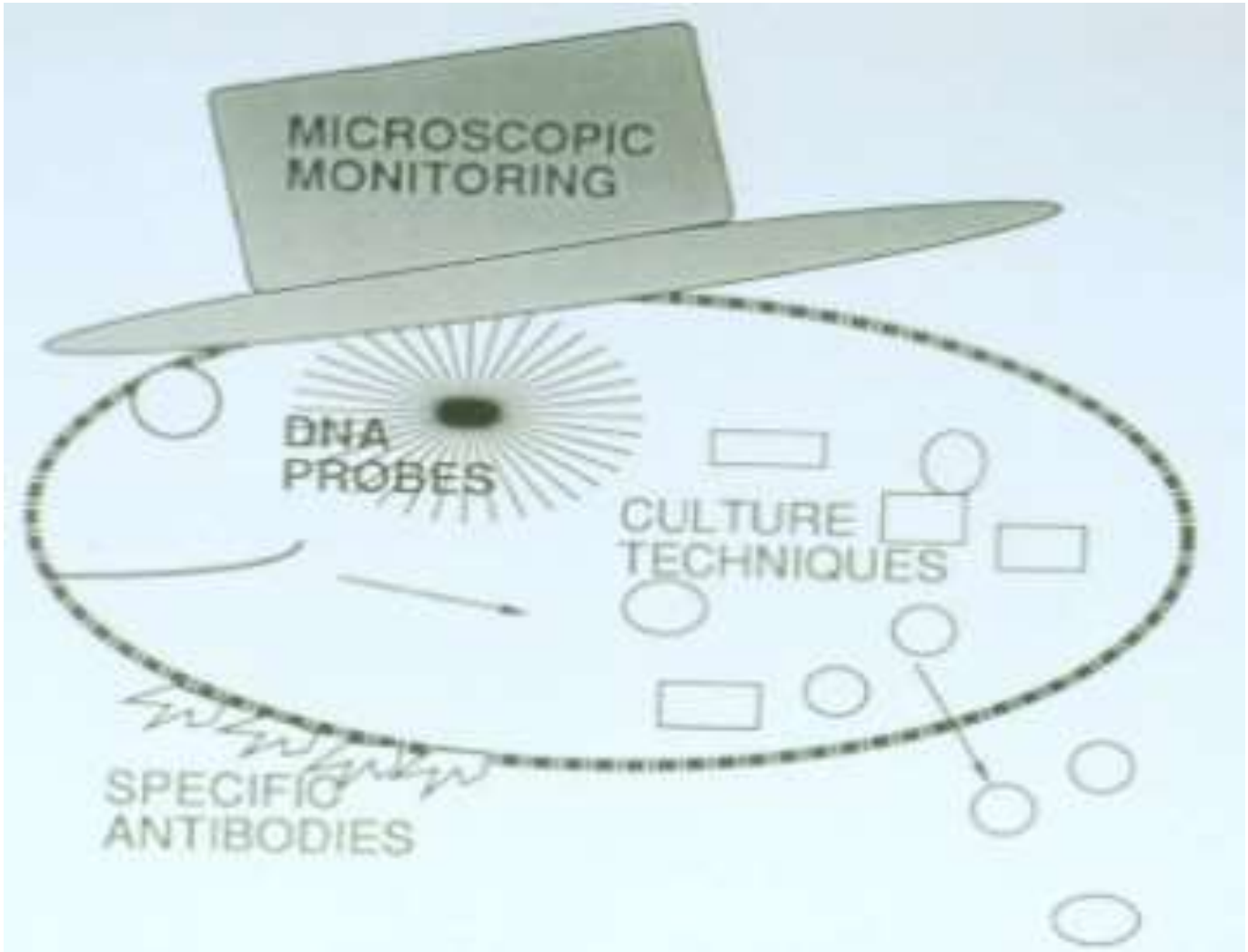
- Obtain definitive identification and characterization
- Grow and isolate all bacteria present in an infection
- Determine which bacteria is most likely causing infection
- Determine which bacteria is likely a contaminant or colonizer

Why Cultivate Bacteria?

Obtain sufficient growth of clinically relevant bacteria to:

- Test antimicrobial susceptibility
- Measure response to treatment
- Characterize the agent
- Bank strain for future use including vaccine development

Approaches for Identifying Bacteria



General Approach for Bacterial Identification

4 steps :

Step 1 : Sampling

Step 2 : Culturing
on isolation media

Step 3 : Performing an identification technique

Step 4 : Results
= name of bacteria

General Approach for Bacterial Identification

Different identification techniques

Physical methods
→ Based on the characterization of proteome of the bacteria

Genetical methods
→ Based on the characterization of specifics genes of the bacteria

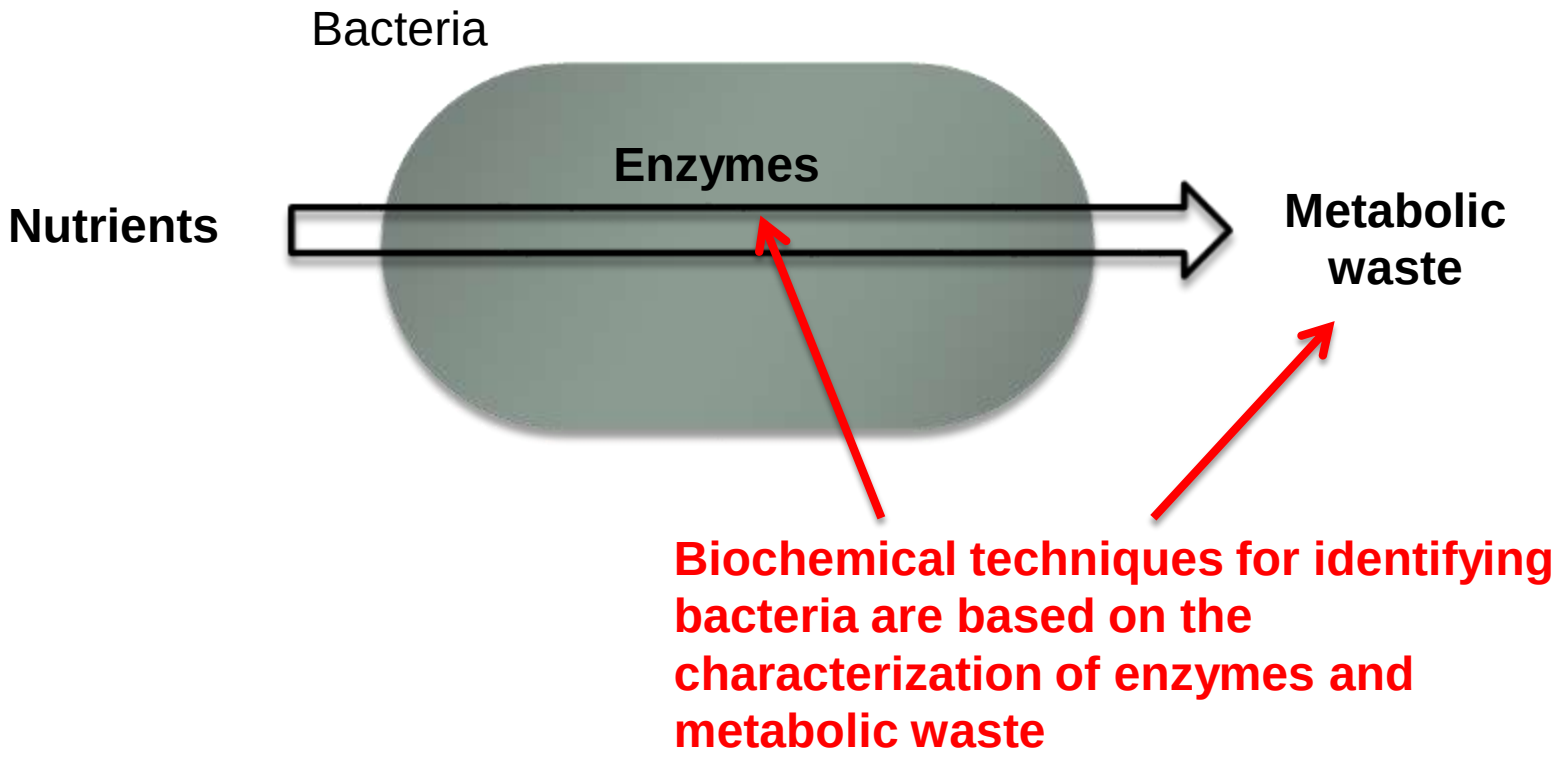
Biochemical methods
→ Based on the characterization of metabolic pathways of the bacteria

To identify unknown bacteria , results are compared to databases

Bacterial Identification

Metabolism of the Bacteria and Biochemical Bacterial Identification

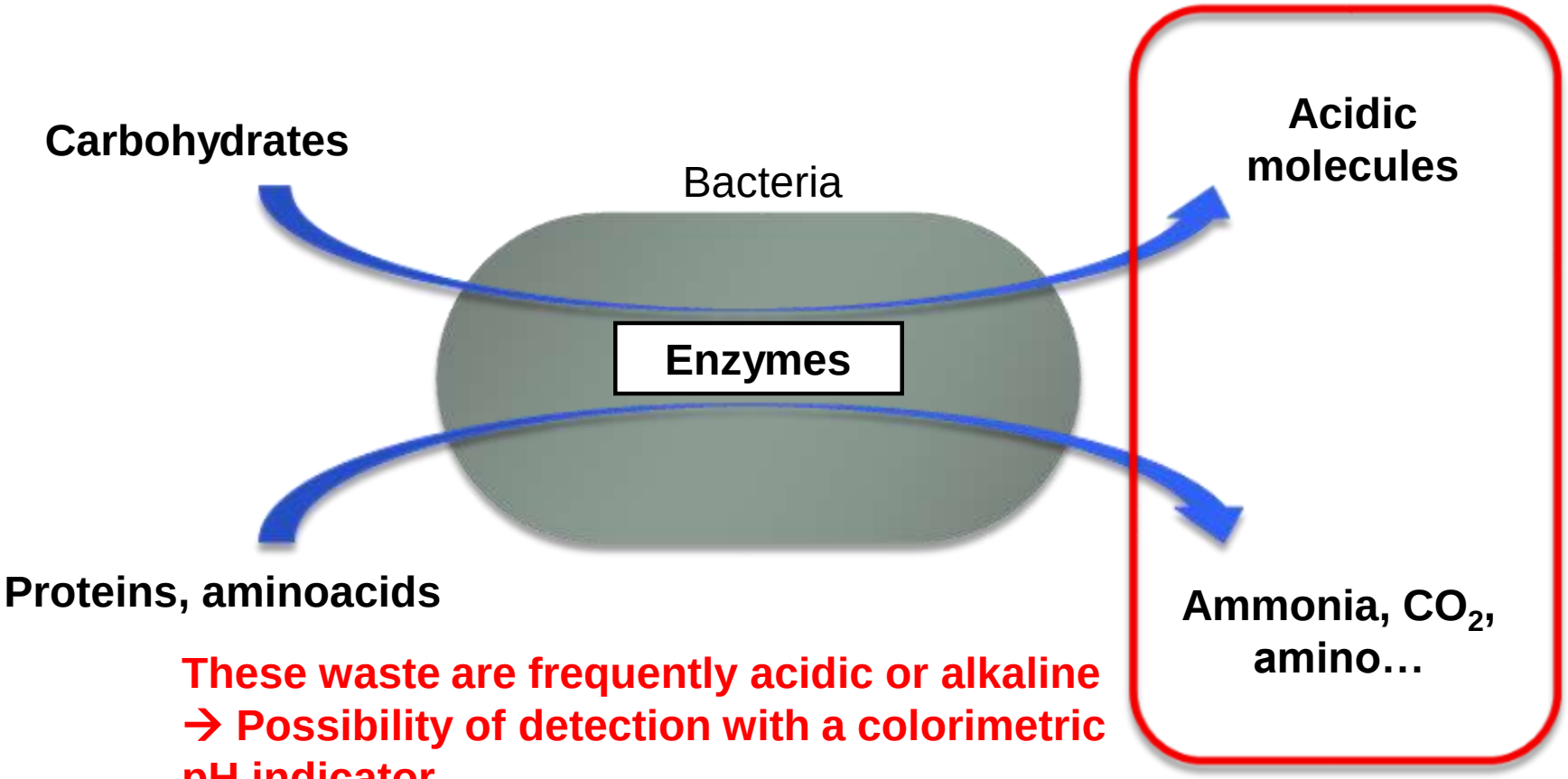
Bacteria are living cells who:
- consume nutrients (carbohydrates, proteins)
- reject metabolic waste.



Bacterial Identification

Metabolism of the Bacteria and Biochemical Bacterial Identification

The main metabolic pathways:



These waste are frequently acidic or alkaline → Possibility of detection with a colorimetric pH indicator

Application to the Identification

Example: research the ability to use glucose

Bacteria inoculation

incubation

Yellow color :

→ Acid pH

→ Production of acidic waste by bacteria

→ Proves the presence of enzymes which allow the use of glucose as nutrient by bacteria

→ **Bacteria «glucose +»**

Medium + **glucose** + pH indicator :
- green color for pH = 7
- yellow color for acid pH (pH <7)

Application to the Identification

How to differentiate bacteria ???

Examples with two bacteria:

- *E.coli* can use as nutrient glucose, mannose, and arabinose but not amylose

→ *Profile* = GLU + MAN + ARA + AMY –

- *E.tarda* can use as nutrient glucose, but non mannose, arabinose and amylose

→ *Profile* = GLU + MAN - ARA - AMY -

Each bacteria has a specific biochemical profile

Application to the Identification

How to identify an unknown bacteria with a biochemical method ??

4 steps :

1. Incubate bacteria to test in media with different nutrient (usually 5 to 20)
→ **one** medium for **one** nutrient

2. After incubation, for each medium, determine the results positive or negative for each nutrient

3. Write the complete biochemical profile (+ - + + + - - + …)

4. Compare the profile with a database to identify your bacteria

Microbe Identification

- The successful identification of microbe depends on:
  - Using the proper aseptic techniques
  - Correctly obtaining the specimen
  - Correctly handling the specimen
  - Quickly transporting the specimen to the lab
  - Once the specimen reaches the lab it is cultured and identified
  - Use care and tact to avoid patient harm

Challenges in Bacterial Identification

- Traditional methods of bacterial identification rely on phenotypic identification of the causative organism
- These methods of bacterial identification suffer from two major drawbacks.
  - They can be used only for organisms that can be cultivated *in vitro*
  - Second, some strains exhibit unique biochemical characteristics that do not fit into patterns that have been used as a characteristic of any known genus and species.

Identification Methods

- The methods fall into three categories :
  - Phenotypic - morphology (micro and macroscopic)
  - Immunological - serological analysis
  - Genetic techniques

Phenotypic Criteria

- Microscopic morphology and staining characteristics
- Macroscopic (colony) morphology
  - Appearance of colony-size, shape, color.
  - Pigment
  - Speed of growth
- Environmental requirement for growth
- Resistance or susceptibility to antibacterials agents
- Nutritional requirement and metabolic capabilities

Phenotypic Methods

- Stages (Step 3)
  - 1st stage tests will identify the genus of an unknown bacterium or at least, will narrow it down to 2 closely related genera
  - 2nd stage tests will identify the species of an unknown bacterium
  - 3rd stage tests will further differentiate the species into sub-species or sub-types
- All the tests require pure cultures

Phenotypic Methods
1st Stage

- Colony morphology and Gram stain (reaction and cell shape)
  - Acid fast stain
  - Spores
  - Motility
  - Catalase
  - Oxidase
- It may not be necessary to perform all the first stage tests, depending on the Gram stain result

Phenotypic Methods
2nd Stage

- The choice of second stage tests depends on the genus of the bacterium
- These tests are used to identify most species of clinically relevant bacteria (pathogens and normal flora) with as few tests as possible
- Common second stage tests include:
  - Carbohydrate fermentation
  - Haemolysis
  - Growth in the presence of inhibitors – high salt, bile
  - Species-specific tests – e.g., coagulase for *S. aureus*

Principles of Bacterial Cultivation

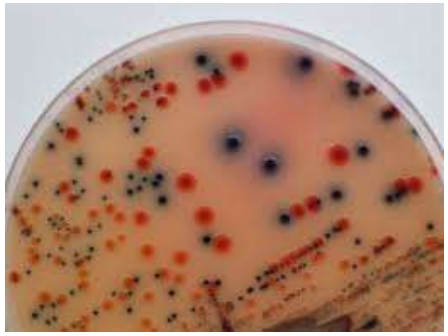
- To grow and isolate all bacteria present in a clinical specimen
- To determine which of the bacteria that grow are most likely causing infection and which are likely contaminants or colonizers
- To obtain sufficient growth of clinically relevant bacteria and characterization

Bacterial Identification
Identification Media

Different packaging of identification media

Tubes media

Agar plate media



Classics tubes

Multi-test miniaturized systems

Culture Methods

Streak culture

- Isolation of bacteria in pure culture from clinical specimen

Lawn culture

- Antimicrobial susceptibility testing (disc diffusion), bacteriophage typing

Liquid cultures

Stroke culture

- To obtain pure growth for slide agglutination; biochemical tests

Stab culture

- Maintenance of stock cultures

Pour-plate culture

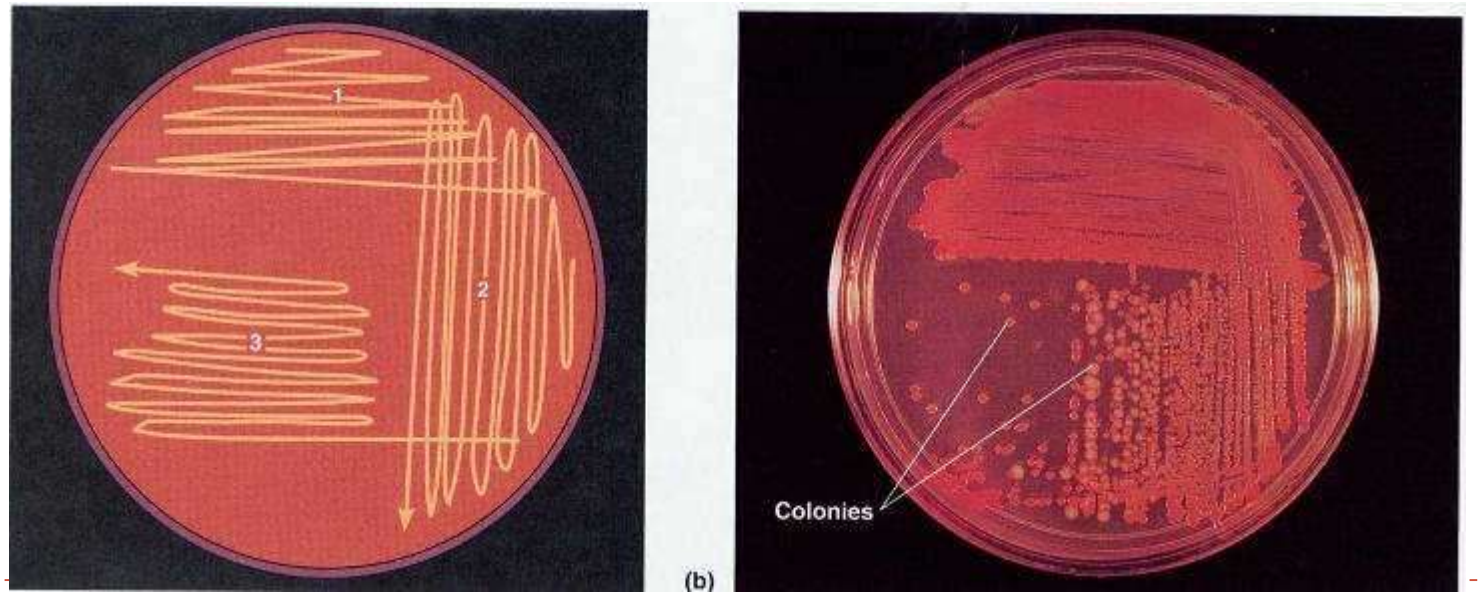
- Quantification of bacteria in liquid cultures, urine sample

Culture Methods

- Continuous bacterial culture
- Maintain a bacterial population at a constant density
  - Keeping a constant environment (oxygen, nutrient etc.)
  - Imitates the growth in the environment

Pure Cultures, Plate or any of the Others

- In theory, each colony (~10^7) bacteria arises from a single bacterium deposited on the surface of the Petri dish
- Each colony consists of a pure clone of cells
- Best obtained on solid media; less sure in liquid media



Cultural Characters

- Bacteria need nutritive culture media to multiply *in vitro*
- An undefined medium (basal or complex medium), that contains:
  - A carbon source such a glucose for bacterial growth
  - Water
  - Various salts needed for bacterial growth
- Defined media (chemically defined media or synthetic media)

Cultural Characters

1. Minimal media (simple medium)
    1. Contains the basic nutritive requirements, e. g. *nutrient broths* and agar media
2. Selective media are used for the growth of only selective microbes
    1. Contains antibiotics, dye, or specific chemicals that inhibits the growth of most types of microbe and stimulate the isolation of one type
        1. Mannitol salt agar (MSA) is selective for G+ bacteria
        2. Blood-free, charcoal-based selective medium agar (CSM) for isolation *Campylobacter*
        3. Lowenstein-Jensen medium – enriched selective media for TB
        4. TCBS (Thiosulphate-Citrate-Bile-Sucrose agar)- selective for *vibrio cholera* due to alk. pH

Cultural Characters

3. Differential media or indicator media distinguish one microorganism type from another growing on the same media

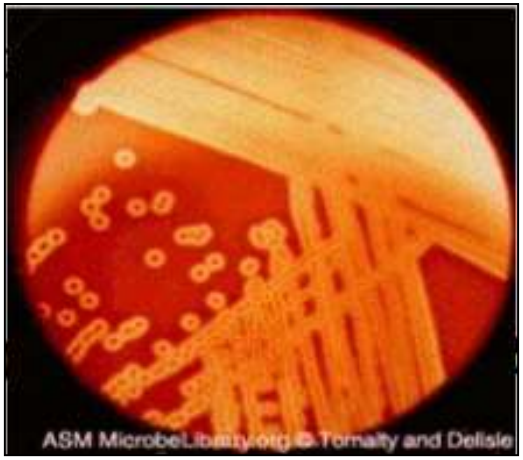
- Indicators (neutral red, phenol red, eosin y, methilen blue)
- Eozin methylene blue (EMB is differential for lactose and sucrose fermentation
- McConkey (MCK) is differential for lactose fermentation

Cultural Characters

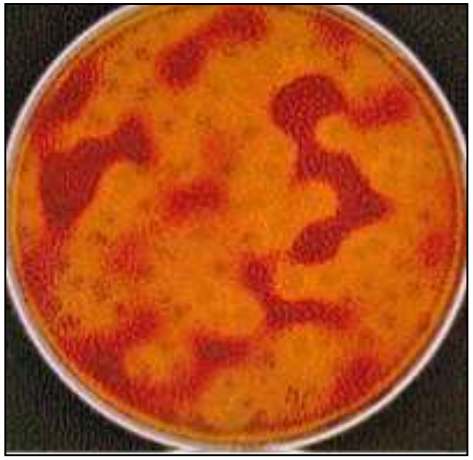
4. Enriched media contains the nutrients required to support the growth of a wide variety of organisms, including some of the more **fastidious** ones:

- Blood agar is an enriched medium in which nutritional rich whole blood supplements the basic nutrients. It contains 5-10% human or animal blood. It shows the type of hemolytic activity of bacteria (complete, partial or non-hemolytic)

Complete Haemolysis of RBCs (β-haemolytic Streptococci)



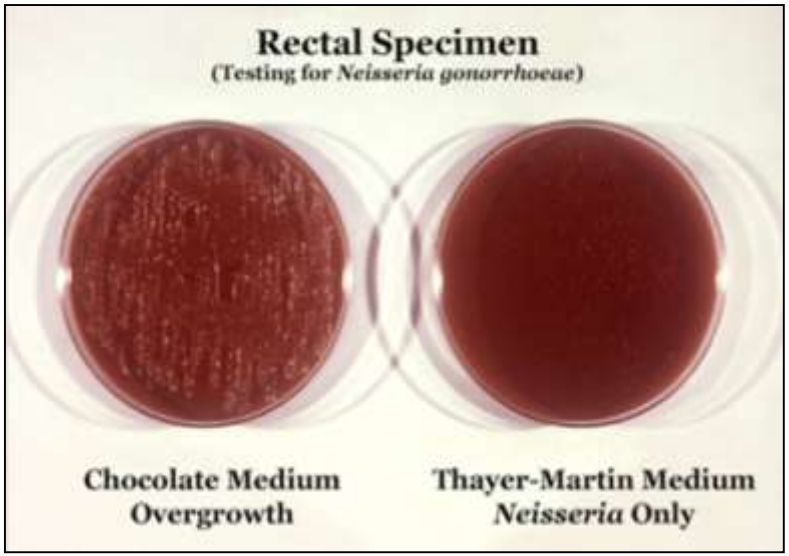
Partial Haemolysis of RBCs (α-haemolytic Streptococci)



Cultural Characters

4. Enriched media contains the nutrients required to support the growth of a wide variety of organisms, including some of the more **fastidious** ones:

- Chocolate agar (heated blood agar) is enriched with heat-treated blood (40-45°C)

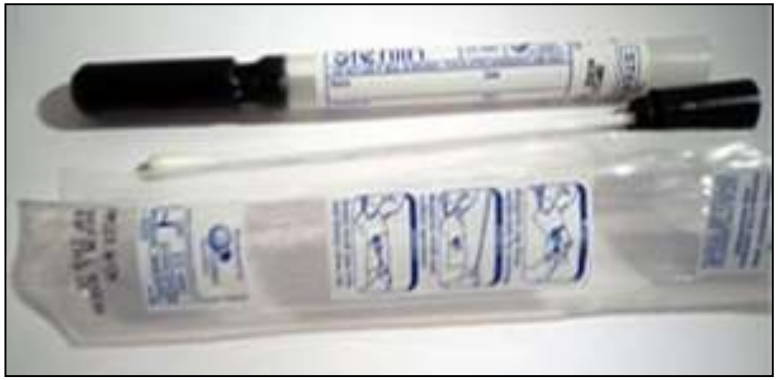


- Lofflers serum media (Horse serum + glucose in a ratio 3:1) is used for cultivation of a *Corynebacterium dyphteria*

Cultural Characters

5. Transport medium is a simple organic medium that maintain the viability of all organisms in the specimen without altering their concentration

- Is mainly used for temporary storage of specimens being transported to the laboratory for cultivation
- e. g. Thioglycolate broth for strict anaerobes



Macroscopic Morphology

- Provides additional information for the identification of the bacterium. The characters revealed in different types of media are noted
- On solid media: Size, Shape, Margins, Surface, their Elevations, Edge, Colour, Structure, Consistency, Pigmentation, Haemolysis
- In fluid media:
  - Degree of growth – absence/scanty/moderate/abundant
  - Presence of turbidity and its nature
  - Presence of deposit and its character
  - Nature of surface growth and odour

The Colonial Appearance on Culture Media

- Size
- Shape
- Surface
- Pigment production
- Consistency
- Edge (margin)
- Elevation
- Opacity
- Effect on Blood agar
- Lactose fermentation

Some

People

Come

Early

Other

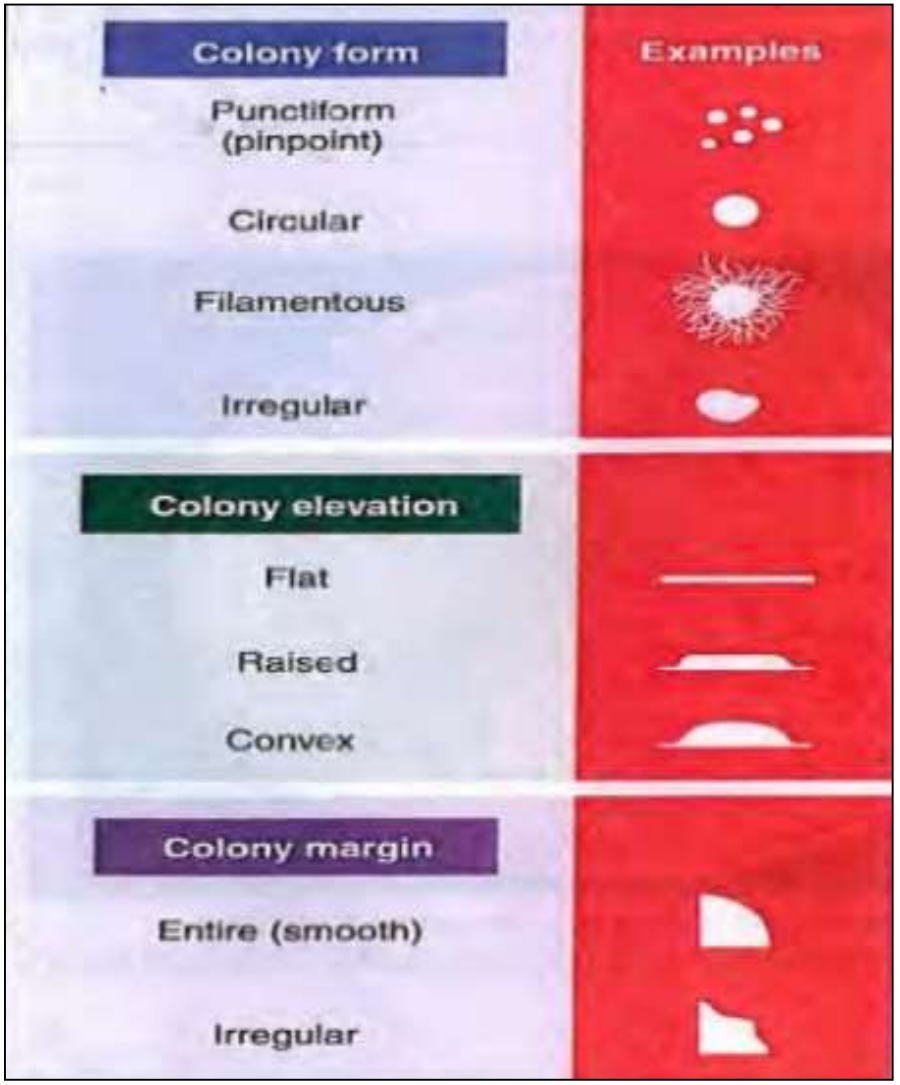
are Badly

Late

The Colonial Appearance on Culture Media

Shape

Figure 7-11 Colony morphologic features and descriptive terms for commonly encountered bacterial colonies.

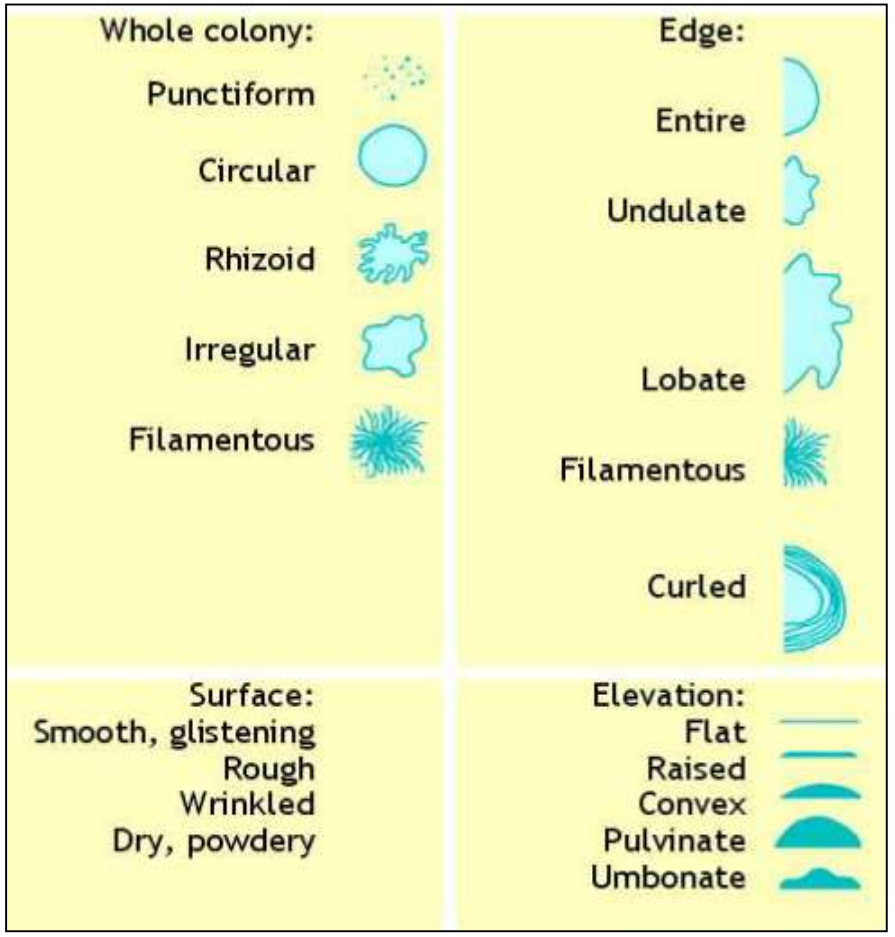


Colour

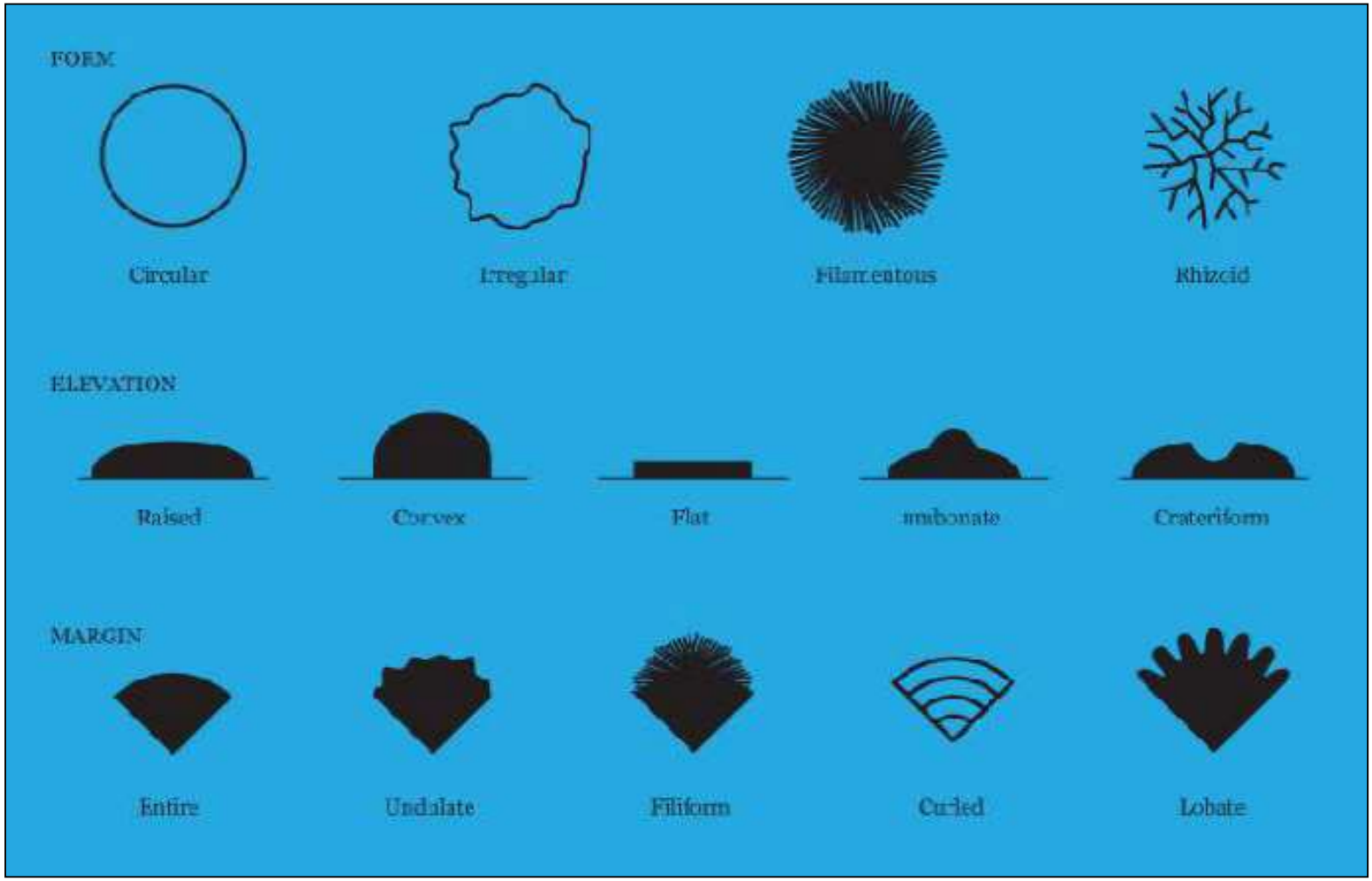
- Colourless or bacteria produce endopigments which give the colonies a characteristic colour:
  - *S. aureus* – golden-yellow colony
  - *S. albus* – white endopigment
  - S. Citreus – lemon-yellow endopigment
- Bacteria produce exopigments
  - *P. aeruginosa* – green exopigment in the surrounding media

The Colonial Appearance on Culture Media

- Form – shape of colon, colour, surface, texture and size
- Elevation – side view
- Edge – margin

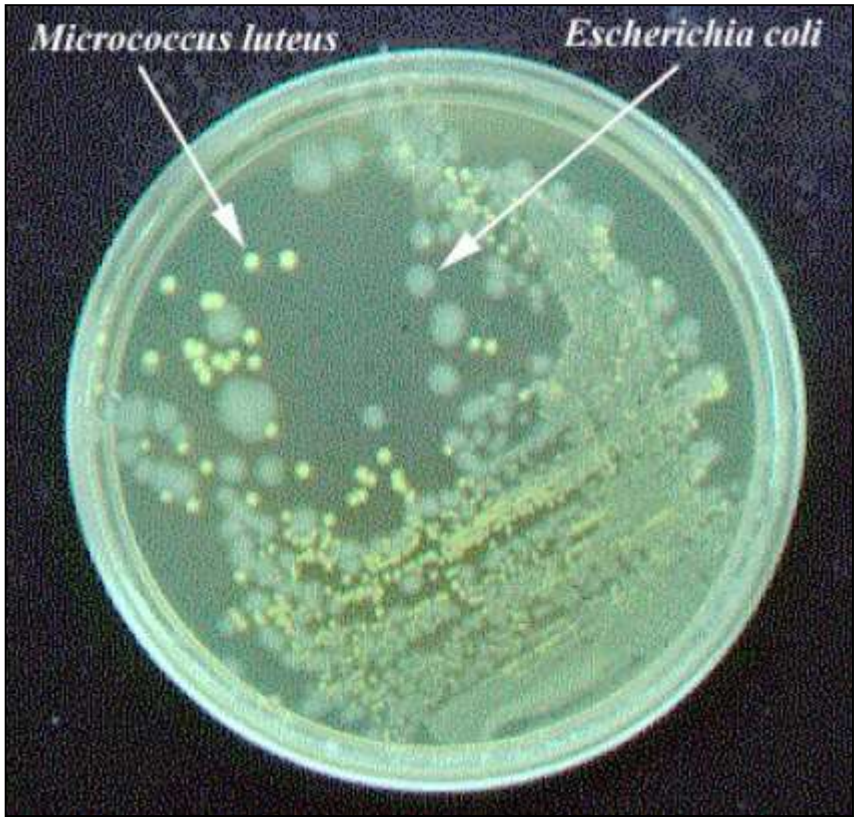


The Colonial Appearance on Culture Media



Size

- Pinpoint
- Small
- Medium
- Large



Shape

- The shapes of colonies describes the entire colony, while the margin describes the edges
- Colonies may be circular or regular, irregular and punctiform
- A punctiform colony is too small to describe as either regular or irregular

Edge (Margin)

- Entire
  - This means that the edge of the colony is distinct all of the way around the colony, and many of the colonies found on plates will be described as circular with entire margins.

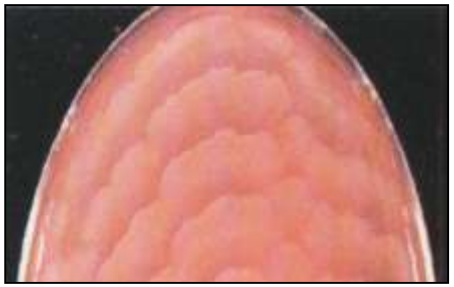
- Serrate
  - This means a saw-toothed edge to a colony.

- Undulate
  - Undulate margins are a regular pattern of waviness around the colony.

Edge (Margin)

- Lobate
  - This means the colony forms lobes at irregular intervals. It may be difficult to distinguish between undulate and lobate at times – they are imprecise distinctions.

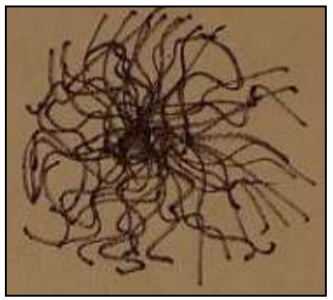
- Diffuse edge (Swarming growth)
  - This means the colony fades away from the densest part making it difficult to tell where the edge of the colony is
  - Usually denotes highly motile organism (proteus)



Rhizoid and Filamentous

- Both are used to describe both the shape and the margin of a colony.
- Rhizoid
  - This means root-like, and describes thick branchlike growths originating from the center of a colony becoming thinner as a result of sequential branching

- Filamentous
  - They are the result of growth filaments of equal width that pile on top of one another forming a thicker part in the center



Elevation

Flat

Raised

Convex

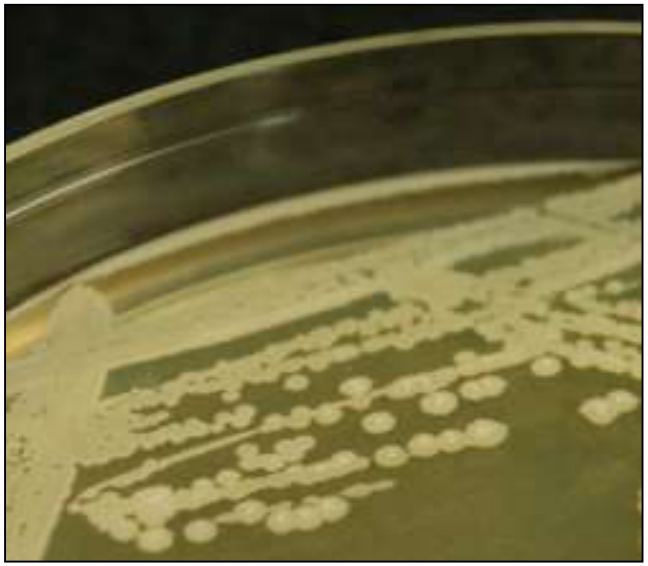
Umbonate

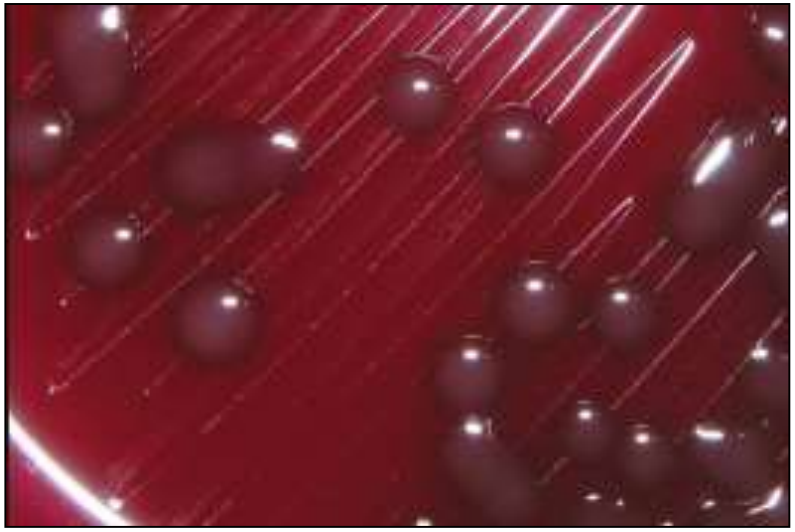
Consistency

- Soft, Mucoid, Sticky
- Tough, Hard

Surface

- Smooth, Shiny
- Rough, Dull

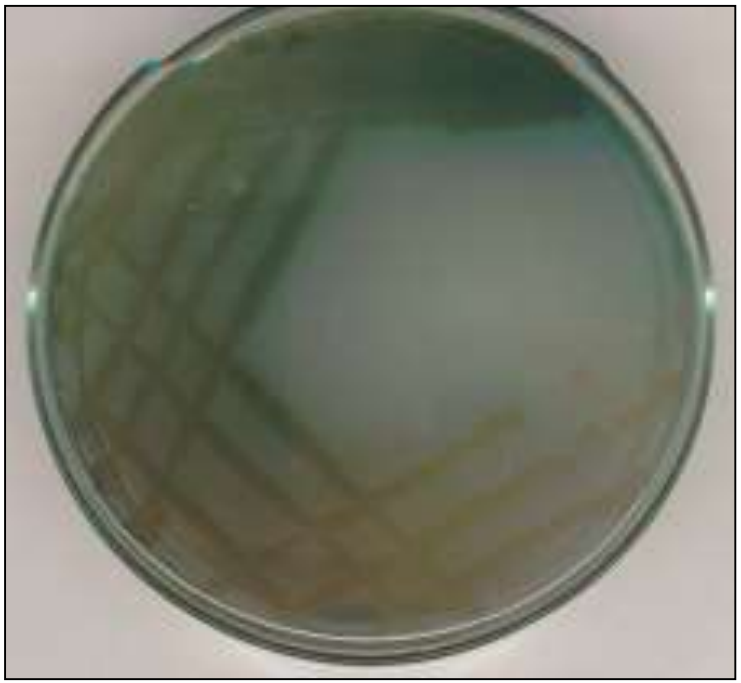




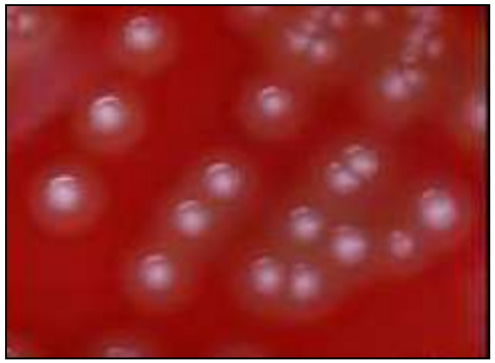
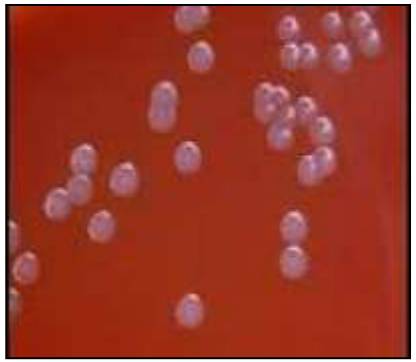
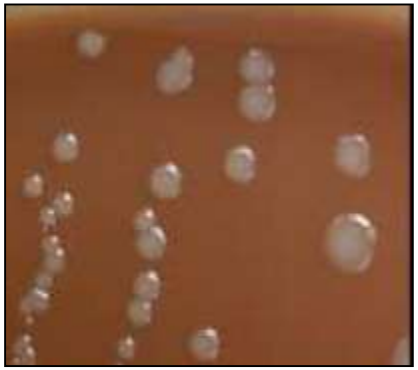
Pigment Production

- Non-pigmented (nonchromogenic)
- Pigmented (Chromogenic)

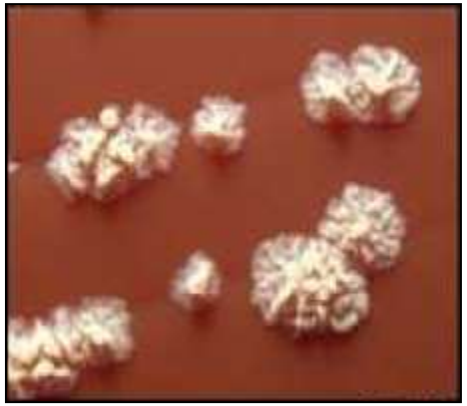
Endopigment



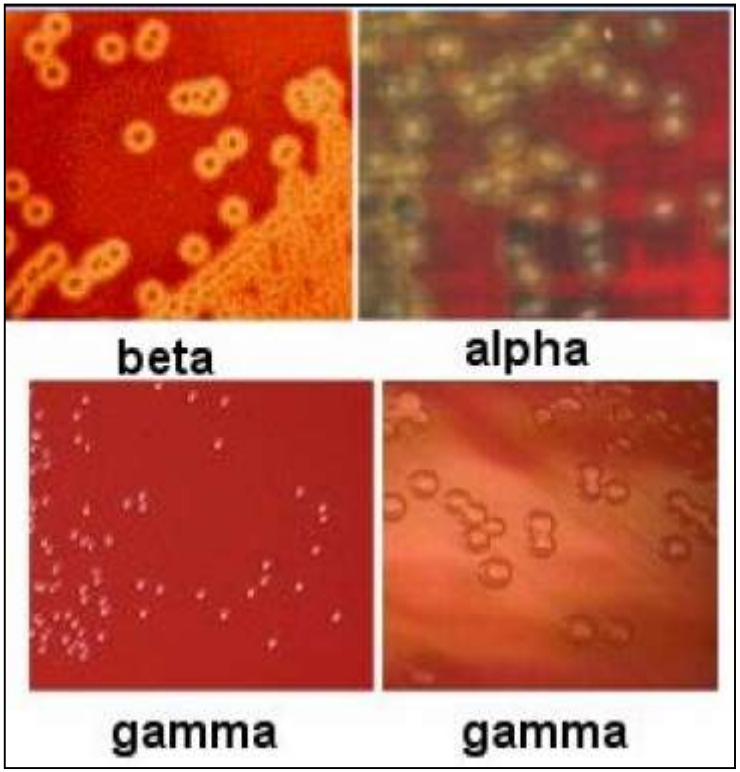
Exopigment

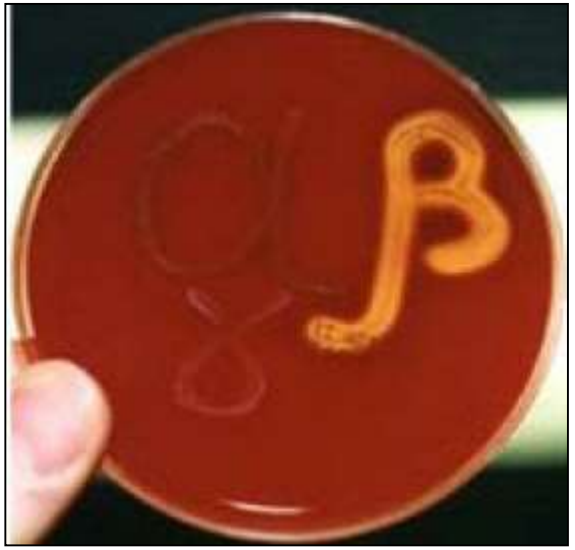






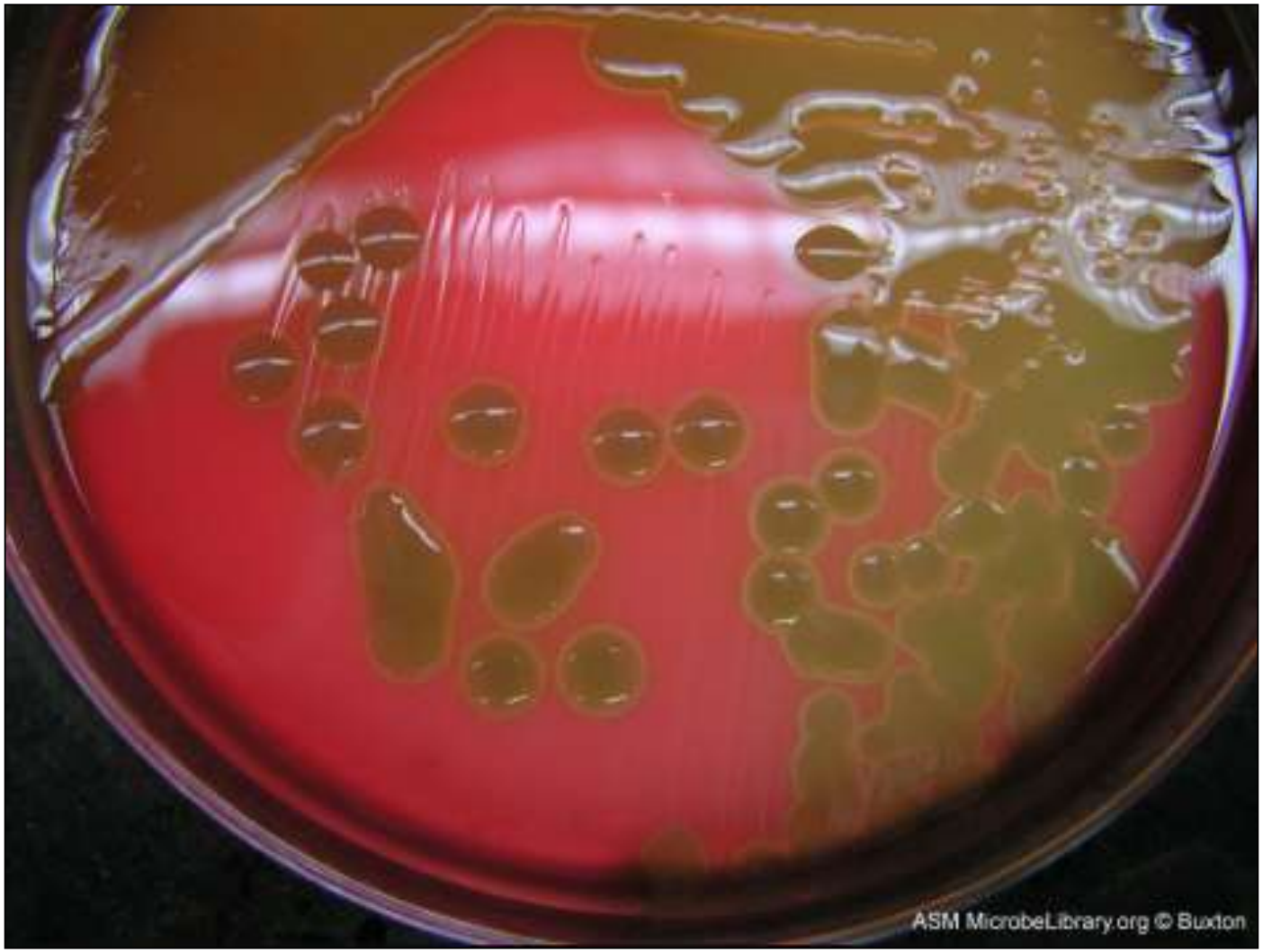



Effect on Blood Agar (Hemolysis Types)

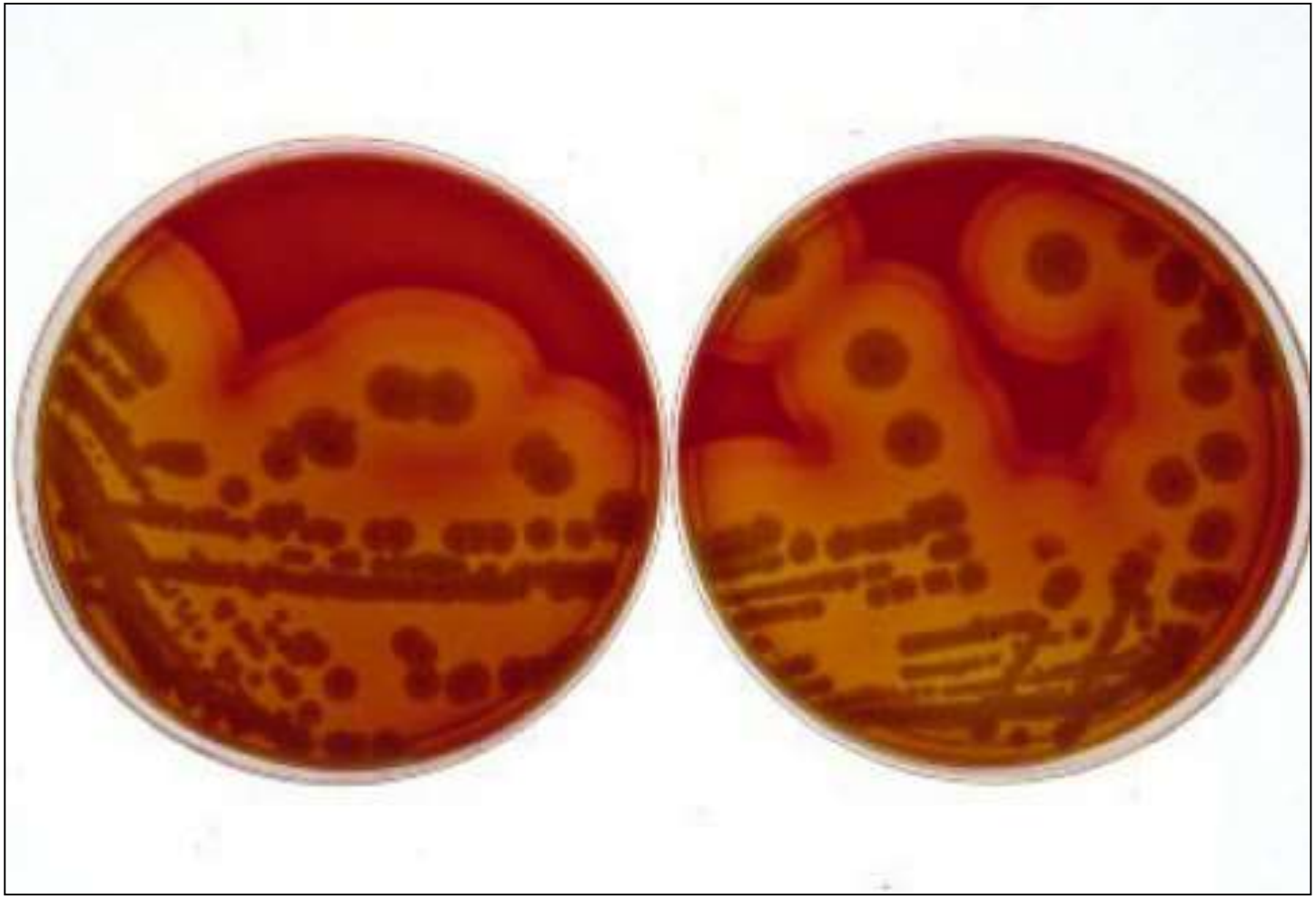




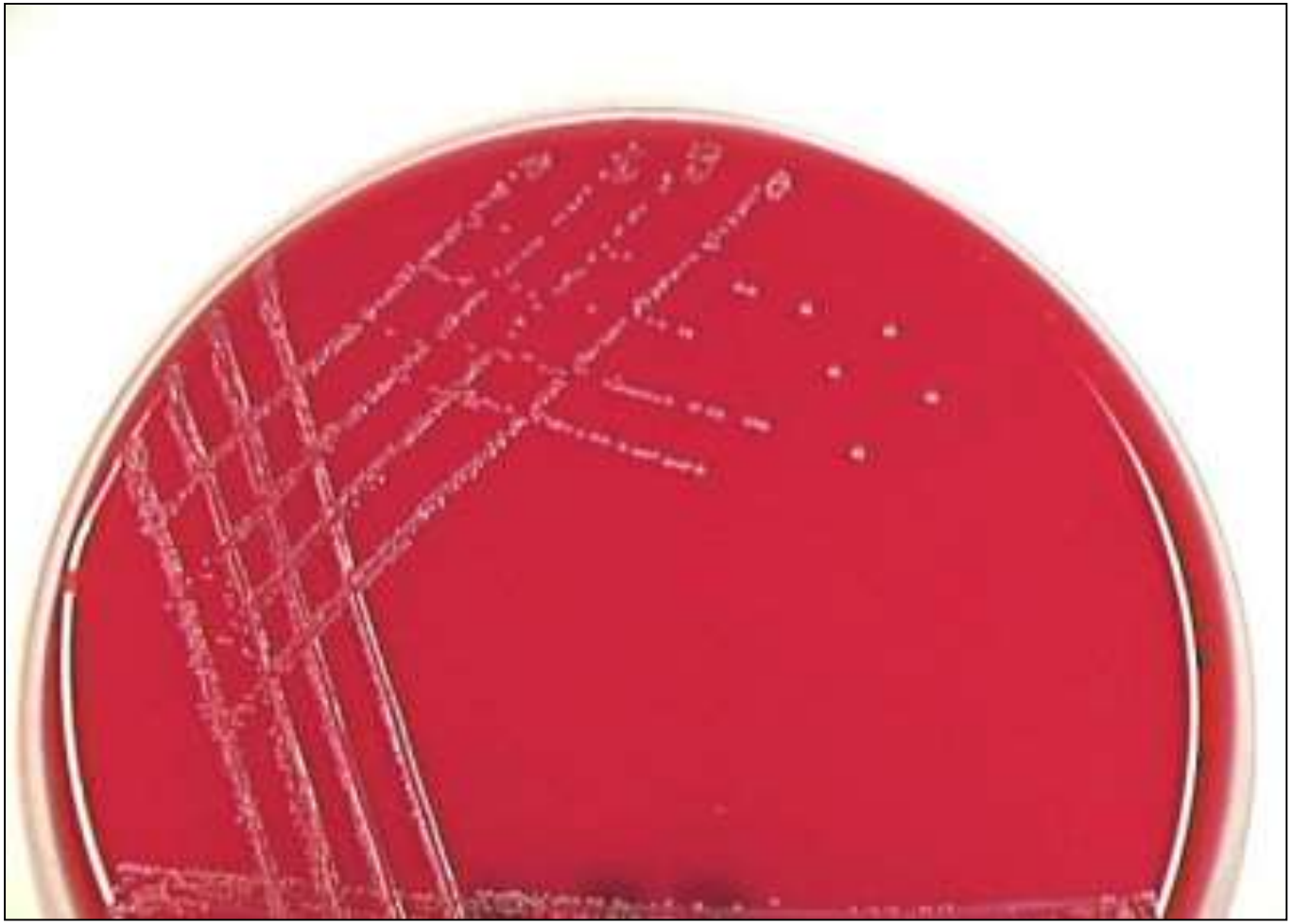
Partial Hemolysis α



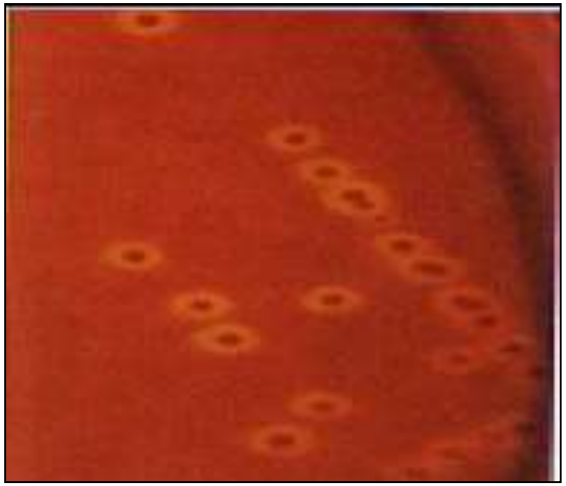
Complete Hemolysis β

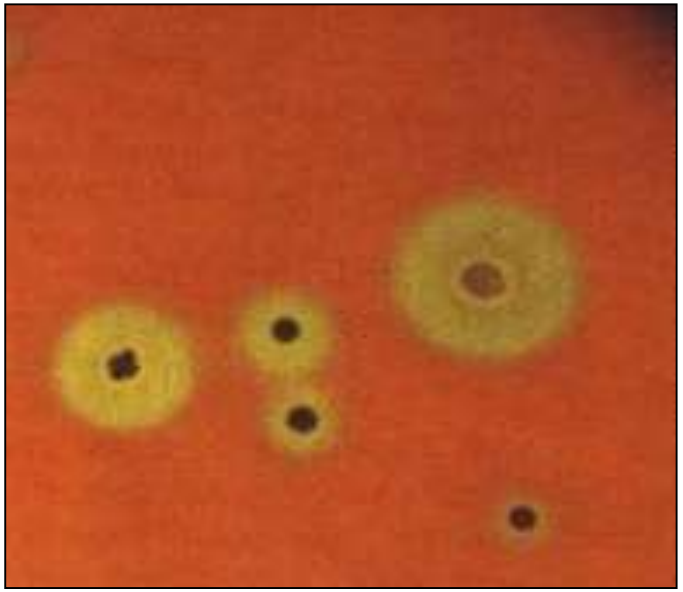


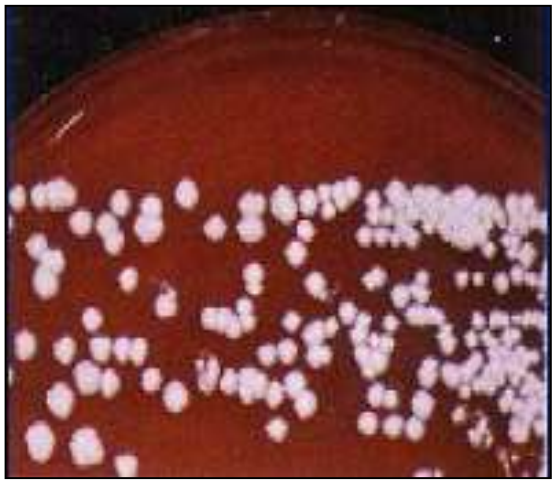
No hemolysis γ



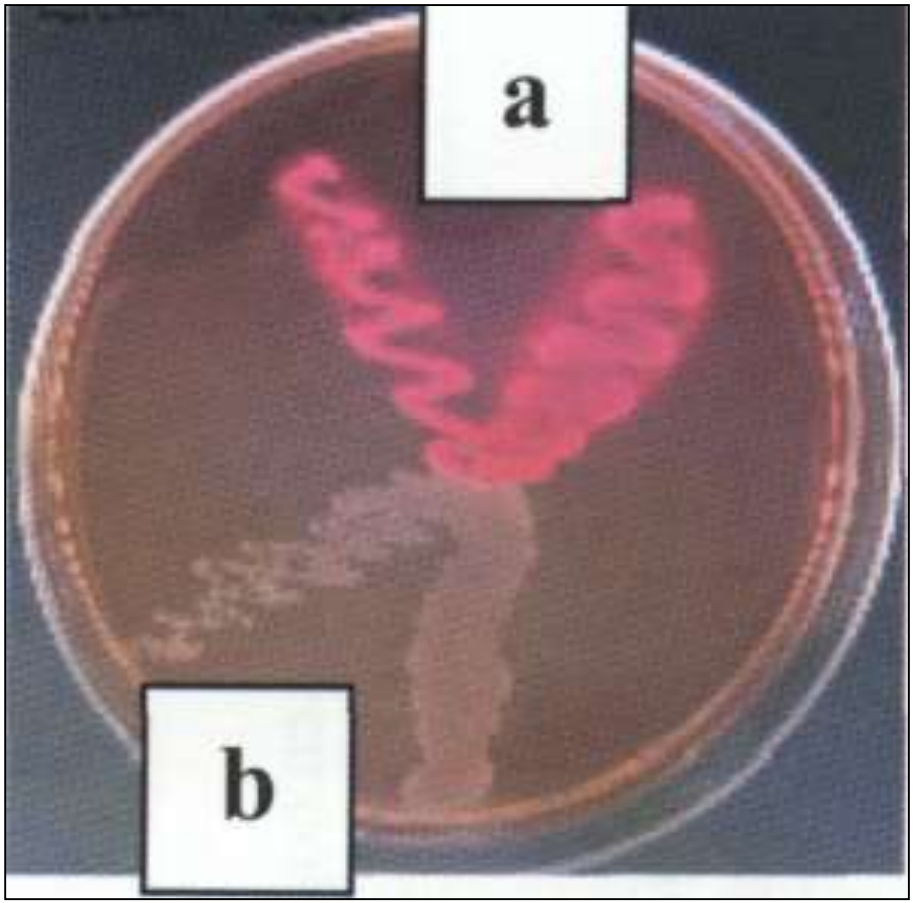
Identify







Growth on MacConkey's Media



LFC

NLFC

Identify

Identify

LACTOSE FERMENTERS: RED/PINK COLONIES

Escherichia coli: *Enterobacter cloacae*: *Klebsiella pneumoniae*

NON-LACTOSE FERMENTERS: COLOURLESS COLONIES

Salmonella typhi: *Shigella sonnei*: *Proteus vulgaris*

Establishing Inhibitor Profiles

- The ability to grow in the presence of one or more inhibitory substance can provide valuable identification information

Growth in the presence of high concentration NaCl	Enterococci
Optochin susceptibility and bile solubility	*Str. pneumoniae*
Ethanol survival	*Bacillus* spp.

Limitation of Phenothypic Methods

- Inability to cultivate on artificial medium
  - *Treponema pallidum*
- Fragility of organisms and failure to survive when transport
- Fastidious nature of some microorganisms
  - *Bartonella*
  - *Leptospira*
- Administration of antibiotic before specimen is obtained

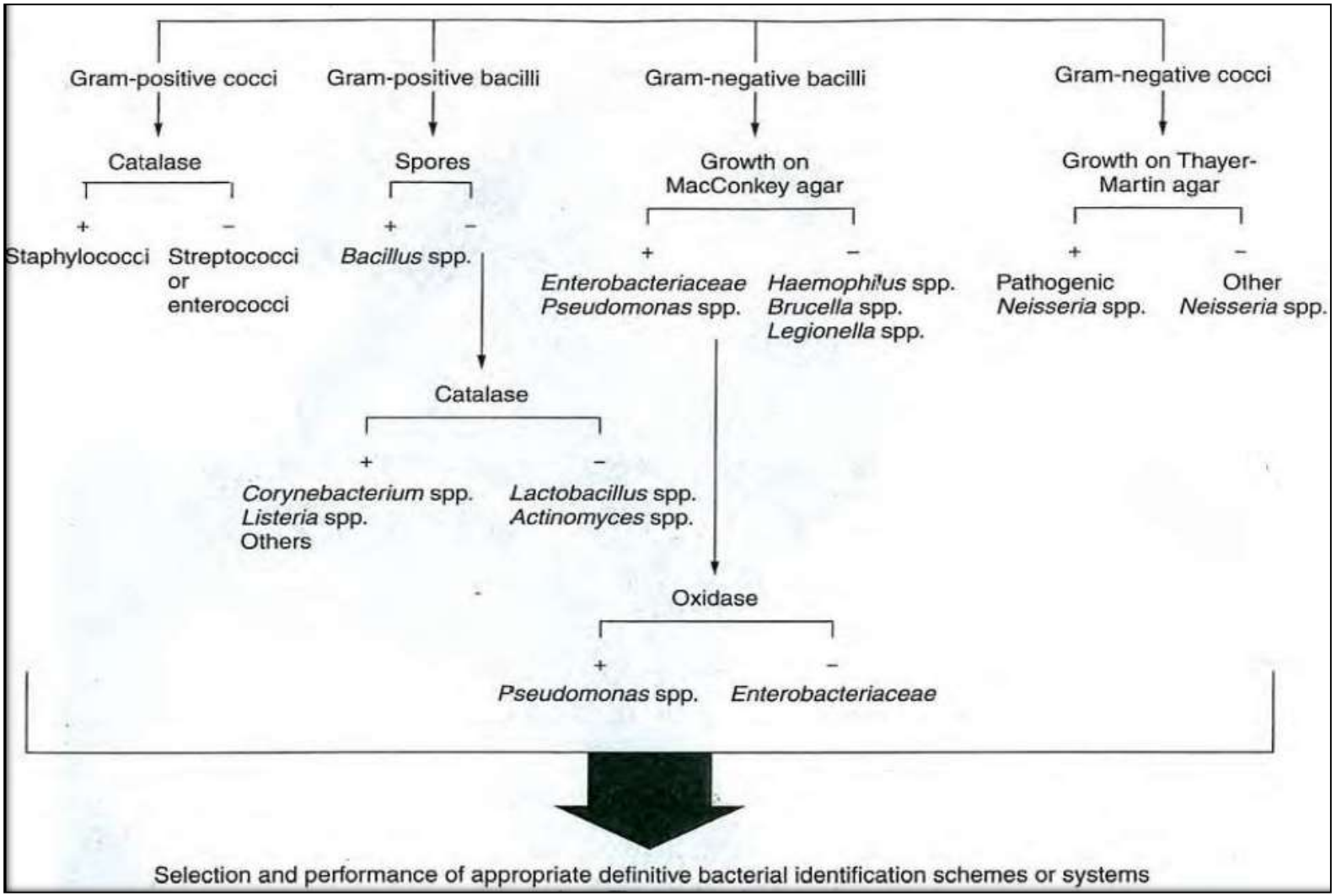
Phenotypic Methods
Microscopic Appearance

- Microscopic Morphology include a combination of
  - Cell shape
  - Size
  - Gram stain
  - Acid fast reaction
  - Special structures
    - e.g. Endospores, Granules and Capsules can be used to give an initial putative identification

Common Used Stains

1. Simple stains (methylene blue stain)
2. Differential stains
    1. Gram stain: G+ and G-
    2. Acid fast stain (Ziehl-Neelsen stain): acid fast and non acid fast
3. Special stains are necessary to bring out characteristics like flagella, capsules, spores and methachromatic granules
    1. EXCEPTION: only staining characteristics alone are used to definitively identify a bacteria species
    2. Fluorescent dyes bring out special characteristics and fluorescent antibody technique enables to identify *Legionella pneumophilia, Bordetella pertusis*

Gram Stain Morphology

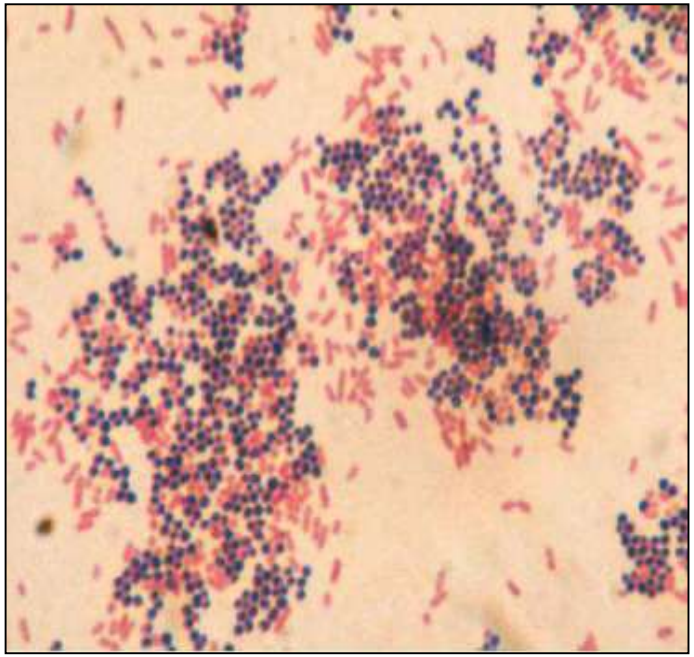


Differential Stains

Gram Stain Differential Stain Distinguishing between G + and G-

1. Primary stain (methyl violet – iodine mixture)
2. Decolourization with Alcohol
3. Counter stain: diluted carbol fuchsin

- Narrows possible identities of an organism
  - Excludes many possibilities
- Generally insufficient alone for diagnosis
  - *E. coli* and *Salmonella* gram stains look alike



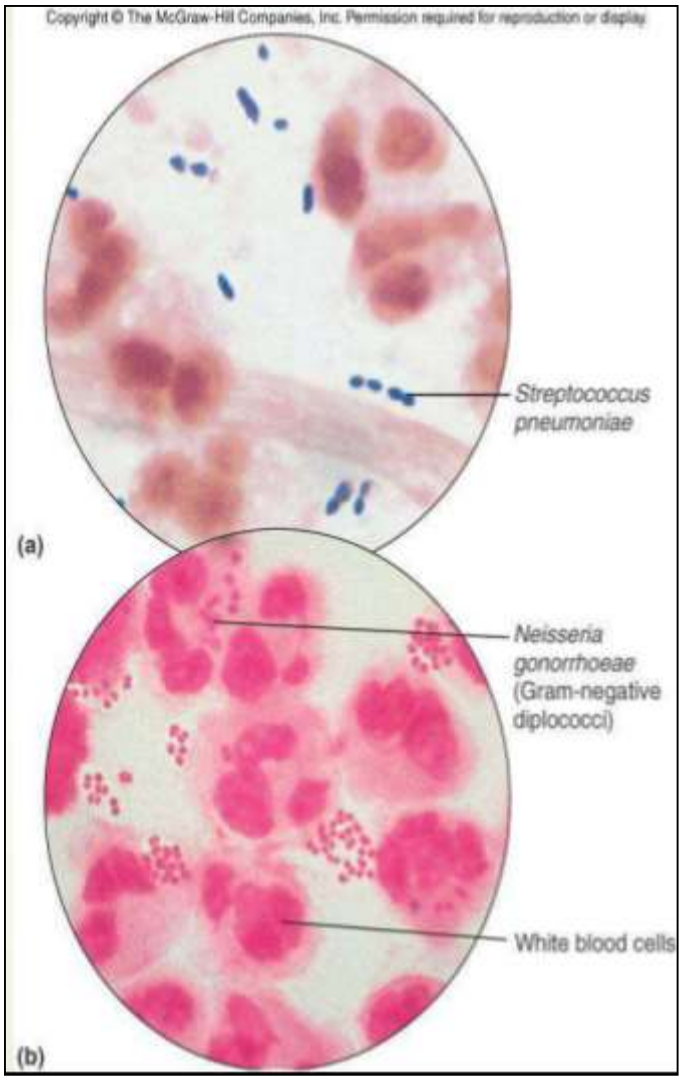
Results:
G+ **Purple**
G- **Red**

Difference – due to structure of cell wall
G+ thick cell wall
G- thin cell wall

Differential Stains

Gram Stain Differential Stain Distinguishing between G + and G-

- Sometimes highly suggestive of a particular microorganism
  - Gram- rods in ♀ urine *E. coli* UTI
  - Gram+ encapsulated diplococci and numerous white blood cells in sputum *Streptococcus pneumoniae*
- Sometimes enough for complete diagnosis
  - Gram- diplococci clustered in white blood cells of male urethral secretions *Neisseria gonorrhoeae*



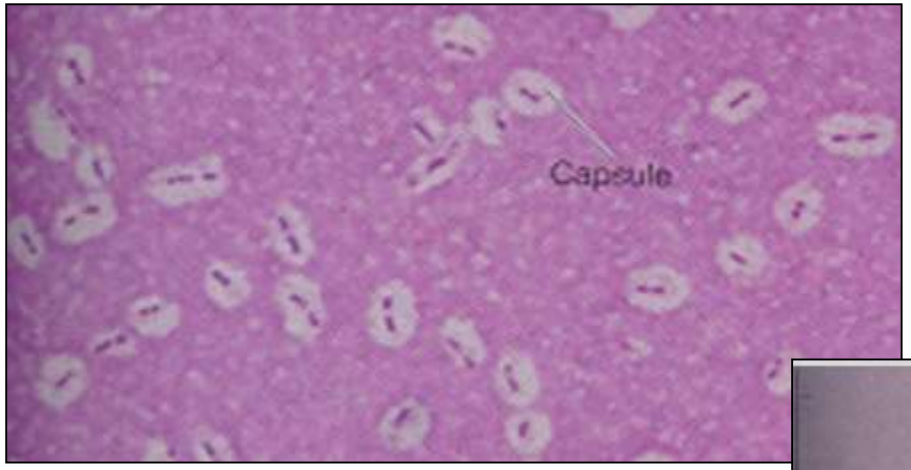
Differential Stains
Acid Fast Stain

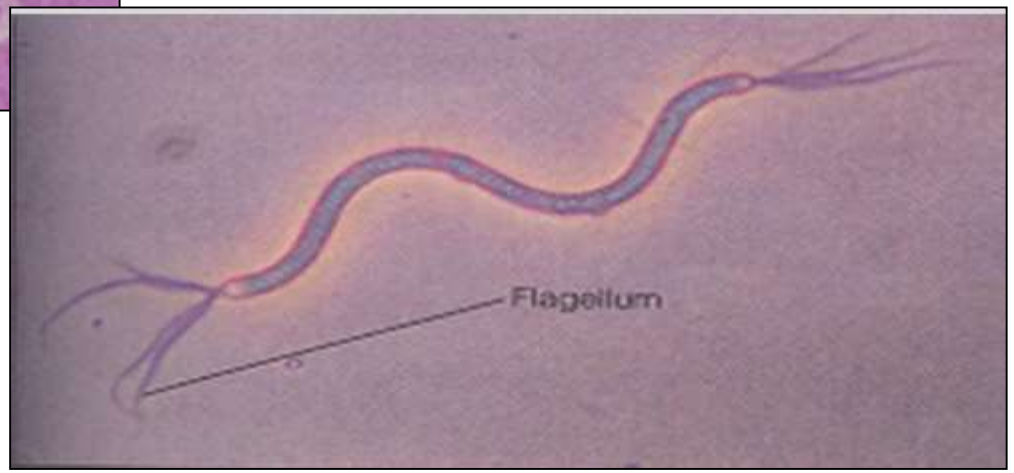
- Differential stain divides bacteria into 2 groups
  - Acid-fast (red as *M. tuberculosis)*
  - Non acid-fast (blue)

- Used to identify organisms in the Genera Mycobacterium (high lipidand wax content in cell wall)
  - Carbol fuchsin – red
  - Decolourization by sulphuric acid 20%
  - Counter stain with Methylene blue

Differential Stains
Special Stain

- Capsule stain
- Flagella stain





Phenotypic Methods
Motility

- If bacteria is motile, there will be growth going out away from the stab line, and test is positive.
- If bacteria is not motile, there will only be growth along the stab line.
- A colored indicator can be used to make the results easier to see.

Phenotypic Methods
Biochemical Tests

- The microbe is cultured in a media with a special substrate and tested for an end product.
- Prominent biochemical tests include:
  - Enzymes
    - Catalase test
    - Oxidase test
    - Urease test
    - Coagulase test
  - Carbohydrate fermentation
  - Acid production
  - Gas production
  - Sensitivity to drugs

Special Tests for Enzymatic Production

1. Catalase test
2. Oxidase test
3. Gelatinase test
4. Urease test
5. Tryptophanase (Indole test)
6. Cysteinase (H2S production)

Nutritional Requirements and Metabolic Capabilities

Single enzyme test	
Catalase: H_2O_2 + catalase = O_2 and H_2O	Staph vs. Strep *Listeria & Corynebacterium* vs. other non spore forming G+ bacilli
Oxidase: detection of cytochrome oxidase that participates in nitrate metabolism	*Pseudomonas, Aeromonas, Neisseria*
Indole: tryptophanase degrades tryptophan into pyruvic acid, ammonia, indole(is detected by aldehyde indicator)	Presumptive id for *E. coli*
Urease: hydrolyzes urea into ammonia, water and CO_2; increase pH changes causes bright pink colour of indicator	
PYR: hydrolysis of PYR, indicators turns pink	Group A Strep and enterococci are +

Nutritional Requirements and Metabolic Capabilities

- Oxidation and fermentation:
  - Oxidation of glucose requires oxygen, fermentation does not
  - pH decreases causing yellow color
- Amino acid degradation:
  - Detection of amino acid decarboxylase enzymes

Phenotypic Methods
Biochemical Tests - Enzymes

- CATALASE TEST
  - Catalase is present in most aerobic and facultative anaerobic bacteria (**except** *Streptococcus* spp)
  - Hydrogen peroxide forms as one of the oxidative end product of aerobic carbohydrate metabolism
  - If this is allowed to accumulate in the bacterial cells it becomes lethal to the bacteria
  - Catalase thus helps in converting H2O2 to H2O and O2
  - The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide and the rapid effervescence of O2 bubbles occurs

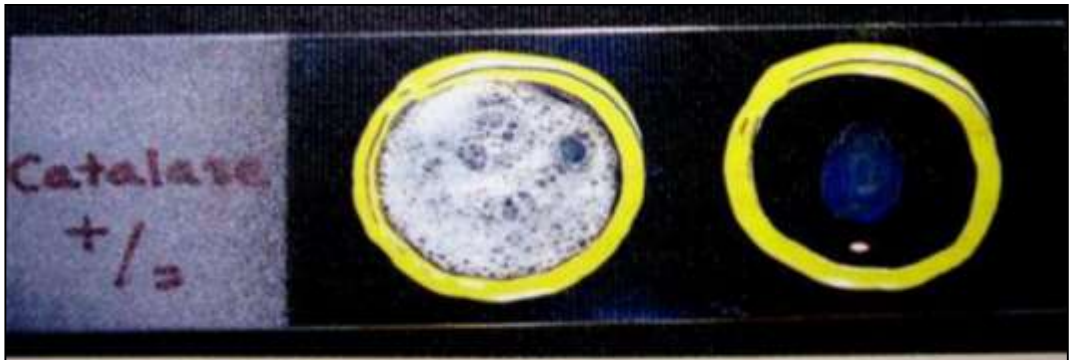
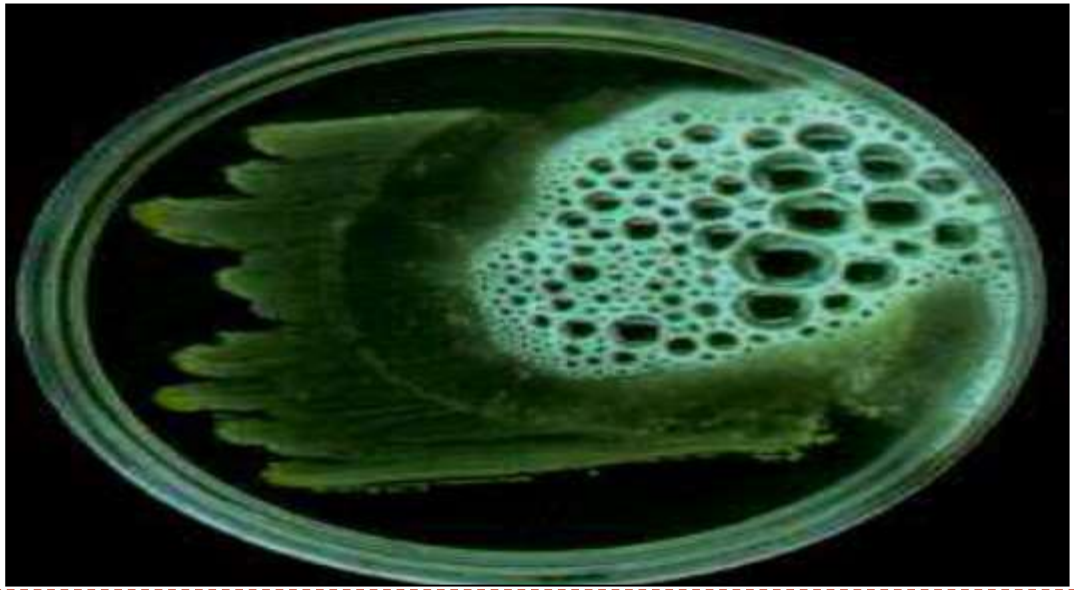
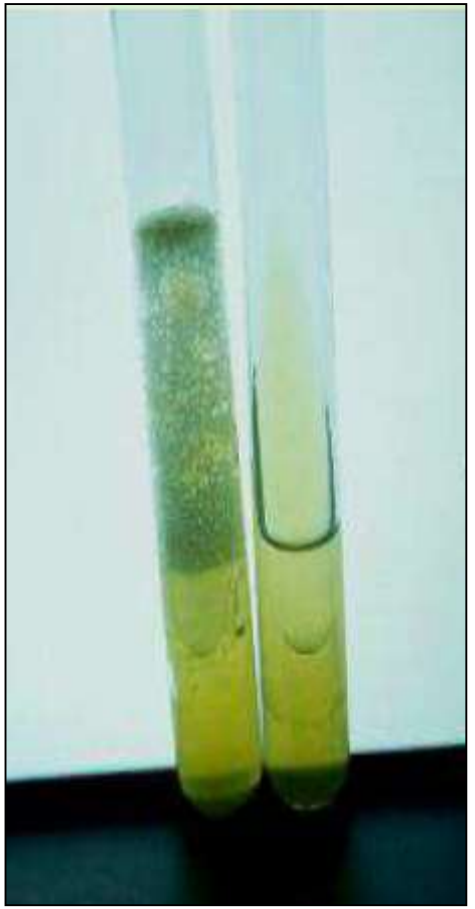


Figure 13-8 Catalase test. **A**, Positive. **B**, Negative.



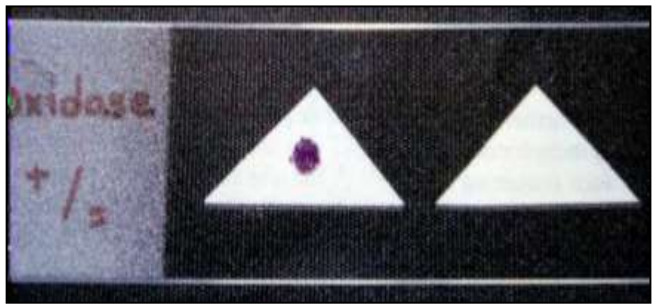


Catalase Test

Positive	Negative
• Micrococcus • Staphylococcus • Bacillus • Listeria monocytogenes • Enterobacteriacae • Gonococcus & Meningococcus • Vibriocholerae • Pseudo/Aero/Plesiomonas	• Streptococcus spp. • Clostridium

Phenotypic Methods
Biochemical Tests - Enzymes

- OXIDASE TEST
  - This test depends on the presence of cytochrome oxidase in bacteria
  - Procedure - place a piece of filter paper in petri dish and add 3 drops of freshly prepared oxidase reagent (1% solution of tetramethyl-p-phenylene diamine)
  - Using a sterile glass rod or toothpick, remove a colony of test organisms from a culture plate and smear it on the filter paper
  - Oxidase positive organisms give blue/ dark purple color within 5-10 seconds, and in oxidase negative organisms, color does not change.



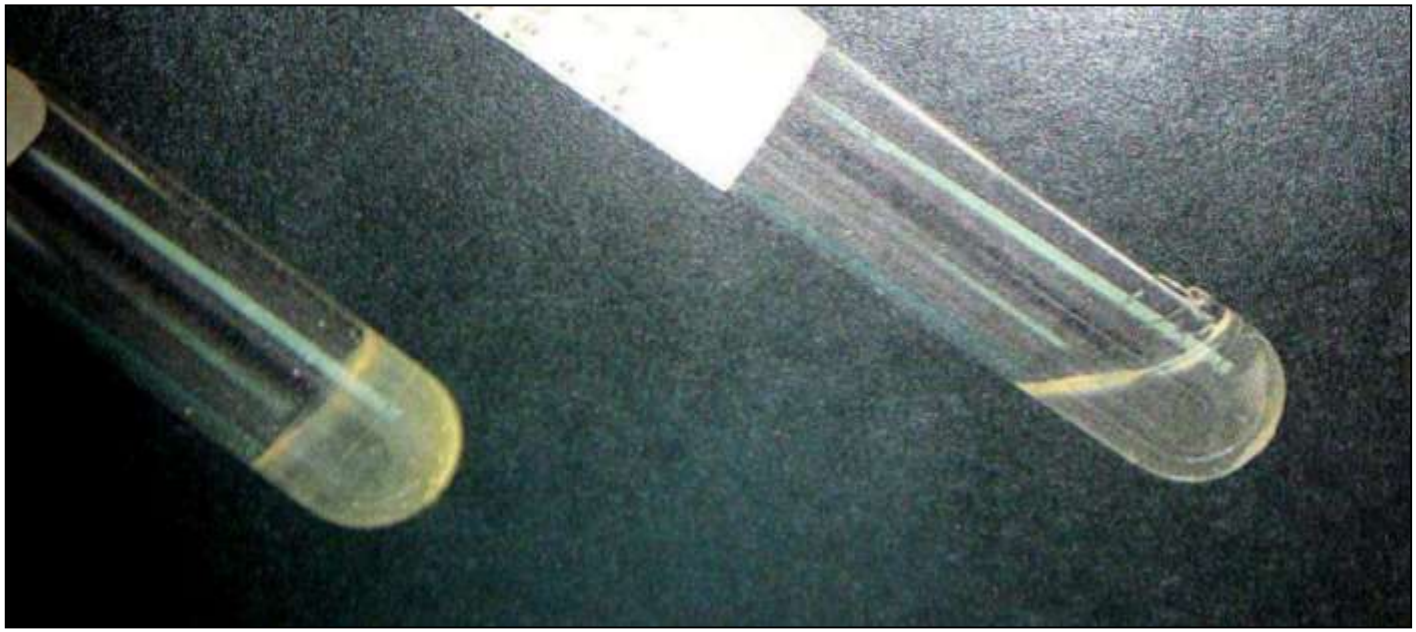
Oxidase Test

Positive	Negative
• Pseudomonas spp. • Acenitobactoer spp. • Vibrio spp. • Alcaligenes spp. • Neisseria spp. • Haemophilus spp.	• Enterobacteriaceae • Aeromonas spp.

Phenotypic Methods
Biochemical Tests - Enzymes

- COAGULASE TEST
  - This test is used to differentiate *Staphylococcus aureus* (positive) from coagulase negative Staphylococci
  - When a bacterial suspension is mixed with plasma, this enzyme causes alteration in fibrinogen.
    - Leading to precipitation on the staphylococcal cells, causing the cells to clump.
    - Slide test: Positive when there is Macroscopic clumping in 10 seconds or less in a plasma drop and no clumping in a saline or water drop.
    - Tube test: Positive when there is a Clot of any size

Coagulase Test



- Tube test:
  - Positive – clot of any size (a) – *Staphylococcus aureus*
  - Negative – no clot (b) – *Staphylococcus epidermidis*

Phenotypic Methods
Biochemical Tests - Enzymes

- UREASE TEST
  - Some bacteria produce urease (hydrolyzes urea into NH_3 and CO_2)
  - The test for urease production relies on the fact that the NH_3 produced upon hydrolysis is alkaline
  - The test organism is inoculated into a urea broth that contains phenol red, a pH indicator, and has a pH of 6.8. When the pH >8.1 phenol red turns a cerise (hot pink) color
  - The urease test is useful for differentiating Salmonella which is urease negative, from Proteus which is urease positive

Phenotypic Methods

Biochemical Tests - Fermentation of Sugars and Gas Production

- TRIPLE SUGAR IRON AGAR (TSI) TEST
  - TSI agar is used to determine whether a G- rod utilizes glucose and lactose or sucrose fermentatively and forms hydrogen sulphide (H2S).
  - TSI contains 10 parts lactose : 10 parts sucrose : 1 part glucose and peptone. Phenol red and ferrous sulphate serves as indicators of acidification and H2S formation, respectively.
  - The formation of CO2 and H2 is indicated by the presence of bubbles or cracks in the agar or by separation of the agar from the sides or bottom of the tube.
  - The production of H2S requires an acidic environment and is indicated by blackening of the butt of the medium in the tube.

Phenotypic Methods

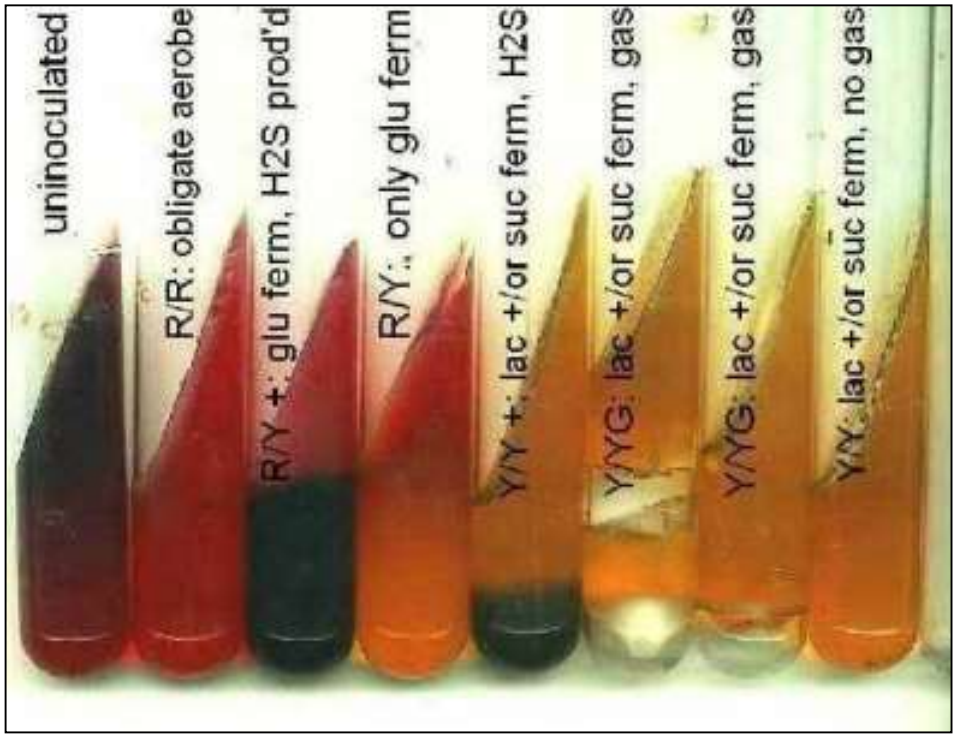
Biochemical Tests - Fermentation of Sugars and Gas Production

- Results interpretation:
  - Acid slant/acid butt (A/A), with gas production = Glucose, sucrose, and/or lactose fermenter
    - E. coli
  - Alkaline slant/acid butt (K/A), H2S production = Glucose fermentation only
    - Salmonella typhi
  - Alkaline slant/no change in the butt (K/NC) = Glucose, lactose and sucrose non-utilizer (alkaline slant/alkaline butt)
    - Pseudomonas aeruginosa

Triple Sugar Iron Media (TSI)

Slant

Butt

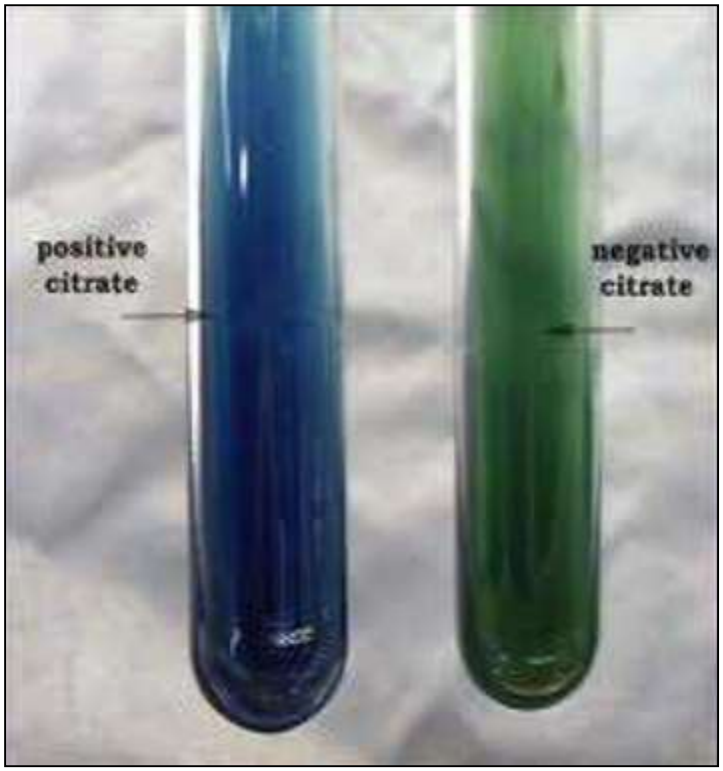


Phenotypic Methods
Biochemical Tests - Enzymes

- LIA – Lysine Iron Agar
  - Lysine Iron Agar was developed to detect lactose fermenting Salmonellae which are known to decarboxylate lysine rapidly and produce large amounts of hydrogen sulfide.
  - This medium is a sensitive medium for the detection of Lactose fermenting and lactose non-fermenting Salmonella species.
  - Many strains of this group, ferment lactose very rapidly thus suppressing H2S production on Triple Sugar Iron Agar
  - It is recommended to use LIA and TSI together for better discrimination between coliform organisms
    - Escherichia and Shigella

Phenotypic Methods
Biochemical Tests - Enzymes

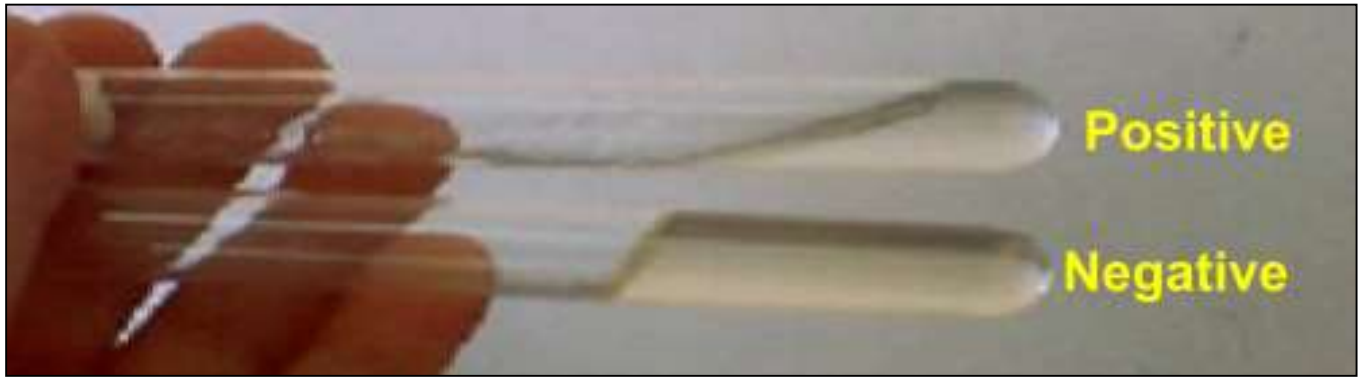
- CITRATE



III.5 Citrate test. Utilization of citric acid as the sole carbon source in Simmons citrate agar causes the indicator to turn blue (**2**). **1** is citrate-negative (Exercise 14).

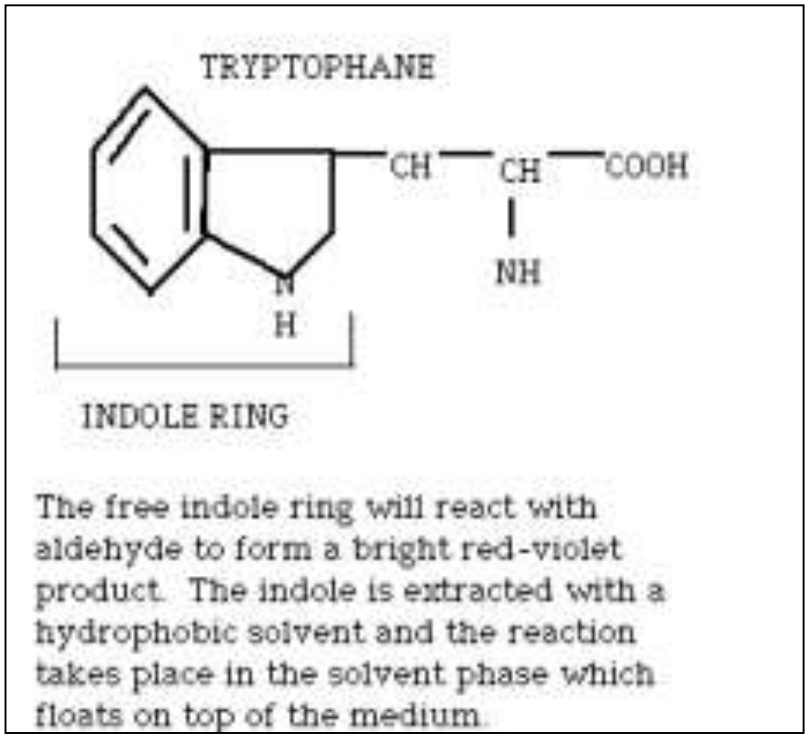
Phenotypic Methods
Biochemical Tests - Enzymes

Gelatinase (Proteinases)



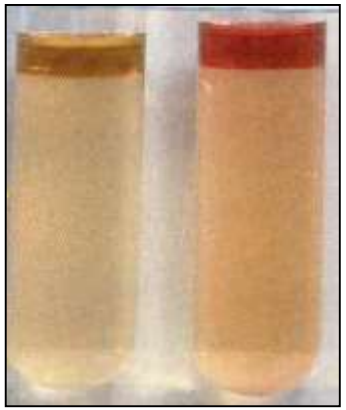
Phenotypic Methods
Biochemical Tests - Enzymes

Indole Test



The free indole ring will react with aldehyde to form a bright red-violet product. The indole is extracted with a hydrophobic solvent and the reaction takes place in the solvent phase which floats on top of the medium.

Negative

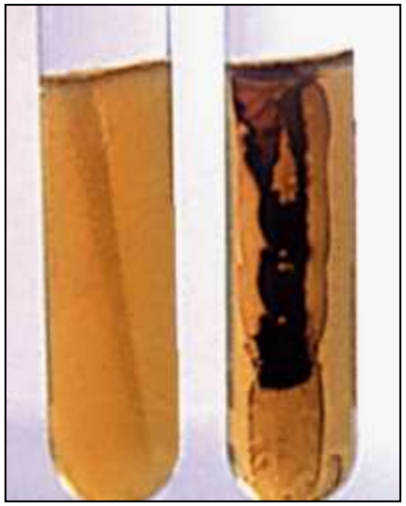


Positive

Phenotypic Methods
Biochemical Tests - Enzymes

H2S Production

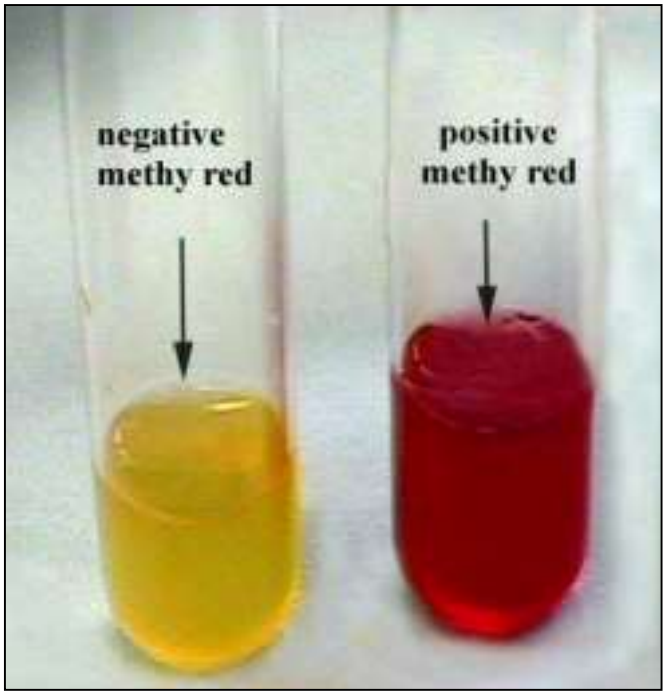
Negative

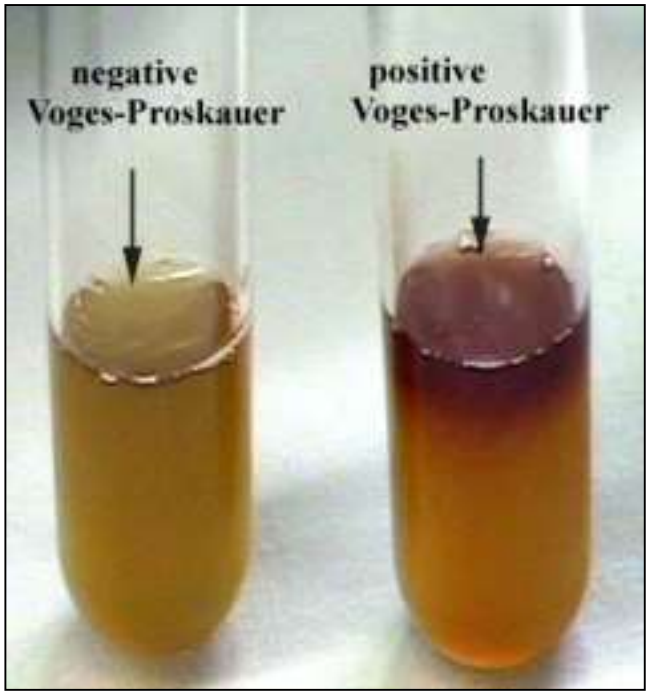


Positive

Phenotypic Methods
Biochemical Tests - Enzymes

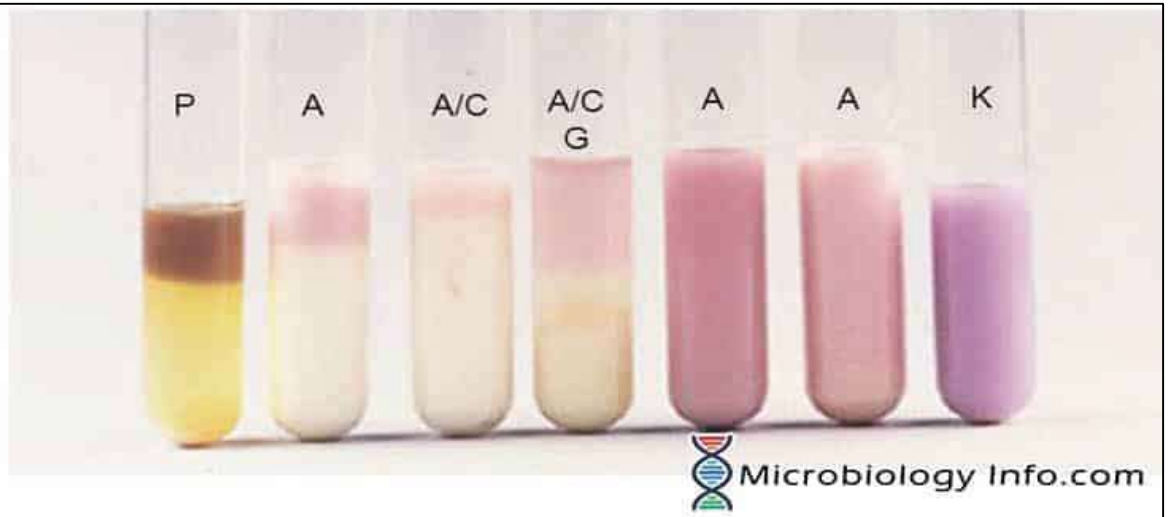
Test for End Product of Fermentation





Phenotypic Methods
Biochemical Tests - Enzymes

Litmus Milk



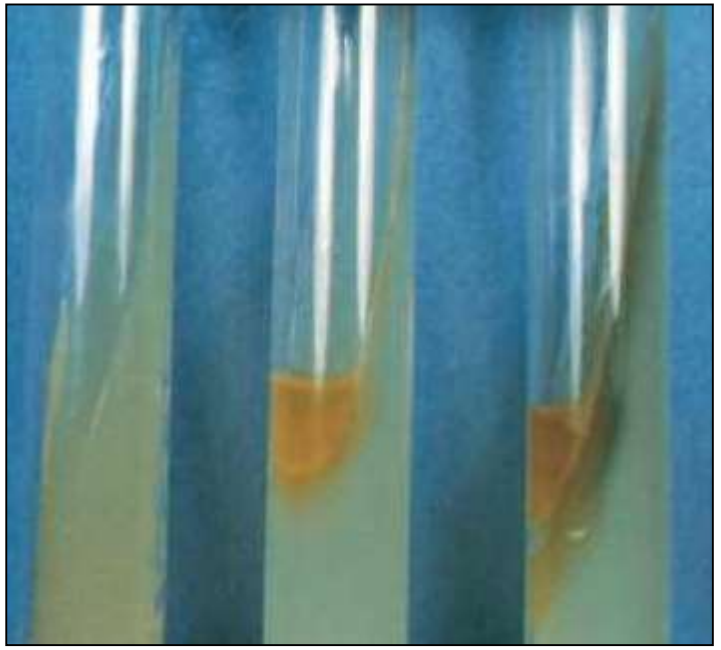
White (R) means litmus was reduced and became clear.
Pink (A) means it fermented lactose and is acidic.
Bluish (K) means the pH is basic, so lactose was not fermented
Curds formed (C)
Gas in the curd (G)
Brown ring (P) means the casein protein in the milk was broken down (proteolysis)

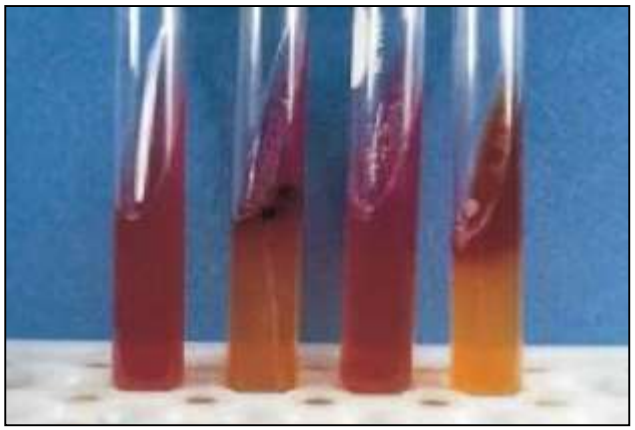
The test itself tells whether the bacterium can ferment lactose, reduce litmus, form clots, form gas, or start peptonization

Indole test

Gelatin hydrolysis or liquefaction

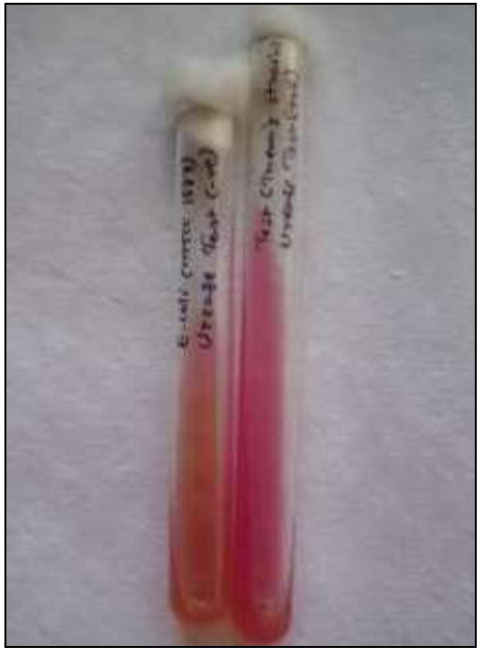
Phenylalanine deamination test



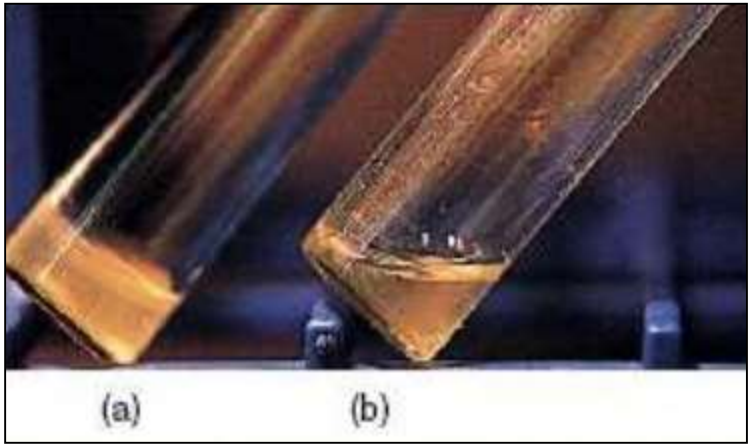


Test for amino acid decarboxylase. The tube on the left is an uninoculated control; the 2nd tube is lysine decarboxylase negative; the 3rd tube is lysine decarboxylase positive; and the last tube is lysine deaminase positive

Urease



Coagulase test

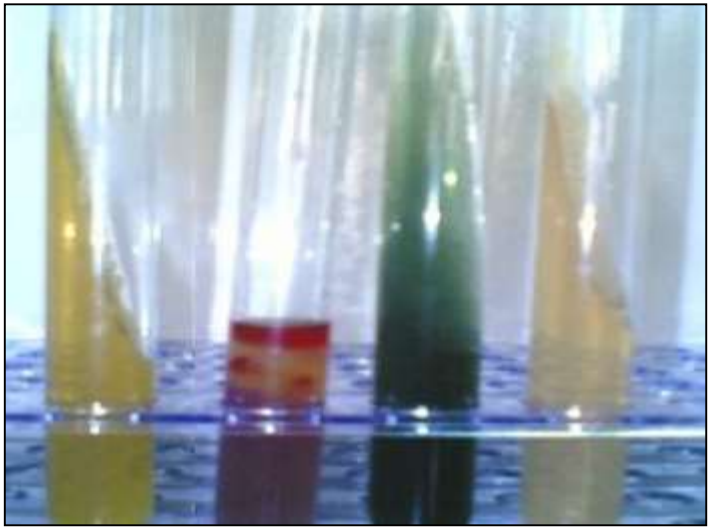


Tube and slide catalase test

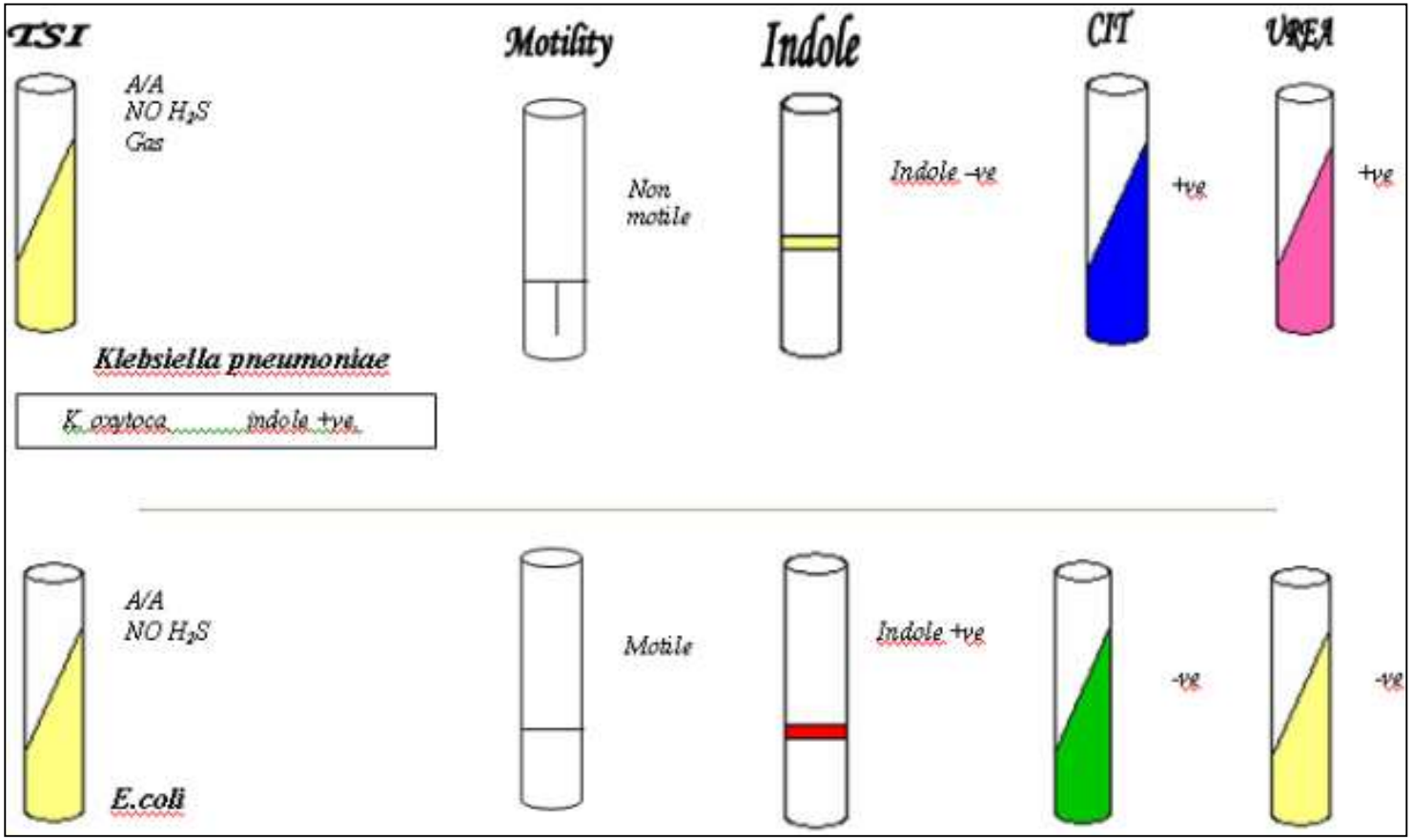
Salmonella typhi

Klebsiella

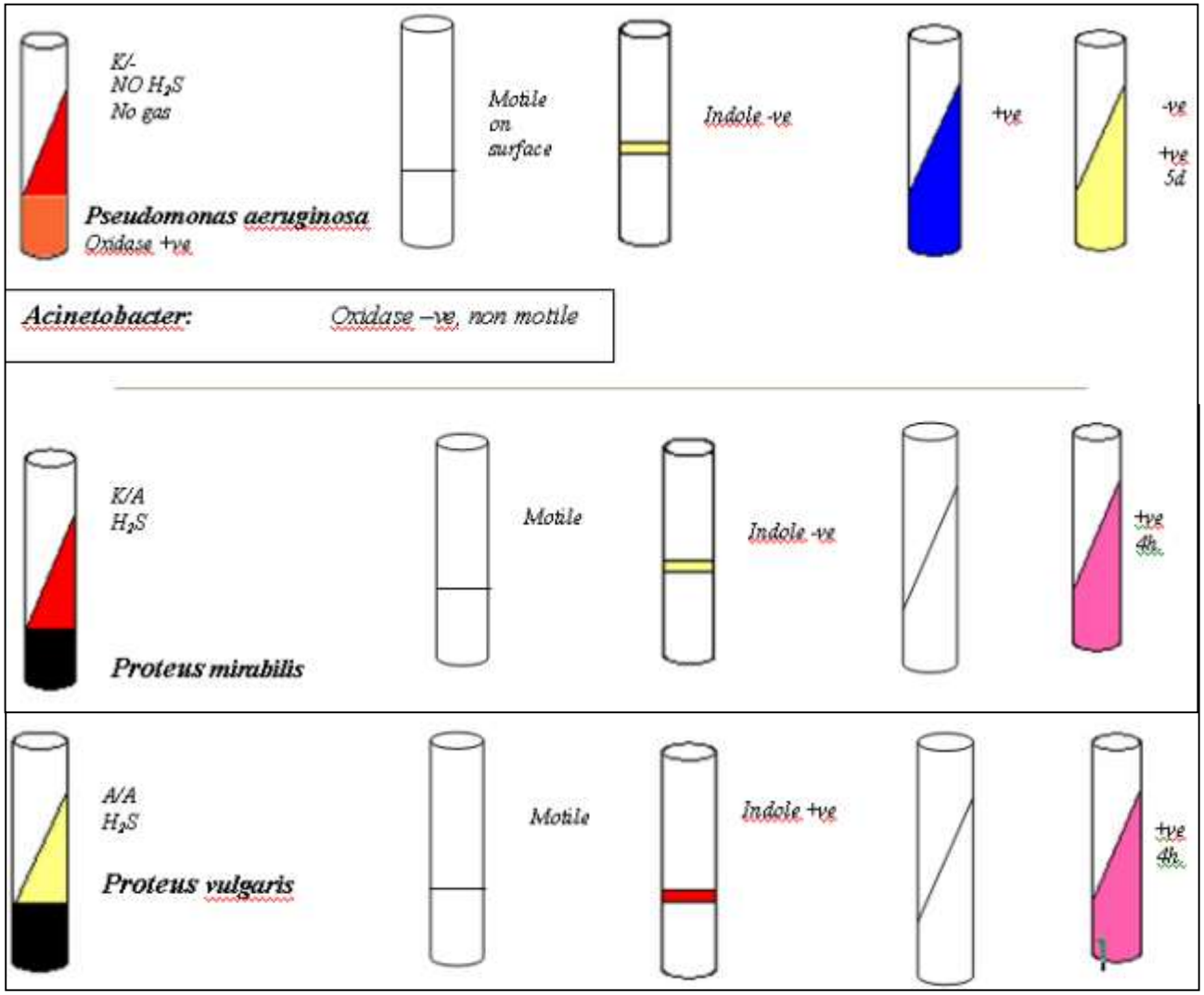
E.coli



Identification of Some Microorganism



Identification of Some Microorganism



Phenotypic Methods
Biochemical Tests - Others

- Bile solubility
- CAMP
- MSA
- Hemolysis
- Cholera red reaction
- Bacitracin
- Serology for strep groups

Immunological Methods

- Immunological methods involve the interaction of a microbial antigen with an antibody (produced by the host immune system).
- Testing for microbial antigen or the production of antibodies is often easier than test for the microbe itself.
- Lab kits based on this technique are available for the identification of many microorganisms

Immunological Tests and Some of Their Uses

Table 17.3 Immunological Tests and Some of Their Uses

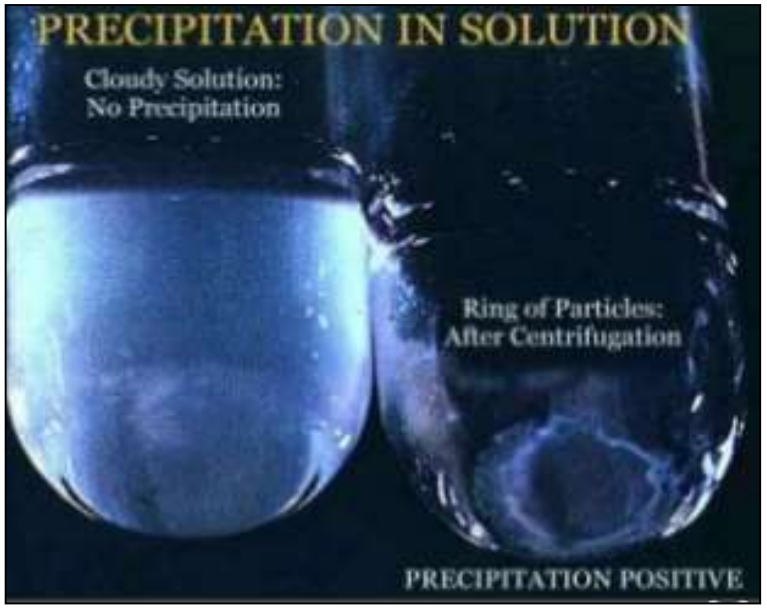
Test	Use
Immunodiffusion (precipitation)	Diagnosis of syphilis, pneumococcal pneumonia
Immunoelectrophoresis (precipitation)	Assay production of particular classes of antibodies
Agglutination	Blood typing; pregnancy testing; diagnosis of salmonellosis, brucellosis, gonorrhea, rickettsial infection, mycoplasma infection, yeast infection, typhoid fever, meningitis caused by *Haemophilus*
Viral neutralization	Diagnosis of infections by specific strains of viruses
Viral hemagglutination inhibition	Diagnosis of viral infections including influenza, measles, mumps, rubella, mononucleosis
Complement fixation	Diagnosis of measles, influenza A, syphilis, rubella, rickettsial infections, scarlet fever, rheumatic fever, infections of respiratory syncytial virus and *Coxiella*
Direct fluorescent antibody	Diagnosis of rabies, infections of group A *Streptococcus*
Indirect fluorescent antibody	Diagnosis of syphilis, mononucleosis
ELISA	Pregnancy testing; presence of drugs in urine; diagnosis of hepatitis A, hepatitis B, rubella; initial diagnosis of HIV infection
Western blot	Verification of infection with HIV, diagnosis of Lyme disease

Immune Testing

- Numerous types of serologic test – differ in their speed and sensitivity
  - Precipitation tests
  - Agglutination tests
  - Neutralization
  - Complement fixation
  - Immunofluorescence
  - RIA (radioimmunoassay)
  - ELISA (Enzyme-Linked Immuno-Sorbent Assay)
  - Western Blotting

Precipitation Reactions

- Precipitation is the interaction of a soluble Ag with an soluble Ab to form an **iinsoluble com**
- The complex formed is anan aggregate of Ag and Ab
- Precipitation reactions occur maximally only when the **optimal proportions** of Ag and Ab present
- RPR, VDRL test



Double Immuno-diffusion

- Precipitation tests – done in gels
- The precipitate is easily seen in gels yield visible **precipitin lines**
- But no visible precipitate forms in regions of Ab or Ag excess
- Disadvantages
- Techniques is too slow

Counterimmunoelectrophoresis (CIE)

- Modification of double immuno-diffusion
- Speeding up migration applying electric current

- Disadvantage:
  - Precipitin band difficult to see sometimes
  - Agargose gel required overnight washing
  - More expensive
  - Less sensitive to particle agglutination test (detecting antigen approx. 01 to 05 mg/ml)

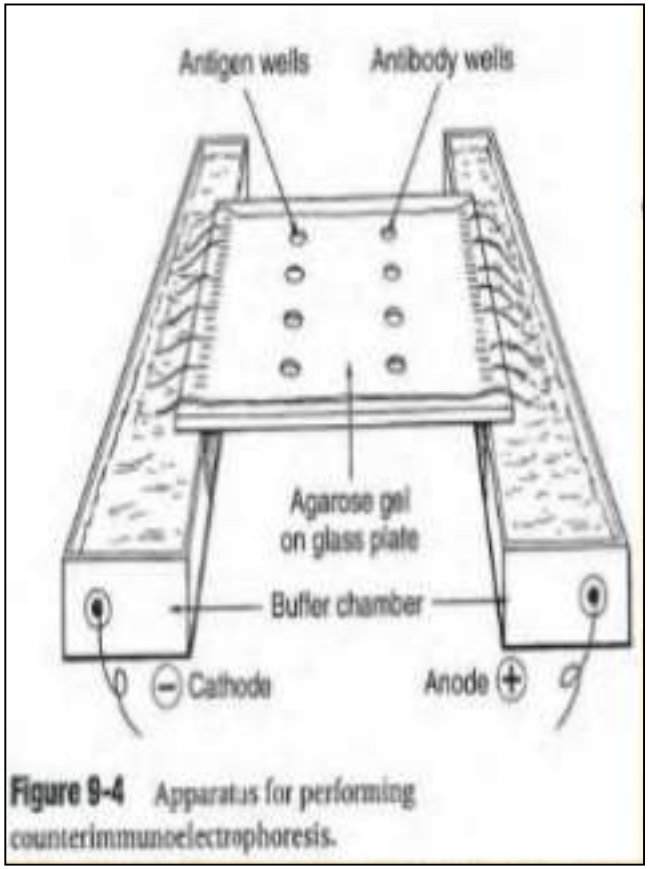


Figure 9-4 Apparatus for performing counterimmunoelectrophoresis.

Agglutination Tests

- Agglutination occurs due to the cross-linking of particulate antigens by antibody molecules
- Agglutination is the visible clumping of insoluble particles, whereas precipitation involves the aggregation of soluble molecules

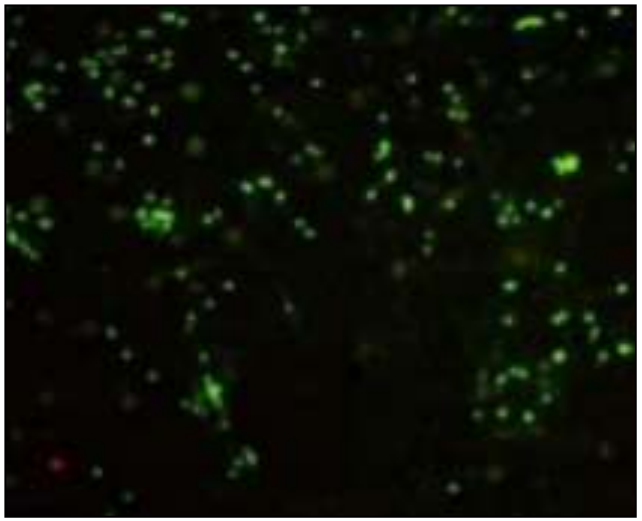
- Types of agglutination reactions:
  - Direct agglutination reactions
  - Indirect or passive agglutination tests
  - Hemagglutination reactions

Agglutination Test
Use

- Bacterial agent difficult to cultivate *in vitro*:
  - Yersinosis, Leptospirosis, Brucellosis, Tularemia
- Microhemagglutination test for antibody to extracellular antigens of *streptococci*
- CDC also perform indirect hemagglutination test for some bacteria
  - *Clostridia, Burkholderia, Pseudomallei, B. anthracis, C. diphtheriae, Leptospira*

Immuno-fluorescence

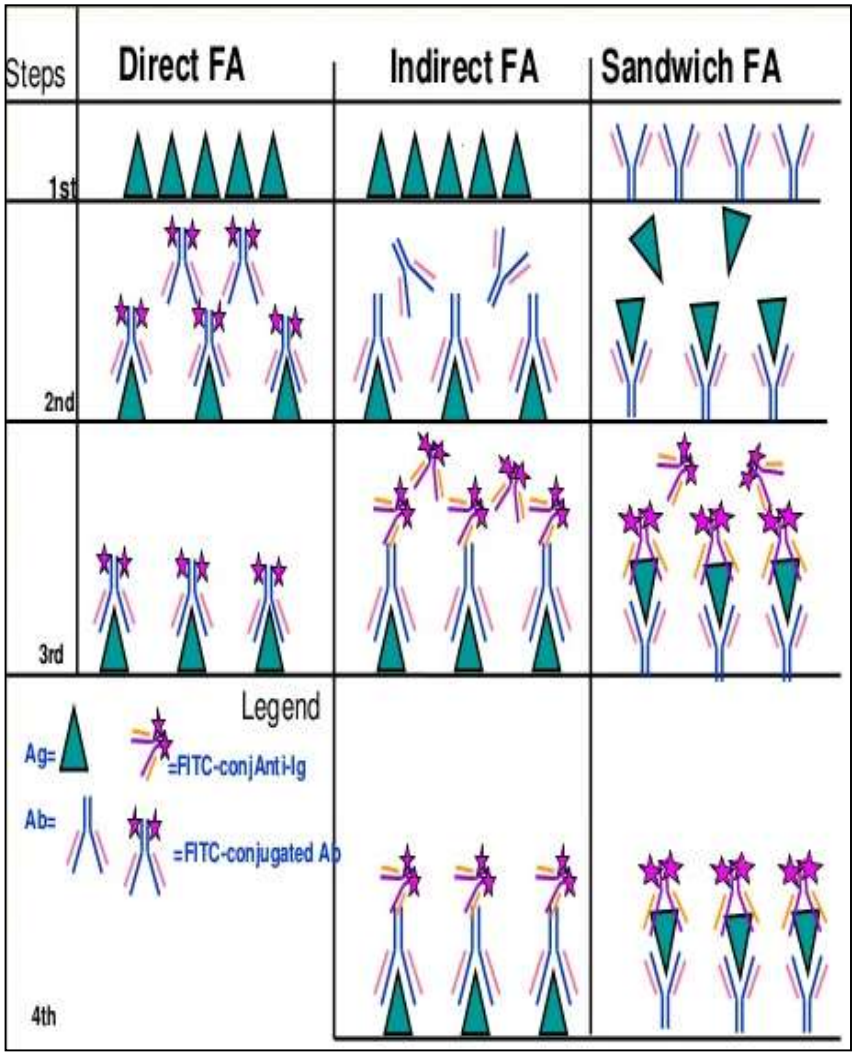
- Principle
  - Use fluorescein isothiocyanate labeled-immunoglobulin to detect antigens or antibodies according to test systems
  - Requires a fluorescent microscope



V. Cholerae

Immuno-fluorescence
Types

- Direct immuno-fluorescence
  - Used to detect antigen
- Indirect and sandwich immuno-fluorescence
  - Antigen detection
  - Antibody detection



Immuno-fluorescence
Performance, applications

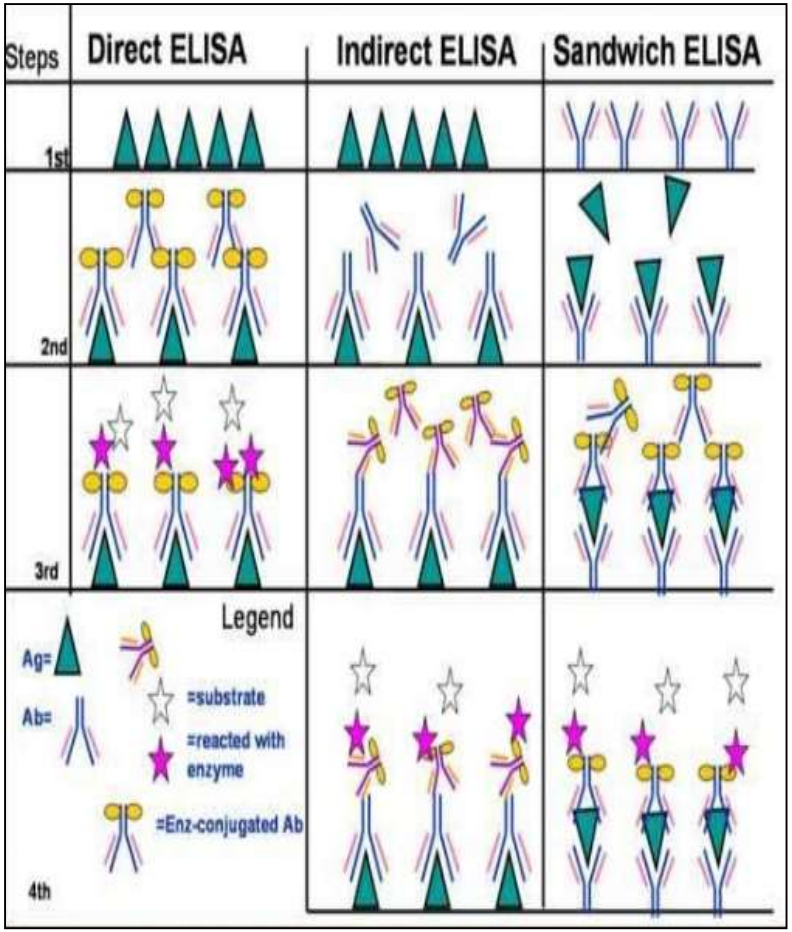
- Advantages
  - Sensitive and specific
  - Can be usedfor discrepant analysis
- Limitations
  - Expensive (reagents and equipment)
  - Subjective
  - Cross reactivity
  - Non-specific immuno-fluorescence
- Time-taken
  - Few minutes to few hours

ELISA

- Principle:
  - Use of enzyme-labeled immunoglobulin to detect antigens or antibodies
  - Signals are developed by the action of hydrolyzing enzyme on chromogenic substrate
  - Optical density measured by micro-plate reader

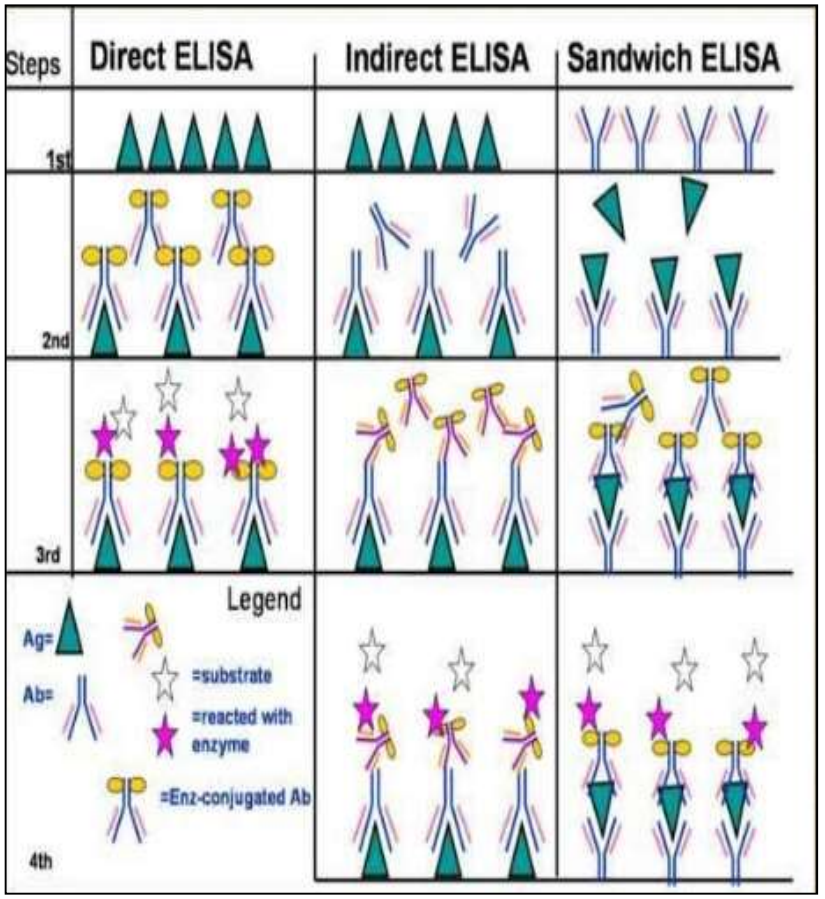
ELISA
Types – Ag-Ab Test

- Competitive
  - Ag or Ab are labeled with enzyme and allowed to compete with unlabeled ones (in patient serum) for binding to the same target
  - Hydrolysis signal from Ag-Ab complex (enzyme-labeled) is measured
  - Antigen or antibody in serum is then calculated
  - No need to remove the excess/unbound Ag or Ab from the reaction plate or tubes)



ELISA
Types – Ag-Ab Test

- Non-competitive - must remove excess/unbound Ag or Ab before every step of reactions:
  - Direct ELISA
  - Indirect ELISA
  - Sandwich ELISA
  - Ab Capture ELISA (similar to sandwich ELISA but in 1st step, anti-Ig (M or G) is coated on the plate
  - Then Ab in patient serum are allowed to capture in next step



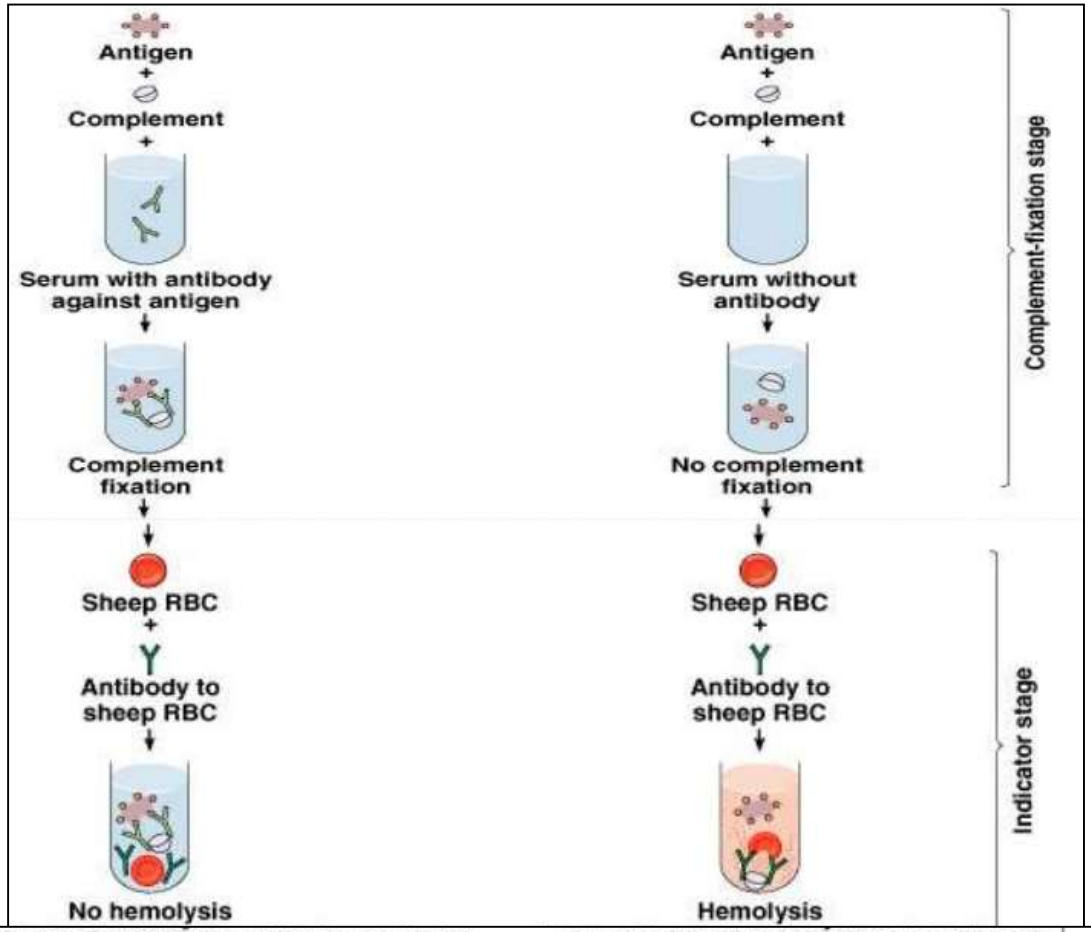
ELISA
Performance, Applications

- Advantages
  - Automated, inexpensive
  - Objective
  - Small quantities required
  - Class specific Ab measurable
- Limitations
  - Expensive initial investment
  - Variable sensitivity / specificity of variable tests
  - Cross contamination
- Time taken – 1 day
- USE-commercial kit available to detect antibody for *Mycoplasma*, *Chlamydiae*, *Borrelia*

Complement-Fixation Reactions

- Complement-fixation
  - Complement (group of serum proteins) binds to Ag-Ab complex
- Complement-fixation can be used to detect very small amounts of Ab
  - Wasserman test for syphilis (in the past)
  - Certain viral & fungal diseases

Complement-Fixation Reactions



(complement tied up in antigen–antibody reaction)

(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(uncombined complement available)

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Problems with Serological Tests

1. Cross reacting Ab
2. Presence of Rheumatoid factors
3. Delay in Ab response (Lyme disease – Legionnaire's disease)
4. Competition for Ag binding site of Ab
    1. IgM binds to the Ag IgG site
    2. IgG binds to the Ag IgM site

Problems with Serology Other Health Conditions Interfere

- Immunocompromised patients often give a reduced or absent humoral immune response
- Patients with infectious mononucleosis and those with connective tissue diseases such as SLE may react non-specifically giving a false positive result
- Patients receiving blood or blood products may give a false positive result due to the transfer of Ab

Bacterial Identification

Multi-test Miniaturized System: the API System

The most used API system:

API Strep
→ Identification of *Streptococcus* species

API Staph
→ Identification of *Staphylococcus* species

API 20NE
→ Identification of **N**on **E**nterobacteria (*Pseudomonas* for example)

API 20E
→ Identification of **E**nterobacteria

The API System

With an unknown bacteria to identify, which API system use ?

… before use API system, it's necessary to perform preliminary tests on bacteria to identify

- **Preliminary test 1** : Gram stain + microscopic observation

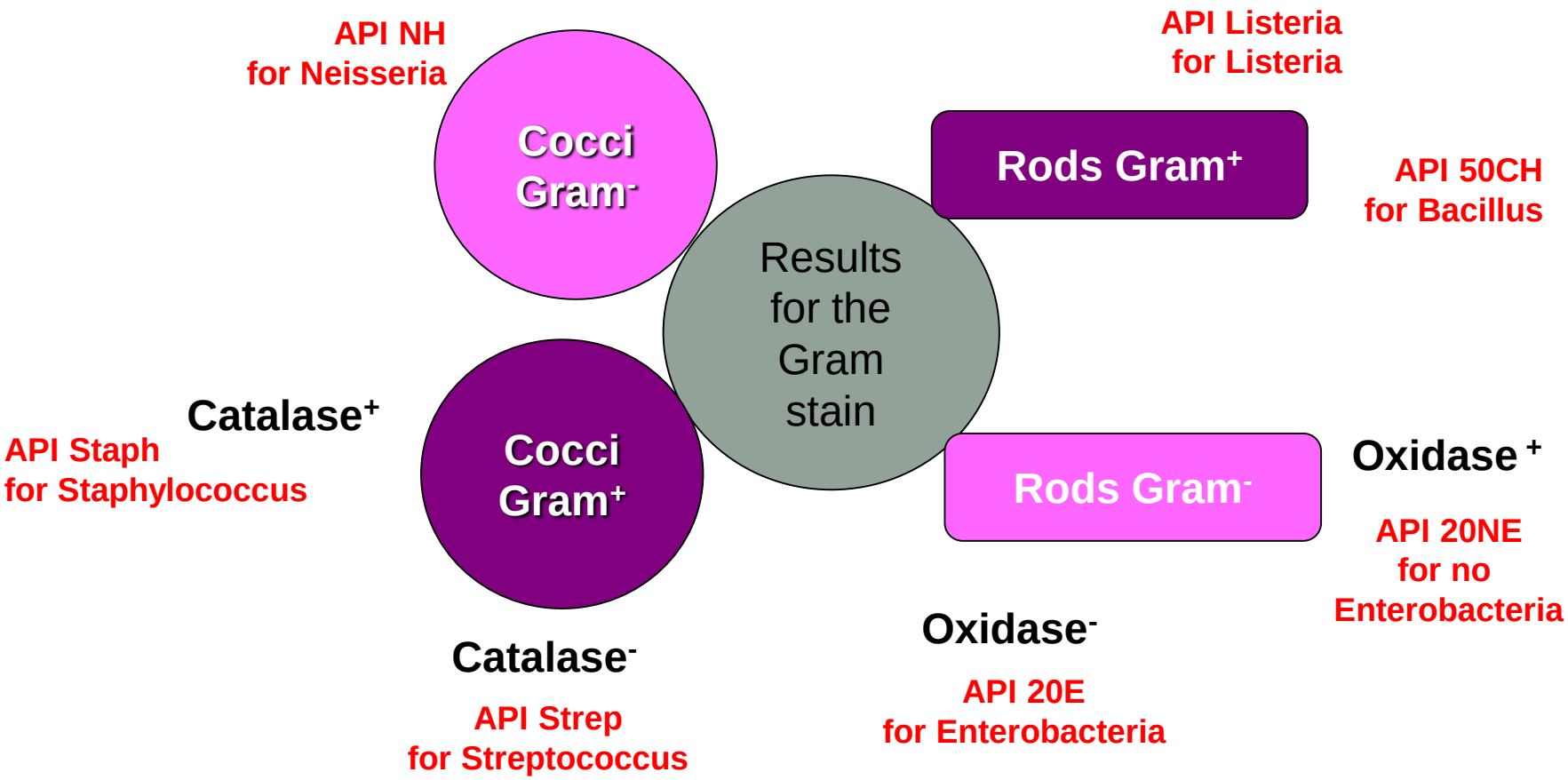
→ Distinguishes bacteria according to their form (cocci, rods) and their response to color (purple = Gram + bacteria, pink = Gram – bacteria)

- **Preliminary test 2** : Bacteria respiratory enzymes test

→ Distinguishes bacteria into groups according to the existence of two enzymes, « oxidase » enzyme, or « catalase » enzyme

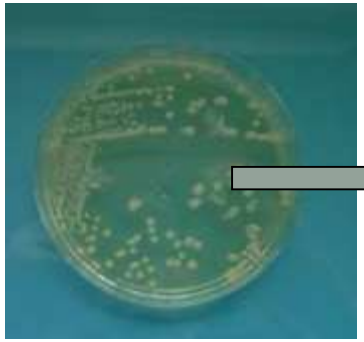
The API System

With an unknown bacteria to identify, which API system use ?



The API System

Introducing the API 20E system for Enterobacteria identification



1 bacterial colony

5 mL of sterile water

Fill microtubes gallery with the bacterial suspension

The API System

View of API 20E just after seeding

Nitrogen metabolisms and specific enzymes

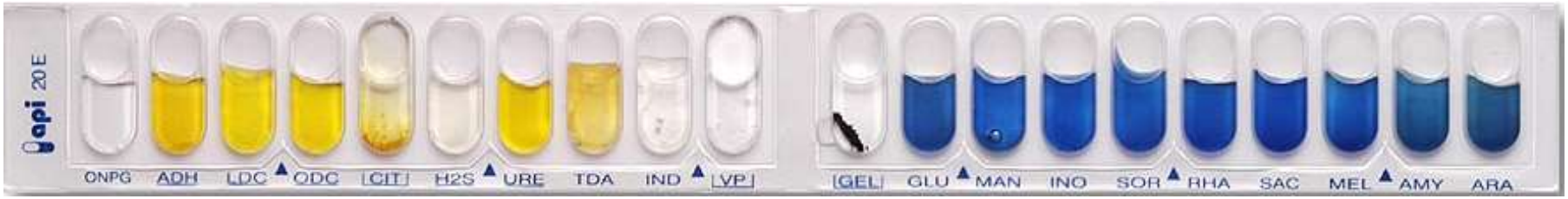
Metabolism of carbohydrates

24h / 37oC

The API System

API 20 E after incubation. Positive results for all tests:

API 20 E after incubation. Negative results for all tests :

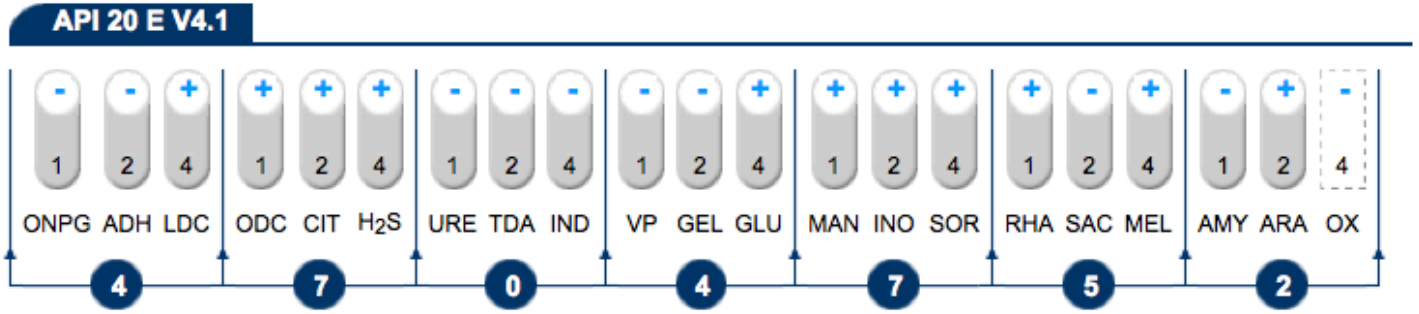


The API System

Example of results for bacteria to test :

1 – reading results:

2 – entering results in the database software:



The API System

Example of results for bacteria to test :

3-Expression of results by software :

EXCELLENTE IDENTIFICATION	
Galerie	API 20 E V4.1
Profil	4 7 0 4 7 5 2
Note(s)	CONFIRMER PAR DES TESTS SEROLOGIQUES

Taxons significatif(s)	% ID	T	Test(s) à l'encontre			
Salmonella spp	99.9	0.95				

Name of the identified bacteria

Quality identification

In this example we have a very good identification of bacteria *Salmonella spp*

The API System

From the time when bacteria was collected and the results…**it took two days**

… there are new and more rapid methods !!

Bacterial Identification

Innovative and Rapid Methods: Andromas

Andromas = automated system of bacterial identification using **mass spectrophotometry**

→ developed by a French team of researchers from Necker hospital in Paris

Principle

Physical detection of molecules (usually **proteome**) contained in bacteria cytoplasm by **mass spectrometry**

+ obtaining a **specific detection profile** which is **compared to a database** for identification

Bacterial Identification

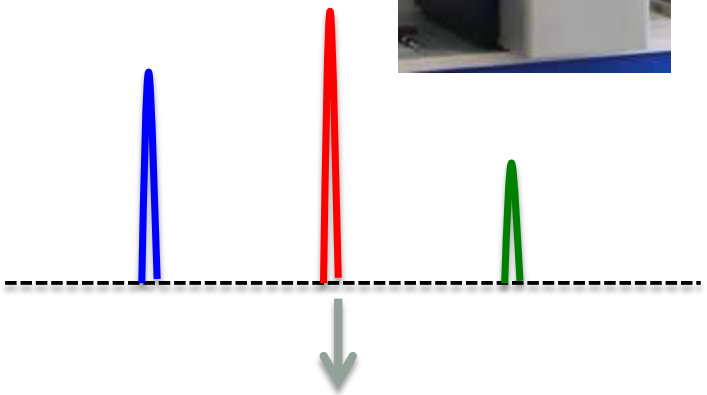
Innovative and Rapid Methods: Andromas

Bacteria

Lysis of cell

Extraction of molecules from cytoplam

Detection of molecules by mass spectrometry whitch creates a characteristic spectrum :



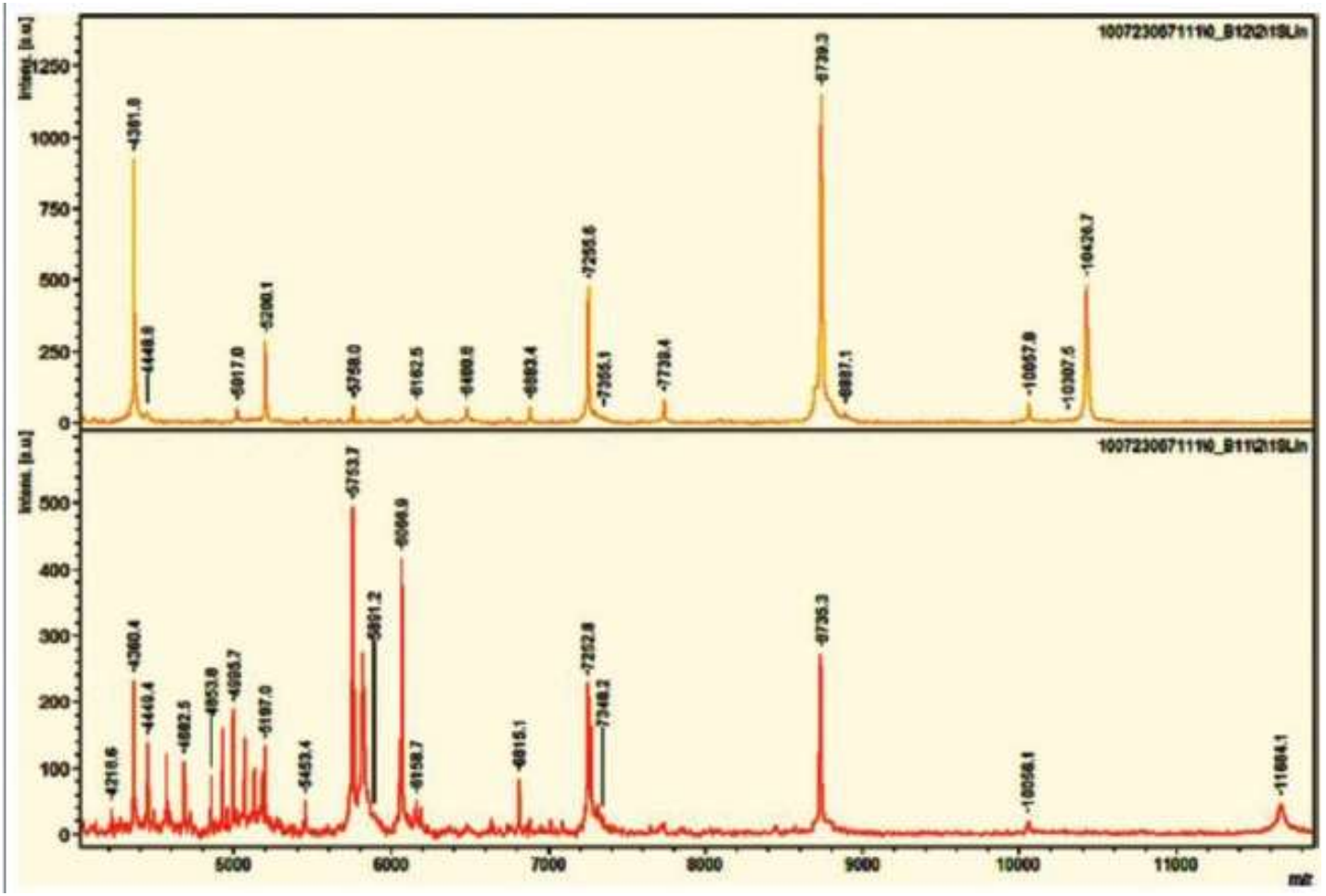
Comparison of the spectrum with spectrum of known bacteria in database

→ **Bacteria are identified**

Bacterial Identification

Innovative and Rapid Methods: Andromas

Examples of real spectrum of bacteria



Bacterial Identification

Innovative and Rapid Methods: Andromas

Comparison Andromas / Automated API system

Automated API system

Sampling + isolation

Seeding API system + incubation

Andromas analysis (less than 10 min)

Andromas system

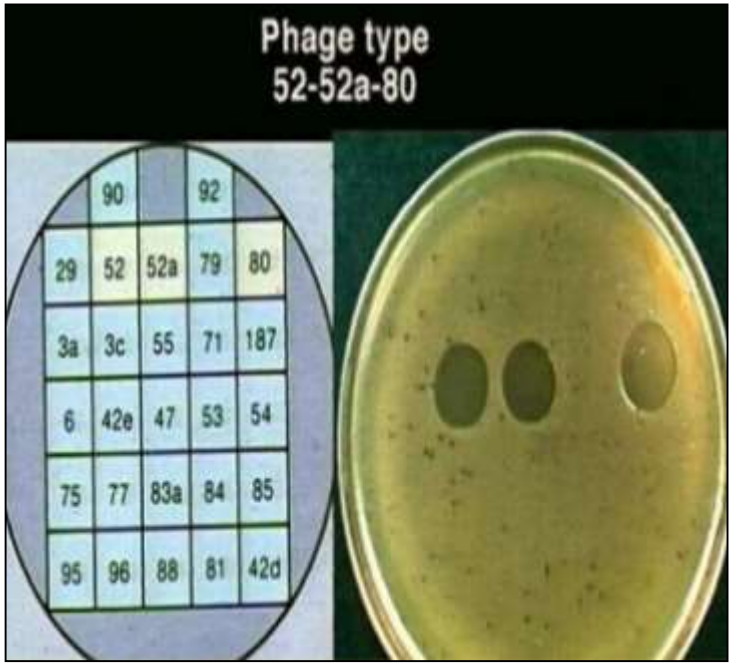
Sampling + isolation

Day 0 | **Day +1** | **Day +2** → Analysis time

Faster analysis system = interesting for doctors to get results quickly

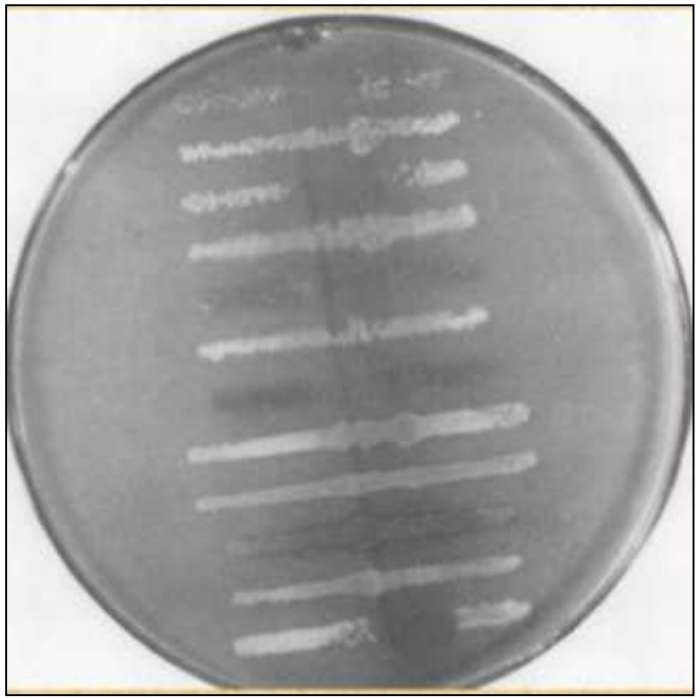
Phage Typing

- Classifies bacterial organisms according to susceptibility of the bacteria to lysis by the panel of bacteriophage
- Interspecies differentiation of some bacteria
- Phage typing has played useful in epidemiologic roles for *S. aureus* & *S. enterica* serotype Typhi



Bacteriocin Typing

- Bacteriocin is protein products produced by bacteria that inhibit growth of the strains of the same genus
- **Classifies** bacteria according to their **susceptibility to bacteriocin**
- Used in reference laboratories for typing *K. pneumoniae, P. aeruginosa*



Genotypic Methods

- The initiation of new molecular technologies in genomics is shifting traditional techniques for bacterial classifications, identification, and characterization in the 21st century toward methods based on the elucidation of specific gene sequences or molecular components of a cell
- Genotypic methods of microbe identification include the use of:
  - Nucleic acid probes
  - PCR
  - Nucleic acid sequences analysis
  - 16s rRNA analysis
  - RFLP
  - Plasmid fingerprinting
- Genotypic techniques are becoming the sole means of identifying many microorganisms because of their speed and accuracy

Specimen Collection

- Preserve viability/nucleic acid integrity of target microorganisms
- Avoid contamination
- Appropriate time and site of collection (blood, urine, other)
- Use proper equipment (coagulant, wood, or plastic swab shafts)
- Commercial collection kits are available
- The Clinical and Laboratory Standards Institute (CLSI) has guidelines for proper specimen handling

Sample Preparation

- Consider the specimen type (stool, plasma, CSF)
- More rigorous lysis procedures are required to penetrate cell walls
- Consider the number of organisms in the sample
- Inactivate inhibitors (acidic polysaccharides in sputum or polymerase inhibitors in CSF)
- Inactivate RNases

Target Microorganisms for Molecular-Based Testing

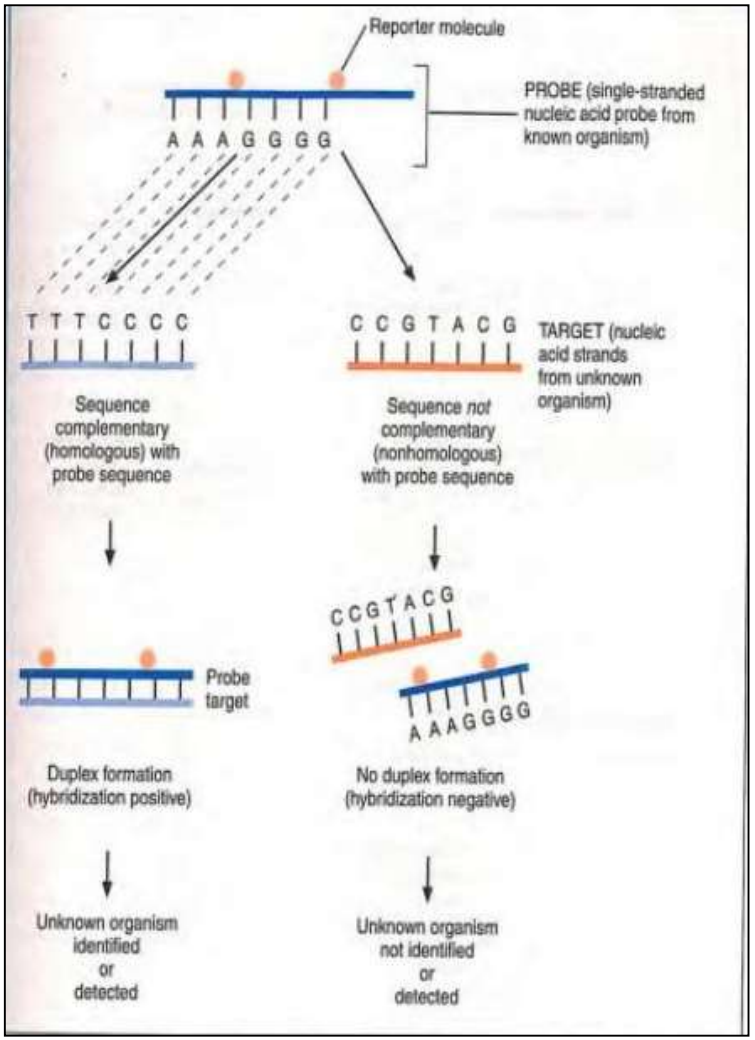
- Those that are difficult or time-consuming to isolate
  - *Mycobacteria*
- Hazardous organisms
  - *Histoplasma, Coccidiodes*
- Those without reliable testing methods
  - *HIV, HCV*
- High-volume tests
  - *S. pyogenes*, *N. gonorrhoeae, C. trachomatis*

Applications of Molecular Based Testing in Clinical Microbiology

- Rapid or high-throughput identification of microorganisms
- Detection and analysis of resistance genes
- Genotyping
- Classification
- Discovery of new microorganisms

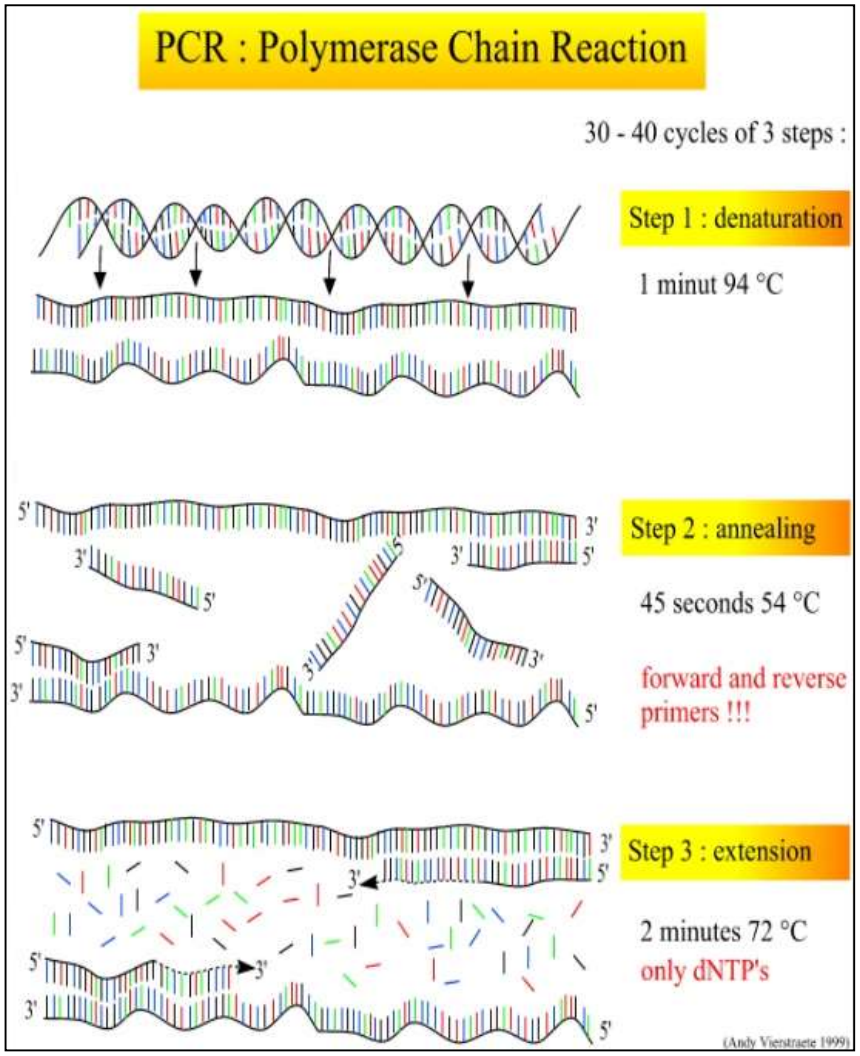
Nucleic Acid Hybridization

- It is technique involves using a labeled nucleic acid probe, which is known DNA or RNA fragment, to bind with the target nucleic acids in the heterogenous population of nucleic acids
- A probe labeled with detectable tracer is the prerequisite for determining a specific DNA sequence or gene in a sample
- Types:
  - Filter hybridization
  - Southern hybridization
  - Sandwich hybridization
  - *In situ* hybridization



PCR

- Is widely used for the identification of microorganisms
- Sequence specific primers are used in PCR for the amplification of DNA or RNA of specific pathogens
- PCR allows for the detection even if **only a few cells are present** and can also be used on **viable non-culturables**
- The presence of the **appropriate amplified PCR product** confirms the presence of the organisms

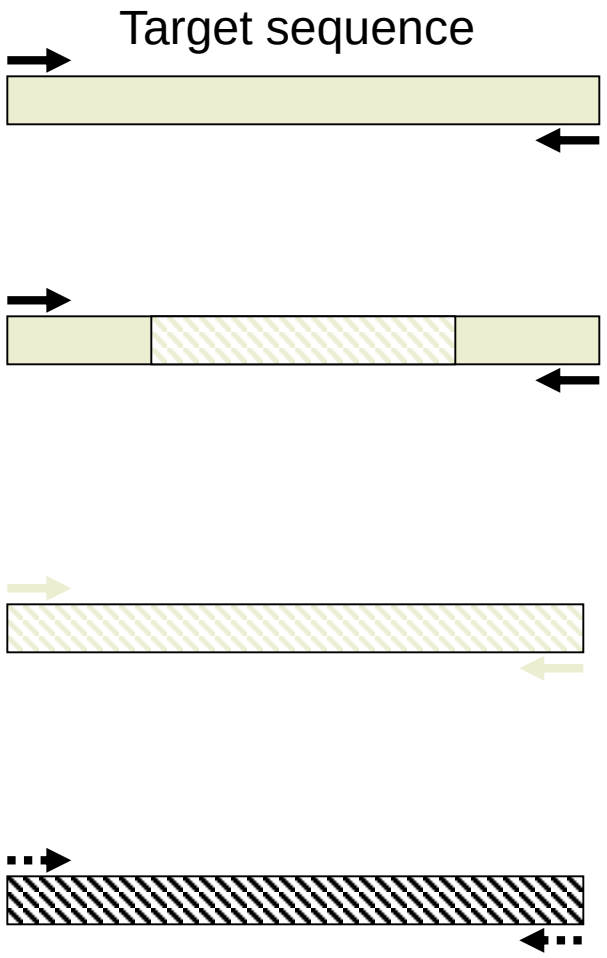


PCR Detection of Microorganisms
Quality Control

- PCR and other amplification methods are extremely sensitive and very specific. For accurate test interpretation, use proper controls.
  - Positive control: positive template
  - Negative control: negative template
  - Amplification control: omnipresent template unrelated to target
  - Reagent blank: no template present

PCR Quality Control
Internal Controls

- Homologous extrinsic
  - Controls for amplification
- Heterologous extrinsic
  - Controls for extraction and amplification
- Heterologous intrinsic
  - Human gene control



PCR Quality Control
False Positives

- Contamination: check reagent blank
- Dead or dying organisms: retest 3–6 weeks after antimicrobial therapy
- Detection of less than clinically significant levels
- Improper collection, specimen handling
- Extraction/amplification failure: check internal controls
- Technical difficulties with chemistry or instrumentation: check method and calibrations

Variations of the PCR

- Colony PCR
- Nested PCR
- multiplex PCR
- AFLP PCR
- Hot Start PCR
- *In situ* PCR
- Inverse PCR
- Asymmetric PCR
- Long PCR
- Long accurate PCR
- Reverse transcriptase PCR
- Allele specific PCR
- Real time PCR

Examples of Non-PCR-based Nucleic Amplification Tests

Table 8-3 Examples of Non–Polymerase Chain Reaction–Based Nucleic Amplification Tests

Amplification Method	Manufacturer/Name	Method Overview	Examples of Commercially Available Assays	Additional Comments
Nucleic acid sequence-based amplification (NASBA)	bioMérieux Inc. NucliSens technology: nucleic acid release, extraction, NASBA amplification, product detection.	(1) Isothermal amplification achieved through coordination of 3 enzymes (avian myeloblastosis, RNAseH, T7 RNA polymerase) in conjunction with 2 oligonucleotide primers specific for the target sequence. (2) Amplification based on primer extension and RNA transcription.	NucliSens HIV-1 QT NucliSens CMV pp67 NucliSens EasyQ HIV-1 NucliSens EasyQ enterovirus	(1) Can be adapted to real-time format using molecular beacons (2) Can develop in-house assays (3) Automated extraction available (NucliSens extractor) (4) Easy Q System = incubator, analyzer, and computer
Transcription-mediated amplification (TMA)	Gen-Probe Inc.: Sample processing, amplification, target detection by hybridization protection or dual kinetic assays for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Also, ASRs for hepatitis C virus (HCV), Bayer Inc., Tarrytown, NY; Gen-Probe/Chiron Corp.: TMA for screening donated blood products for HIV-1 and HCV.	(1) Autocatalytic, isothermal amplification utilizing reverse transcriptase and T7 RNA polymerase and 2 primers complementary to the target. (2) Exponential extension of RNA (up to 10 billion amplicons within 10 minutes).	Gen-Probe: *Mycobacterium tuberculosis* Direct Test; APTIMA Combo 2 for dual detection of *C. trachomatis* and *N. gonorrhoeae*; Bayer ASR reagents for HCV; Gen-Probe/Chiron: Procleix HIV-1/HCV	(1) Second-generation TMA assays of Gen-Probe better at removing interfering substances • Less labor-intensive • Uses target capture after sample lysis using an intermediate capture oligomer • TMA performed directly on captured target (2) Automated system for TMA-based assays: TIGRIS DTS system (Gen-Probe) • Instrument handles specimen processing through amplification and detection • 500 tests in 9 hours; 3.5 hours to first result (3) Real-time TMA-based assays using molecular beacons under development by Gen-Probe that will be compatible with a variety of thermal cyclers (e.g., iCycler, ABI Prism)
Standard displacement amplification (SDA)	BD ProbeTec ET System: SDA coupled with homogeneous real-time detection.	(1) Isothermal process in which single-stranded target is first generated. (2) Exponential amplification of target.	BDProbe Tec ET System for *C. trachomatis* and *N. gonorrhoeae*; Panel assays for *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *L. pneumoniae*, *Chlamydiaceae*; Assay that detects *C. trachomatis*, *C. pneumophila*, and *C. psittaci*; BD ProbeTec *M. tuberculosis* Direct	(1) Reagents dried in separate disposable microwell strips (2) All assays have internal control to monitor for inhibition (3) Automated system for sample processing: BD Viper Sample Processor

Advantages of Molecular Detection of Resistance to Antimicrobial Agents

- Mutated genes are strong evidence of resistance
- Rapid detection without culturing
- Direct comparison of multiple isolates in epidemiological investigations

Advantage of Genotypic Methods over Phenotypic Methods

- Speed, accuracy, cost
- Ability to detect nonviable organisms that are not retrievable by cultivation based method
- Identification of bacteria grown in culture
  - Slow growing bacteria
  - Common pathogen exhibit unusual phenotypic traits
- Detection of antimicrobial resistance

Other Genotypic Methods Used to Type Organisms

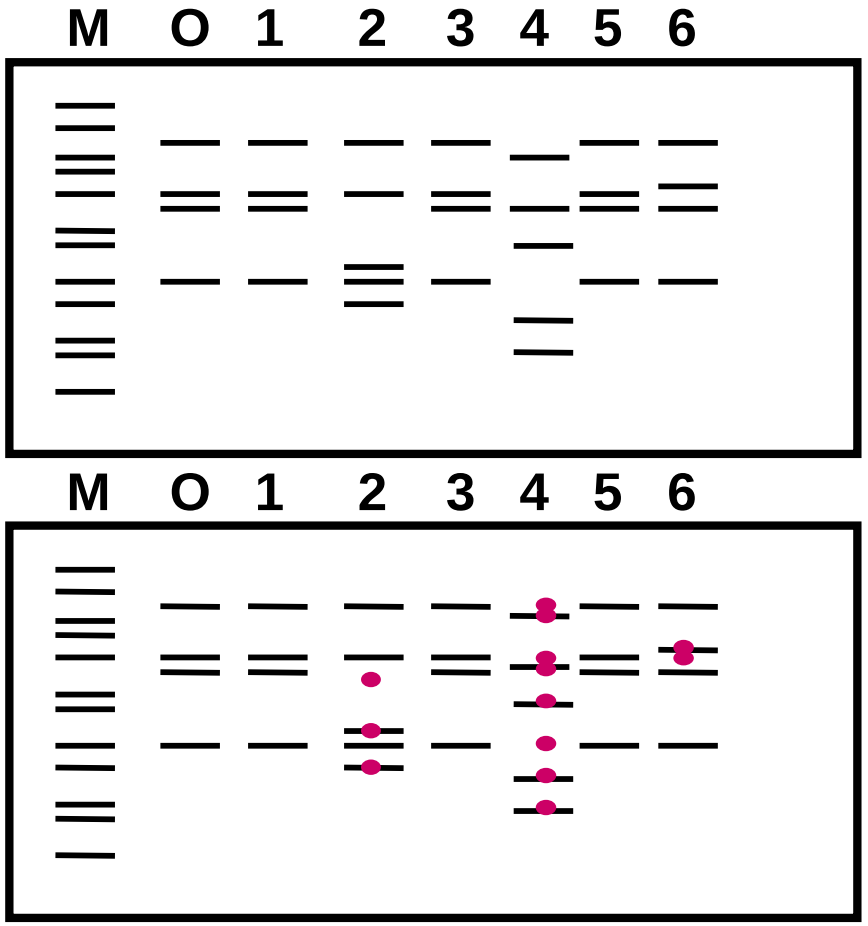
- Plasmid fingerprinting with restriction enzymes
- RFLP analysis
- Amplified Fragment Length Polymorphism (AFLP)
- Interspersed repetitive elements
- Ribotyping
- *spa* typing
- Multilocus sequence typing

Pulsed-field Gel Electrophoresis (PFGE)

O = Outbreak strain

1-6 = Isolates

● = Changes from outbreak strain

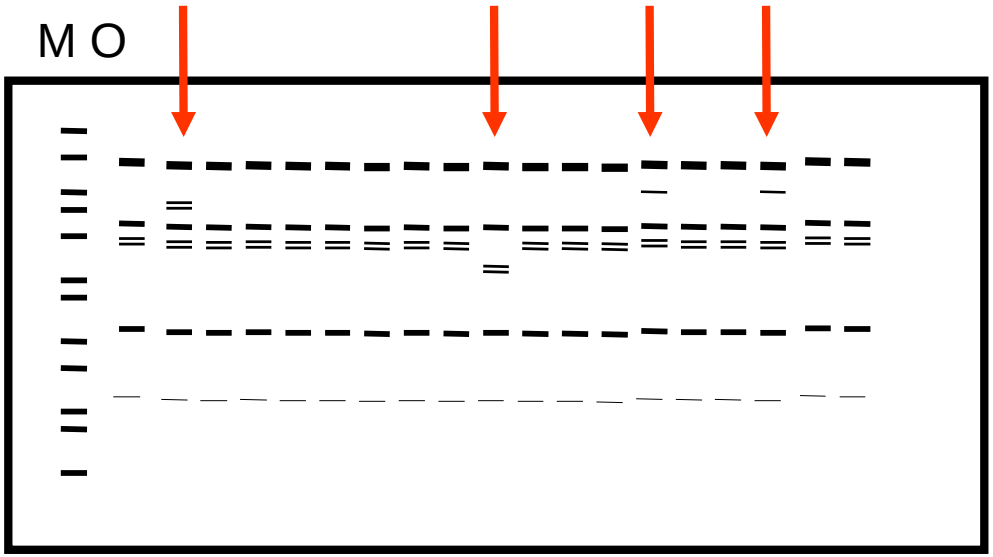


Criteria for PFGE Pattern Interpretation
Rule of Three

Category	Genetic differences*	Fragment differences*	Epidemiological interpretation
Indistinguishable	0	0	Test isolate is the same strain as the outbreak strain.
Closely related	1	2–3	Test isolate is closely related to the outbreak strain.
Possibly related	2	4–6	Test isolate is possibly related to the outbreak strain.
Different	≥3	≥6	Test isolate unrelated to the outbreak.

*Compared to the outbreak strain.

Arbitrarily Primed PCR: Random Amplification of Polymorphic DNA (RAPD)



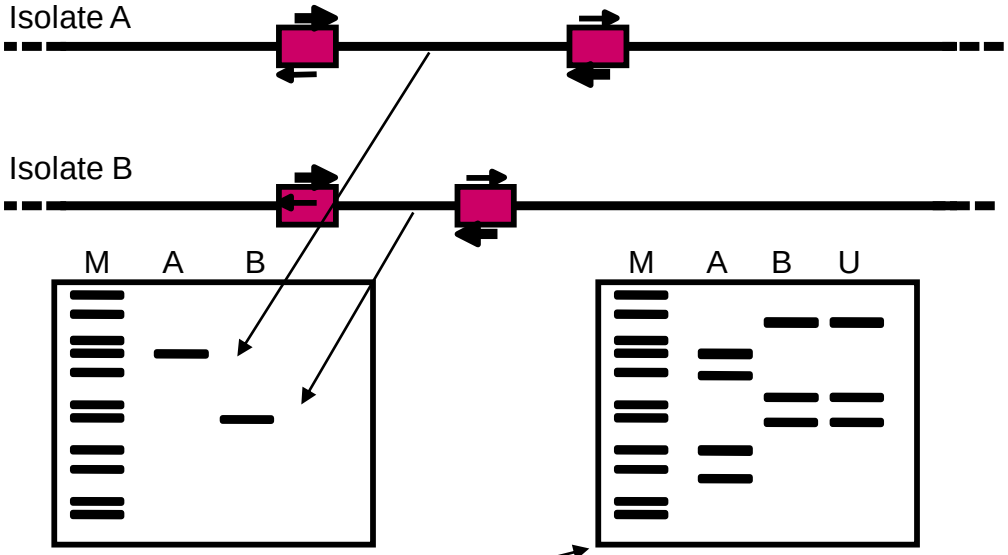
M = Molecular weight marker
O = Outbreak strain
Four isolates differ from the outbreak strain.

Interspersed Repetitive Elements

REP sequence inverted repeat

ERIC sequence inverted repeat

PCR amplification priming outward from repetitive elements generates strain-specific products.



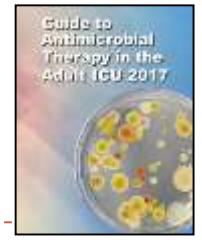
Is the unknown (U) strain A or B?

Comparison of Molecular Epidemiology Methods

Method	Typing capacity	Discriminatory Power	Reproducibility	Ease of use	Ease of interpretation
Plasmid analysis	Good	Good	Good	High	Good
PFGE	High	High	High	Moderate	Good moderate
Genomic RFLP	High	Good	Good	High	Moderate–poor
Ribotyping	High	High	High	Good	High
PCR-RFLP	Good	Moderate	Good	High	High
RAPD	High	High	Poor	High	Good–high
AFLP	High	High	Good	Moderate	High
Repetitive elements	Good	Good	High	High	High
Sequencing	High	High	High	Moderate	Good–high

Summary

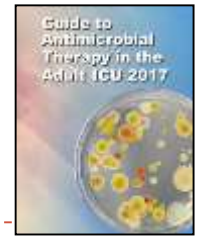
- Molecular-based methods offer sensitive and direct detection of microorganisms
- Due to high sensitivity and specificity, proper quality control is critical for molecular testing
- Several molecular methods are used to type bacterial strains in epidemiological investigations



Microbiological Investigations

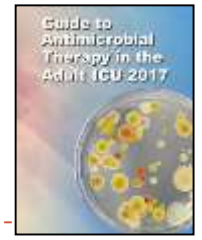
- Some common microbiological investigations that are relevant to the intensive care practice:
  - Blood specimen
    - All septic patients should have blood cultures taken prior to commencement of antimicrobials. Blood cultures must be taken with proper skin antisepsis to prevent contamination with skin commensals (*Corynebacterium* spp. and *Propionibacterium* spp.). Coagulase-negative *Staphylococcus* (CoNS) isolated from peripheral blood alone is usually a contaminant. The recommended skin antiseptic is 2% chlorhexidine in 70% isopropyl alcohol. A venipuncture is the preferred site and collection from an intravascular device is to be avoided unless for the purpose of diagnosing catheter-related bloodstream infection (CRBSI)
    - A minimum of 20 mls of blood should be drawn; 10 mls for each aerobic and anaerobic bottle. Increasing the volume to 40-60 mls from different venipuncture sites (obtaining 2-3 pairs of blood cultures) has been shown to increase the yield further.
    - If CRBSI is suspected simultaneous blood sampling from the peripheral blood and catheter hub need to be taken.
    - Blood cultures are routinely incubated for 5 days. Longer incubation times if HACEK organisms, Legionella, Brucella, Bartonella or Nocardia spp. are suspected. Incubation up to 4-6 weeks is needed for *M. tuberculosis*

Microbiological Investigations

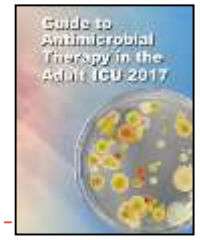


- Respiratory specimen
  - A good specimen of sputum or tracheal aspirate for Gram-stain and cultures should have less than10 epithelial cells per low power field reflecting a lower respiratory tract sample. Special stains can be requested to diagnose *Pneumocystis jiroveci* or *M. tuberculosis*.
  - Most laboratories report the results of sputum and tracheal aspirate cultures semiquantitatively (light, moderate or heavy growth). A positive culture does not differentiate true pathogens from colonisers. Results must be interpreted in the context of the clinical condition to prevent unnecessary antimicrobial use
  - Nasopharyngeal swabs and the above respiratory specimens can be sent using appropriate viral transport media for viral serology and PCR tests. Viral cultures are not routinely performed. The viruses commonly investigated are influenza, parainfluenza, respiratory syncytial virus, adenovirus, CMV and measles
  - Of note, some respiratory infections can be diagnosed with urinary specimens e.g. *Legionella* Serotype 1 (legionella urinary antigen test) and pneumococcal infections (pneumococcal urinary antigen test)

Microbiological Investigations



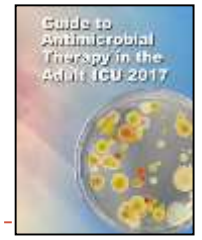
- Pleural fluid specimen
  - In diseased conditions, pleural fluid can be classified into exudate or transudate. According to Light's criteria, pleural effusion is likely to be an exudate if at least one of the following exists:
    - Pleural fluid/serum protein >0.5 or absolute pleura protein >30 g/L
    - Pleural fluid/serum LDH >0.6
    - Pleural fluid LDH level > 2/3 upper limit of normal serum value
  - A parapneumonic effusion is an exudative pleural effusion formed secondary to pneumonia (bacterial or viral) or lung abscess, with a predominance of neutrophils



Microbiological Investigations

Characteristics of parapneumonic effusions

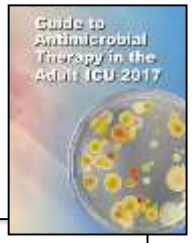
Characteristics	Normal	Parapneumonic effusion		
		Uncomplicated	Complicated	Empyema
Appearance	clear	Clear, slightly turbid	Cloudy	Pus
Biochemistry				
pH	7.60 - 7.64	> 7.30	< 7.20	NA
Glucose, mmol/L	similar to plasma	> 3.3	< 2.2	
Ratio of pleural fluid to serum glucose	1.0	> 0.5	< 0.5	NA
Lactate dehydrogenase, U/L	< 50% of plasma	< 700	> 1000	NA
Polymorphonuclear count, cells/mL	< 1000 leucocytes/mm^3	< 15,000	> 125,000	NA
Microbiological test result	-	Negative	May be positive	May be positive
Treatment	-	Antibiotics	Antibiotics and drainage	Antibiotics and drainage



Microbiological Investigations

- Cerebrospinal fluid specimen
  - Lumbar puncture should only be performed after a neurological examination but should never delay the administration of antimicrobials
  - Cerebrospinal fluid (CSF) should be analysed within an hour of collection. If there is a delay, it should be stored between 4-8°C. Do not allow CSF to sediment before partitioning into separate tubes
  - The standard for the diagnosis of bacterial meningitis is CSF Gram stain and culture. Gram stain has sensitivity between 60-90%, provided the patient has not received antibiotics prior to lumbar puncture

CSF analysis	**Minimum volume (ml)** (may vary from lab to lab)
Microscopy and stain (Gram, Indian ink and Ziehl-Neelsen)	1
Biochemistry	1
Culture and sensitivity (aerobic and anaerobic)	2
Latex agglutination test: *Streptococcus pneumoniae*, group B streptococcus, *Haemophilus influenzae* type B, *Neisseria meningitidis* group A, B, C, Y and W135, *Escherichia coli* K1, *Listeria monocytogenes*	1
Viral: PCR and/or serology Herpes simplex type 1 & 2, varicella zoster virus, Japanese B encephalitis virus, cytomegalovirus, Epstein-Barr virus, Nipah virus, human herpesvirus 6, enterovirus, human parechovirus	3
Parasite PCR *Toxoplasma gondii*	3
Mycobacterium tuberculosis PCR and culture	10
Fungal antigen and culture *Aspergillus fumigatus, Cryptococcus neoformans*	3

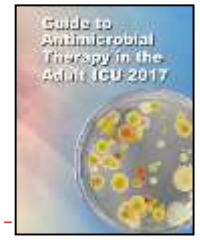


Microbiological Investigations

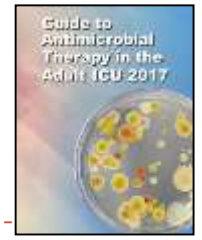
Characteristics of CSF in CNS infections

	Normal	Bacterial meningitis	Viral meningitis/ encephalitis	Tuberculous meningitis	Fungal meningitis	Meningitis or ventriculitis in the presence of drains or shunts
Pressure, cmH_2O	10-20	> 30	N or ↑	↑	↑	-
Appearance	Clear	Turbid	Clear	Fibrin web	Clear or turbid	Clear or turbid
Protein, g/L	0.18-0.45	> 1.0	N or ↑	1.0-5.0	0.2-5.0	N or ↑
Glucose, mmol/L	2.5-3.5	< 2.2	N or ↓	1.6-2.5	↓	↓
CSF:serum glucose ratio	0.6	< 0.4	> 0.6	< 0.5	< 0.5	< 0.5
Lactate, mmol/L	< 2.9	↑↑	N	↑	↑↑	↑
Cell count/mm^3 (predominant cell type)	0-5 lymphocytes (70%) and monocytes (30%)	> 1000 polymorphs	5-1000 lymphocytes and monocytes	< 500 lymphocytes	10-500 lymphocytes	> 15 polymorphs WBC: RBC ratio is less than 1:100 (normal 1:500)
Notes		Partial treatment with antibiotics may alter CSF parameters. Neutropenics may not have characteristic polymorph responses in the CSF.	Neutrophils may predominate early in the illness.			Cell count index > 1 (ratio WBC: RBC in CSF to blood) Positive CSF culture may represent contaminant and clinical correlation is needed.

Microbiological Investigations



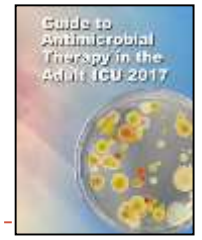
- Urine specimen
  - Urine collection must be taken under aseptic technique to minimise the degree of bacterial contamination
  - The sample should be sent within an hour of collection since bacteria will continue to proliferate. Urine samples not sent immediately should be stored at 4°C, however this may affect leukocyte counts
  - If the patient needs catheterisation, discard the first few mls of urine and collect the rest in the sterile container
  - If the patient is already catheterised, clamp the catheter and clean the sampling port with 70% alcohol and collect a 10 mls sample of urine
  - Do not take urine samples from the drainage bag due to high risk of bacterial overgrowth leading to false positive results
  - In and out catheterisation for urine samples in an uncatheterised patient can be done
  - In patients on long-term catheters, replace the catheter before collecting specimens



Microbiological Investigations

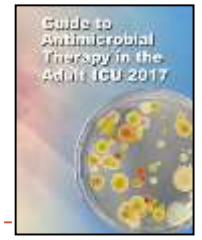
- Most cases of urinary tract infection (UTI) can be diagnosed using the criteria below. Catheter-associated UTI is the presence of bacteriuria in a catheterized patient (≥48 hours) who has signs and symptoms that are consistent with UTI.
- Pyuria is common in catheterised patient and it has no predictive value.

	Symptom	Bacteriuria cfu/mL	Pyuria WBC/mm^3	No. of species	Nitrite	Comments
With catheter	Present	≥ 10^3	Pyuria is common in patients with catheters. Its level has no predictive value.	≤ 2	undetected	Treat as UTI. Replace catheter if in place for > 7 days.
	Absent	Routine urine culture in asymptomatic catheterised patient is not recommended. Asymptomatic significant bacteriuria: - a single specimen ≥ 10^5 cfu/mL - specimen collected by in and out catheter ≥ 10^2 cfu/mL				Treat asymptomatic significant bacteriuria in • pregnancy • prior to genitourinary manipulation • post renal transplant • neutropenics



Microbiological Investigations

	Symptom	Bacteriuria cfu/mL	Pyuria WBC/mm^3	No. of species	Nitrite	Comments
Without catheter	Present	≥ 10^3 in pregnant women and acute uncomplicated cystitis in women ≥10^4 in acute uncomplicated pyelonephritis in women. ≥ 10^4 in complicated UTI in men ≥ 10^5 in complicated UTI in women	> 10	≤ 2	detected (only positive in nitrite producing bacteria e.g. *E. coli, Serratia spp, Klebsiella spp and Proteus spp*)	Treat as UTI For definition of complicated and uncomplicated UTI refer to the chapter on genitourinary tract infection.
	Absent	Asymptomatic significant bacteriuria if 2 consecutive (> 24h apart) mid-stream urine grows ≥ 10^5 cfu/ml of the same bacterial species in women and ≥ 10^3 cfu/ml in men.				Treat asymptomatic significant bacteriuria in • pregnancy • prior to genitourinary manipulation • post renal transplant • neutropenics • urinary obstruction or abnormal genitourinary tract



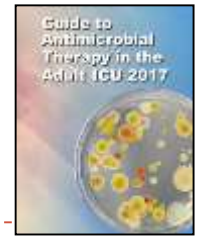
Microbiological Investigations

- Peritoneal fluid specimen
  - Analysis of peritoneal fluid obtained through paracentesis should be carried out to determine if there is presence of ascitic fluid infection in septic patients with ascites. Do not take specimens for culture from *in-situ* abdominal drains due to risk of contamination
  - The decision to begin early empirical antibiotic treatment of suspected ascitic fluid infection is based largely on the absolute neutrophil count rather than the culture, which takes 24-48hours to demonstrate growth

Characteristics of ascitic fluid infections

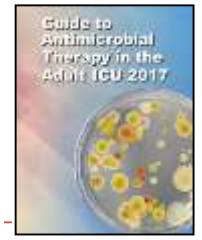
	Polymorphs count (/mm³)	Bacterial culture	Glucose mmol/L	Protein g/dL	LDH IU/L	Treatment	Notes
Spontaneous bacterial peritonitis (SBP)	≥ 250	Positive (usually 1 type of organism) Poor yield for Gram-stain	> 2.7	< 1.0	< 225	Antibiotics	Inoculate peritoneal fluid into blood culture bottles at bedside to improve sensitivity.
Culture negative neutrocytic ascites	> 250	Negative	NA	NA	NA	Treat as SBP	Causes include: prior antibiotics, peritoneal carcinomatosis, pancreatitis, tuberculous peritonitis

Microbiological Investigations



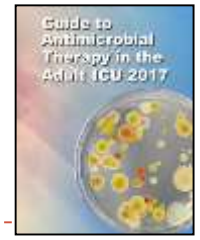
	Polymorphs count (/mm3)	Bacterial culture	Glucose mmol/L	Protein g/dL	LDH IU/L	Treatment	Notes
Monomicrobial non-neutrocytic bacteriascites	≤ 250	Positive (1 type of organism)	NA	NA	NA	Treat as SBP in presence of sepsis	May be early stage of SBP. In asymptomatic patients, repeat paracentesis.
Polymicrobial bacteriascites	< 250	Positive (polymicrobial)	NA	NA	NA	Antibiotics if develops peritonitis	Usually due to inadvertent puncture of the intestines during paracentesis.
Secondary bacterial peritonitis	> 250 (> 10,000 WBC/ml)	Positive (polymicrobial)	< 2.7	> 1.0	> 225	Antibiotics and source control	
Tuberculous peritonitis	150-4000 WBC/ml (>70% lymphocytes)	-	Lower than serum	> 2.5 (SAAG < 1.1)	> 90	-	Acid-fast bacilli - Ziehl-Neelsen stain is positive in only 3% of cases.

Microbiological Investigations

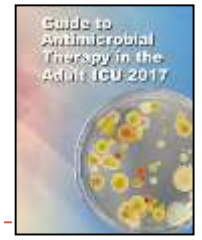


- Stool specimen
  - Stool culture should not be done routinely in all patients presenting with diarrhoea unless in the immunocompromised, elderly, those with underlying inflammatory bowel disease and with severe or bloody diarrhoea
  - Three specimens should be sent on consecutive days since parasite excretion may be intermittent
  - At least 5 mls of diarrheal stool per rectal or per stoma is collected in a clean leak-proof container. The specimen should be transported to the laboratory and processed as soon as possible after collection
  - Culture of a single stool specimen has a sensitivity of >95% for detection of the enteric bacterial pathogen. A negativeculture for *Salmonella*, *Campylobacter* and *Shigella* usually rules out infection by these organisms as excretion of these pathogens is continuous. Repeat specimens are not required
  - Stools should be sent for *Clostridium difficile* toxin assay for patients who develop new onset of diarrhoea while in hospital

Wound swabs



- Wound infections should be diagnosed clinically.
- Chronic wounds have colonized microorganisms but this does not necessarily mean that the wound is infected.
- Wounds should only be cultured when signs and symptoms of a deep infection are present.
- Culturing uninfected wounds may only be used as part of an infection control surveillance protocol.



Wound swabs

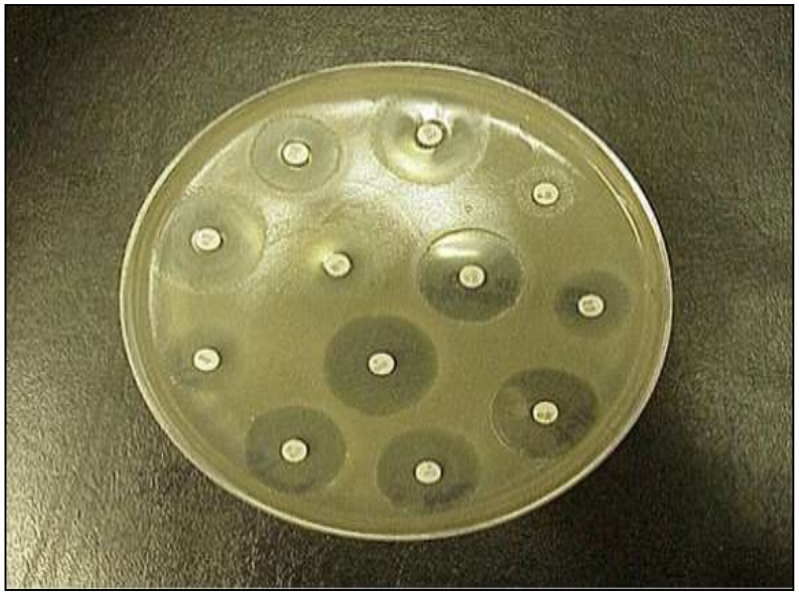
- Wound culture and susceptibility testing may be done using a swab, tissue biopsy or needle aspiration
  - Needle aspiration and tissue biopsy are preferred methods of specimen collection, however swab cultures are acceptable as they are practical, non-invasive and cost effective. Wound infection occurs in viable wound tissue; therefore viable wound tissue must be swabbed rather than necrotic tissue or pus. At least 1cm 2 area of viable tissue is required
  - To obtain wound swabs, clean the wound with sterile saline to irrigate purulent debris and ensure that skin around the wound is cleaned. Moisten the swab with sterile saline to increase the adherence of bacteria. Rotate the swab while moving it across the entire wound in a zigzag manner. Alternatively Levine's technique can be used where one rotates the swab over 1cm2 of the cleansed wound exerting enough pressure to express exudates from within the tissue

Antibiotic Sensitivity

- Antibiotic sensitivity is a term used to describe the susceptibility of bacteria to antibiotics
- Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*
  - AST is often done by the Kirby-Bauer method (disc-diffusion method)
  - The E-test (also based on antibiotic diffusion)
  - Agar and Broth dilution methods for MIC determination

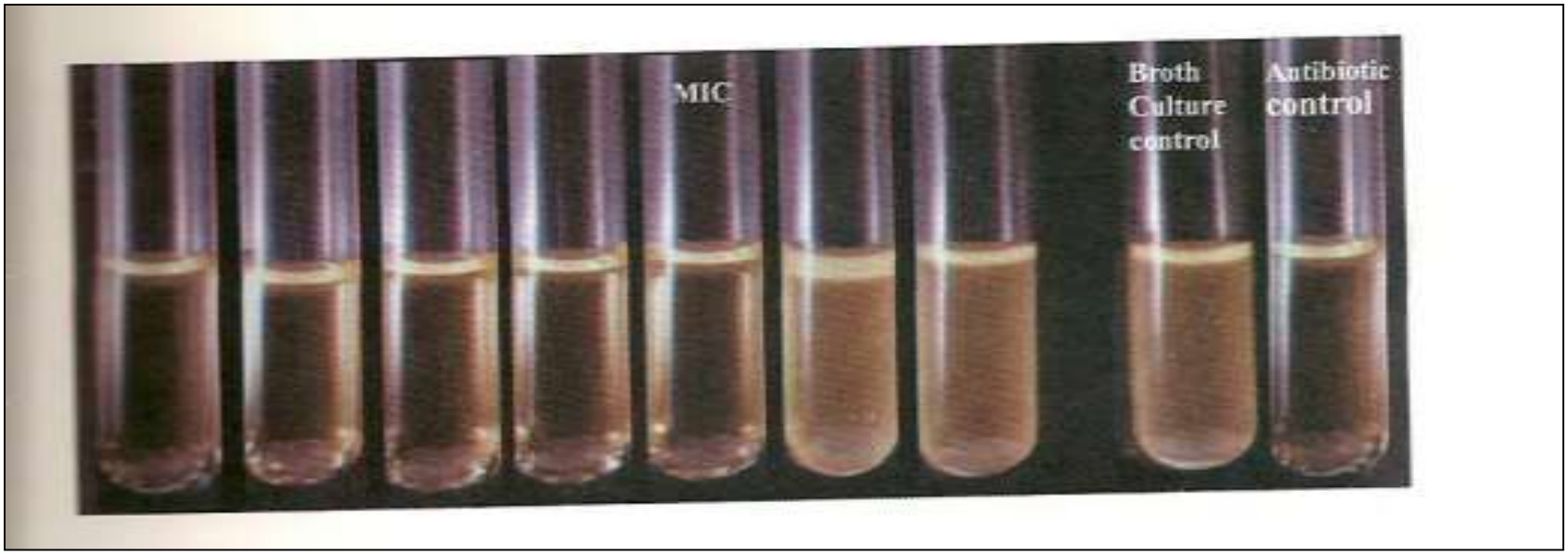
Antibiotic Sensitivity
AST

- Different antibiotics have been placed on an agar plate growing bacteria and the plate is incubated
  - The degree of inhibition of growth around the discs is measured
- The more zone of inhibition, the more the sensitivity to the antibiotics

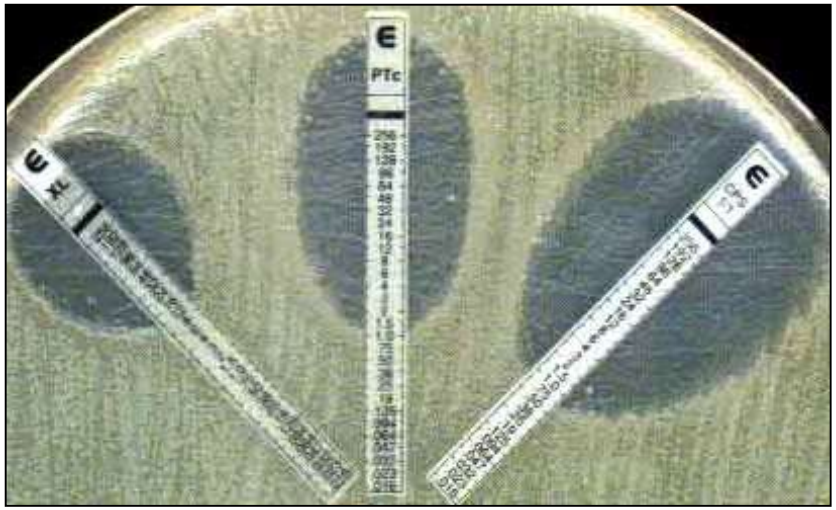


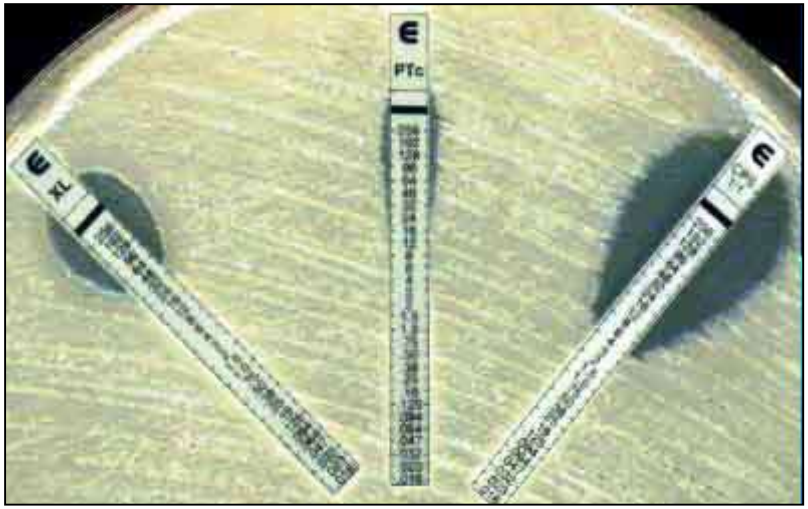
Antibiotic Sensitivity
The Dilution Method

- Serial dilutions of antibiotics are incorporated in agar containing or broth culture media
- The MBC may be determined in broth dilution tests by subculturing the containers that show no growth on to antibiotic-free agar containing media



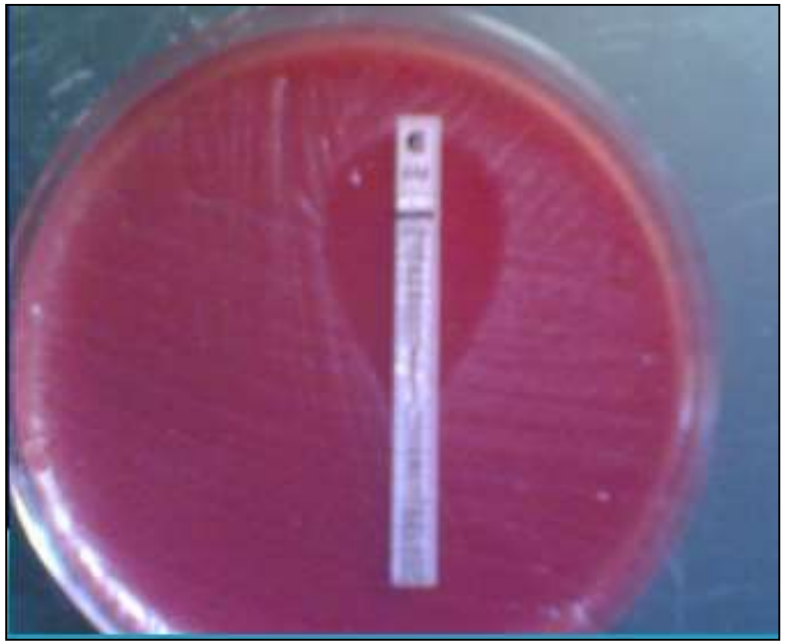
E-test





E-test

E-Test for exopigment producing organism



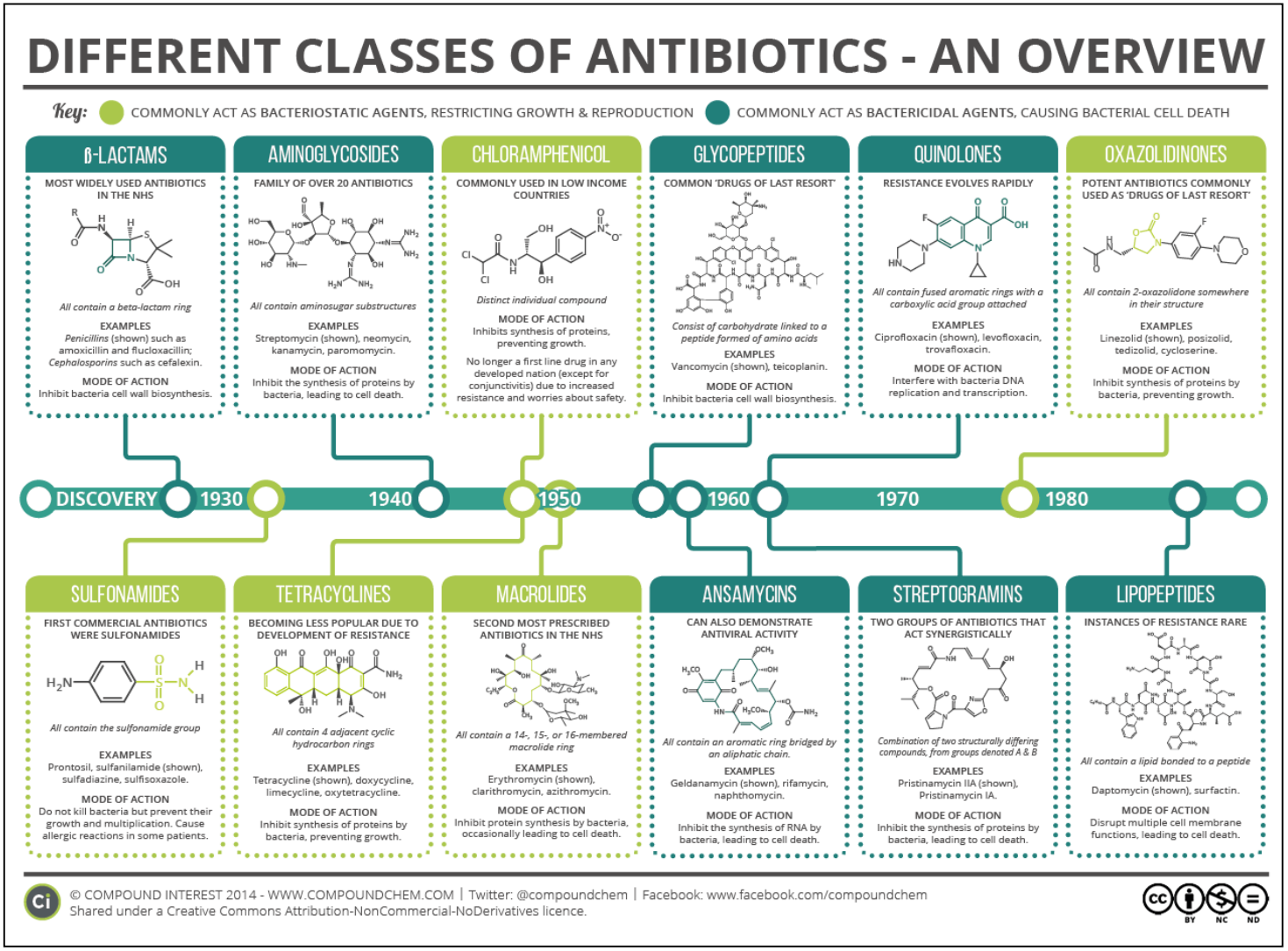
E-Test for fastidious microorganism

Highly Resistant Bacteria

Antimicrobial Drugs

- **Antibiotic**. This term was originally coined to refer to only those compounds produced by microorganisms and capable of inhibiting bacterial growth (*Waksman & Woodruff 1940*)
- However the term “antibiotic” is now commonly used to refer to any drug (natural or synthetic) that is used for treating bacterial infections

Antimicrobial Drugs



Antibiotic producing microbes

TABLE 20.1 Representative Sources of Antibiotics

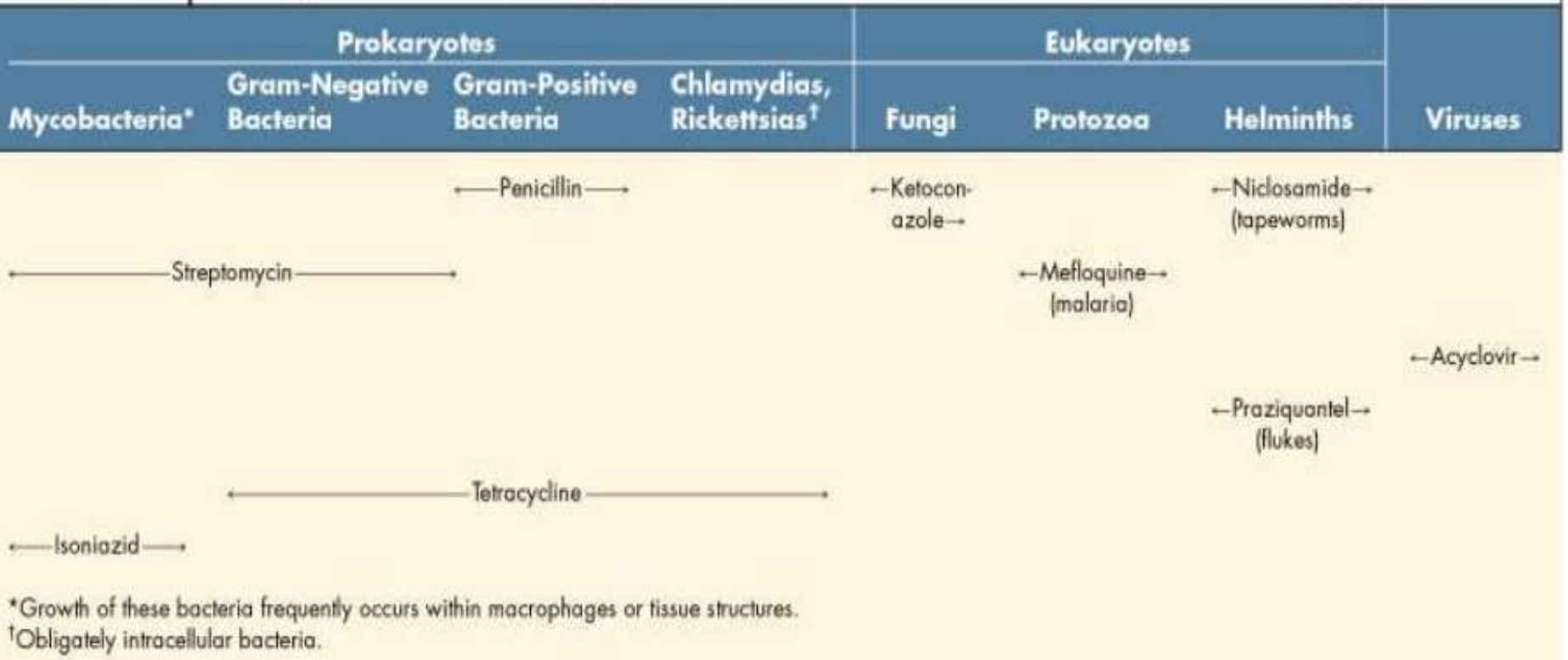
Microorganism	Antibiotic
Gram-Positive Rods	
Bacillus subtilis	Bacitracin
Bacillus polymyxa	Polymyxin
Actinomycetes	
Streptomyces nodosus	Amphotericin B
Streptomyces venezuelae	Chloramphenicol
Streptomyces aureofaciens	Chlortetracycline and tetracycline
Streptomyces erythraeus	Erythromycin
Streptomyces fradiae	Neomycin
Streptomyces griseus	Streptomycin
Micromonospora purpureae	Gentamicin
Fungi	
Cephalosporium spp.	Cephalothin
Penicillium griseofulvum	Griseofulvin
Penicillium notatum	Penicillin

Antibiotic Spectrum of Activity

- Narrow-spectrum
  - Effective against a subset of bacteria (either gram positive and negative)
  - e. g. Isoniazid (Mycobacteria only)
- Extended-spectrum
  - Effective against gram-positive organisms and a significant number of gram-negative orgaisms
  - e.g. Ampicillin
- Broad-spectrum
  - Effective against many different types of bacteria (e.g. both gram positive and negative)
  - e. g. Tetracylin & Chloramphenicol
  - Can alter the nature of intestinal flora = super infection

Antibiotic Spectrum of Activity

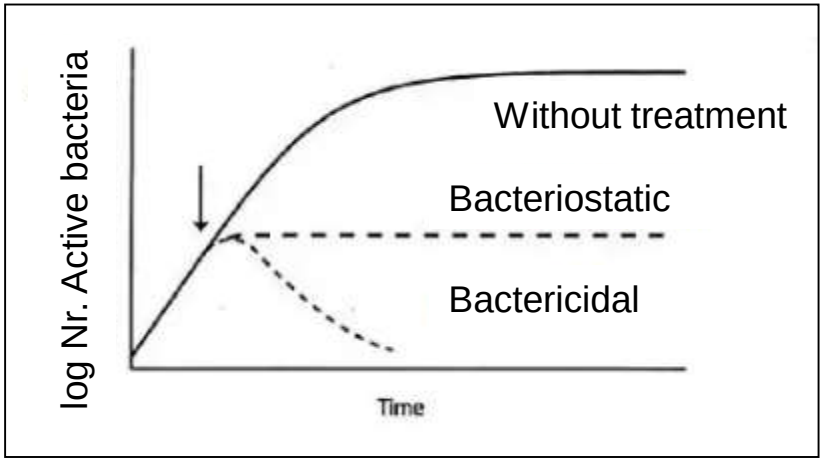
TABLE 20.2 The Spectrum of Activity of Antibiotics and Other Antimicrobial Drugs



*Growth of these bacteria frequently occurs within macrophages or tissue structures.
†Obligately intracellular bacteria.

Mechanisms of Antibiotic Action

- Bacteriostatic
  - Kill bacteria directly



- Penicillinis
- Cephalosporins
- Fluorquinolone
- Glycopeptides
- Monobactams
- Carbapenems

- Bactericidal
  - Prevent bacteria from growing

- Tetracyclines
- Spectinomycin
- Sulphonamides
- Marolides
- Chloramphenicol
- Trimethoprim

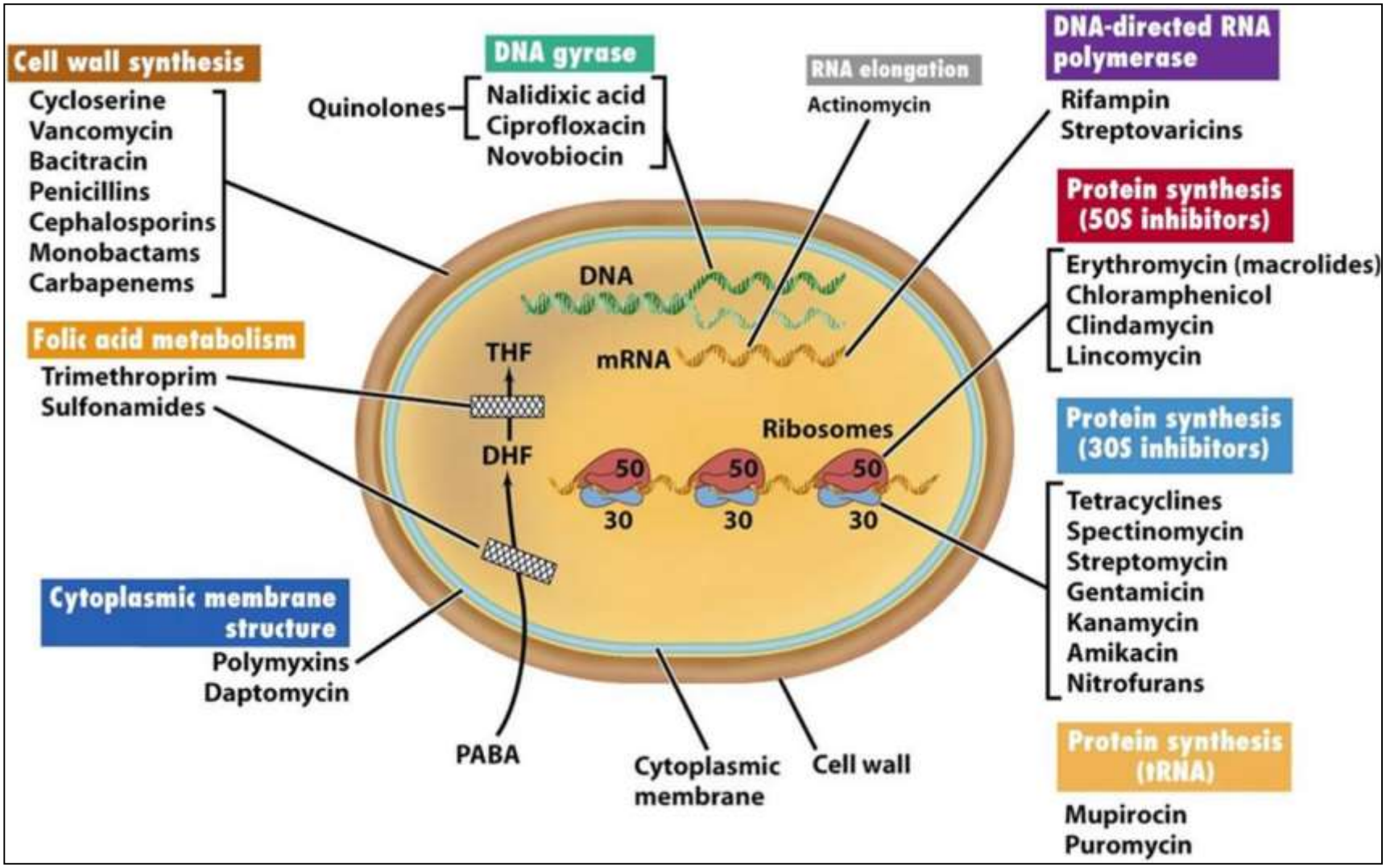
- Growth on bio-films can dramatically reduce the effectiveness of antibiotic therapy

Mechanisms of Antibiotic Action

- Bacteria have their own enzymes for:
  - Cell wall formation
  - Protein synthesis
  - DNA replication
  - RNA synthesis
  - Synthesis of essential metabolites

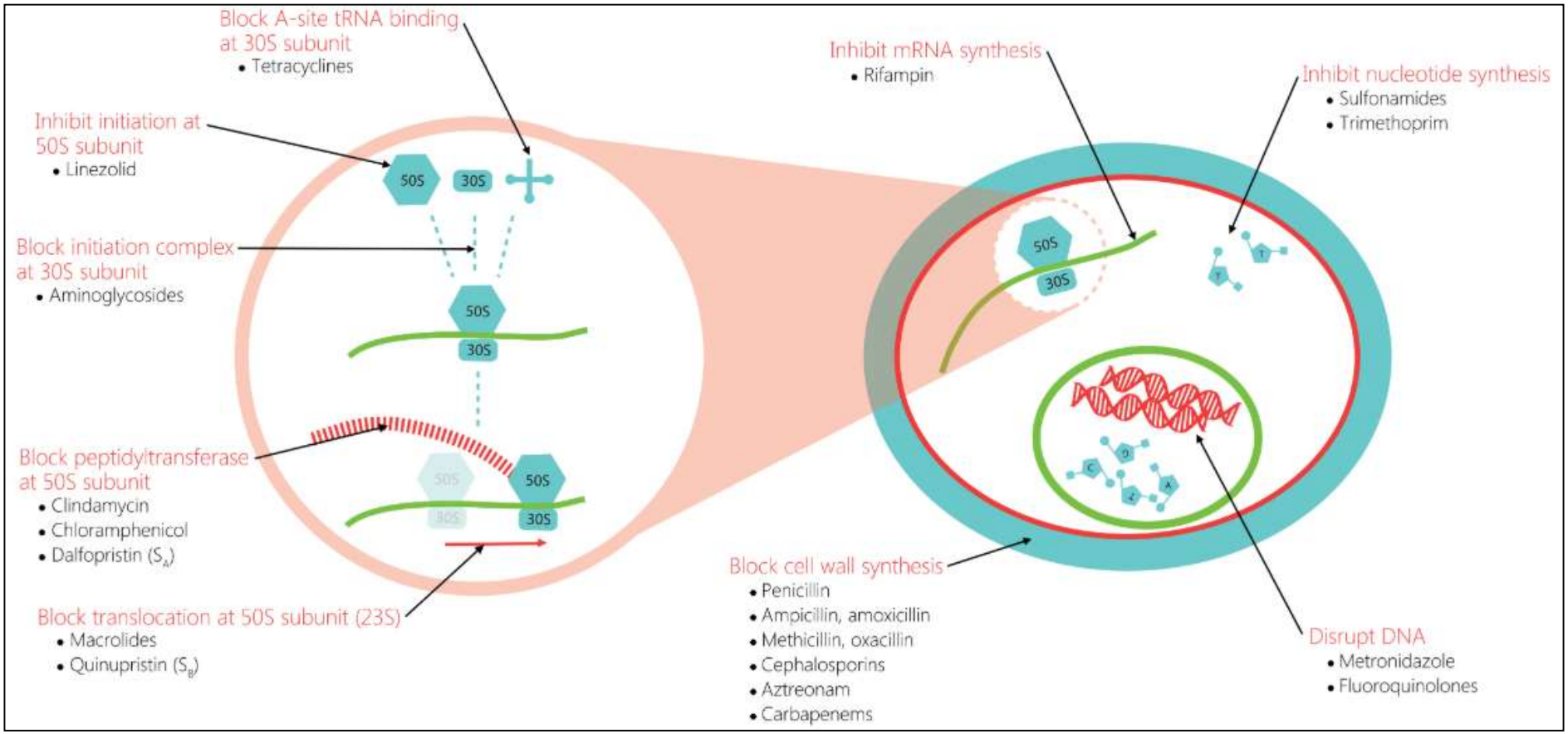
The more similar the pathogen and host enzymes, the more side effects the antibiotics will have

Mechanisms of Antibiotic Action



- Antibiotic target sites (Madigan and Martinko, 2006)

Mechanisms of Antibiotic Action

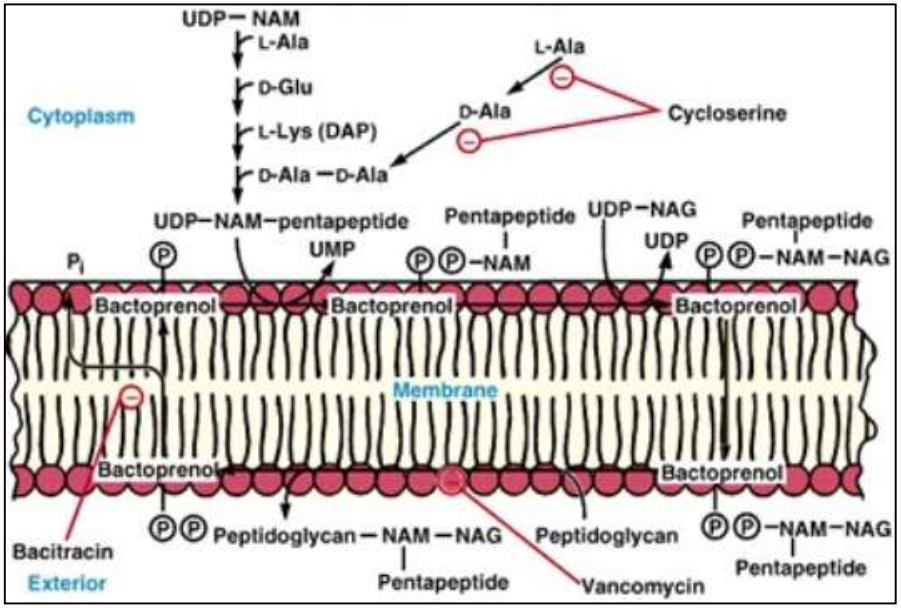


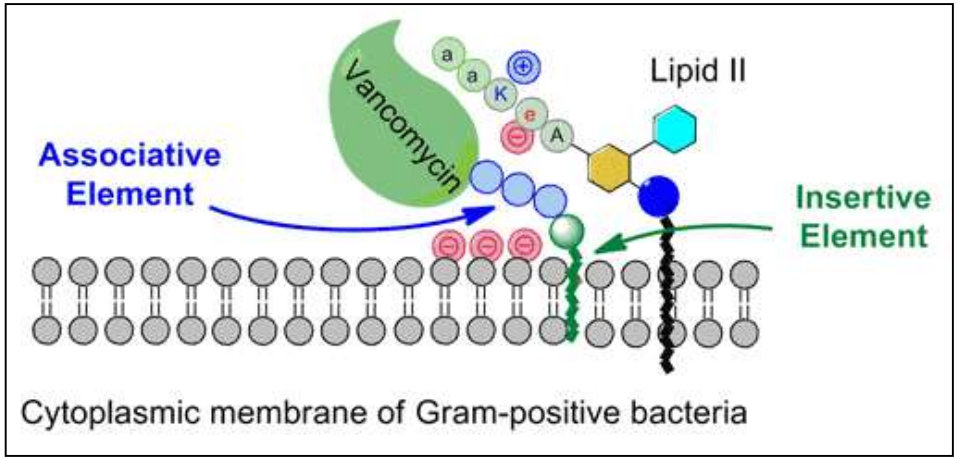
De sarro A, De sarro GB, Ascioti C, Nisticó G. Epileptogenic activity of some beta-lactam derivatives: structure-activity relationship. Neuropharmacology. 1989;28(4):359-65.

Rodloff AC, Goldstein EJ, Torres A. Two decades of imipenem therapy. J Antimicrob Chemother. 2006;58(5):916-29.

Mechanisms of Antibiotic Action

- **Inhibition of cell wall synthesis – interfere with peptide <u>glycan synthesis</u>**
  - Result in **cell lysis**
  - **Low toxicity**



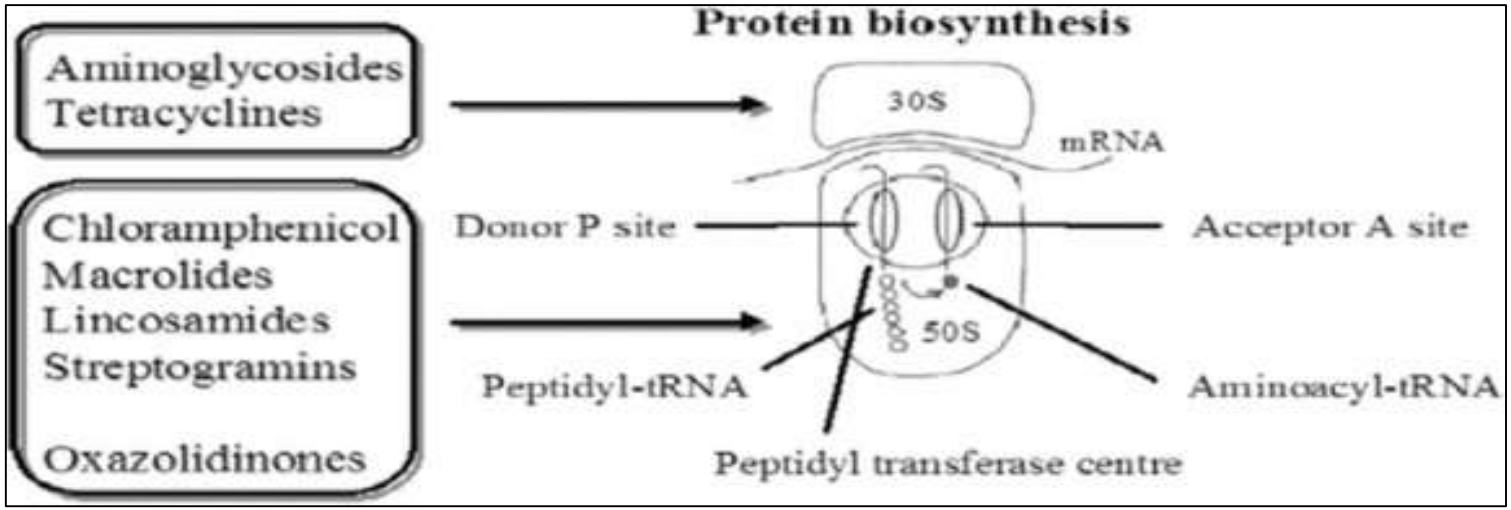


e.g. **penicillin** and **vancomycin**
Other antibiotics: cycloserine, bacitracin

1. Boucher, HW et al., *Clin. Infect. Dis.* **2009**, 48,1
2. Hamad, B., *Nat. Rev. Drug Discov.* **2010**, 9, 675

Mechanisms of Antibiotic Action

- **Inhibition of protein synthesis – interfere with procaryotic (70S) ribosomes, also found in mitochondria**
  - Most have broad spectrum of activity

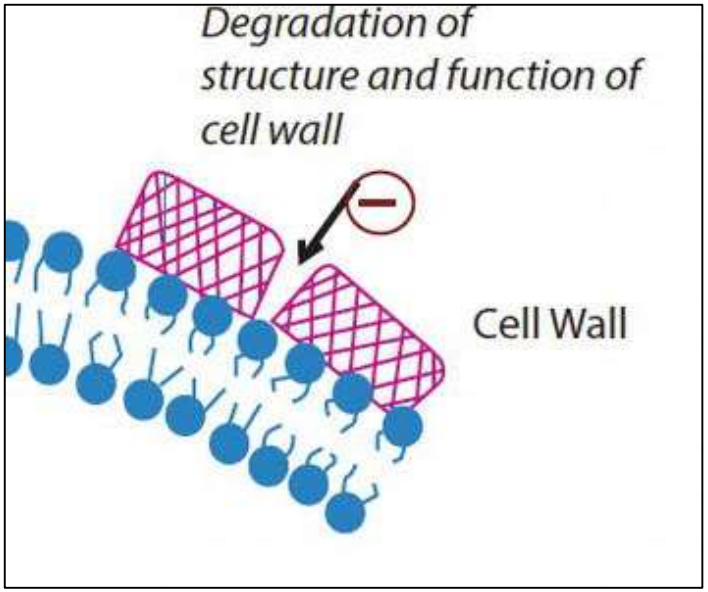


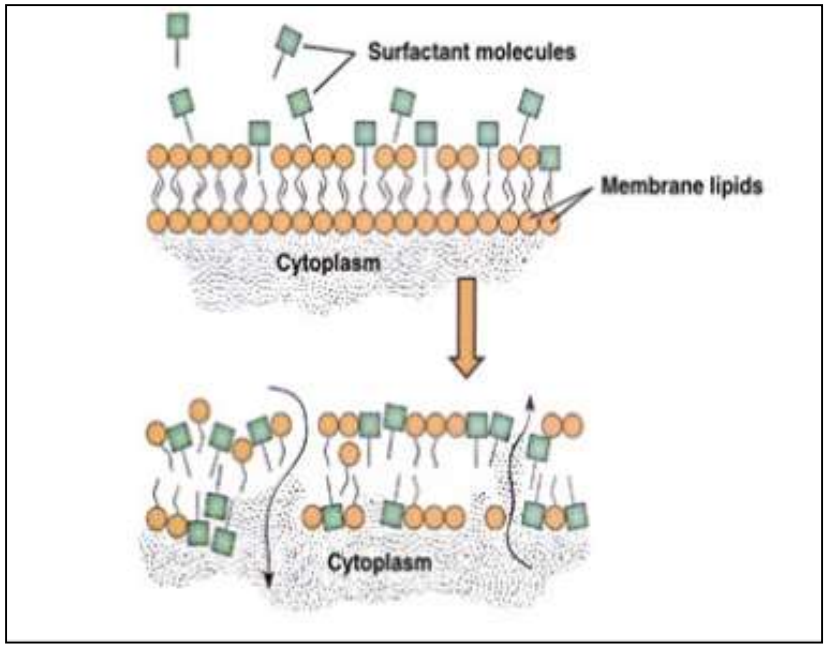
Reversible inhibition (bacteriostatic)	
Chloramphenicol	Tetracyclines
Macrolides (Erythromycin)	Clindamycin
Linezolid	Streptogramins
Irreversible inhibition	
The bactericidal aminoglycosides	

Kapoor G., Saigal S., Elongavan A. Action and resistance mechanisms of antibiotics: A guide for clinicians. JOACP, 2017, Vol. 33, Nr 3, 300-305

Mechanisms of Antibiotic Action

- **Injury to the plasma membrane (disruption of cell membranes) – cause changes in membrane permeability**
  - Result in loss of metabolites and/or cell lysis
  - Many polypeptide antibiotics



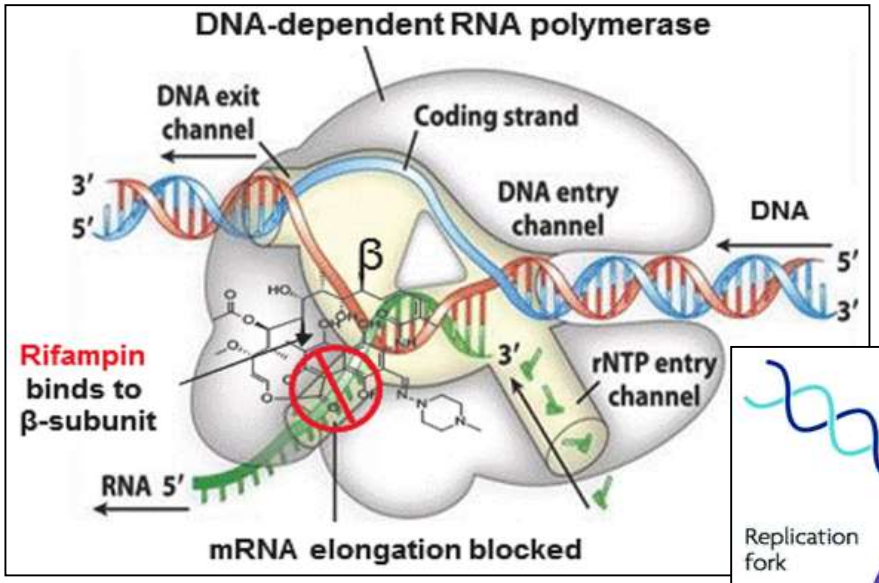


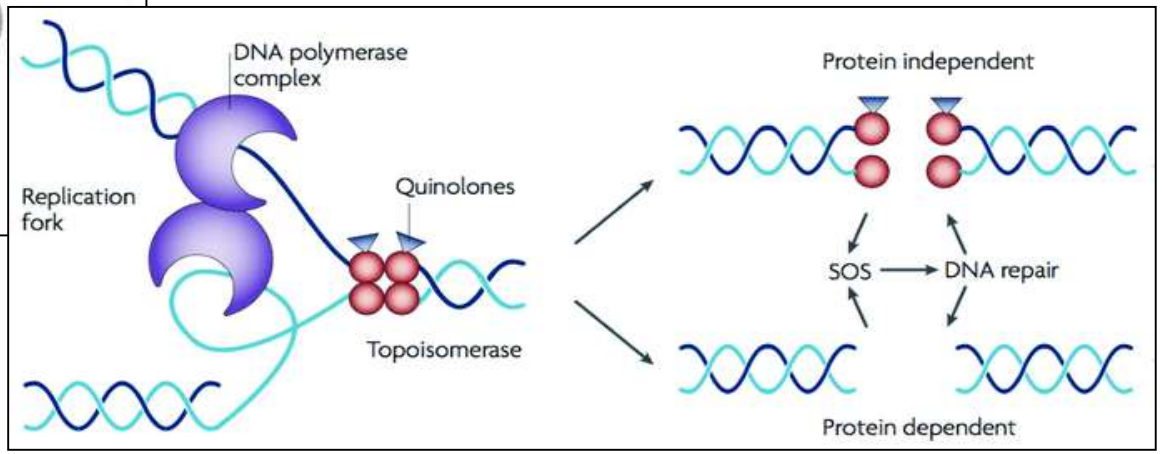
e. g. polymyxin B, Polyenes (acid fungal agents)

Kırmusaoğlu S., Gareayaghi N.and Kocazeybek B.S. Introductory Chapter: The Action Mechanisms of Antibiotics and Antibiotic Resistance, 02/27/2019 DOI: 10.5772/intechopen.85211

Mechanisms of Antibiotic Action

- **Inhibition of Nucleic Acid (DNA/RNA) synthesis – interfere with DNA replication and transcription**
  - May be toxic to human cells



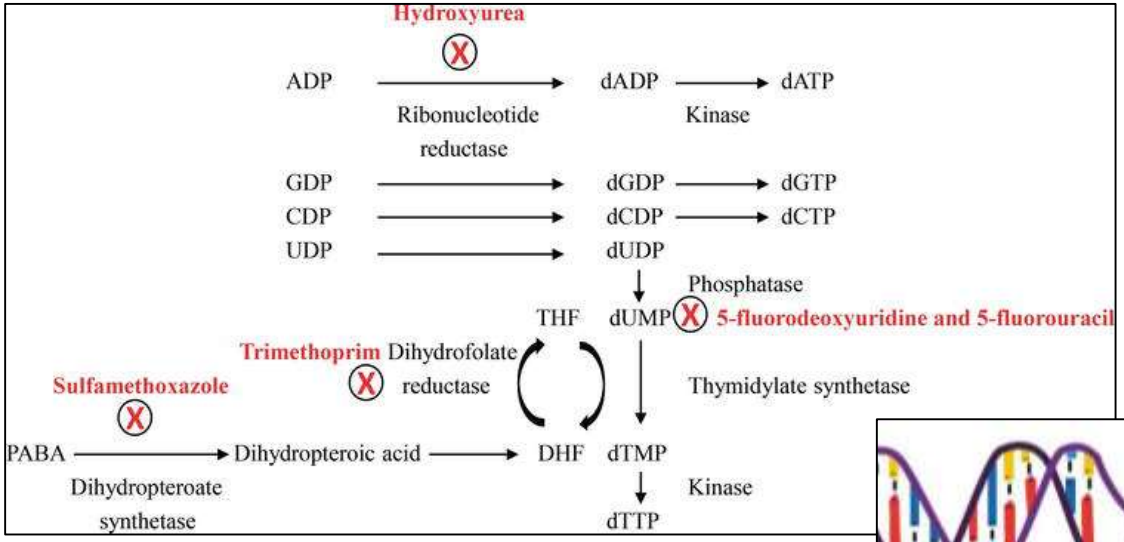


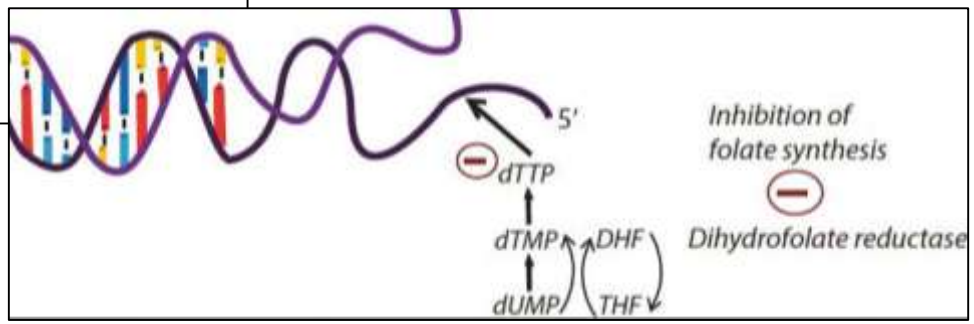
e. g. rifampin (RNA-polymerase) and quinolones (DNA-gyrase)

1. Modified from Figure 15-14, Molecular Biology: Principles and Practice, 2012. W.H. Freeman and Co.
2. Adapted from Kohanski et al 2010 Nature Review Microbiology.

Mechanisms of Antibiotic Action

- **Inhibition of synthesis of <u>essential metabolites</u> – involve <u>competitive inhibition</u> of <u>key enzeme</u>**
  - Closely <u>resemble substrate</u> of <u>enzyme</u>





e.g. <u>trimethoprim</u> and <u>sulfonamides</u>

Kırmusaoğlu S., Gareayaghi N.and Kocazeybek B.S. Introductory Chapter: The Action Mechanisms of Antibiotics and Antibiotic Resistance, 02/27/2019 DOI: 10.5772/intechopen.85211

Selection of Antimicrobial Agent

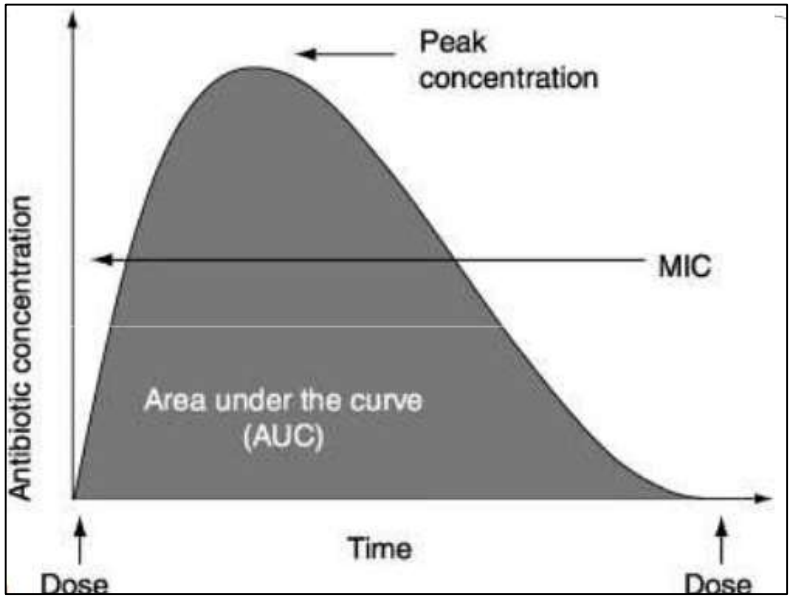
- **Empiric therapy** – prior to identification of organism – critically ill patients
- **Organism's susceptibility** to the antibiotic
- **Patient factors** – immune system, renal/hepatic function
- Effect of **site of infection** on therapy – blood brain barrier
- **Safety** of the agent
- **Cost** of therapy

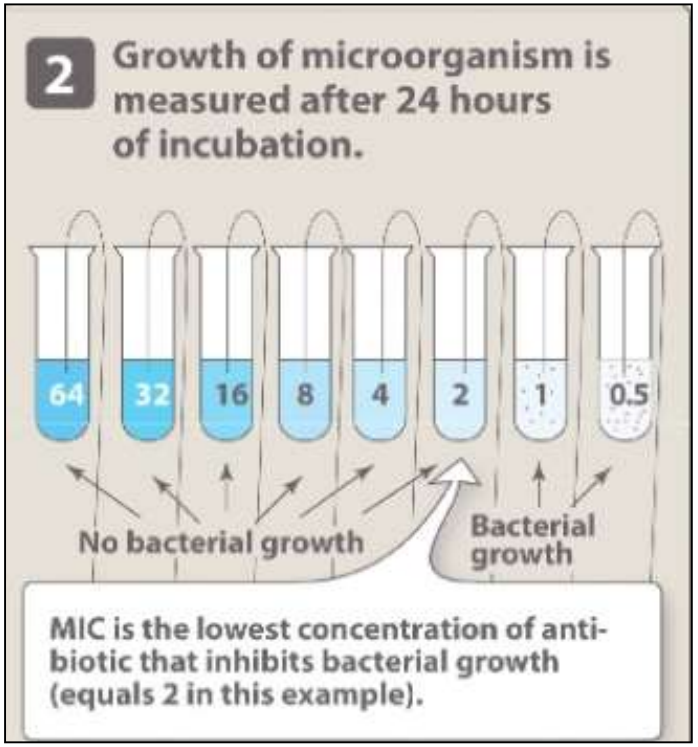
Properties Influencing Frequency of Dosing

- **Concentration dependent killing** - antibiotics including aminoglycosides = significant increase in rate of bacterial killing as the drug concentration increases
- **Time-dependent killing** – β-lactams, glycopeptides, macrolides, clindamycin & linezolid – dependent on the % of time that blood concentrations remain above minimum inhibitory concentration (MIC)
- **Post antibiotic effect (PAE)** - persistent suppresion of microbial growth after levels of antibiotic have fallen below MIC
  - **Antibiotic with a long PAE – aminoglycosides and fluoroquinolones**

Properties Influencing Frequency of Dosing

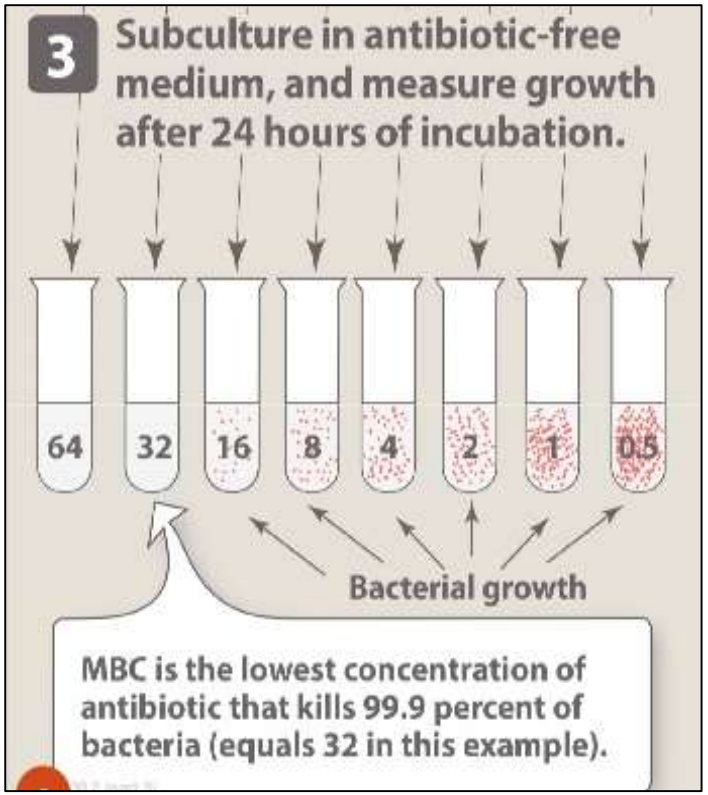
- Minimum inhibitory concentration (MIC)
  - The lowest concentration that inhibits the growth of bacterial population





Properties Influencing Frequency of Dosing

- Minimum bactericidal concentration (MBC)
  - The lowest concentration that kills 99.9% of the bacterial population



Antimicrobial Stewardship

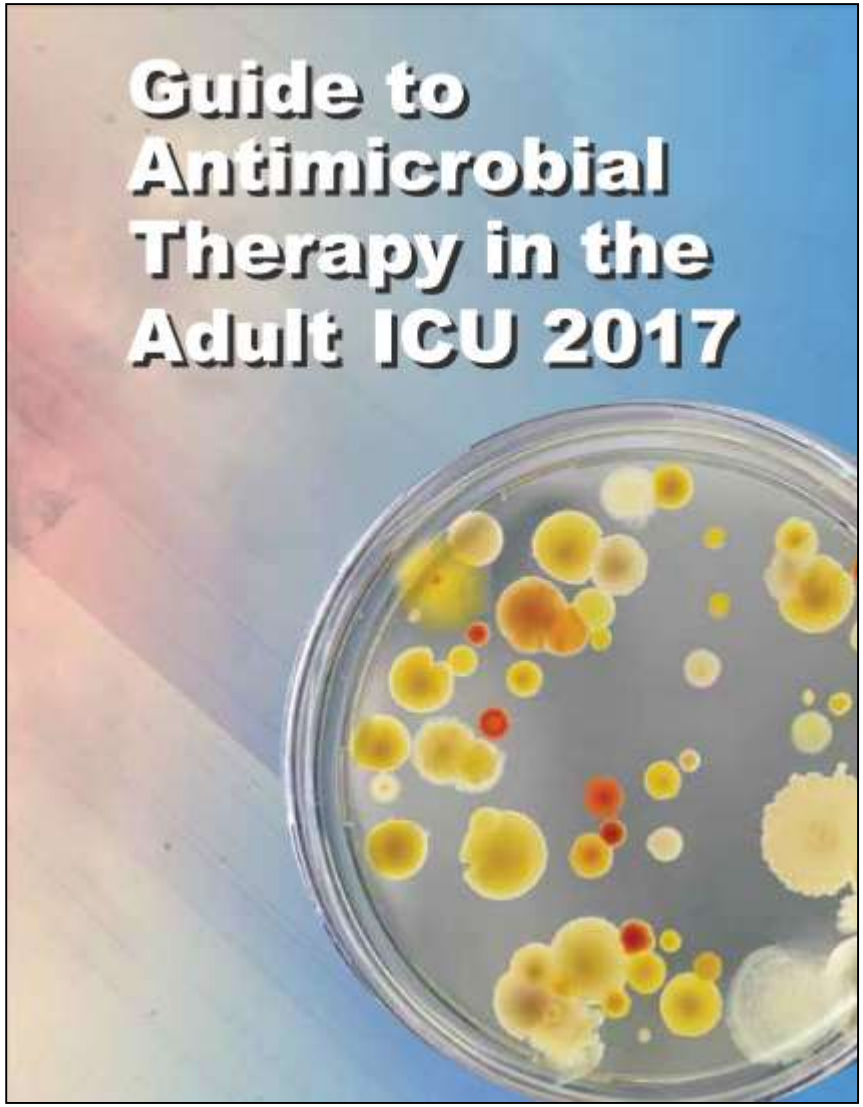
1. Rapid identification of patients with bacterial infections, while reducing the numbers of patients treated unnecessarily
2. **Appropriate empirical treatment selection**
3. Using PK-PD characteristics to optimize antimicrobial dosing and administration modalities
4. **De-escalation once culture results become available**
5. Shortening therapy duration

Safety Concerns with the Use of Antibiotics

- Toxicity
  - Kidney damage
  - Liver damage
  - Bone marrow (Chloramphenicol and aplastic anemia)
- Interactions with other medications
  - May neutralize effectiveness of contraceptive pills
- Hypersensitivity reactions
  - Anaphylactic reactions to penicillin
  - Triple antibiotic ointment (rashes and neomycin B)
- Fetal damage / risk to pregnant women
  - Tetracyclin causes discoloration of teeth in children and may cause liver damage in pregnant women
  - Fluoroquinolones may cause cartilage damage
- Dysbiosis
  - Host's normal beneficial flora killed off, causing various symptoms such as diarrhea, constipation, gas, yeast infection
- Antibiotic Resistance
  - Multiple antibiotic resistant is becoming a huge problem – MRSA

Complications of Antibiotic therapy

- **Resistance** – inappropriate use of antibiotics
- **Hypersensitivity** – penicillin
- **Direct toxicity** – aminoglycosides (ototoxicity)
- **Super infections** – broad-spectrum antibiotics cause alteration of the normal flora; difficult to treat



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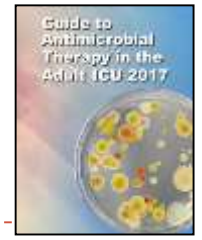
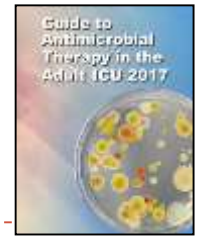


Summary of Important Changes

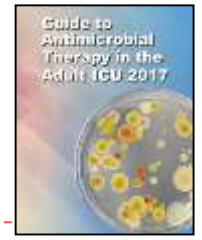
- In line with more evidence and better understanding of pharmacokinetics and pharmacodynamics of antibiotics, some new dosing has been introduced:
  - Loading doses recommended for certain antibiotics (fluconazole, ceftriaxone)
  - Polymyxin and high-dose sulbactam dosing has changed
  - Expanded appendix on dose adjustment for renal failure
  - Appendix on drugs affected by hypoalbuminaemia
  - Appendix on drug dosing in obesity
  - The use of cefoperazone/sulbactam to deliver high dose sulbactam has been omitted, as it would exceed daily dose limits of cefoperazone (increases the risk of coagulopathy)
  - Other new recommended dosing:
    - IV cefepime 2g q8h
    - IV ceftriaxone 1g q12h
    - IV ceftazidime 2g q6h in melioidosis
    - IV cloxacillin 2g q4h
    - IV gentamicin 3mg/kg q24h in endocarditis
    - IV imipenem 1g q8h for severe infections
  - Potential use of inhaled polymyxin and amikacin as adjunct to IV therapy in the treatment of ventilator-associated pneumonia caused by certain MDR organisms

Antibiotic Stewardship in ICU



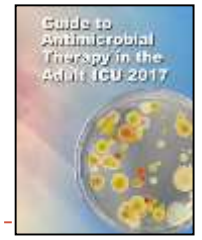
- The dilemma of current antibiotic use in ICUs is the balance between providing adequate coverage against likely pathogens and at the same time minimising selection of antibiotic resistant organisms. Antibiotic stewardship refers to activities that help optimise antibiotic therapy, ensuring the best clinical outcome for the patient while lowering the risk of development of antimicrobial resistance and minimising adverse effects and costs.
- Appropriate measures in antibiotic stewardship in ICUs include:
  1. Rapid identification of patients with infections
     - An accurate diagnosis of bacterial infection should be made before administration of any antibiotics.
     - Obtaining specimens for appropriate cultures before antibiotic administration is essential to confirm infection, identify responsible pathogens and enable de-escalation therapy. However, the results are often unavailable for the first 24 hours.
     - The decision to start antibiotics in a possibly infected critically ill patient needs to be balanced between the uncertainty of infection and risk of delaying treatment against the overuse of antibiotics. Observational data suggested that delaying antibiotics in haemodynamically stable surgical patients with suspected ICU-acquired infections could be an option when exercised with sound clinical judgement.
     - Currently, there is no biomarker of infection that clinicians can rely exclusively on.
     - Molecular diagnostic testing has the potential to be used for timely and rapid identification of causative microorganism.

Antibiotic Stewardship in ICU



2. Ensure appropriate empiric antibiotic therapy
   - Appropriate therapy is defined as the use of antibiotics at its correctdose, in which the organism is susceptible to.
   - Studies have shown that early use of appropriate antibiotic therapy improves outcome.
   - Empirical therapy should be based on regularly updated local data on the incidence of causative organisms and their susceptibility to antimicrobial agents. This applies to both community and hospital-acquired infections.
   - Another consideration in selection of antibiotics includes previous antibiotic use within the preceding 2 weeks. Whenever possible, do not use the same class of antibiotics.
3. Minimise time to initial antibiotic dose
   - The timing of initial therapy should be guided by the urgency of the situation.
   - In critically ill patients with septic shock, patients with febrile neutropenia or bacterial meningitis, empiric therapy should be initiated immediately after obtaining microbiological specimens.
   - In more stable patients, antimicrobial therapy can be withheld until appropriate specimens or investigations have been obtained.

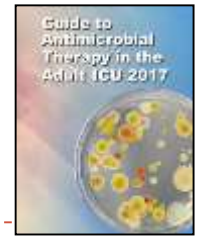
Antibiotic Stewardship in ICU



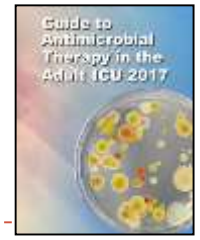
4. Optimise antibiotic dose and interval

- Factors affecting dosing in the critically ill include:
- Increase in volume of distribution (Vd) of hydrophilic antibiotics Vd of hydrophilic antibiotics (aminoglycosides, β-lactams, vancomycin, linezolid, polymyxins) is increased in patients with sepsis and burns. These increases in Vd can cause lower than expected plasma concentrations during the first day of therapy. Larger Vd will require the administration of loading doses to saturate body tissues where the drug distributes to whilst still achieving appropriate concentrations at the site of infection.
- Hypoalbuminaemia is likely to increase the Vd and clearance of highly protein-bound antibiotics (ceftriaxone, cloxacillin, ertapenem). In the presence of increased clearance, the increased Vd still causes a significant prolongation of half-life of the antibiotic, which is beneficial for sustaining concentrations throughout the dosing interval for highly susceptible pathogens. However, the decreased concentrations from the increased Vd may cause therapeutic failure against pathogens with higher MICs. In these situations, higher loading doses followed by an increase in frequency of administration or continuous infusion is required.
- Augmented renal clearance (CrCl > 130ml/min/1.73m 2) is common in patients with sepsis, burns, polytrauma, traumatic brain injury and febrile neutropenia. Significant correlations between subtherapeutic concentrations of β-lactams or vancomycin and augmented renal clearance were observed. Hence, the dose of antibiotics may have to be increased and levels to be monitored.
- Extracorporeal therapies in patients with renal failure, the time to achievement of steady-state is increased for antibiotics cleared by the kidneys.

Antibiotic Stewardship in ICU



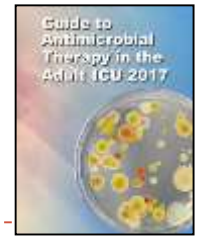
5. De-escalation therapy
   - De-escalation refers to the modification of an empirical antibiotic regimen to an alternate regimen with a narrower spectrum of activity.
   - Stop antibiotic therapy on day 3 if infection becomes unlikely based on negative cultures and clinical course.
   - De-escalate the empirical antibiotic regimen once the etiological pathogen is identified
   - Switch to monotherapy after 3 to 5 days, provided that the initial therapy was appropriate and the clinical response was good.
   - Benefits from combination therapy have been inconsistent.
6. Shorten treatment duration
   - Duration of antibiotic therapy can be shortened to 7 days for most patients including septic shock, based on therapeutic response and microbiological data. The exceptions are the immunosuppressed, those infected with multi-resistant organisms, those whose course deteriorates despite appropriate antibiotic or those whose initial therapy was inappropriate for the responsible organism
   - Studies have shown that procalcitonin-guided therapy resulted in shorter duration of antibiotics in units where antibiotic duration exceeds 10 days



Principles of Antimicrobial Therapy

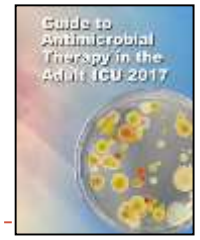
- Prophylactic Antimicrobial Therapy
  - Antimicrobial prophylaxis (AP) can be primary, secondary or for eradication of colonising organisms. It is often for surgical or nonsurgical indications. Examples of nonsurgical AP include prevention of infective endocarditis in valvular heart disease undergoing dental procedures and prevention of infection by encapsulated organisms in asplenic patients.
  - Perioperative antimicrobial prophylaxis is to prevent surgical site infections (SSI). Optimal agents for prophylaxis should be bactericidal, inexpensive and active against the typical pathogens that can cause SSI postoperatively.
  - IV prophylaxisshould be given within 30 to 60 minutes before the surgical incision to maximise its effectiveness.
- Empirical Antimicrobial Therapy
  - Sepsis in the critically illremains a diagnosticand management challenge. Besides adequate fluid resuscitation, vasopressor therapy and the support of the failing organ systems, the use of appropriate antimicrobial therapy and source control are equally important for good clinical outcomes. The aim of antimicrobial therapy is to achieve effective concentration at the target sites while minimising adverse events.

Principles of Antimicrobial Therapy



- Inappropriate and/or delayed antimicrobial use in the ICU is associated with poor outcomes. Moreover this can lead to the emergence of resistant organisms, antimicrobial-related adverse events and increase in healthcare costs. Antibiotic stewardship has been suggested to overcome these problems.
- When initiating empirical antimicrobials in patients with sepsis, consider the likely organisms, patient factors and antimicrobial profiles.

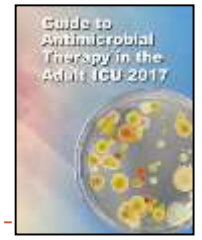
1. Likely causative organism
   - Decide if community or nosocomial infection.
   - Identify the most likely source of infection.
   - Consider local epidemiological data and laboratory-oriented surveillance.
   - Evaluate risk factors for MDR organisms (MRSA, VRE and MDR Gram-negative bacilli)
   - Obtain source control as rapidly as is practical to ensure success of therapy.



Principles of Antimicrobial Therapy

2. Patient factors
   - Exposure history
     - Take a travel history (e.g. malaria in endemic areas), occupational exposure e.g. rice farmers (*Burkholderia pseudomallei*), fishermen (*Vibrio vulnificus*), IV drug users (*Staphylococcus aureus*), activities in contaminated soil/water (leptospirosis)
   - Co-morbidities
     - Examples in diabetes mellitus (melioidosis), chronic lung diseases (Pseudomonas aeruginosa) and valvular heart diseases (endocarditis)
   - Severity of illness
     - Patients in sepsis and septic shock require emergent and broad-spectrum antimicrobial therapy. Every one hour delay of intravenous antimicrobials is associated with significant increased mortality
   - Prior antimicrobial use or prolonged hospitalisation
     - Both are risk factors for the presence of resistant organisms.
   - Immunosuppressive states
     - Patients with underlying malignancy, post-splenectomy, unvaccinated, malnourished, on steroid or immunosuppressive drugs may require broad-spectrum therapy including antifungal.
   - Presence of renal or hepatic dysfunction Drug clearance may be affected. The risk-benefit ratio of the antimicrobials must be determined on a case-to-case basis.
   - Pregnancy and lactation. As certain the risk categories of antimicrobials in pregnancy.
   - Others
     - Adjust drug dosage in obesity and consider alternatives in drug allergies

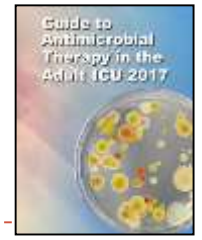
Principles of Antimicrobial Therapy



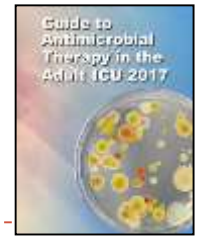
3. Antimicrobial profile

- Route of administration
  - The IV route should be used in severe infection as oral absorption is unpredictable even for drugs with good oral bioavailability. In addition to IV administration; intrathecal or inhalational routes may be considered to improve target site concentrations.
- Dose and interval
  - ICU patients often have an increased Vd for hydrophilic antibiotics. Lower antibiotic concentrations can be potentiated by hypoalbuminaemia and augmented renal clearance for renally excreted drugs. Understanding exposure-effect relationships is required to optimise antibiotic dosing in the critically ill.

Principles of Antimicrobial Therapy



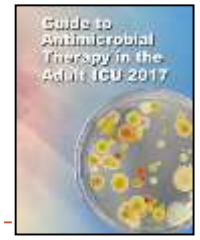
- Achievable antimicrobial concentrations at target tissue
  - Dose optimisation strategies should be taken to increase the antimicrobial activities at the target sites as illustrated in the table above
  - Aminoglycosides and glycopeptides penetrate tissues poorly. Aminoglycosides should not be used as monotherapy while a higher plasma level of glycopeptides is recommended to ensure adequate tissue penetration. Both ß-lactams and quinolones have good tissue penetration. Even then higher doses are required to achieve adequate concentrations in infections of the central nervous system.



Principles of Antimicrobial Therapy

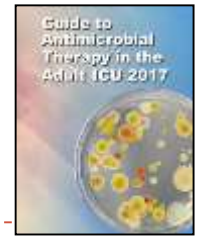
PD kill characteristics	Optimal PK parameter	Goals of therapy/application	Examples
Time-dependent (Refer to **Appendix D**)	T>MIC Percentage of time where drug concentration remains above MIC during a dosing interval	Maximise duration of exposure → administer continuous infusion	Penicillins Cephalosporins Carbapenems
Concentration-dependent	C_{max}/MIC Ratio of peak concentration to MIC	Maximise concentration of drug → use higher maintenance dose	Aminoglycosides Polymyxin
Concentration-dependent with time dependence	$AUC_{0\text{-}24}$/MIC Ratio of area under concentration-time curve (AUC) during a 24-h period to MIC	Optimise amount of drug → administer loading dose	Fluoroquinolones Vancomycin

Principles of Antimicrobial Therapy



- Post antibiotic effect (PAE)
  - This is defined as persistent suppression of bacterial growth even after the serum antibiotic concentration falls below the MIC of the target organism. Aminoglycosides and fluoroquinolones have post-antibiotic effect against Gram-negative bacteria.
- Adverse events
  - Risk-benefit of antimicrobials with potential serious adverse events should be considered on a case-to-case basis. If unavoidable, serum levels should be monitored for toxicity (e.g. aminoglycosides).
- Ecological profile
  - Limit the use of antimicrobials with potential for selecting resistant organisms e.g. third generation cephalosporins result in selection pressure for ESBL producing Enterobacteriaceae.
  - Empirical therapy should be re-evaluated after 48-72hours or when culture results become available. Once a causativepathogen is identified, narrow the spectrum of antimicrobial therapy (de-escalation). Sensitivity tests should be interpreted carefully. In vitro sensitivity does not equate with clinical effectiveness (e.g. ESCAPPM organisms: *Enterobacter* spp, *Serratia* spp, *Citrobacter freundii*, *Aeromonas* spp, *Proteus vulgaris*, *Providencia* spp, *Morganella morganii*)

Principles of Antimicrobial Therapy



- If there is no clinical response within 48-72 hours, consider:
  - Possibility of a secondary infection
  - Presence of resistant organisms
  - Inadequate source control (e.g. abscesses not drained, infected prosthesis not removed)
  - Inadequate penetration of antimicrobial to the site of infection
  - Inadequate spectrum of antimicrobialcoverage
  - Inadequate dose or interval
  - non-infectious causes (e.g. deep vein thrombosis, acute myocardial or pulmonary infarctions, acute pancreatitis, hyperthyroidism, Addisonian crisis, malignancies and central nervous system hemorrhages)

Choosing the Right Antibiotic
Is It Really Needed?

- Nature of the illness
  - Is it a bacterial infection or something else?
- Presumptive diagnosis (based on history and clinical symptoms)
  - Empiric therapy – broad spectrum drug
  - Specific therapy – narrow spectrum drug

When Antibiotics are Needed

Viruses or Bacteria
What's got you sick?

Antibiotics are only needed for treating certain infections caused by bacteria. Viral illnesses cannot be treated with antibiotics. When an antibiotic is not prescribed, ask your healthcare professional for tips on how to relieve symptoms and feel better.

Common Condition	Common Cause			Are Antibiotics Needed?
	Bacteria	Bacteria or Virus	Virus	
Strep throat	✓			Yes
Whooping cough	✓			Yes
Urinary tract infection	✓			Yes
Sinus infection		✓		Maybe
Middle ear infection		✓		Maybe
Bronchitis/chest cold (in otherwise healthy children and adults)*		✓		No*
Common cold/runny nose			✓	No
Sore throat (except strep)			✓	No
Flu			✓	No

* Studies show that in otherwise healthy children and adults, antibiotics for bronchitis won't help you feel better.

BE ANTIBIOTICS AWARE
SMART USE, BEST CARE

To learn more about antibiotic prescribing and use, visit www.cdc.gov/antibiotic-use.

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CDC

Choosing the right antibiotic
Pharmacokinetic consideration

- Location of infection
  - Some antibiotics may or may not reach therapeutic concentrations in certain bodily fluids (e.g. CSF and urine)
- Degree to which antibiotic binds serum proteins
  - Excesssive binding will affect passive diffusion of antibiotic from serum to tissue

Deranged Physiology

A free online resource for Intensive Care Medicine.

An unofficial Fellowship Exam (CICM Part 2) preparation resource.

Factors which influence antibiotic choice

Disease factors	Host factors	Organism factors	Drug factors
• Travel history • Occupation • Recreational exposure • IVDU • Severity of illness, urgency of therapy • Reliability of cultures	• Age • Clearance organ function • Allergies • Immune status, HIV • Pregnancy and lactation	• Source control • Susceptibility • Empiric vs specific • Intra vs. extracellular • Duration of therapy • Assessment of response	• Cost • Toxicity • Bioavaiability • Source site penetration • Drug synergy • Bacteriostatic vs bactericidal

Factors which Influence the Choice of Antibiotic Therapy

Factors		Discussion and examples
Disease specifics	**Travel history**	• Geography of endemic regions (eg. leptospirosis) • Known ongoing outbreaks (eg. Ebola, H1N1, MERS)
	Occupational exposure	• Abbatoir workers (*Coxiella burnetii*) • Fisherman (*Vibrio vulnificus*) • Cattle farmers (*Brucella sp.*)
	Recreational exposure	• IV drug use (endocarditis) • Pets or animal exposure (eg. psittacosis or toxoplasma) • Bushwalking (eg. tick-borne disease) • Alcoholism (prognostic importance in community-acquired pneumonia)
	Recent antimicrobial use	• Was it the right antibiotic? i.e. was the course of antibiotics ineffective because of poor agent choice? • Did it select for a specific group of organisms? • Prophylaxis vs. endemic pathogens (eg, malaria)
	Empiric vs. definitive	• Are we convinced of the diagnosis? • Is there a need to cover broadly?
	Urgency and timing	• Septic patient (every hour delay is associated with a 1% mortality increase)
	Reliability of cultures	• Are we sure we cultured the correct pathogen? • Is a polymicrobial infection possible (eg. diabetic foot)?

Host factors	**Clearance**	• Decreased renal clearance (by renal failure) • Increased renal clearance (by dialysis, or in pregnancy) • Decreased hepatic clearance (eg. cirrhosis) • Exotically altered clearance (eg. plasma exchange, haemoperfusion, adsorption on to ECMO circuit surfaces, and so forth).
	Age	• Paediatric dosing needs to be adjusted to weitght • Geriatric dosing needs to account for change in volume of distribution and clearance
	Genetic variation	• Genetic differences in side effects from antibiotics • Congenital idiosyncracies preventing the use of certain antibiotics (eg. G6PD deficiency resulting in haemolysis when exposed to dapsone or nitrofurantoin) • Hepatic enzyme defects
	Pregnancy and lactation	• Early pregnancy teratogenesis (eg. nitrofurantoin, chloramphenicol, sulfonamides) • Late pregnancy teratogensis (eg. tetracyclines)
	Immunocomptence	• Steroid use • Post-splenectomy, unvaccinated (susceptible to encapsulated organisms) • Chemotherapy • Solid organ or bone marrow transplantation
	Allergies	• Fatal hypersensitivity reaction vs. some sort of mild scaly rash with a little itching.

Choosing the right antibiotic
Host Factors

- Status of host immune system (cidal vs. static)
- Local environment of infected site (pus, foreign bodies)
- Age (organ function in newborns and elderly)
- Inherited metabolic disorder
- Pregnancy (fetal or neonatal development)

Choosing the Right Antibiotic
Host Factors

- Drug Allergies
  - Rashes
  - Anaphylaxis
  - SJS Syndrome
- Co-morbid conditions are aggravated by some antibiotics
  - Seizures
  - Blood disorders

Organism factors	**Susceptibility**	• ESCAPPM, MRO, etc • Community prevalence of drug resistance • Tendency to develop resistance during treatment
	Biology	• Intracellular pathogen vs. extracellular • Unusual life cycle (eg. helminthes, malaria) - need to kill the eggs or dormant cocoons or whatnot
	Source control	• Success of therapy overall is largely determined by this
	Duration of therapy	• Short course, eg. in urosepsis • Long course, eg. osteomyelitis
	Assessment of response	• To repeat the cultures, or not? • Is there a point in monitoring serology?

Drug factors	**Cost**	• Fluconazole: $57.99 AUD for 28 capsules (200mg) • Anidulafungin: ~$ 300 AUD per single 200mg dose. • Cost of monitoring the drug levels • How much is a life worth? you amoral monsters, etc.
	Toxicity	• Risk vs benefit • Some drugs (eg. chloramphenicl) are uniformly "too toxic for use", as there are less toxic alternatives in almost every situation.
	Bioavailability	• Convenience of oral dosing • Certainty of IV dosing • Altered absorption via GI tract in context of critical illness, shock states, low flow, what have you.
	Site penetration	• Basic chemistry of the drug influences this aspect. Eg: • Penetration to the CSF (lipophilicity) • Exclusive distribution into the circulating volume, (hydrophilicity, or high serum protein binding) • Weird organ preference (eg. the strange affinity of fluoroquinolones for the prostate) • Exclusion of a drug from a specific organ (eg. the inactivation of daptomycin by lung surfactant)
	Bactericidal vs bacteriostatic	• Some agents are bacteriostatic against one pathogen and bactericidial against another • There may not be any in-vivo difference
	Synergistic combination	• Need for multiple agent therapy (eg. in *Pseudomonas*) • Unquestioned need for synergy (eg. cocktail for TB) • Advantage from synergy (eg. ampicillin with gentamicin for enterococci) • Need for broad-spectrum coverage (in which case you use multiple agents to start with, and then narrow the spectrum of cover) • Need for polymicrobial coverage (eg. surgical triple therapy, or in context of bone marrow transplant) • Need to prevent emergence of resistance (eg. the argument offered to defend the use of selective digestive tract decontamination; also, a genuine argument for the use of rifampicin and fusidic acid together)

Review

Patient specific risk stratification for antimicrobial resistance and possible treatment strategies in gram-negative bacterial infections

Matteo Bassetti, Alessia Carnelutti & Maddalena Peghin

Pages 55-65 | Received 15 Oct 2016, Accepted 19 Oct 2016, Accepted author version posted online: 21 Oct 2016, Published online: 07 Nov 2016

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Table 2. Risk factors for antimicrobial-resistant Gram-negative bacteria.

Risk factors for MDR pathogens				
Baseline characteristics	Epidemiological background	Recent (<3 months) antibiotic therapy	Prior colonization	Indwelling devices
• Age >70 years	• Prior hospital admission (in the last 12 months)	• Recent aminopenicillins	• Gut colonization with ESBL	• Urinary catheter
• Diabetes mellitus	• Prolonged hospitalization	• Recent cephalosporins	• Gut colonization with CRE	• Gastrostomy or jejunostomy
• Charlson index ≥3	• Transfer from another health-care facility	• Recent fluoroquinolones	• Colonization with MRSA	• Nasogastric tube
• Recurrent or obstructive UTIs	• Current or prior ICU admission	• Recent carbapenems	• Colonization with *Acinetobacter*	• CVC
• Use of corticosteroids	• Local epidemiology, outbreak	• Recent aminoglycosides	• Endotracheal colonization with *P. aeruginosa*	• Mechanical ventilation
• Immunosuppression	• Travel from high endemic area[a]			• Hemodialysis
• Trauma				
• Malignancy				
• Organ transplantation				
• COPD				
• Neutropenia				
• Recent surgery				

Requirements for Effective Antibiotherapy

- The drug must reach the site of action
- The drug's concentration at the site of action must be sufficient to inhibit bacteria
- The duration of antibiotherapy must be sufficient to allow the drug to act

Local Delivery of Antibiotics

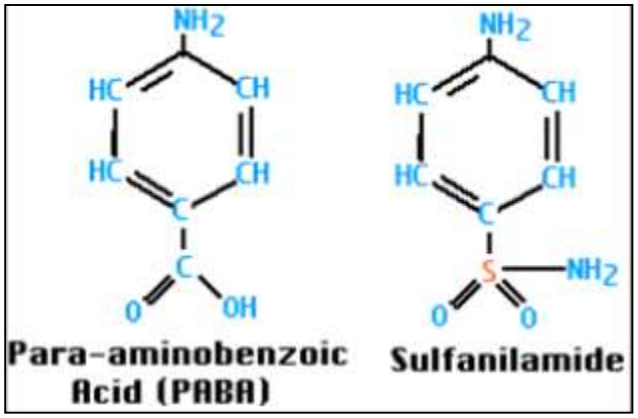
- Advantages
  - Higher local drug concentrations
  - Sustained therapeutic drug levels (independent of patient compliance)
  - Effective drug levels can be attained at sites that are difficult to reach
  - Adverse side effects are minimazed

Empirical “blind” therapy

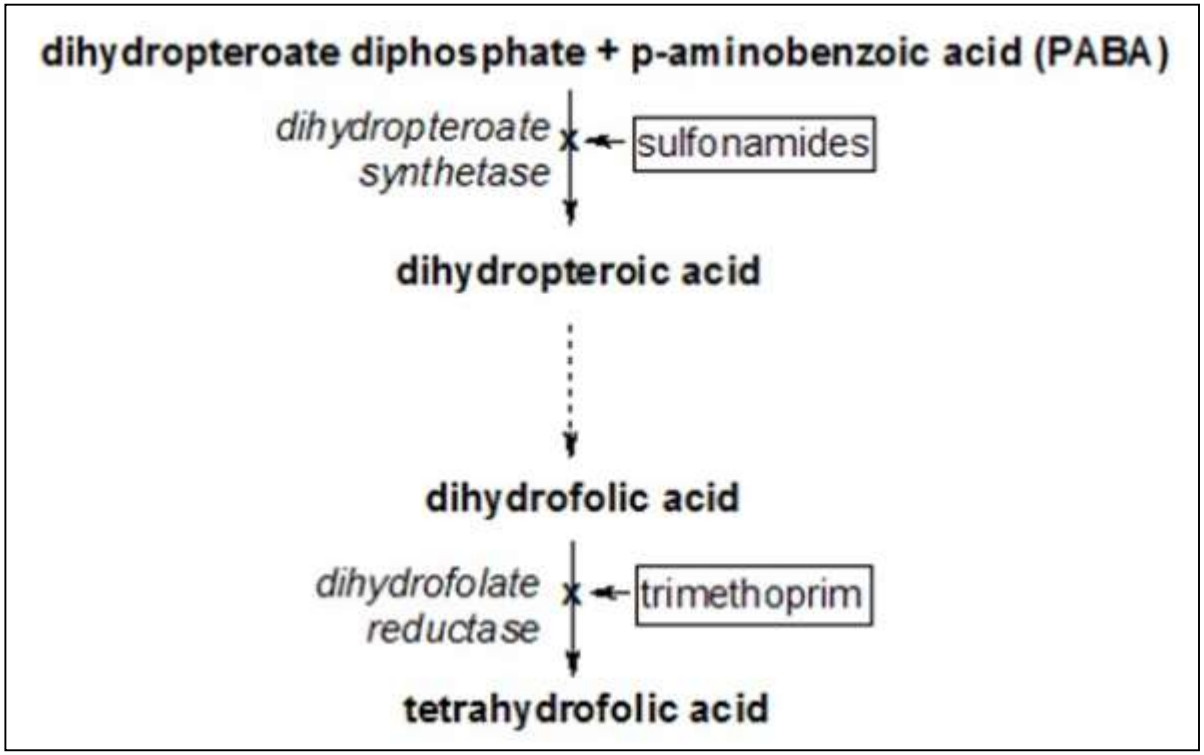
- Most antibiotic prescribing, especially in the community, is empirical. Even in hospital practice, microbiological documentation of the nature of an infection and the pathogen is generally not available for a day or two
- Initial choice of therapy relies on a clinical diagnosis and, in turn, a presumptive microbiological diagnosis. Such “blind” therapy “ is directed at the most likely pathogen(s) responsible for a particular syndrome such as meningitis, urinary tract infection or pneumonia
  - Examples of “blind therapy” for these three conditions are ceftriaxone, trimethoprim and amoxicillin + erythromycin, respectively. Initial therapy in the severely ill patients often broad spectrum in order to cover the range of possible pathogens but should be targeted once microbiological information becomes available

Sulfonamides

- Analogues of para-aminobenzoic acid
- Broad spectrum
- Competitive inhibitors of dihydropteroate synthase – needed for folic acid synthesis

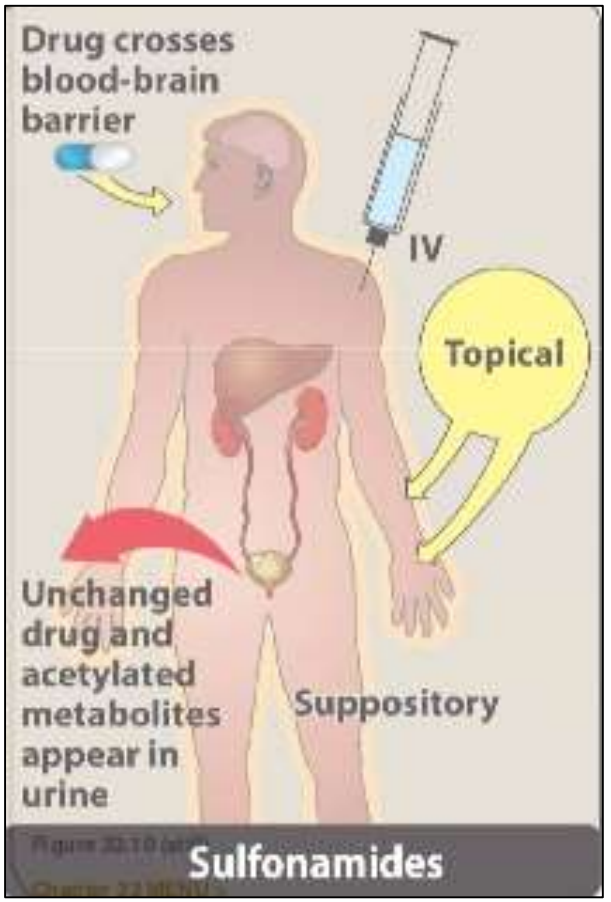


Sulfonamides



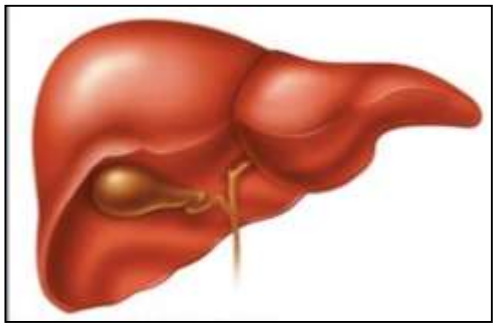
Sulfonamides

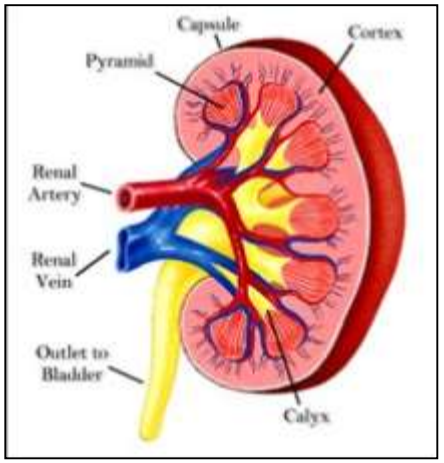
- Cell membrane inhibitors
- Seldom prescribed on their own
- Resistance limits spectrum of antimicrobial activity
- Trimethoprim – similar activity to sulphonamides – in combination with
  sulphonamides is synergistic



Sulfonamides

- Mostly absorbed from GI tract
- Binds variably to serum albumin
- Wide tissue distribution, including transplacentally
- Variably inactivated in liver by acetylation and then excreted in urine
- Some agents can precipitate I acid urine

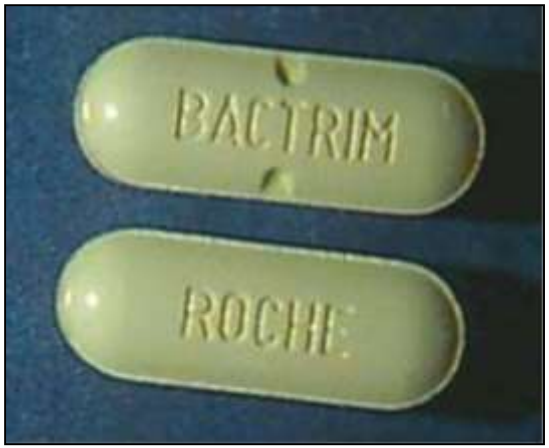




Sulfonamides
Rapidly Absorbed and Eliminated

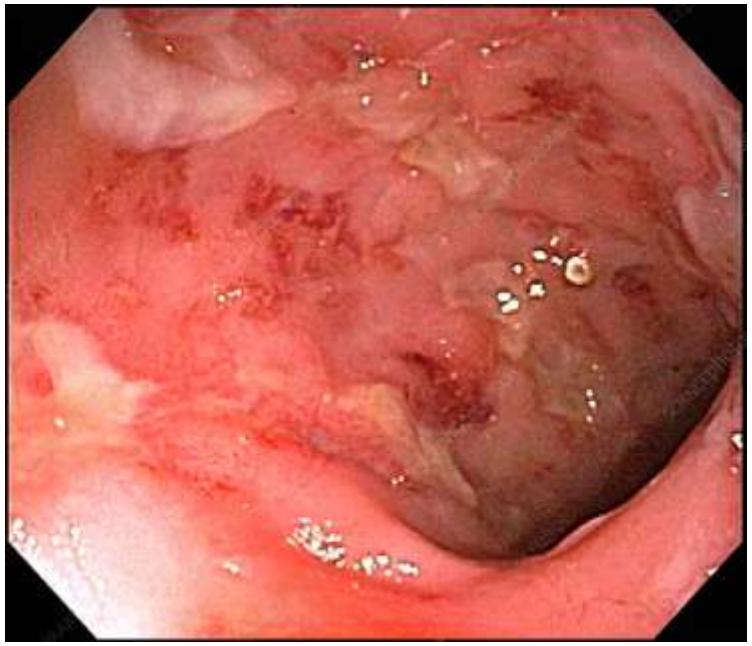
- Sulfisoxazole, sulfamethoxazole, sulfadiazine
  - Bind extensively to plasma proteins
  - Highly concentrated in urine (cidal)

Sulfamethoxazole combined with trimethoprim (Bactrim) is widely used to treat a variety of infection (esp. UTI)



Sulfonamides
Poorly Absorbed

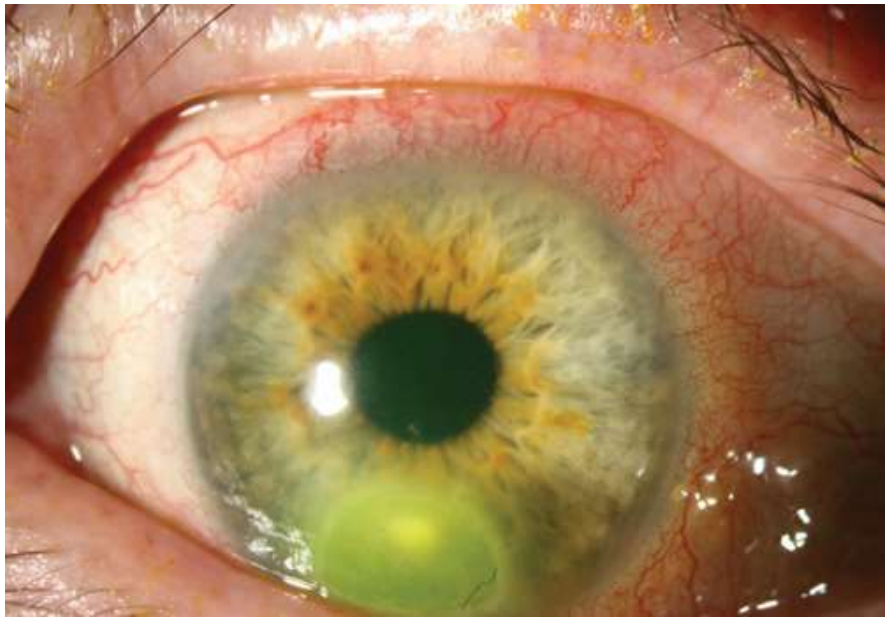
- Sulfasalazine
  - Used to treat ulcerative colitis and irritable bowel syndrome
  - Gut flora metabolize drug into 2 compounds: 1 toxic and 1 therapeutic (5-aminosalicylate)



David Musher / Science Photo Library

Sulfonamides
Topical Use

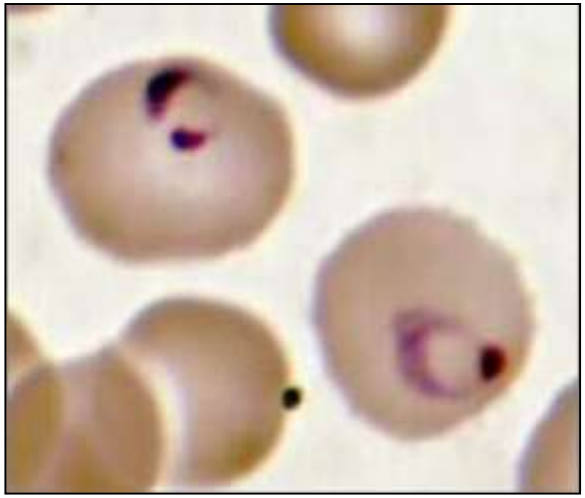
- Sulfacetamide
  - Good penetration in eye
  - Non-irritating
- Silver sulfadiazine
  - Prevention and treatment of burn wound infections



Christine W. Sindt, OD

Sulfonamides
Long Acting

- Sulfadoxine
  - Serum half-life is measured in days rather than minutes or hours
  - Combined with Pyrimethamine to treat malaria



Plasmodium vivax

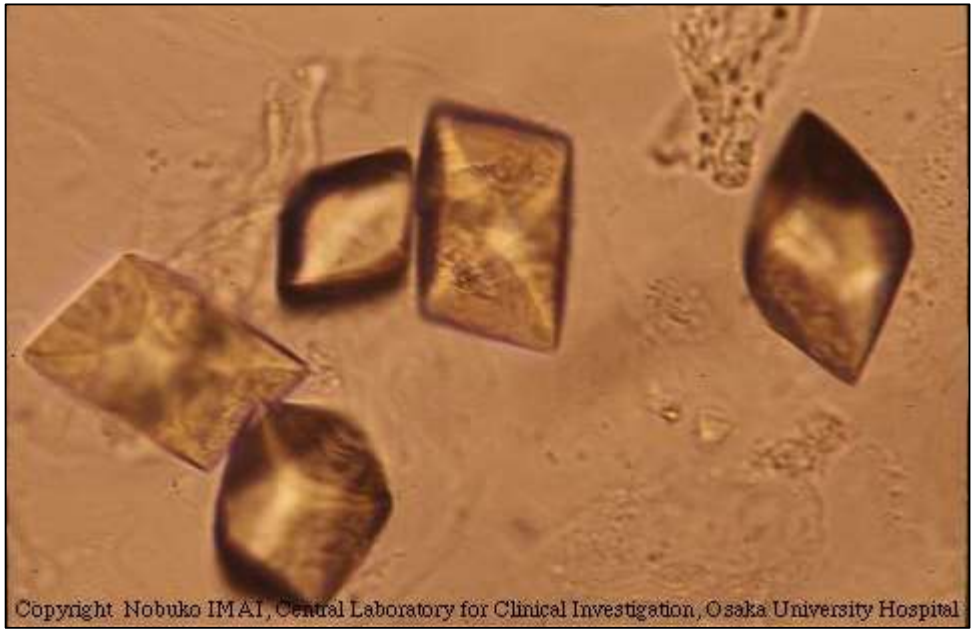
Sulfonamides
Therapeutic Use

- Cidal in urine
- Nocardiosis
  - Nocardia asteroides
  - Nocardia brasiliensis
- Toxoplasmosis (avoid using in pregnant women)

- Mechanisms of resistance
  - Altered affinity of enzyme for drug
  - Decreased permeability or active efflux
  - New pathway of folic acid synthesis

Sulfonamides
Toxicity/Contraindication – Urine

- Crystallization in acid urine
  - Common to uncommon depending on drug
  - Alkaliza urine or increase hydration



Sulfonamides
Toxicity/Contraindication – Blood

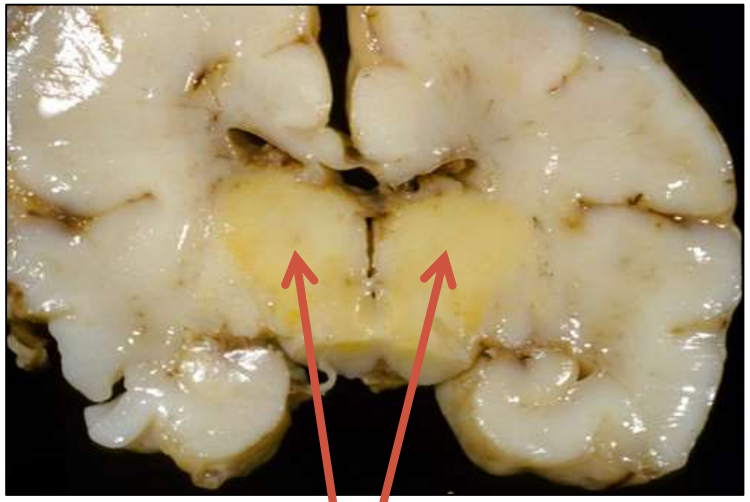
- Acute hemolytic anemia
  - Rare to extremely rare
  - Associated with glucose-6-phosphate dehydrogenase activity in RBC
- Agranulocytosis
  - Extremely rare
- Aplastic anemia
  - Extremely rare

Sulfonamides
Toxicity/Contraindication – Immune

- Hypersensitivity reactions (common to uncommon)
  - Skin and mucous membrane manifestations (rashes)
  - Serum sickness
  - Focal or diffuse necrosis of the liver (rare)

Sulfonamides
Toxicity/Contraindication – Miscellaneous

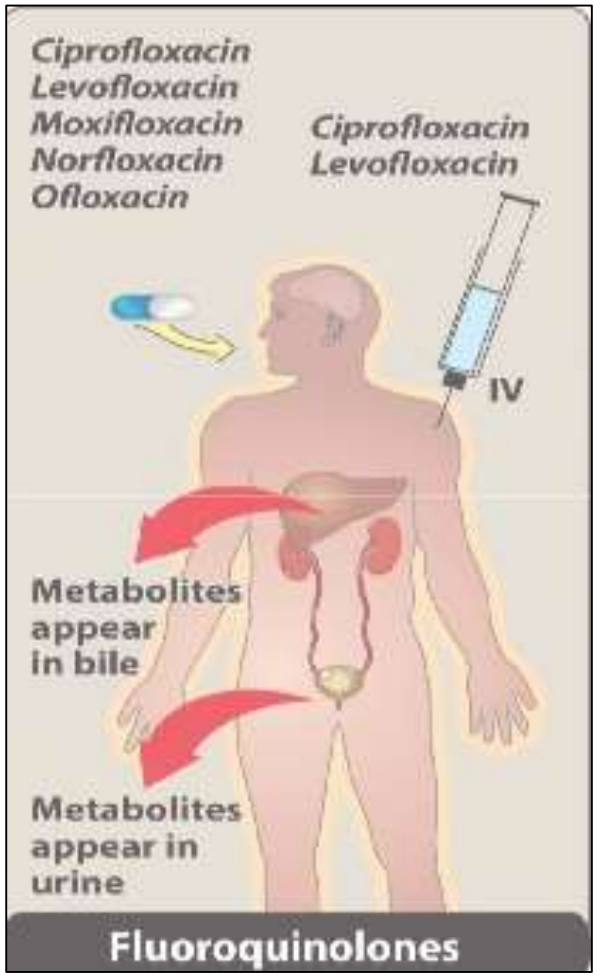
- Nausea, anorexia, vomiting (common)
- Kernicterus
  - Displacement of bilirubin from plasma albumin to brain resulting in encephalopathy
  - Never give sulfa drugs to a pregnant or lactating woman
- Potentiation of oral anticoagulants, sulfinylurea hypoglycemic drugs and hydantoin anticonvulsants



Bilirubin deposits in neonatal brain
From Neuropathology-web.org

Quinolones

- Newer compounds – Ciprofloxacin and Ofloxacin
  - Greater potency
  - Broader spectrum of antimicrobial activity
  - Greater efficacy against resistant organisms
  - Active against G- bacilli & cocci, mycobacteria, mycoplasmas & rikettsiae
  - Some cases better safety profile than older quinolones
- Respiratory quinolones
  - Levofloxacin, gemifloxacin, moxifloxacin
  - Active against G+, typical, atypical, anaerobic pathogens

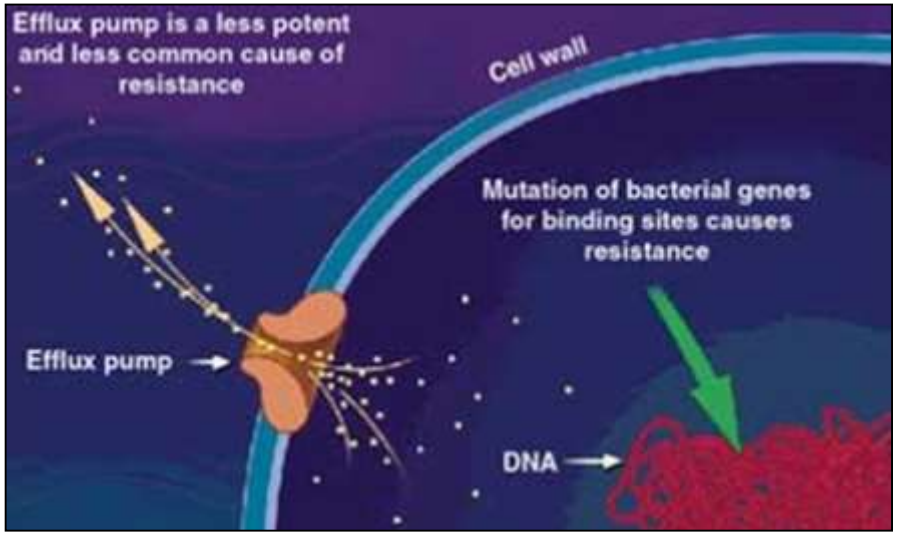


Quinolones

- Nucleic Acid Inhibitors
- Not recommended for children
- May prolong QT interval, not to be used in patients with arrhythmias
- Limited therapeutic utility and rapid development of resistance
- Interfere with absorption
  - Antacids containing aluminium or magnesium
  - Dietary substances containing iron orzinc
  - Calcium, milk or yogurt

Quinolones

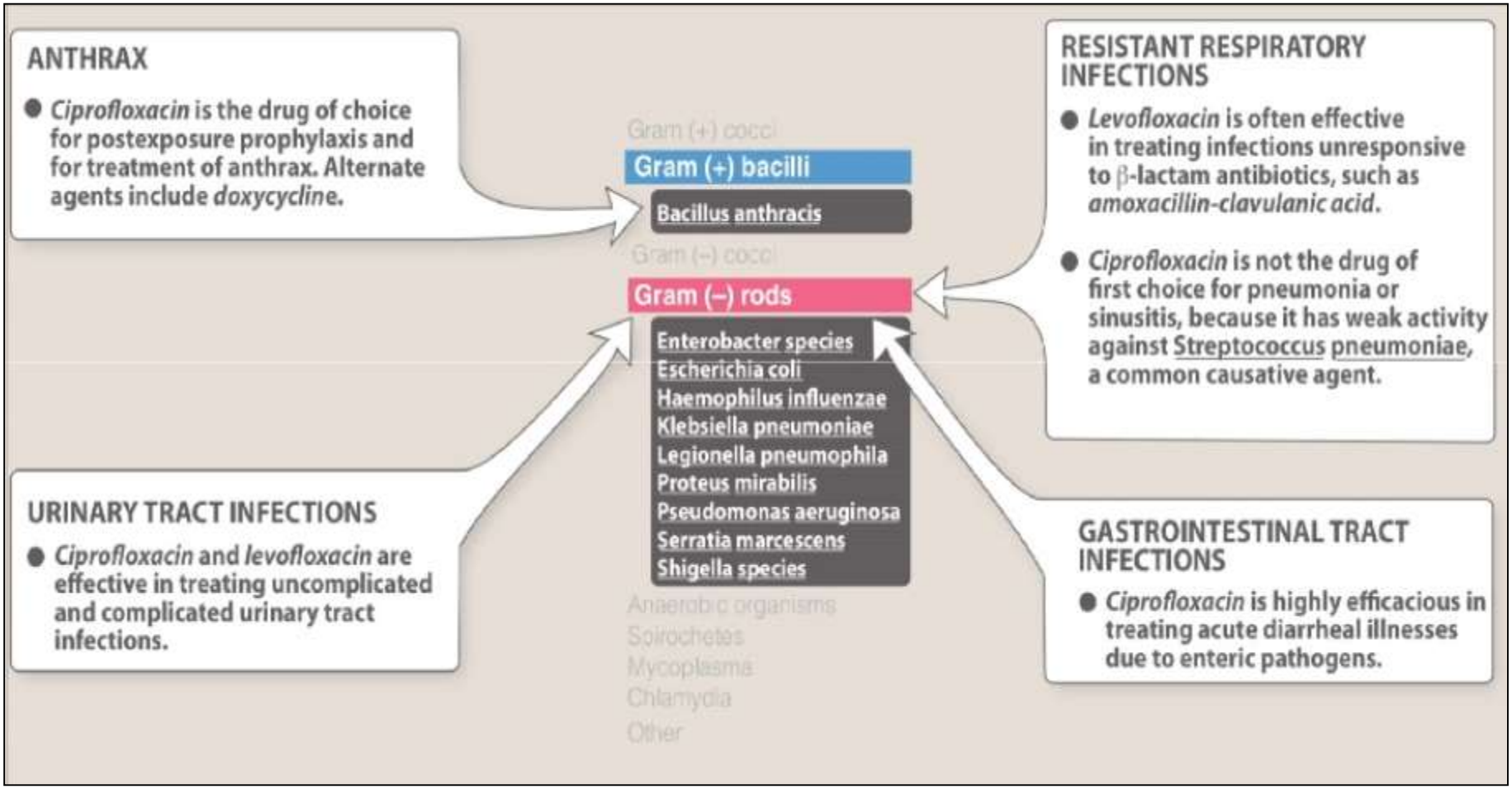
- Naladixic acid was a byproduct of choroquine synthesis
- Current drugs are fluorinated 4-quinolones
- Broad coverage (some broader than others)
- Targets DNA topoisomerase II (DNA –gyrase) (G-) and topoisomerase IV (G+)
- Resistance due to efflux and mutations in targets



Quinolones

- Favorable pharmacological attributes
  - Orally adminestered, quickly absorbed, even with a full stomach
  - Excellent bioavailability in a wide range of tissues and body fluids (including inside cells)
- Mostly cleared by the kidneys
  - Exceptions are pefloxacin and moxifloxacin which are metabolized by liver
- Ciprofloxavin, ofloxacin and pefloxacin are excreted in breast milk

Fluoroquinolones
Therapeutic Application



Quinolones
Therapeutic Uses

- Urinary tract infections
- Prostatitis
- STD's
  - Chlamydia
  - Chancroid
  - Not syphilis or gonorrhea (due to increased resistance)
- GI and abdominal
  - Travelers diarrhea
  - Shigellosis
  - Typhoid fever

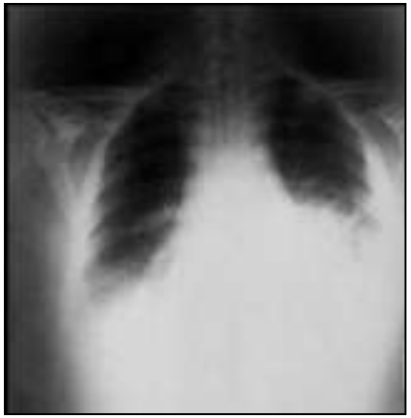
Quinolones
Therapeutic Uses

- Respiratory tract
  - All work well against atypical pneumonia agents (e.g. chlamydia, mycoplasma and legionella)
  - New agents for Str. pneumonia
  - Respiratory fluoroquinolones: levofloxacin, gatifloxacin, gemifloxacin and moxifloxacin
    - Are effective and used increasingly for treatment of upper and lower respiratory tract infections
- Bone, joint, soft tissue
  - Ideal for chronic osteomylitis
- Resistance developing in
  - S. aureus
  - P. aeruginosa
  - S. marcesens
- Good against polymycrobial infections like diabetic foot ulcers

Quinolones
Therapeutic Uses

- Ciprofloxacin for anthrax and tularemia (Francisella tularensis)

- Combined with other drugs, useful for atypical *Mycobacterium* spp. Or for prophylaxis in neutropenic patients

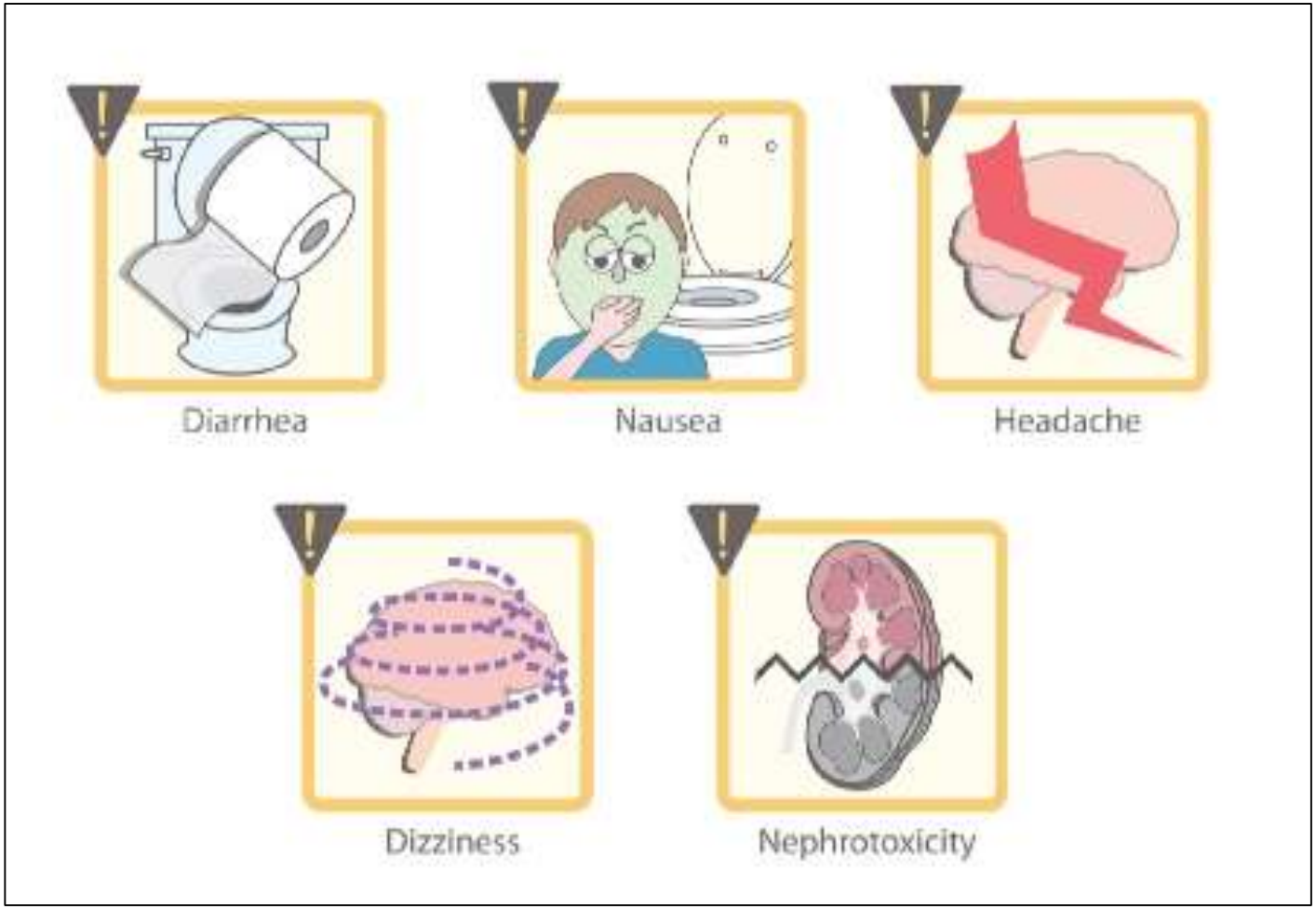


Pulmonary Anthrax

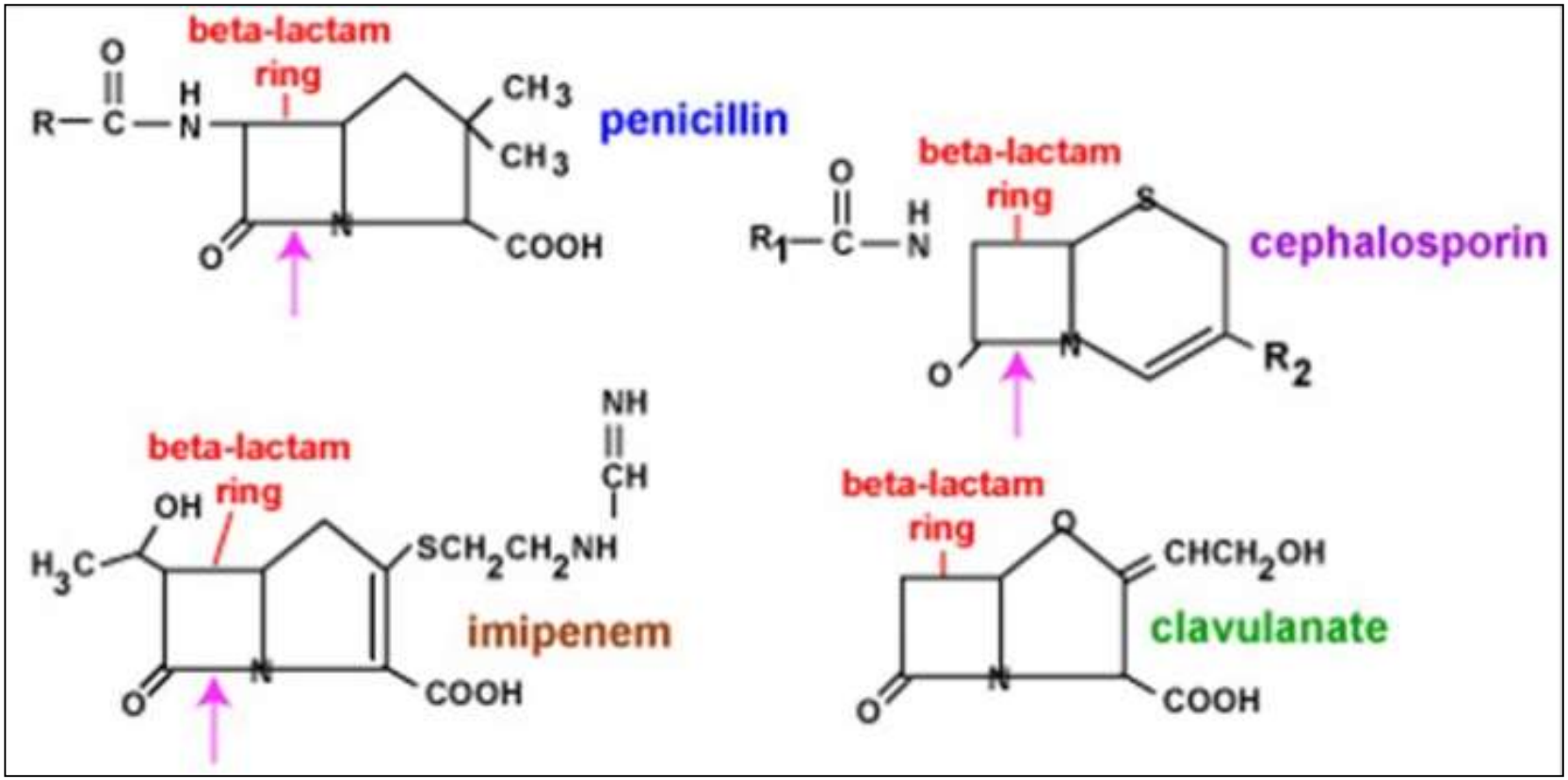
Quinolones
Toxicity/Contraindications

- Nausea, vomiting, abdominal discomfort (common)
- Diarrhea and antibiotic-associated colitis (uncommon to rare)
- CNS side effects
  - Mild headache and dizziness (common ta rare)
  - Hallucinations, delirium, seizure (rare)
- May damage growing cartilage and cause an arthropathy. Thus, these drugs are not routinely recommended for patients under 18 years of age (common)
  - Quinolones not given to children unless benefits outweigh the risks
- Leukopenia, eosinophilia, heart arrhythmias (rare)

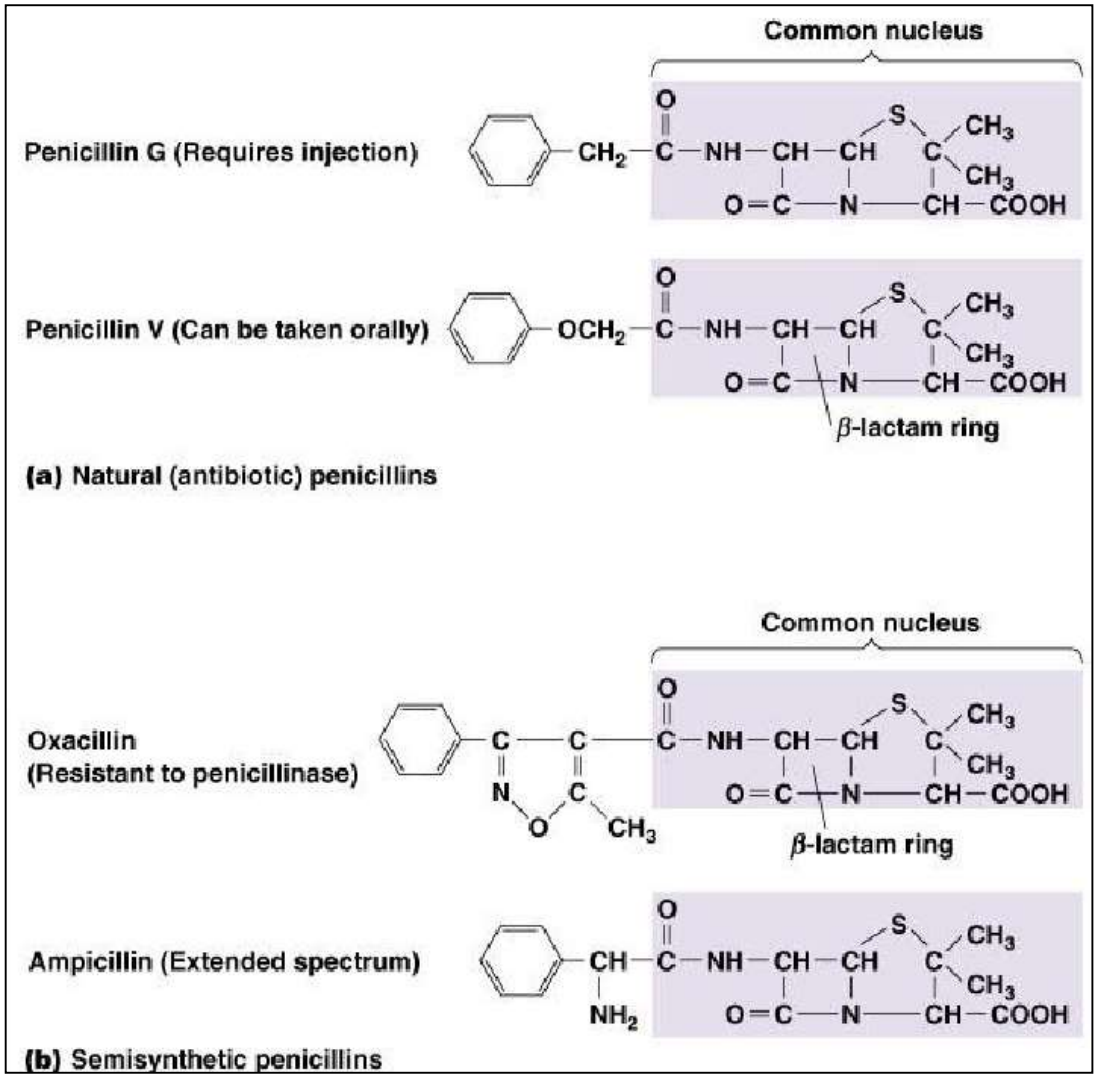
Quinolones
Adverse Reactions



β-lactams


beta-lactam ring
penicillin
beta-lactam ring
cephalosporin
beta-lactam ring
imipenem
beta-lactam ring
clavulanate

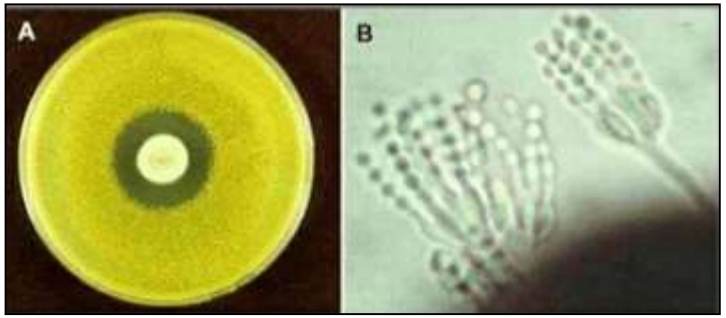
Penicillins



Penicillins

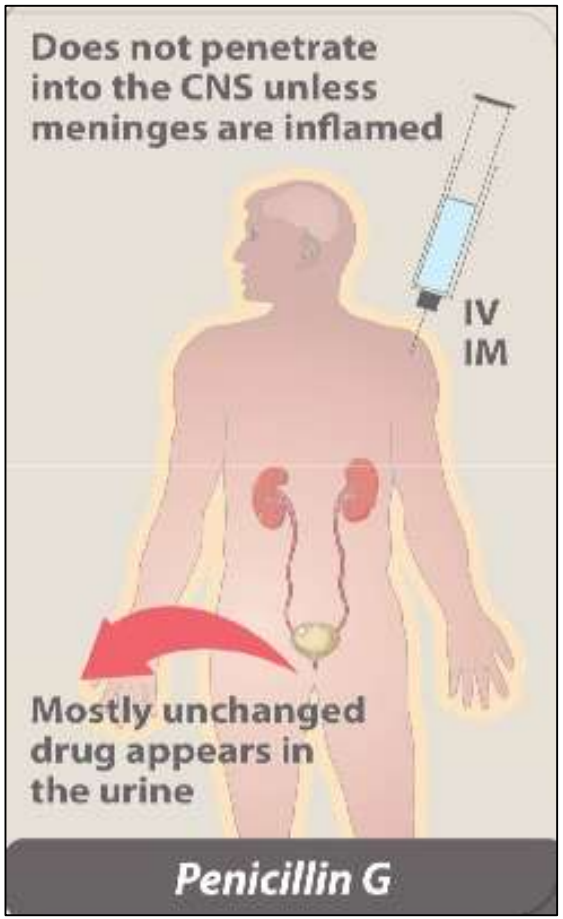
- *Penicillium notatum*
  - Produces the only naturally occuring agent – penicillin G or benzylpenicillin

- *Penicillinum chrysogenum*
  - Produces 6-aminopenicillanic acid, raw material for semi-synthetics



Administration and Fate of Penicillin

- Routes of administration
  - Oral only – Pen V, amoxicillin, amoxicillin with clavulanic acid
  - IV/IM – ticarcillin, piperacillin, ampicillin with sulbactam, ticarcillin with clavulanic acid, piperacillin with tazobactam
- Absorption
  - ↓ by food in the stomach → administer before meals 30-60‘
- Distribution
  - After oral dose, widely distributed in tissues and secretions (except CNS, prostatic fluid and the eye)
  - To bone and CSF insufficient
- Excretion
  - Kidneys



Penicillins

- Spectrum of activity based on R-groups added to 6-aminopenicillanic acid core
- All are bactericidal and inhibit transpeptidases
- Mechanisms of resistance
  - Alter affinity of transpeptidase
  - Enzymatically cleave the β-lactam ring
  - Efflux pump
  - Poor penetration into cell

Penicillins G and V

- Effective against aerobic G+ organisms **except** *Staphylococcus*
  - Pen G active against Neisseria and aerobes
- 2/3 of oral Pen G destroyed by stomach acid, Pen V is more resistant so more is delivered to serum
- Rapid elimination through kidney so probenecid is added to slow excretion
- Procaine or benzathine forms of Pen G (IM)

Penicillins G and V

- Most drug is bound to serum albumin but significant amounts show up in liver, bile, kidney, semen, joint fluid, lymph
- Cautions use in neonates and infants because renal function is not fully established
- Patients with renal failure clear the drugs through liver although at a slow pace

Penicillins G and V
Therapeutic Uses

- *Streptococcus pneumoniae* infections
- *S. pyogenes* infections
- Viridans strep endocarditis (also given prophylactically)
- Anaerobes **except** *Bacteroides fragillis* group
- Meningococcal infections
- Syphilis and other diseases caused by spirochetes

Isoxazol Penicillins

- Oxacillin, cloxacillin, dicloxacillin, nafcillin
- Designed to resist staphylococcal β-lactamases
- Like Pen V, stable in stomach acid but usually given parentaly for serious staph infections
- **MRSA not covered!**
- Absorption and fate of drugs after absorption, excretion similar to Pen G and Pen V

Aminopenicillins

- Ampicillin and amoxicillin
- Broad spectrum
  - **Not effective against β-lactamase producers**
  - β-lactamase inhibitors extend spectrum (clavulanic acid, sulbactam, tazobactam)
- Both are acid resistant but amoxicillin is better absorbed, even with food
- Do not bind plasma proteins as much as predecessors
- Secreted though the kidney

Aminopenicillins
Therapeutic Uses

- Upper respiratory tract infections
- Otitis media
- Uncomplicated UTI
- Acute bacterial meningitis in kids
- Typhoid fever

Carboxypenicillin and Ureidopenicillin

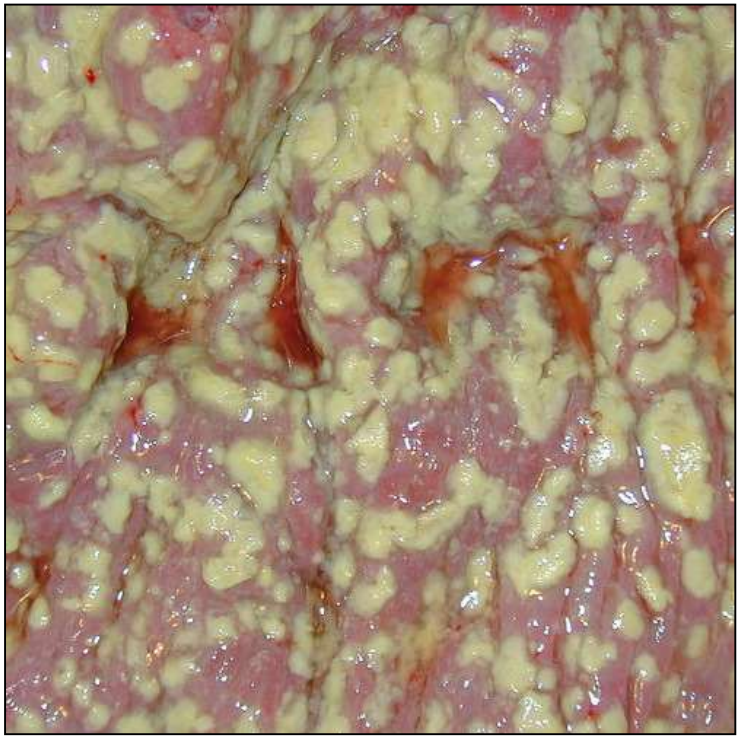
- Ticarcillin and piperacillin
  - Ticarcillin is anti-*Pseudomonas* drug
  - Piperacillin + tazobactam has the broadest spectrum
- Given parentally
- Used for serious infections

Penicillins
Toxicity/Contraindications

- Hypersensitivity reactions (uncommon)
  - Rash, fever, bronchospasm, vasculitis, serum sickness, exfoliative dermatitis, SJS, anaphylaxis
  - Drugs act as haptens when bound to serum proteins
  - Rashes will disappear when drug is withdraw or can treat with antihistamines
  - For patients with allergies, switch to a different class of antibiotics

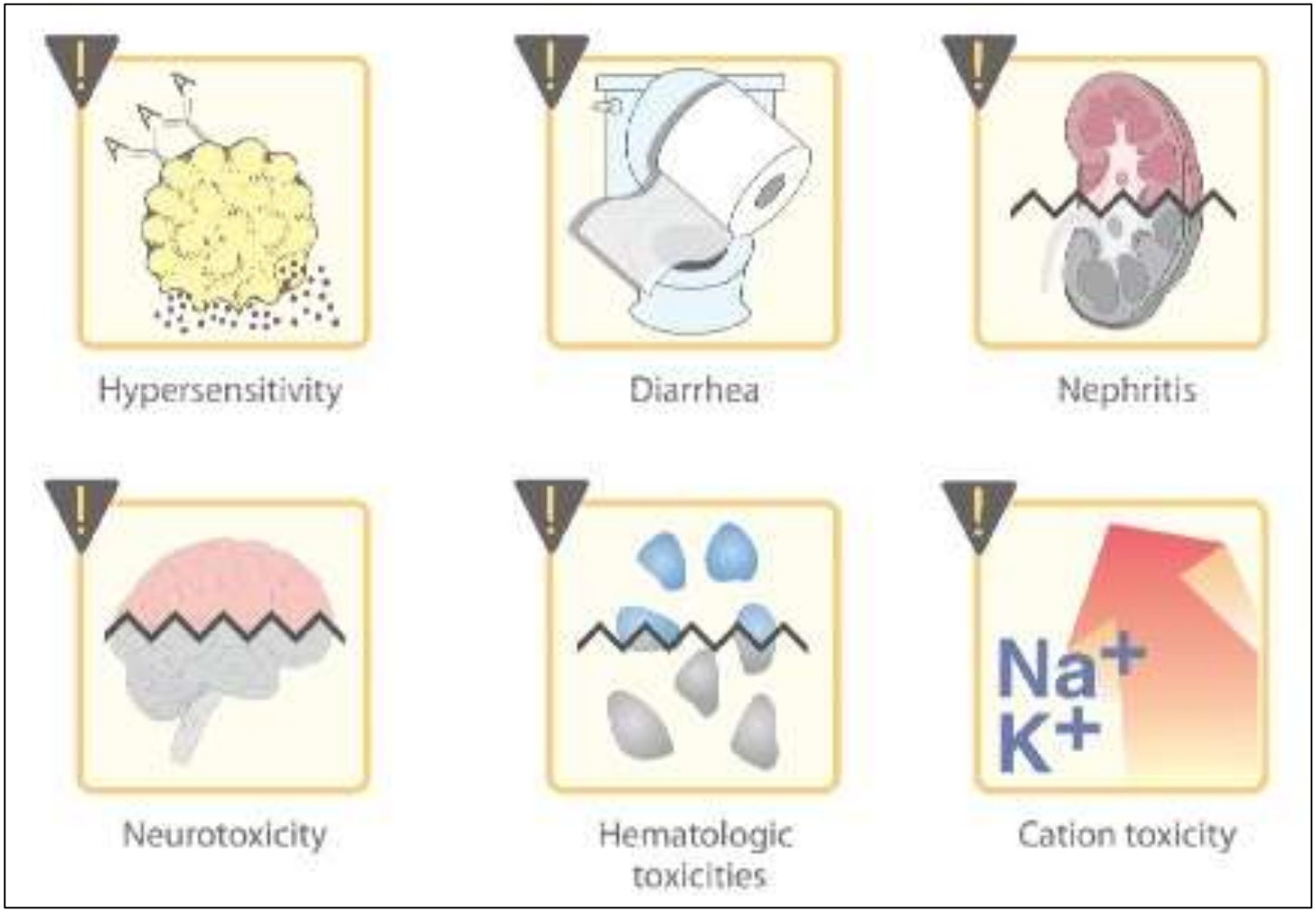
Penicillins
Toxicity/Contraindications

- GI disturbances with oral penicillins
- Large doses given to patients with renal failure can cause lethargy, confusion twitching and seizures
- Sudden release of procaine can cause dizziness, tinnitus, headache, hallucinations



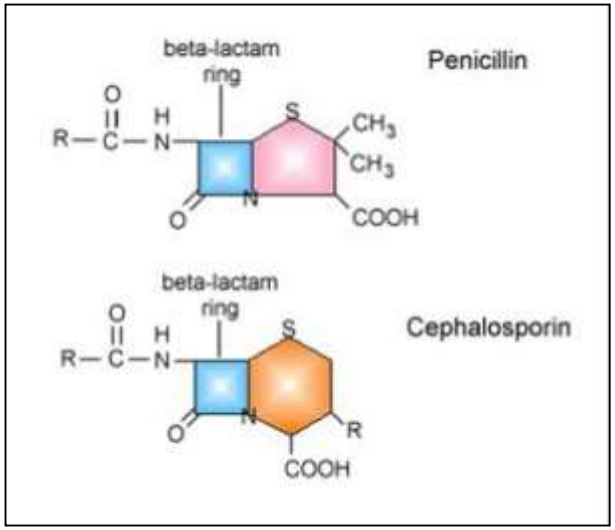
James Heilman, MD

Adverse Effects of Penicillin



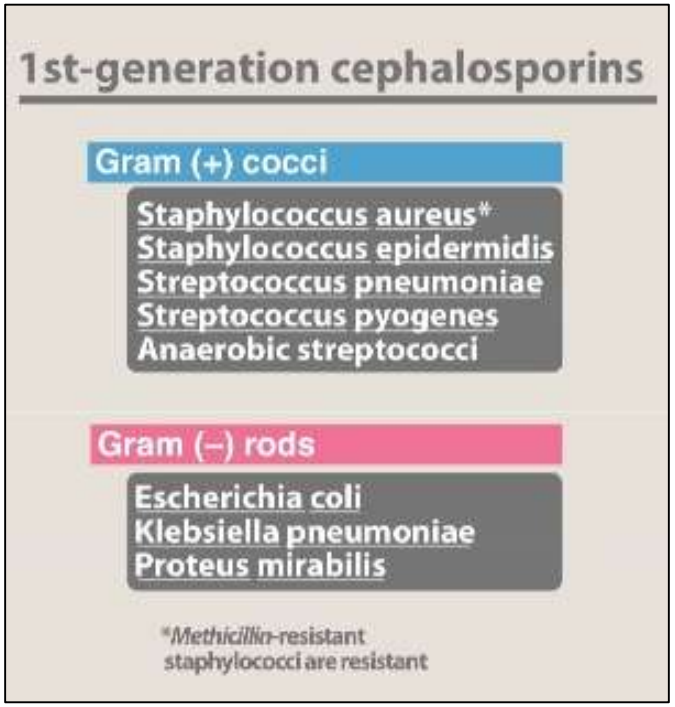
Cephalosporins

- Base molecule is 7-aminocephalosporanic acid produced by a Sardinian sewer mold
- R groups determine spectrum of activity and pharmacological properties
- Mechanism of action/resistance and class pharmacology essentially the same as penicillins



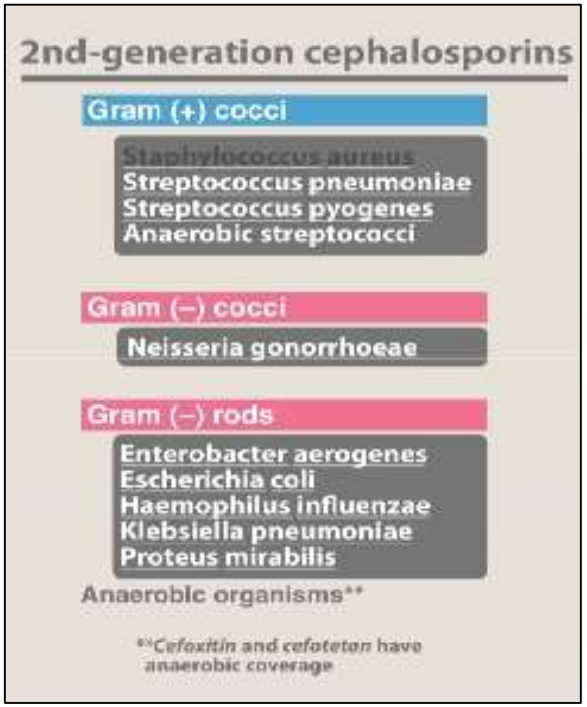
Cephalosporins
1st Generation

- Cefazolin, cephalexin, cephadroxil
- Excellent against susceptible Staph and Strep
- Modest activity against G-
- Act as penicillin G substitutes
- Cefazolin given parentally, others orally
- More than half of the drug is bound to plasma proteins
- Excreted by kidneys unmetabolized
- Good for Staph and Strep skin and soft tissue infections
- Resistant to Staph penicillinase



Cephalosporins
2nd Generation

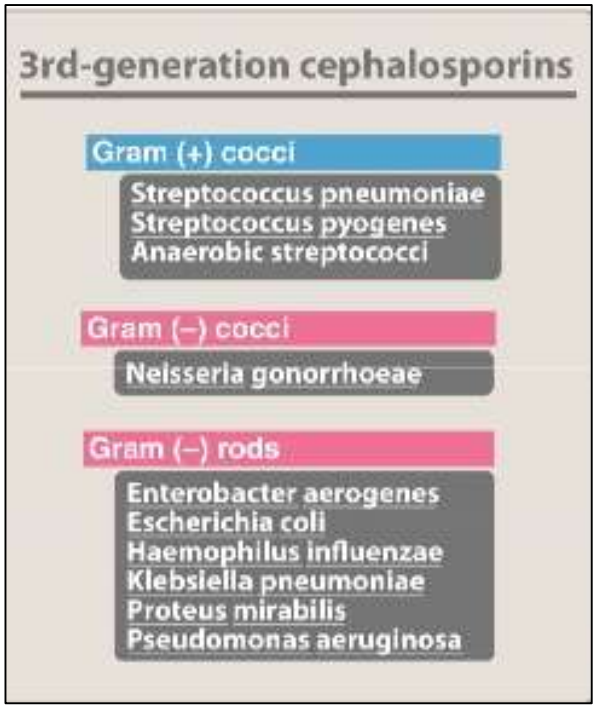
- Cefaclor, cefuroxime, cefprozil, cefotetan, cefoxitine, cefamandole
- Modest activity against G+, increased activity against G-, works against anaerobes
- Cefaclor and cefprozil given orally
- Absorption and excretion same as first gen
- Good for treating
  - Respiratory tract infections
  - Intraabdominal infections
  - Pelvic inflammatory disease
  - Diabetic foot ulcers



Cephalosporins
3rd Generation

- Cefotaxime, ceftriaxone, cefoperazone, cefpodoxime, cefixime
- Broad spectrum killers
- Inferior to 1st gen in activity against MSSA
- Drugs of choice for serious infections
- No effect against *Listeria* and β-lactamase producing peumococci
- Cefpodoxime and cefixime are given orally, others parentally
- Most excreted by kidney

- Therapeutic uses:
  - Bacterial meningitis (2 exception cefoperazone, cefixime)
  - Lyme disease
  - Life-threatening G-sepsis

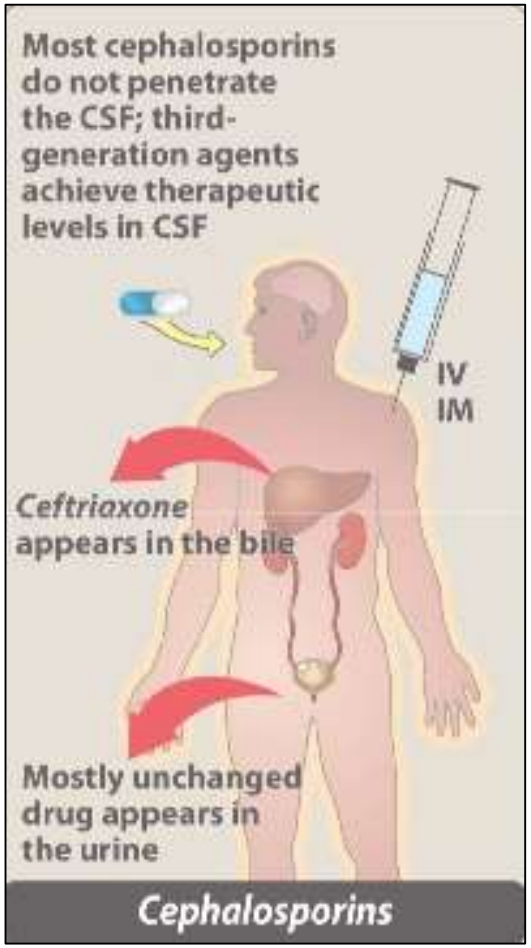


Cephalosporins
4th Generation

- Cefepime
  - Same antimicrobial spectrum as 3rd generation but resist more β-lactamases
  - Given parentally, excellent penetration into CSF
  - Good for nosocomial infections

Administration and Fate of Cephalosporins

- Resistance same as that for penicillins



Cephalosporins
Toxicity/Contraindications

- Hypersensitivity reactions (uncommon) essentially same as for penicillins
- Cross-reaction between 2 classes

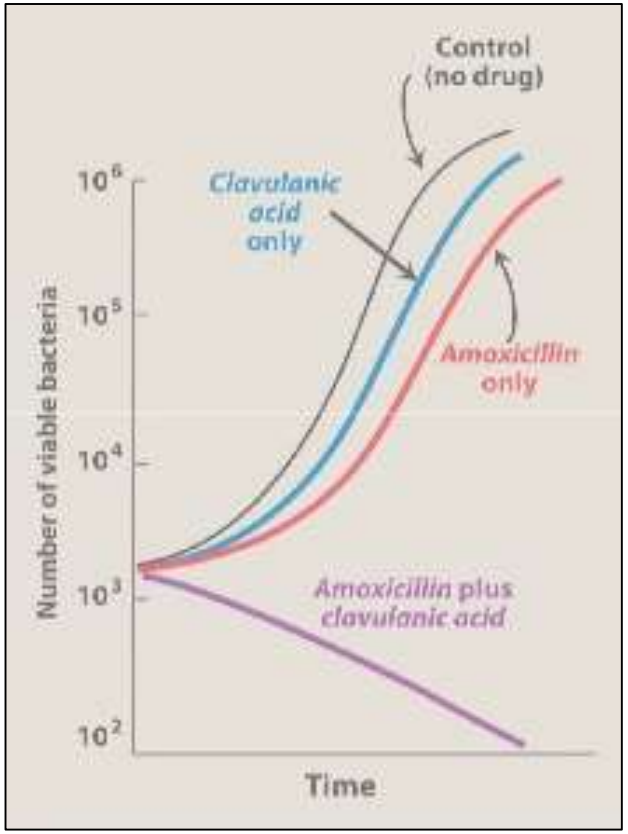
- Adverse effects:
  - Pain at injection site
  - Phlebitis after IV
  - When given with aminoglycosides, may increase nephrotoxicity
  - Drugs containing methylthiotetrazole (e. g. cefamandole, cefaperazone, cefotetan) may cause hypoprothrombinemia and disulfiram-like reaction

Other β-lactam Antibiotics

- Carbapenems
  - Imipenem – broad spectrum of activity against G+ and G- aerobic and anaerobic
  - Meropenem – Important for empirical monotherapy of serious infections
- Monobactams (Aztreonam)
  - Activity restricted to G- aerobic bacteria

β-lactamase inhibitors

- Clavulanic acid – sulbactam and tazobactam
  - Do not have significant antibacterial activity
- Bind to and inactivate the β-lactamases – protect the antibiotics
- Formulated in combination with β-lactamase sensitive antibiotics
- Clavulanic acid and amoxicillin



Growth of *E. coli* in presence of amoxicillin with and without clavulanic acid

Vancomycin

- Tricyclic glycopeptide
- Effective against multiple drug resistant organisms (MRSA) & enterococci
- Resistance is becoming a problem
  - *Enterococcus faecium*
  - *Enterococcus faecalis*

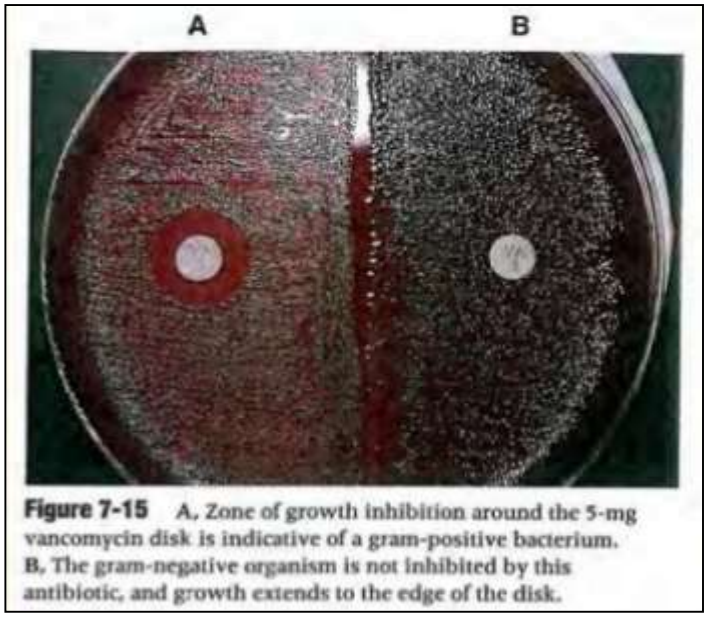
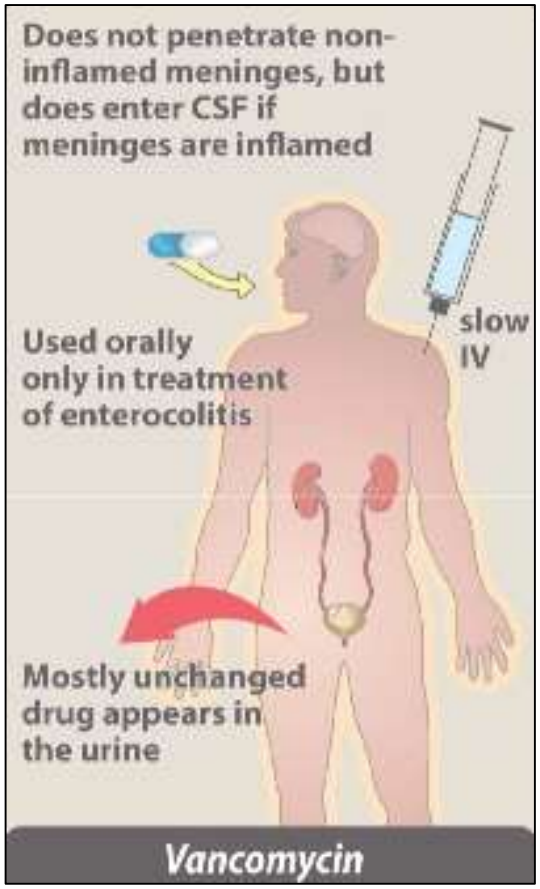
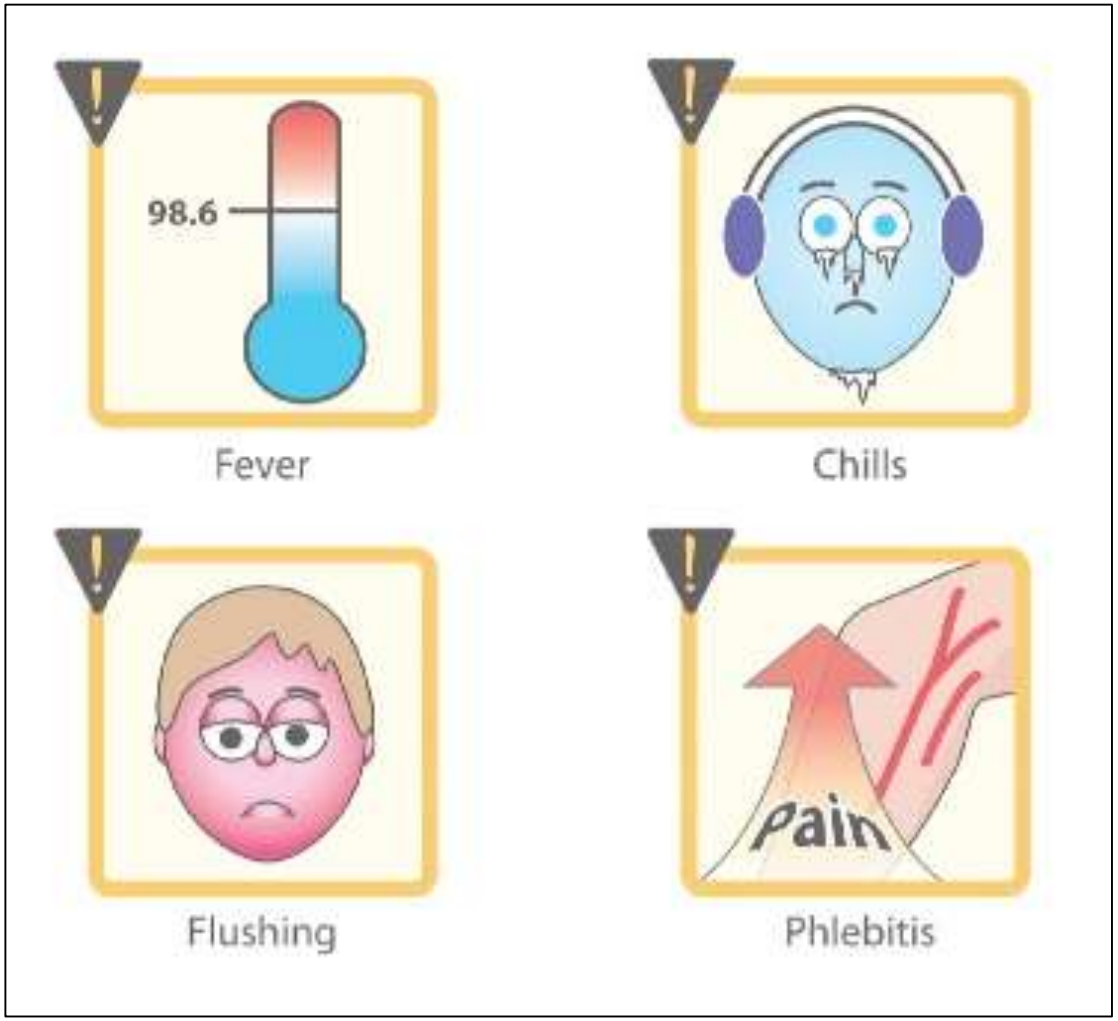


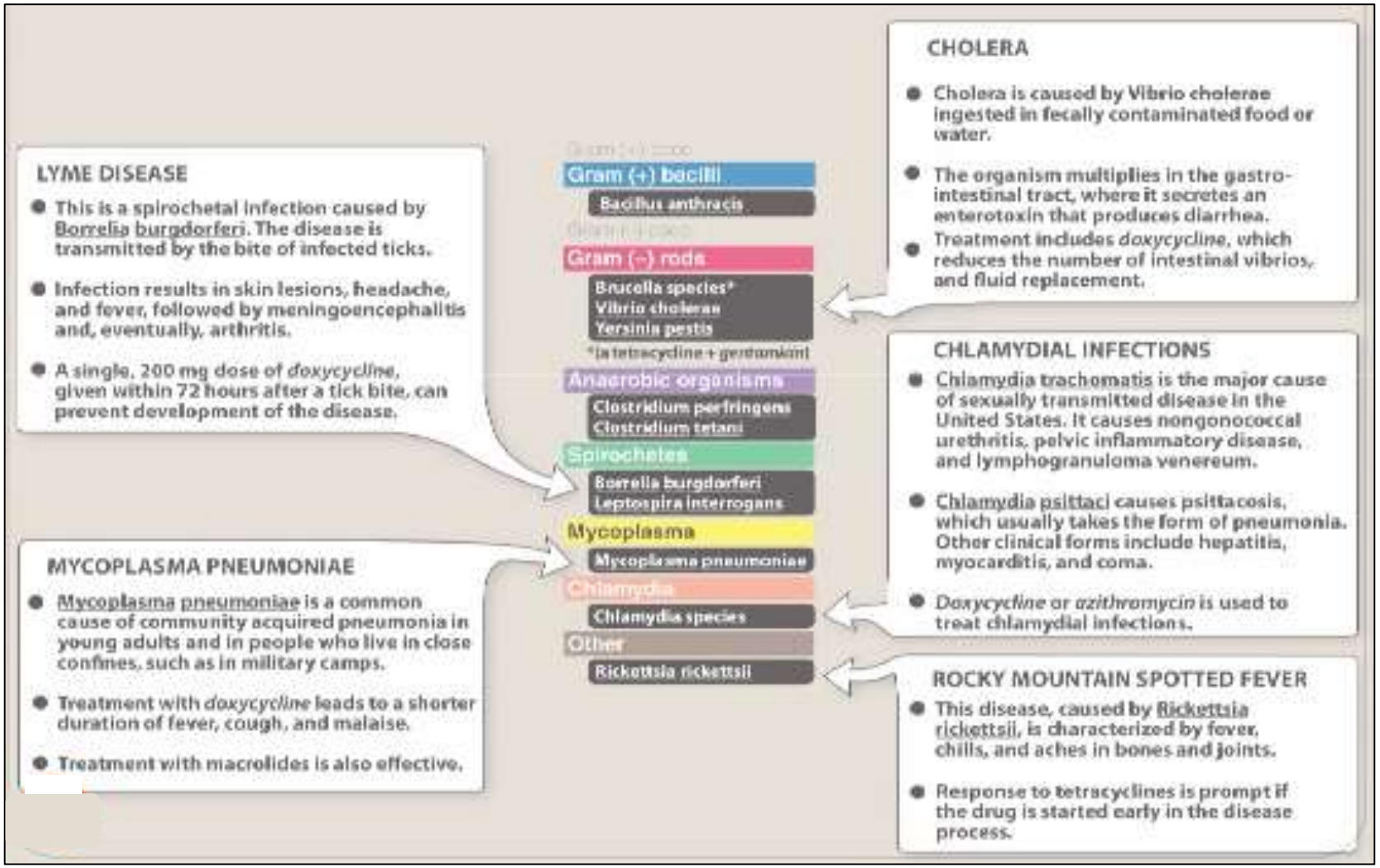
Figure 7-15 A, Zone of growth inhibition around the 5-mg vancomycin disk is indicative of a gram-positive bacterium. B, The gram-negative organism is not inhibited by this antibiotic, and growth extends to the edge of the disk.



Adverse Effects of Vancomycin

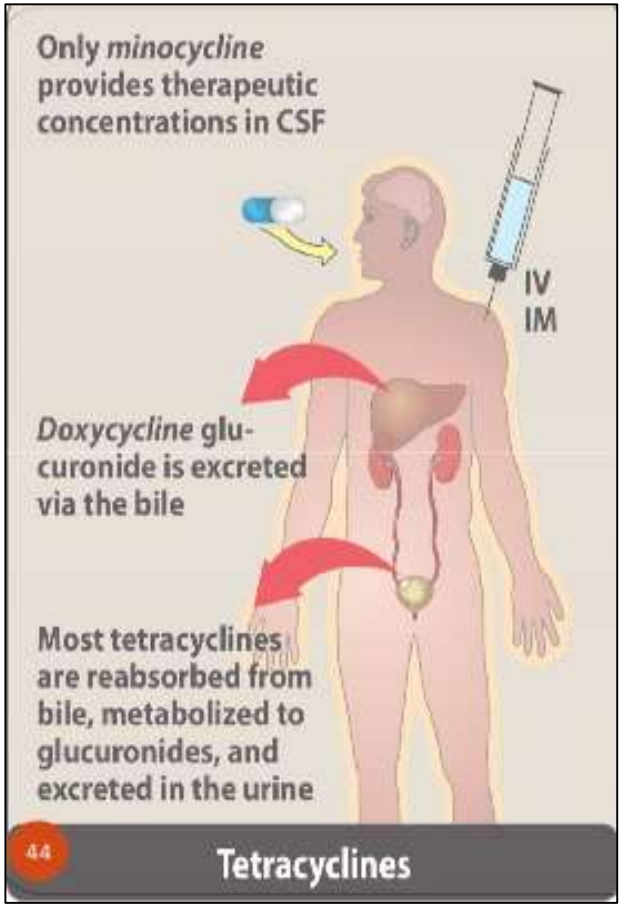


Tetracyclines – drug of choice



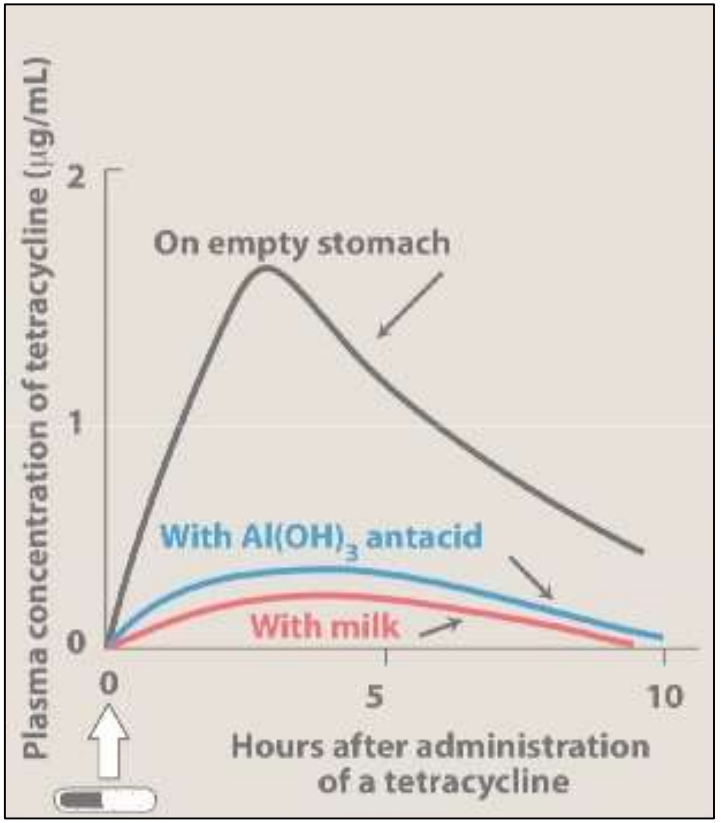
Tetracycline

- Broad-spectrum bacteriostatic antibiotic
- Effective against:
  - G+ and G- bacteria
  - Organisms other than bacteria
- Absorption
  - Adequately but incomplete oral absorption
  - Taking with dairy foods decreases absorption
- Resistance
  - Widespread resistance limits clinical use

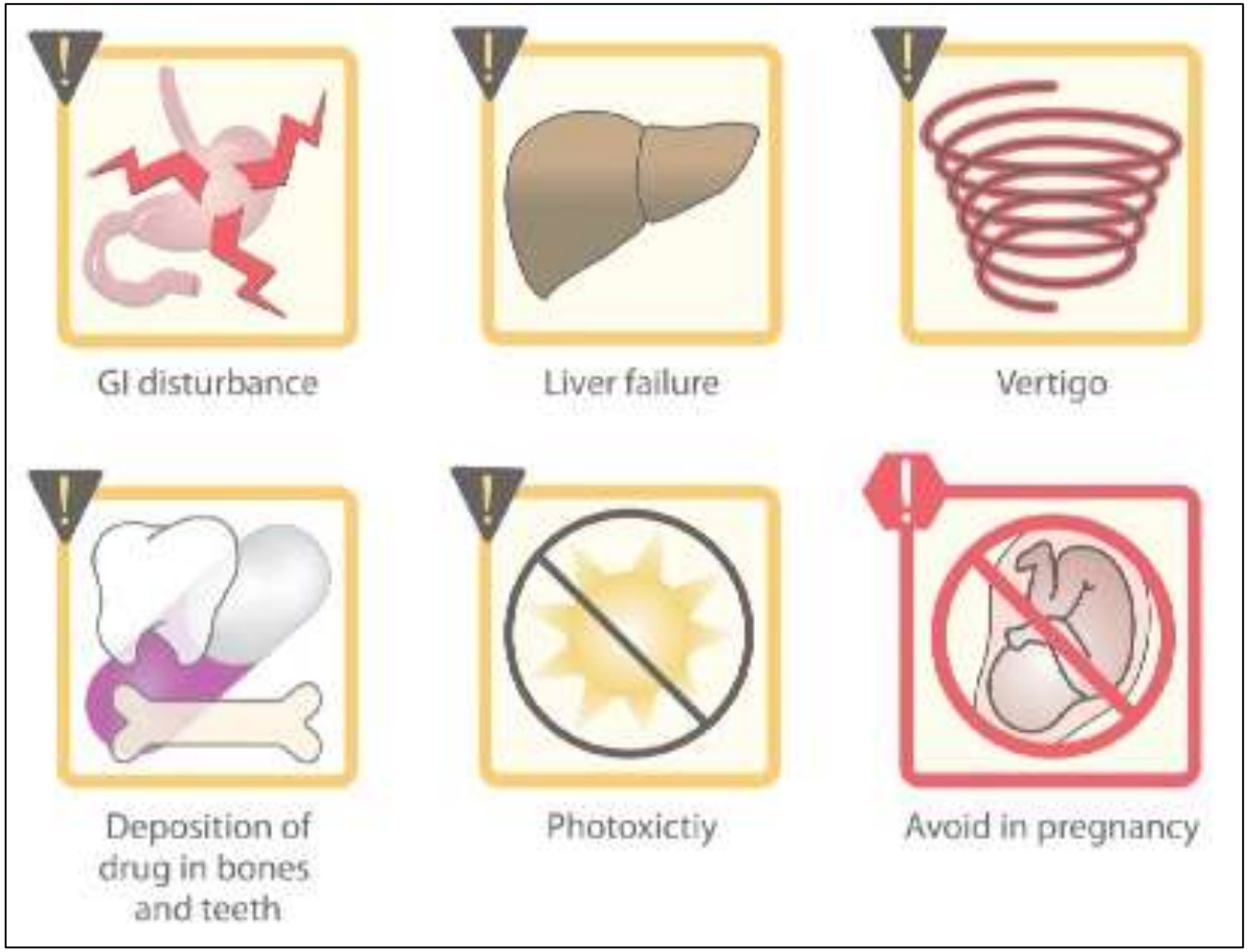


Tetracyclines
Therapeutic Use

- Distribution:
  - Liver, kidneys, liver and skin
  - Blind tio tissue undergoing calcification; bones and teeth, tumours with high calcium
  - Penetrate most body fluids



Adverse Effects of Tetracycline



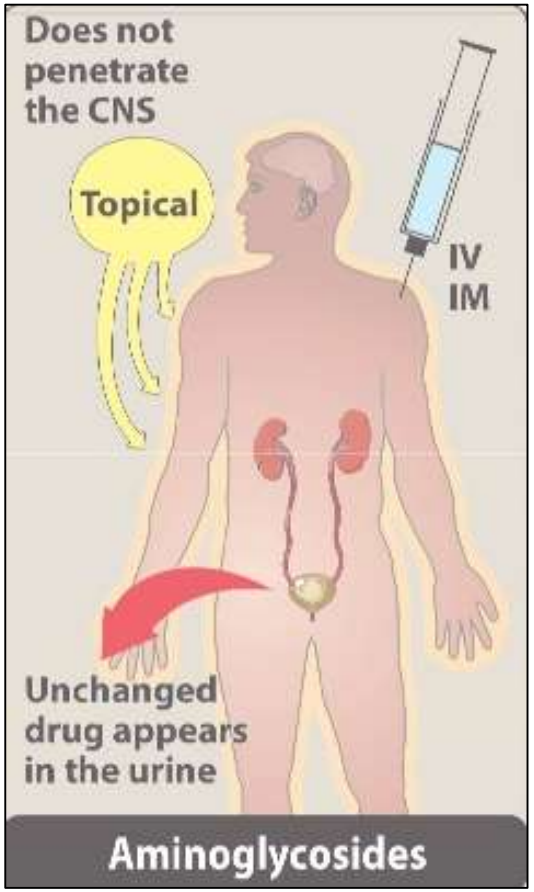
Aminoglycosides

- Similar antimicrobial spectrum to Macrolides
- Relatively toxic but still useful in treatment of infections caused by anaerobic G- bacteria
- Ototoxicity = main limitation
- Inhibit bacterial protein synthesis
- Have a PAE

- **Good to kow:**
  - Only available IV
  - Not absorbed by gut

Aminoglycosides

- Antibacterial spectrum – effective in combination for empirical treatment of aerobic G- bacilli infections (*P. aeruginosa*)
- Combines with β-lactam (Vancomycin), Aminoglycosides and bactericidal amikacin, gentamycin, tobramycin, streptomycin



Adverse Effects of Aminoglycosides

Macrolides (bacteriostatic)

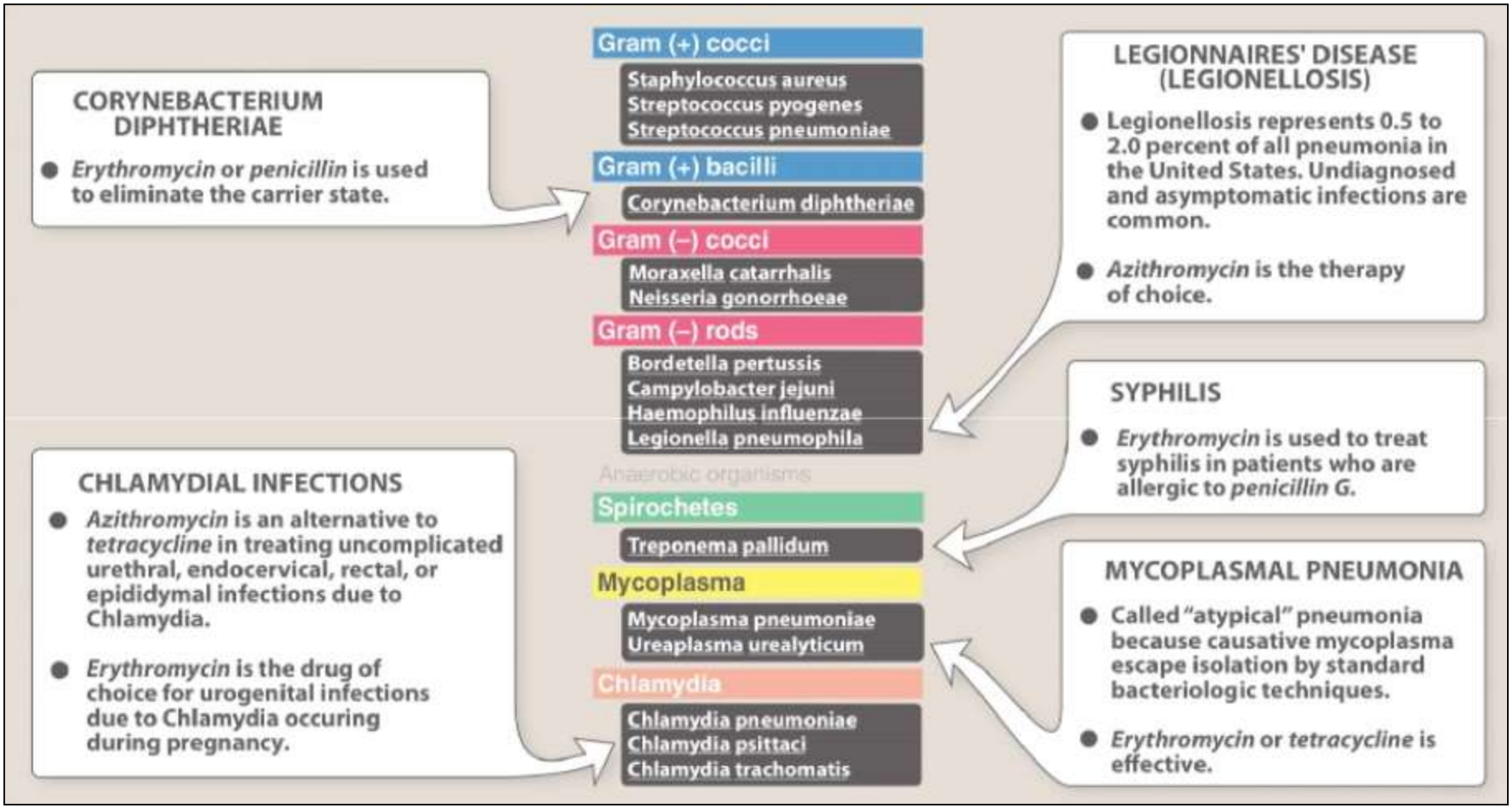
- May also be bactericidal
- Large group of antibacterials
- Low toxicity
- Similar spectrum of activity
- PAE – antibacterial activity continues after concentrations have dropped

- **Good to know:**
  - Take on empty stomach

Macrolides
Antibacterial Spectrum

- Erythromycin – effective against the same organisms as Pen G
- Clarithromycin – apectrum of activity similar to erythromycin also Chlamidia, Legionella, Moraxella & Ureaplasma species & *H. pylori*
- Azithromycin – less active to Strep and Staph. More active against *H. influenzae, M. catarrhalis*
  - Preferred therapy for urethritis caused by *C. trachomatis*
  - Also activity against *M. avium* – intracellulare complex in patients with AIDS
- Telithromycin (ketolite)
  - Spectrum similar to azithromycin, resistance lower = more effective

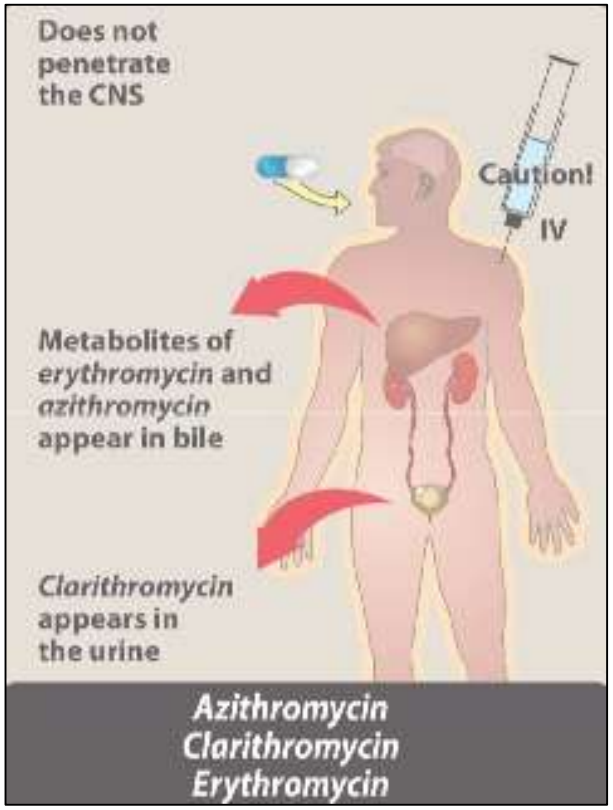
Macrolides
Therapeutic Use



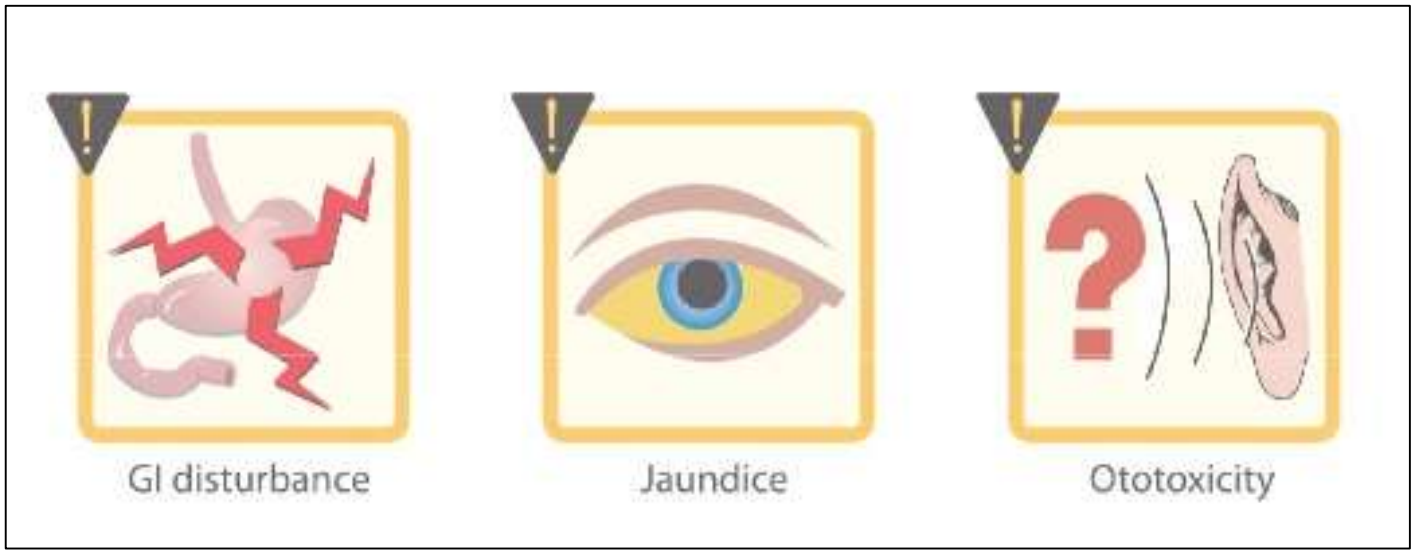
Most strains of staphylococci in hospitals are resistant!

Macrolides

- **Absorption**
  - Food interferes with absorption
  - IV – increased trombophlebitis
- **Distribution**
  - High in all body fluids & prostatic fluids
- **Elimination**
  - Erythromycin & telithromycin interfere with metabolism of drugs such as theophyline & carbamazepine



Macrolides
Adverse Effects



- **Interaction:**
  - Erythromycin, telithromycin and clarithromycin inhibit metabolismof a number of drugs → toxic accumulation

Chloramphenicol

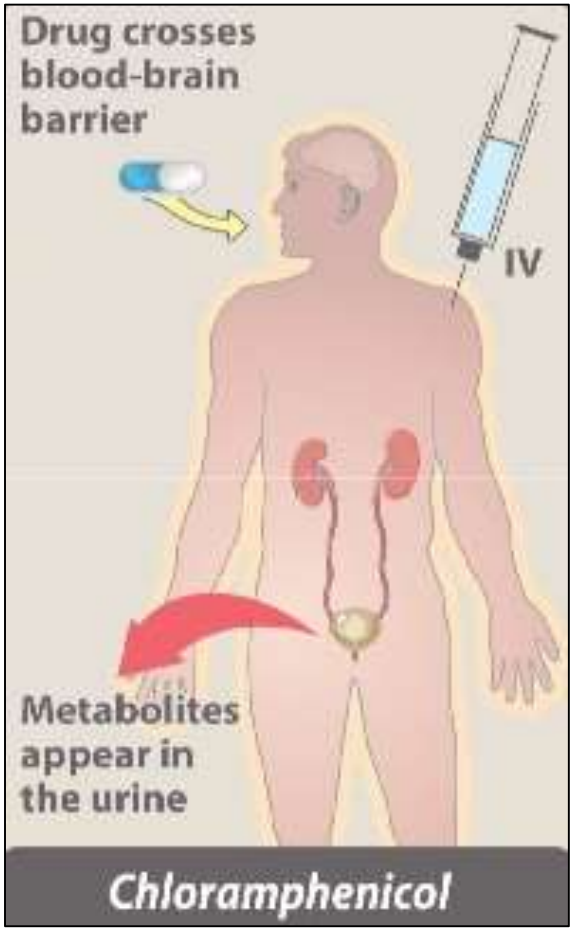
- Active of against a wide range of G+ and G- organisms
- High toxicity – bone marrow toxicity
- Restricted for life –threatening infections where no alternative exists

Chloramphenicol
Spectrum

- Broad spectrum antibiotic
- Activ against bacteria, Rickettsia
- Most affected against *P. aeruginosa* and chlamydiae
- Excellent activity against anaerobes
- Both bactericidal and bacteriostatic

Chloramphenicol
Adverse Effects

- **Clinical use** limited to life threatining infections
  - Serious side effects
  - GI upsets
  - Overgrowth of *C. albicans*
- **Anaemias** – haemolytic anaemia
- **Gray baby syndrome**
  - Poor feeding
  - Deppresed breathing
  - Cardiovascular collapse
  - Cyanosis and death
- **Interactions**
  - Blocks the metabolism of warfarin, phenytoin, tolbutamide, chlopropamide = increasedeffects of the drugs
- **Bone marrow depression**



Clindamycin

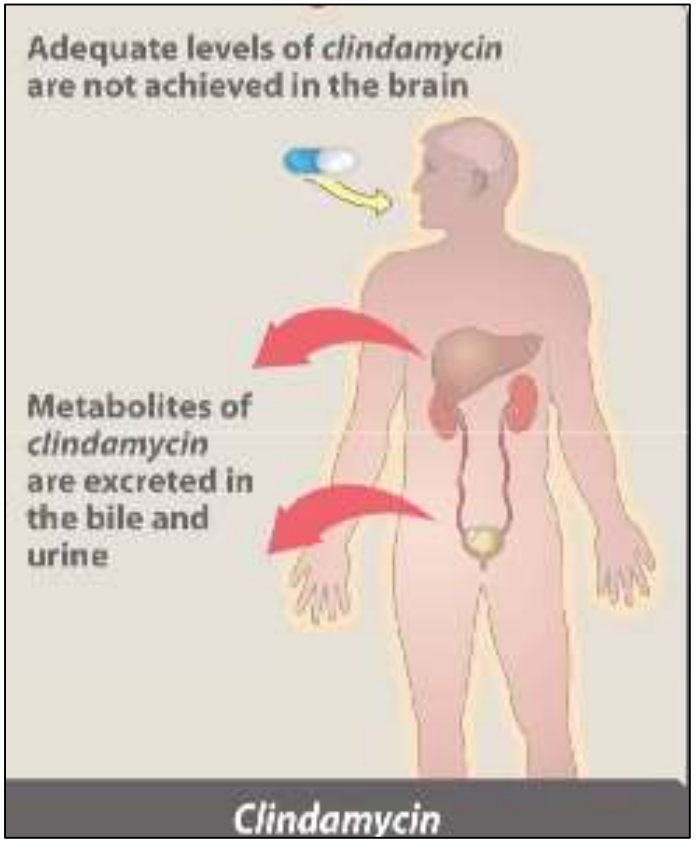
- Mechanism of action same as erythromycin
- Treatment of infections caused by anaerobic bacteria (*Bacteroides fragillis*) – infections associated with trauma, and MRSA
- Resistance same as erythromycin

Clindamycin

- Administration
  - Well absorbed by oral route
  - Adequate levels not achieved in the brain
  - Penetration into bone – good

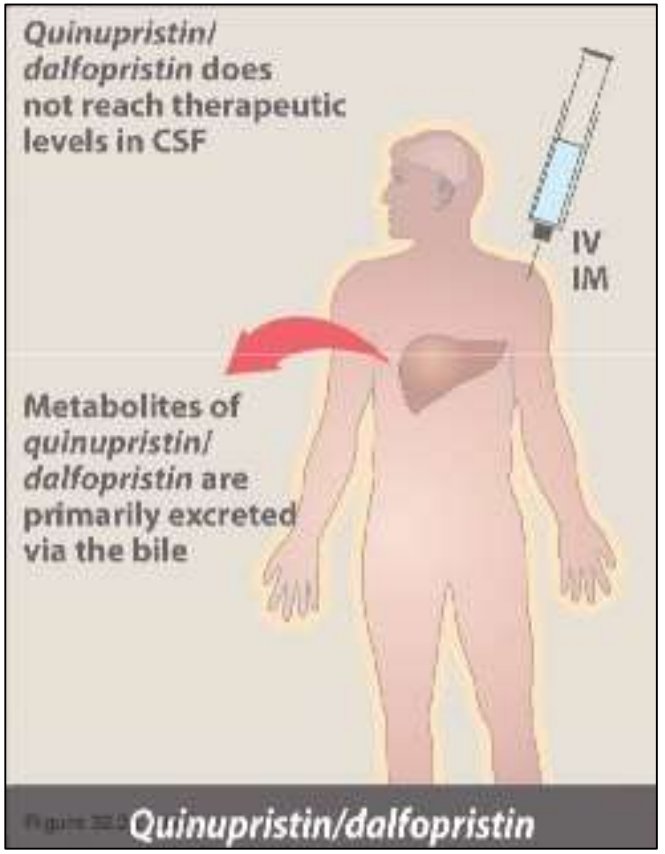
Accumulation of drug in patients with compromised renal function or hepatic failure

- Side Effects
  - Fatal pseudomembraneous colitis



Quinupristin / Dalfopristin

- Reserved for Vancomycin-resistant Enterococcus faecium (VRE)
- Active against G+ cocci including those resistant to other antibiotics, including MRSA
- Primary use treatment of *E. faecium* infections + VRE strains

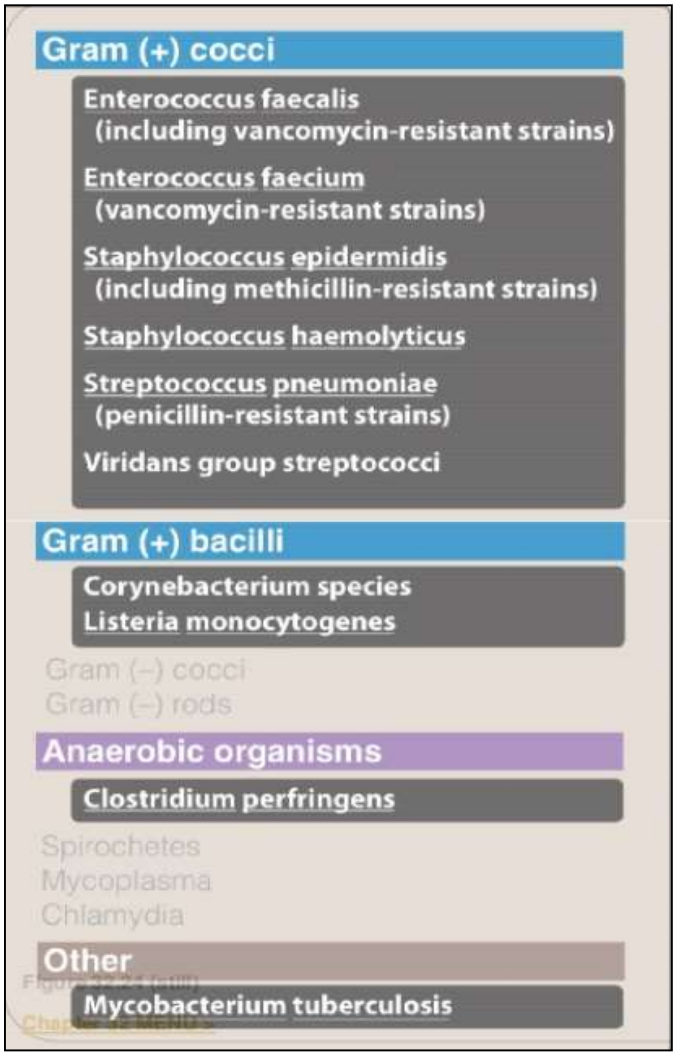


Adverse Effects

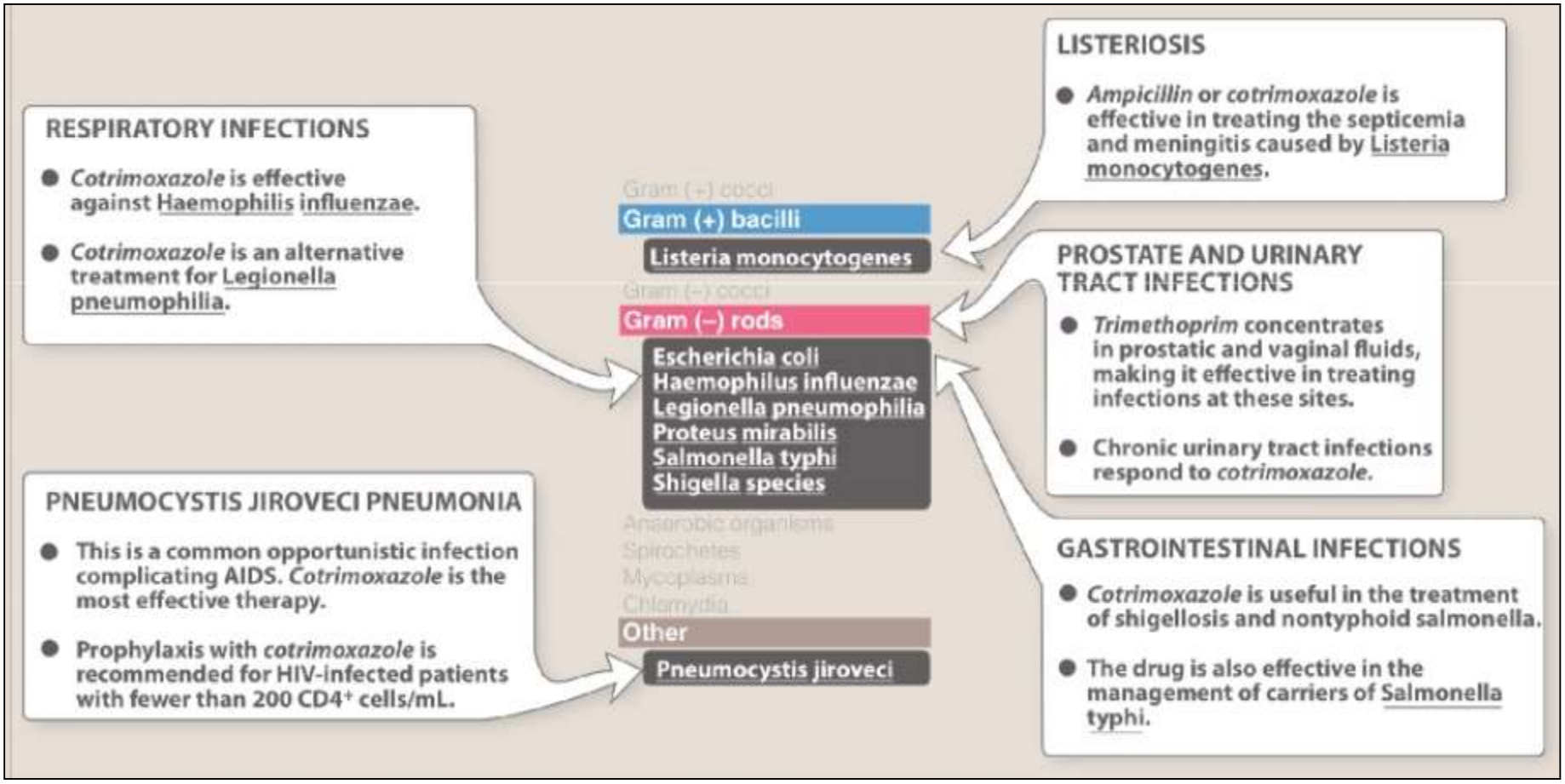
- Venous irritation
- Arthralgia & myalgia
- Hyperbilirubinemia
- Drug interaction

Linezolid

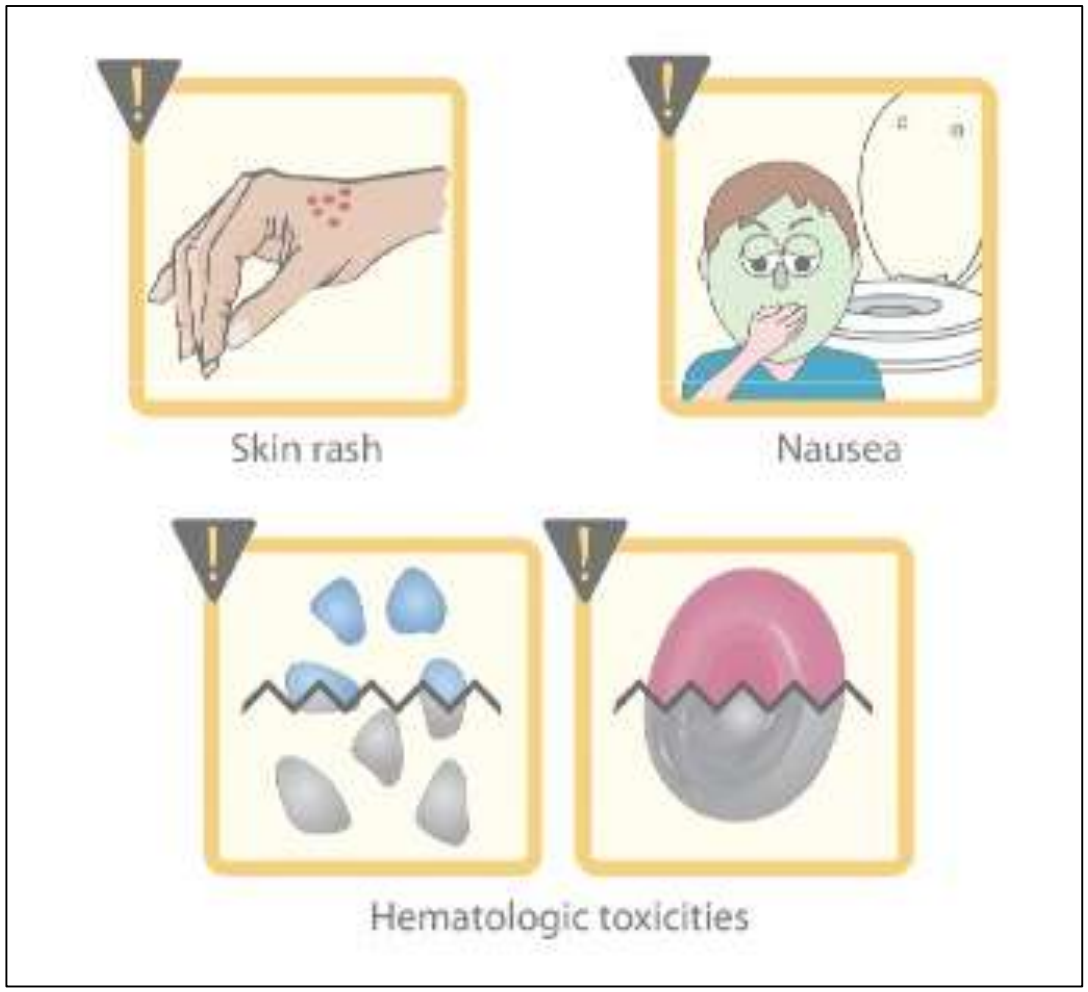
- **Adverse effects**
  - GI upset
  - Diarrhoea
  - Headaches
  - Rash
  - Thrombocytopenia
  - Inhibits MAO activity
  - Precipitate serotonin syndrome in patients taking SSRI's



Therapeutic Application of Cotrimoxazole (sulfamethoxazole plus trimetoprim)

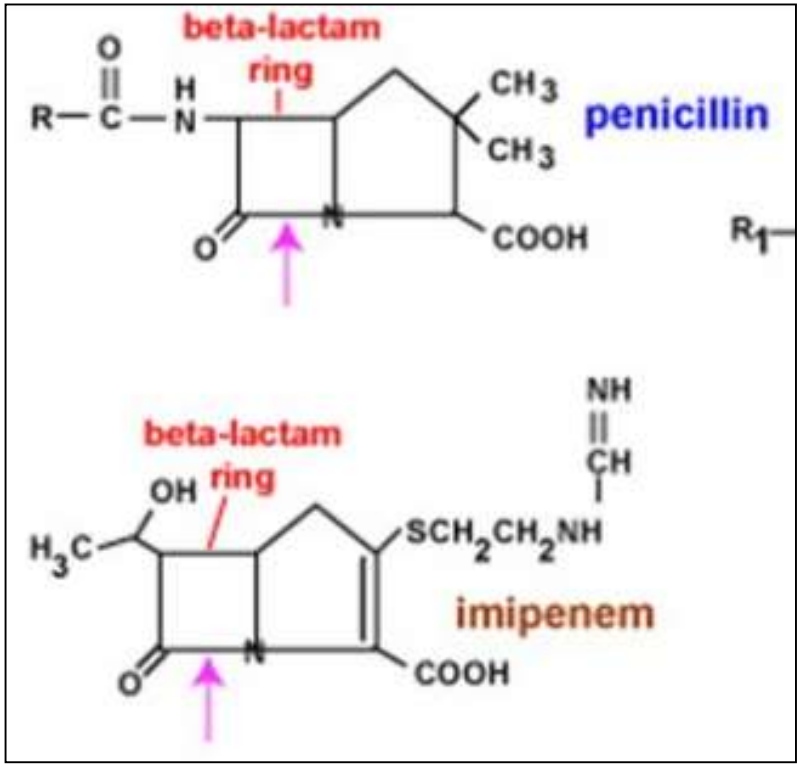


Cotrimoxazole
Adverse Effects



Carbapenems

- Imipenem, Meropenem, Ertapenem
- β-lactam ring is fused to a 5 member ring system



Carbapenems

- Effect on microbes and pharmacology of carbapenems similar to penicillins
- Wider G+ activity, G- and anaerobes
- For pseudomonal infections
  - Given with aminglycosides
- Parenteral administration
- Drugs of choice for infections caused by *Enterobacter*

Carbapenems

- Imipenem
  - Rapidly inactivated by renal dehydropeptidase I
  - Should be given in combination with an inhibitor (Cilastatin)
  - Adverse effects of imipenem-cilastatin :
    - GI distress
    - CNS toxicity
    - Partial cross-allerginicity with penicillins

Carbapenems

- Meropenem
  - Not metabolized by dehydropeptidases
  - Less likely to cause seizures
- Ertapenem
  - Has longer half-like
  - Less effective against pseudomonas
  - Causes pain at site of injection
- Aztreonam – a monobactam
  - Works only on G-, including *Pseudomonas aeruginosa*
  - Useful for treating G- infections that require a β-lactam because it **does not elicit hypersensitivity reactions**

Carbapenems
Toxicity/Contraindications

- Nausea and vommiting (common)
- Hypersensitivity reactions (uncommon)
  - Essentially the same as for penicillins, exception is the monobactam
  - Cross-reactivity is possible, exception is the monobactam

History of Combination Therapy

- Mortality rate
  - Combination therapy – 27%
  - Monotherapy – 47% (p<0,02)
    - Monotherapy was often an aminoglycoside

Hilf M, Yu VL, Sharp J, Zuravieff JJ, Korvick JA, Muder RR. Antibiotic therapy for *Pseudomonas aeruginosa* bacteremia: outcome correlations in a prospective study of 200 patients. Am J Med 1989 Nov; 87(5):540-5.

“Does Combination Antimicrobial Therapy Reduce Mortality in Gram Negative Bacteremia? A Meta-Analysis”

- 17 studies
  - Outcome: mortality
  - Overall gram negative bacteremia – 0,96 (95%CI 0,70-1,32)
  - Pseudomonas – 0,5 (95%CI 0,3-0,79)

Safdar N, Handeisman J, Maki DG. Lancet Infect Dis. Aug 2004; 4(8):519-27.

IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Medical Sciences

2015; 12(9): 695-700. doi: 10.7150/ijms.11988

Research Paper

In vitro Activity of Colistin in Combination with Tigecycline against Carbapenem-Resistant *Acinetobacter baumannii* Strains Isolated from Patients with Ventilator-Associated Pneumonia

Aytekin Cikman[1], Baris Gulhan[1], Merve Aydin[1], Mehmet Resat Ceylan[2], Mehmet Parlak[3], Faruk Karakecili[4], Alper Karagoz[5]

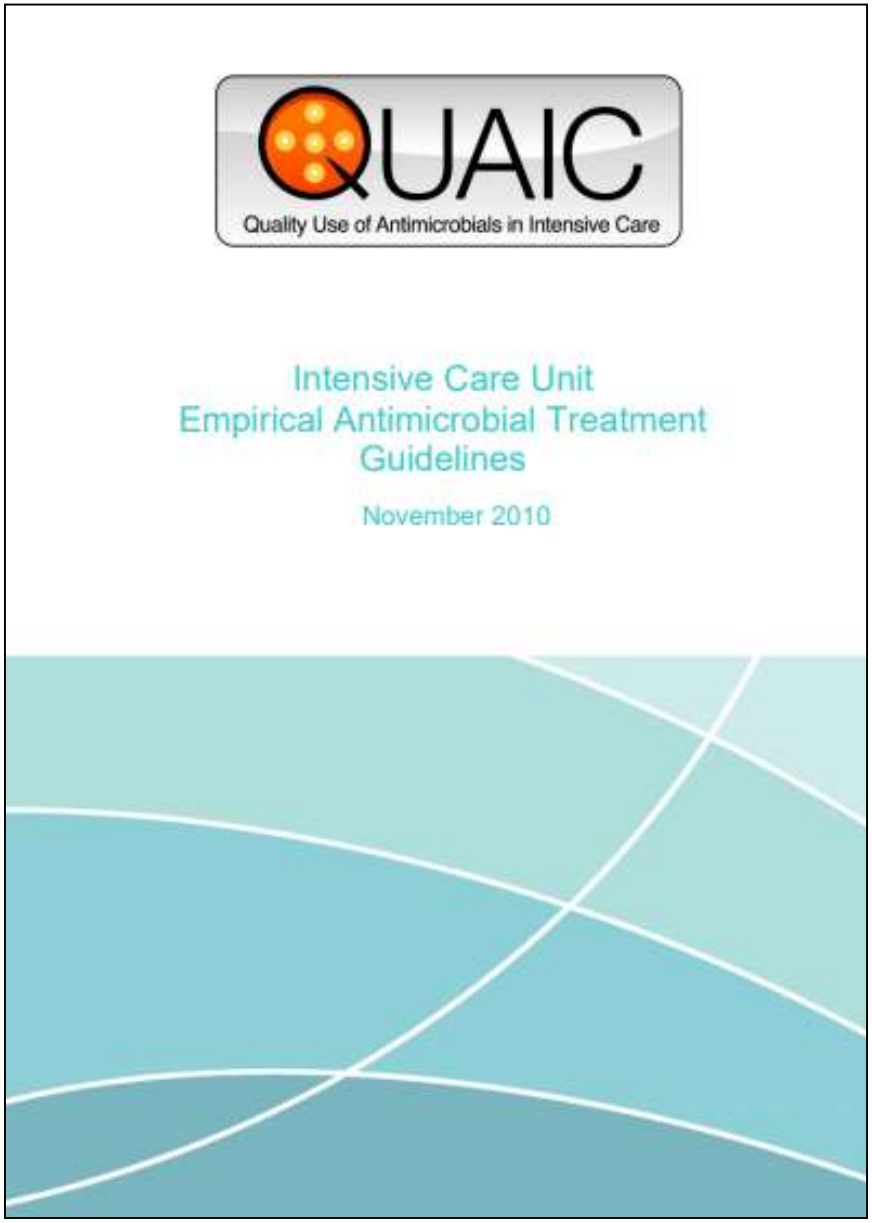
Antagonism in 80% indifference in 20%

Conclusion: In contrast to their synergistic effect against carbapenem-resistant *A. baumannii* isolates, colistin and tigecycline were highly antagonistic to carbapenem-resistant *A. baumannii* strains isolated from patients with VAP when the drugs were administered together. Therefore, alternative treatment options should be used during the treatment of VAP attributed to *A. baumannii*.

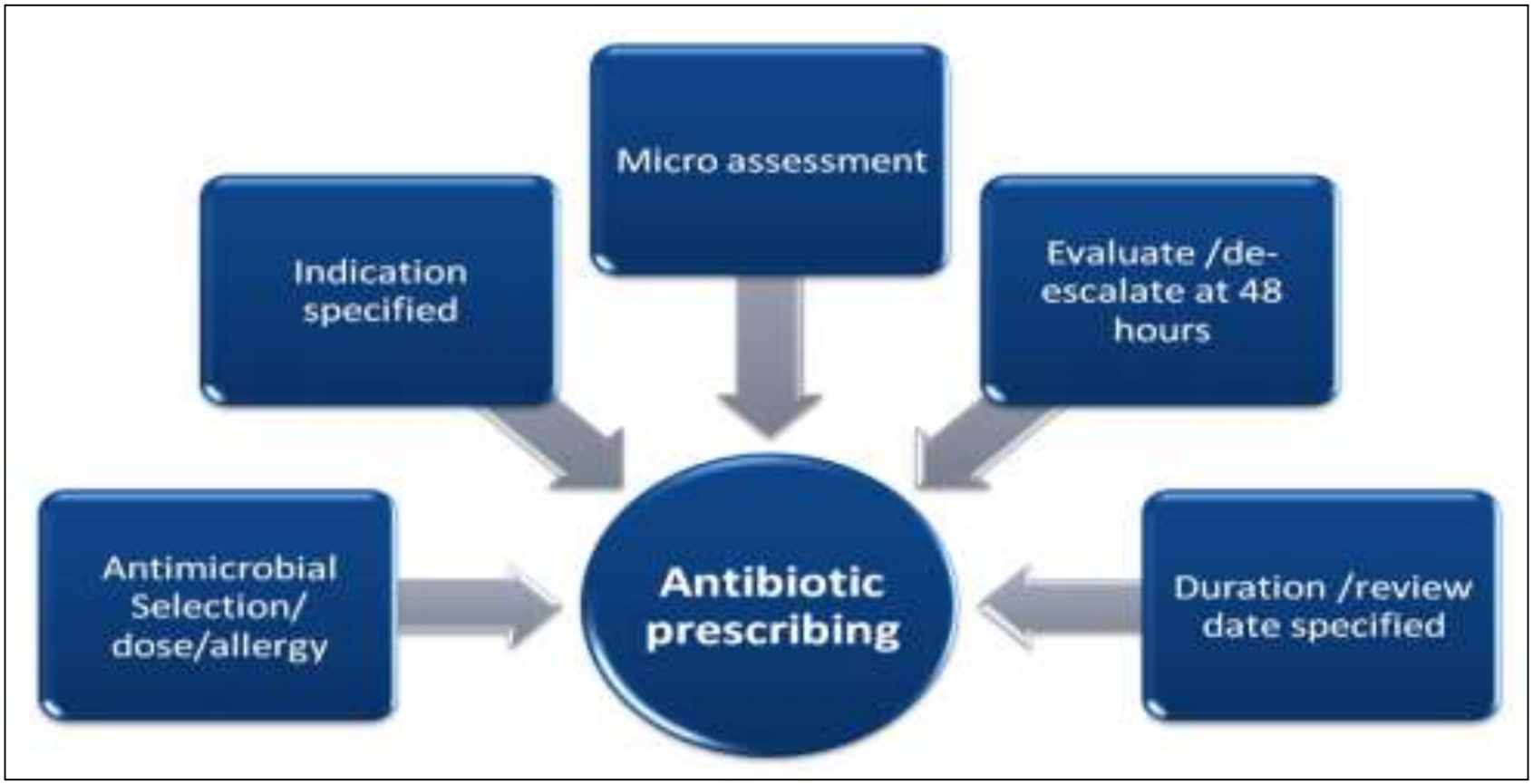
Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates

Antimicrobial agent	n (%) non-susceptibility
Amikacin	48 (47,5)
Cefepime	61 (60,4)
Ceftazidime	70 (69,3)
Ciprofloxacin	74 (73,1)
Colistin	2 (2,0)
Doripenem	76 (75,2)
Gentamicin	51 (50,5)
Fosfomycin	90 (89,1)
Levofloxacin	78 (77,2)
Meropenem	78 (77,2)
Piperacillin/tazobactam	78 (77,2)
Imipenem/cilastatin	76 (75,2)
Tobramycin	58 (57,4)

International journal of Antimicrobial Agents 53 (2019) 408-415


QUAIC
Quality Use of Antimicrobials in Intensive Care
Intensive Care Unit
Empirical Antimicrobial Treatment
Guidelines
November 2010

AIMED Antimicrobial Prescribing Model



Prescribing Principles (AIMED model)

- Select empiric agents in accordance with Antibiotic Guidelines and local antibiogram data.
- Seek Infectious diseases physician /Microbiologist input as required (e. g. life threatening penicillin allergy and de-escalation of therapy).
- Investigate patients appropriately prior to antimicrobial treatment if possible.
- See identification of a potential source and detection of bloodstream events below.
- De-escalate or streamline antimicrobial treatment at 48 hrs based on the clinical picture and relevant microbiological results - where necessary obtain specialist advice.
- Limit duration of therapy according to clinical response, ultimate diagnosis and available evidence by specifying the indication, evaluating at 48 hrs and documenting a specific duration or review date for every antimicrobial course.
- Consider IV to oral switch as soon as clinically feasible.

Prescribing Principles (AIMED model)

- Ensure patients discharged from ICU with antimicrobials still prescribed have a review date provided in the discharge summary.
- When prescribing certain agents the need for ongoing therapeutic drug monitoring should be considered.
  - Aminoglycosides if used beyond 72 hours to detect accumulation.
  - Vancomycin to ensure adequacy of dosing. Adjust dose according to renal function to achieve recommended concentration.
    - For intermittent dosing the target trough concentration is 12–18 mg/L.
    - For continuous infusion the target concentration is 17–23 mg/L.
- If impending renal failure an issue avoid more than 1 dose of gentamicin and consider an antipseudomonal β-lactam such as ticarcillin/ clavulanate or piperacillin/tazobactam as an alternative.
- If sepsis develops when patient has been on antibiotics for more than 48hrs, discuss with an infectious disease physician or microbiologist before changing drugs

Initial Aminoglycoside Dose for Empirical and Directed Therapy

Age	Initial gentamicin/ tobramycin dose [NB1]
10–29 years	6 mg/kg up to 560 mg
30 to 60 years	5 mg/kg up to 480 mg
More than 60 years	4 mg/kg up to 400 mg
10 years or more with severe sepsis (sepsis syndrome) [NB2]	7 mg/kg up to 640 mg
Any age with streptococcal and enterococcal endocarditis	3 mg/kg/day [NB3] (use gentamicin only, in divided doses)

NB1: For subsequent empirical dosing, see Table 2. For subsequent directed dosing, see Antibiotic Guidelines 14, p 361.

NB2: Patients with severe sepsis have higher volumes of distribution and therefore require a higher mg/kg dose.

NB3: Lower doses are used for synergistic treatment in endocarditis (see Streptococcal endocarditis p 57 and Enterococcal endocarditis p 59 in Antibiotic Guidelines 14).

Aminoglycoside Dosing Interval for Subsequent Empirical Doses

Creatinine clearance [NB4]	Dosing interval doses as above	Maximum empiric doses
Greater than 60 mL/min	24 hours	3 (at 0, 24 and 48 hours)
40–60 mL/min	36 hours	2 (0 and 36 hours)
30–40 mL/min	48 hours	2 (0 and 48 hours)
Less than 30 mL/min	Give initial dose then seek expert advice	

NB4: Creatinine clearance estimate should be based on a creatinine measurement obtained as recently as possible (eg within the last 12 to 24 hours); however, this might still overestimate renal function in acute renal failure.

Empiric Antimicrobial Therapy

- Empiric antimicrobial therapy should be initiated early in patients experiencing septic shock (within 1 hour of recognition of septic shock) and sepsis without septic shock, if possible.
- The Surviving Sepsis Campaign guidelines recommend including 1 or more agents that are not only active against the likely organisms but also capable of penetrating “in adequate concentrations into the presumed source of sepsis,” with daily reevaluation of the anti-infective therapy for potential de-escalation.
- Generally, a 7- to 10-day treatment course is followed. Longer treatment regimens may be warranted in the presence of a slow clinical response, undrainable foci of infection, and immunologic deficiencies (e. g. neutropenia). The use of procalcitonin or similar biomarkers may facilitate discontinuance of antibiotics in patients with clinical improvement and no further evidence of infection.

Empiric Antimicrobial Therapy

- Combination empiric therapy is recommended for patients with the following:
  - Difficult-to-treat, multidrug-resistant microorganisms (*Pseudomonas* and *Acinetobacter* species)
  - Severe infections associated with respiratory failure and septic shock
  - Septic shock and bacteremia from pneumococci
- However, combination therapy should be limited to 3-5 days, after which period treatment should switch to the most appropriate monotherapy once the results of the susceptibility profile are available.
- The following points must always be considered:
  - Early broad-spectrum empiric antibiotic therapy is essential; the coverage spectrum will be narrowed later, when culture results become available
  - Waiting until cultures are back is an invalid reason to withhold antibiotics
    - Only 30% of patients with presumed septic shock have positive blood cultures
    - About 25% of presumed septic shock patients remain culture-negative from all sites, but mortality is similar to that for culture-positive counterparts
  - Promptly discontinue antimicrobial therapy if the patient's condition is determined to be from a noninfectious source

Empiric Antimicrobial Therapy

- Antibiotic selection
  - The selection of appropriate agents is based on the patient's underlying host defenses, the potential sources of infection, and the most likely culprit organisms. Antibiotics must be broad-spectrum agents and must cover G+, G-, and anaerobic bacteria because organisms from any of these different classes can produce the same clinical picture of distributive shock.
  - If the patient is "antibiotic-experienced," strong consideration should be given to using an aminoglycoside rather than a quinolone or cephalosporin for G- coverage.
  - Antibiotics should be administered parenterally, in doses adequate to achieve bactericidal serum levels. Many studies have found that clinical improvement correlates with the achievement of serum bactericidal levels rather than with the number of antibiotics given.
  - In the selection of empiric antibiotics, the increasing prevalence of MRSA must be taken into account, and an agent such as vancomycin or linezolid should be included. This is especially true in patients with a history of IV drug use, those with indwelling vascular catheters or devices, or those with recent hospitalizations. Antianaerobic coverage is indicated in patients with intra-abdominal or perineal infections.

Empiric Antimicrobial Therapy

- Antibiotic selection
  - Certain organisms, chiefly Enterobacteriaceae (*E. coli* and *K. pneumoniae*), contain a β-lactamase enzyme and thus inactivates these antibiotics (ESBL-producing bacteria). β-lactam antibiotics that have remained effective against ESBL-producing organisms include cephamycins (cefotetan) and carbapenems (imipenem, meropenem, and ertapenem).
  - In immunocompetent patients, monotherapy with carbapenems (imipenem and meropenem), third- or fourth-generation cephalosporins (cefotaxime, cefoperazone, ceftazidime, cefepime), or extended-spectrum penicillins (ticarcillin and piperacillin) is usually adequate, without the need for a nephrotoxic aminoglycoside. Patients who are immunocompromised or at high risk for MDR organisms typically require dual broad-spectrum antibiotics with overlapping coverage.
  - Within these general guidelines, no single combination of antibiotics is clearly superior to any other.
  - Three additional antibiotics, oritavancin, dalbavancin, and tedizolid, are approved by the FDA for the treatment of acute bacterial skin and skin structure infections. These agents are active against *S. aureus* (including MSSA and MRSA isolates), *S. pyogenes*, *S. agalactiae*, *S. anginosus* group (includes *S. anginosus*, *S. intermedius*, *S. constellatus*), among others.

Empiric Antimicrobial Therapy
Community-acquired pneumonia

- For inpatients with pneumonia who are not admitted to the ICU, the guidelines formulated by the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) recommend administering the following:
  - A respiratory fluoroquinolone, especially in penicillin-allergic patients
  - A β-lactam agent (cefotaxime, ceftriaxone, ampicillin) plus a macrolide; ertapenem may be used for selected patients, and doxycycline may be an alternative to the macrolide
  - Antibiotic therapy for a minimum of 5 days for community-acquired pneumonia; the treatment duration may be increased in complicated cases or in cases where the initial therapy did not provide a clinical response against the identified organism

Empiric Antimicrobial Therapy
Community-acquired pneumonia

- For inpatients with pneumonia who are admitted to the ICU, the IDSA/ATS guidelines offer the following minimal recommendations:
  - Administer a β-lactam (cefotaxime, ceftriaxone, ampicillin-sulbactam) plus either azithromycin or a fluoroquinolone; penicillin-allergic patients may receive a respiratory fluoroquinolone and aztreonam
  - For pseudomonal infections, administer:
    - An antipneumococcal, antipseudomonal β-lactam agent (piperacillin-tazobactam, cefepime, imipenem, meropenem) plus ciprofloxacin or levofloxacin;
    - The β-lactam above plus an aminoglycoside and azithromycin; or
    - The β-lactam above plus an aminoglycoside and an antipneumococcal fluoroquinolone (for penicillin-allergic patients, use aztreonam instead of the above β-lactam)
  - Add vancomycin or linezolid for patients with community-acquired MRSA (CA-MRSA) infection

Principles of Appropriate Use of Antibiotics
Adapted from Lipman

1. Take cultures before administering antibiotics
2. Take 2 sets of cultures, not from a line
3. Timing of blood cultures with fever is not critical
4. Do not delay the administration of antibiotics
5. Use empirical therapy first; narrow the spectrum later
6. Ensure initial doses are sufficient – under-doing must be avoided
7. Use monotherapy where possible (reduces cost and toxicity).
8. If the microbiology results suggest decreased susceptibility, consider whether the antibiotics working clinically. If there is direct bedside evidence that they are working, then continue them in spite of laboratory evidence. *In vitro* sensitivity does not always predict *in vivo* effect!
9. A shorter course (e.g. 7 days) is probably as good as a standard 2-week course in most cases
10. Infectious diseases specialists should be consulted when managing serious infections
11. Know antimicrobial pharmacokinetics and pharmacodynamics; consider tissue penetration and dose adjustment to correct for altered clearance
12. Monitor antibiotic levels when available
13. Limit “prophylactic” use to appropriate situations
14. Consider non-infective causes of inflammation (sepsis mimics are surprisingly common)
15. Adhere to infection control policies
16. Have an antimicrobial stewardship program in the ICU

Common Errors in Antibiotic Use
Adapted from Lipman

1. Delay in antibiotic administration in severe sepsis
    1. It is suggested that within 1 hour from triage is a reasonable target (first 6 hours after the onset of hypotension was associated with >7% decrease in survival)
2. Antibiotics given before cultures taken
3. Contaminated or insufficient blood culture collection
4. Excessively long courses of antibiotics
5. Erratic changes of antibiotics in non-resolving sepsis
6. Inadequate doses
7. Poor choice of empirical antibiotics, failing to account for resident flora
8. Failure to predict toxicity or account for interactions
9. Failure to consider tissue penetration of different antibiotics
10. Inappropriate use of antibiotic polypharmacy or failure to de-escalate to monotherapy

Example of an ICU Antibiotic Guideline General Rules

- Pneumonia
  - ceftraixone + azithromycin (community acquired)
  - ceftriaxone + metronidazole (aspiration)
- Abdominal focus
  - ampicilin + gentamicin + metronidazole
- Neurosurgical infection
  - vancomycin 1g q6h + ceftazidime 2g q6h
  - no need for routine prophylaxis if EVD inserted
- Necrotizing fasciitis
  - meropenem 1g q8h + benzylpenicilin 2.4g q4h + lincomycin 900mg q8h
  - if GPC on biopsy = suspect group A streptococcus -> IvIg 1g/kg on day 1, 0.5 g/kg/d on days 2 and 3

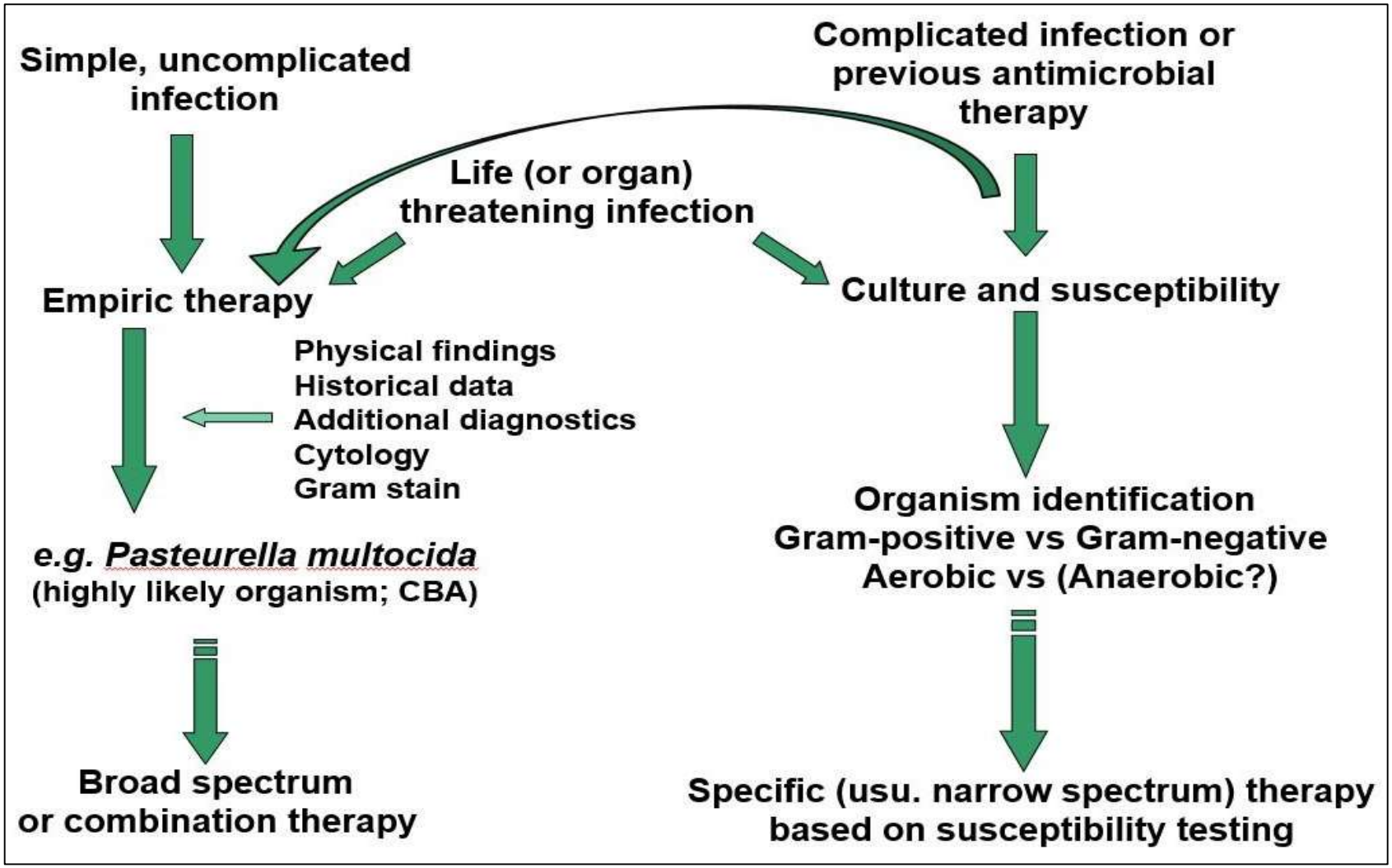
Example of an ICU Antibiotic Guideline General Rules

- Sepsis unknown source or VAP
  - Always cover prior resistant bacteria even if found much earlier (e.g. VRE if previously colonised)
  - Tazocin + gentamicin
    if mild rash to penicillin then cefepime + gentamicin
    if anaphylaxis/ DRESS/ Steven-Johnson to penicillin then vancomycin + aztreonam + gentamicin
    use ciprofloxacin not gentamicin if eGFR <50, age >65y or recently on gentamicin
  - Add vancomycin if:
    hospitalised >7 days or recent admission past 3 months
    prior MRSA
    pre-existing longterm lines
  - Teichoplanin if VRE colonised
  - Add fungal cover in severe sepsis using fluconazole if ECMO or yeast isolated
    if already on fluconazole change to caspofungin
    if already on posaconazole or voriconazle talk to ID!

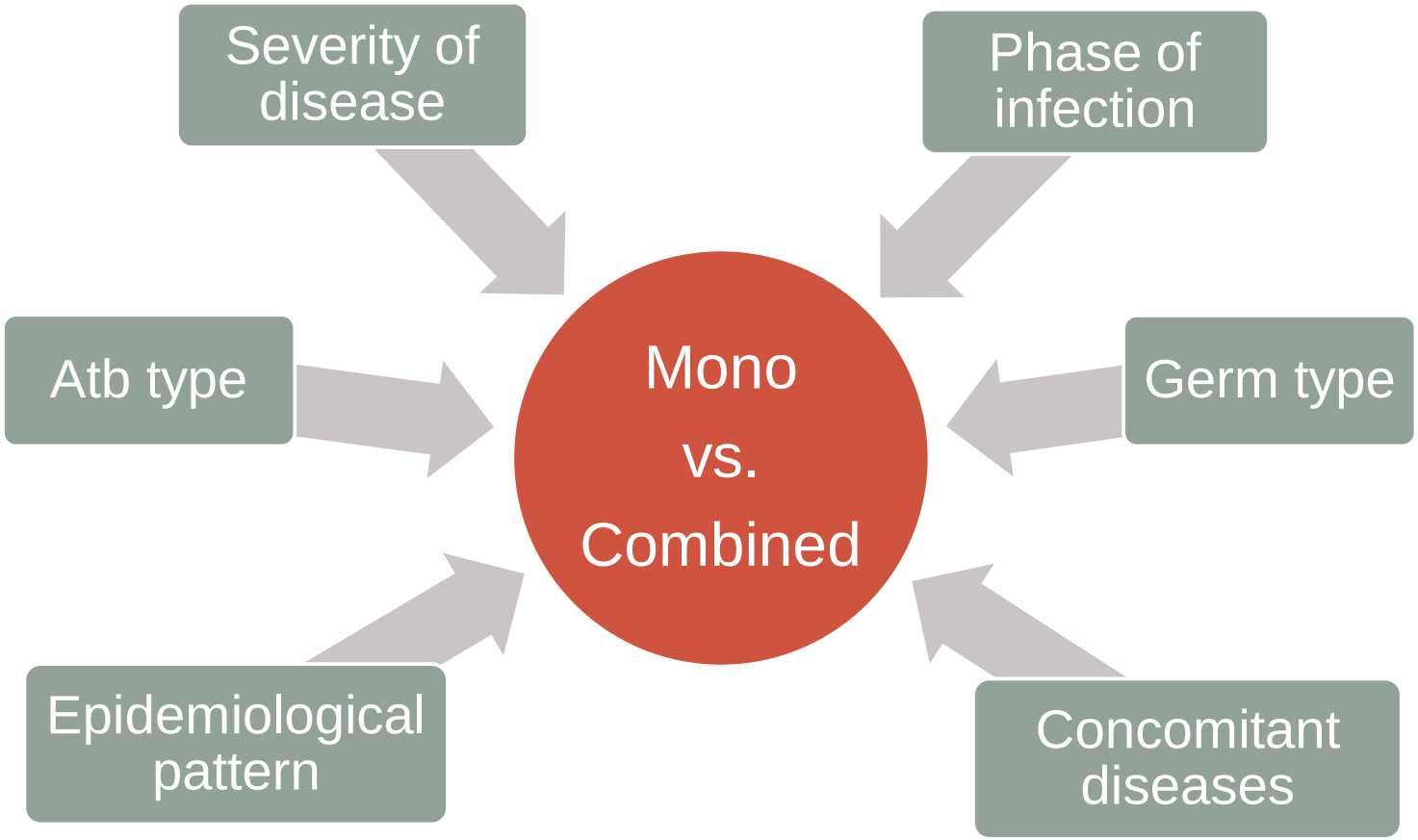
Example of an ICU Antibiotic Guideline General Rules

- When to stop antibiotics
  - Day 2
    vanc/ gent/ cipro/ flucon/ caspo
    if patient improved and no MRSA, resistant GNBs or candida
    streamline tazocin when sensitivities available
    switch gentamicin to ciprofloxacin if 2nd agent still needed
  - Day 6
    stop all antibiotics unless specific diagnosis requires longer – discuss with ID

Determine the Need: Empiric vs. Specific Therapy



Factors Influencing Decision



Antibiotic Combination Therapy

- Indications for combination therapy may include:
  - Infections caused by multiple microorganisms (e. g. abdominal and pelvic infections)
  - Nosocomial infections, which may be caused by many different organisms
  - Serious infections in which a combination is synergic (e. g. an aminoglycoside and an antipseudomonal penicillin for pseudomonal infections)
  - Likely emergence of drug –resistant organisms if a single drug is used (e. g. tuberculosis). Although drug combinations to prevent resistance are widely used, the only clearly effective use is for treatment tuberculosis
  - Fever or other signs of infection in clients whose immune systems are suppresed. Combinations of antibacterial plus antiviral and/or antifungal drugs may be needed

Clinical Indications of Combination

- Polymicrobial infection
- Specific sites (infective endocarditis, meningitis, pneumonia)
- Specific organisms (TB, legionella)
- **Initial/Empirical therapy for severe infection**
- **MDRO**

Initial/Empiric broad spectrum/combination antibiotics

- Severe sepsis (organ impairment/shock)
- Hospital Acquired / Health-Care Associated Infection
- Multiple co-morbidities
- Immunosuppressed
- Exposure to multiple broad spectrum antibiotics in the last 90 days
- Recent procedures or patient has devices in situ
- Elderly >65 years
- Colonized with MDROs

Combined antibacterial therapy

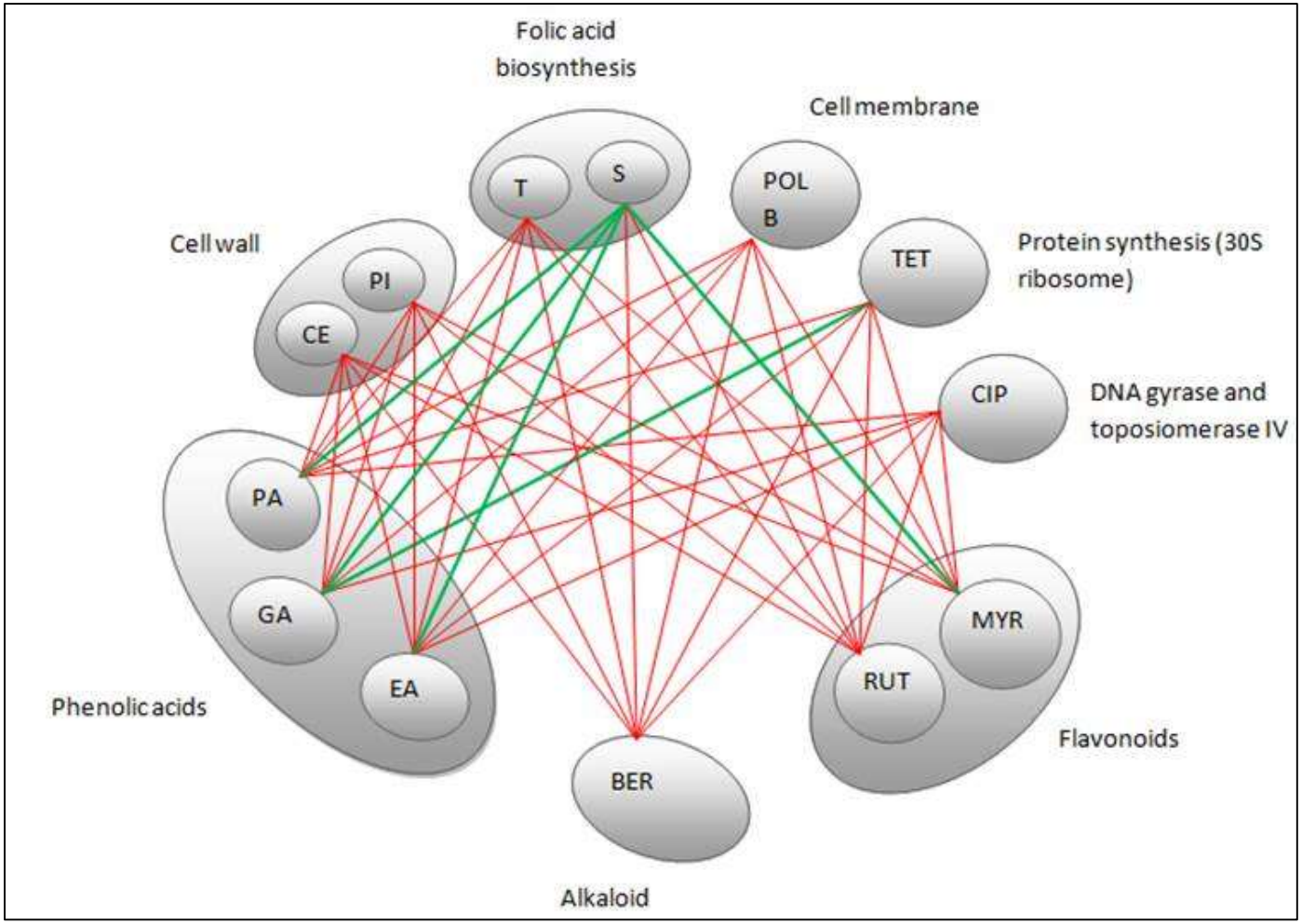
- Advantages
  - Synergism or additive effect (MDRO)
  - Effective against infections of unknown origin
  - Broadened spectrum of antimicrobial activity
  - Prevention of bacterial resistance development

- Disadvantages
  - Antagonism
    - Bacteristatic (tetracyciline) drugs may interfere with bactericidal (penicillin & cephalosporin) drugs
  - Elevated incidence of adverse effects
  - Super infection
  - Antimicrobial resistance
  - Increased cost

Combined antibacterial therapy

- Antibiotic synergism
  - Combination of antibiotics have enhanced activity when tested together compared with each antibiotic alone (2+2=6)
  - E. g. ampicillin + gentamicin in enterococcal carditis
- Additive effect
  - Combination of antibiotics has an additive effect (2+2=4)
  - E.g. combination of two β-lactam antibiotics
- Antagonism
  - Combination in which the activity of one antibiotic interferes with the activity of the other (2+2<4)

Network interactions between antibiotics and phytochemicals against *Pseudomonas aeruginosa in vitro*

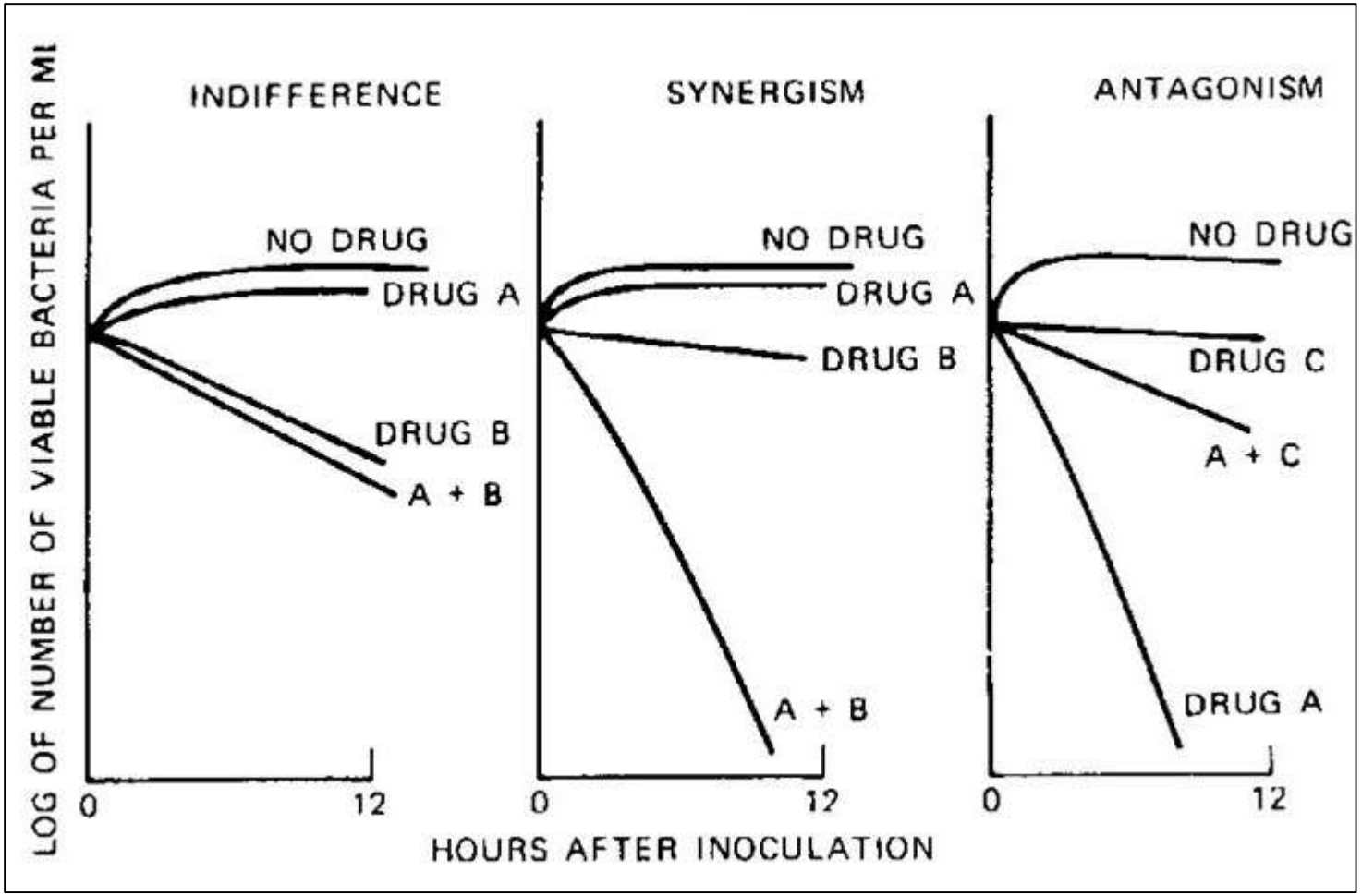


CE – ceftazidime
PI – piperacillin
T – trimethoprim
S – sulfamethoxazole
TET – tetracycline
CIP – ciprofloxacin.

PA – protocatechuic acid
GA – gallic acid
EA – ellagic acid
BER – berberine – alkaloid
RUT – rutin
MYR – myricetin

Jayaraman P, Sakharkar MK, Lim CS, Tang TH, Sakharkar KR. Activity and interactions of antibiotic and phytochemical combinations against *Pseudomonas aeruginosa in vitro*. *Int J Biol Sci* 2010; 6(6):556-568. doi:10.7150/ijbs.6.556

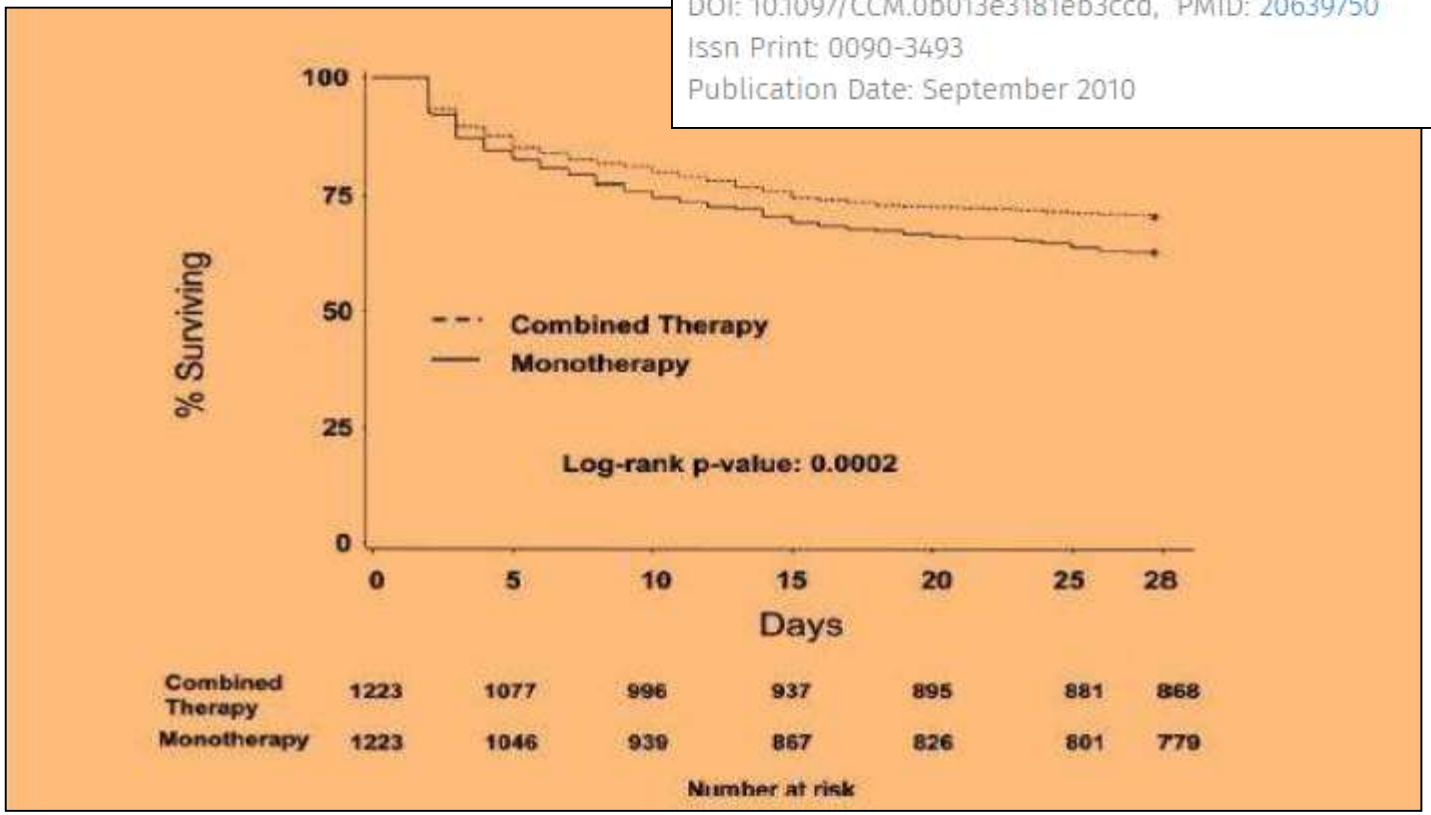
Combined antibacterial therapy



Early combination antibiotic therapy yields improved survival compared with monotherapy in septic shock: A propensity-matched analysis*

Anand Kumar;Ryan Zarychanski;Bruce Light;Joseph Parrillo;Dennis Maki;Dave Simon;Denny Laporta;Steve Lapinsky;Paul Ellis;Yazdan Mirzanejad;Greg Martinka;Sean Keenan;Gordon Wood;Yaseen Arabi;Daniel Feinstein;Aseem Kumar;Peter Dodek;Laura Kravetsky;Steve Doucette;

Critical Care Medicine. 38(9):1773-1785, SEPTEMBER 2010
DOI: 10.1097/CCM.0b013e3181eb3ccd, PMID: 20639750
Issn Print: 0090-3493
Publication Date: September 2010



Synergism

Enhanced Uptake of Aminoglycoside when Combined with β-lactam agents

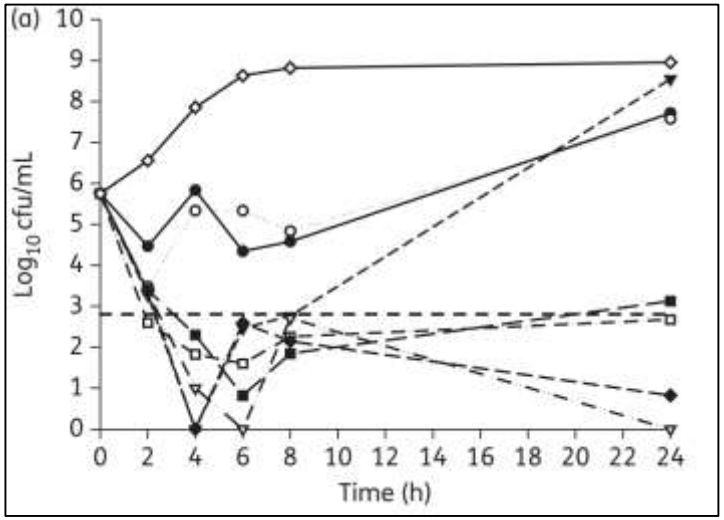
- Usually different classes with cidal activity
  - Enterococcal endocarditis – **ampicillin & gentamicin**
  - Viridans streptococcal endocarditis – **penicillin & gentamicin**
  - Staphylococcal bacteremia – **vancomycin & gentamicin**
  - Pseudomonas infection – **β-lactam agent & aminoglycosides**

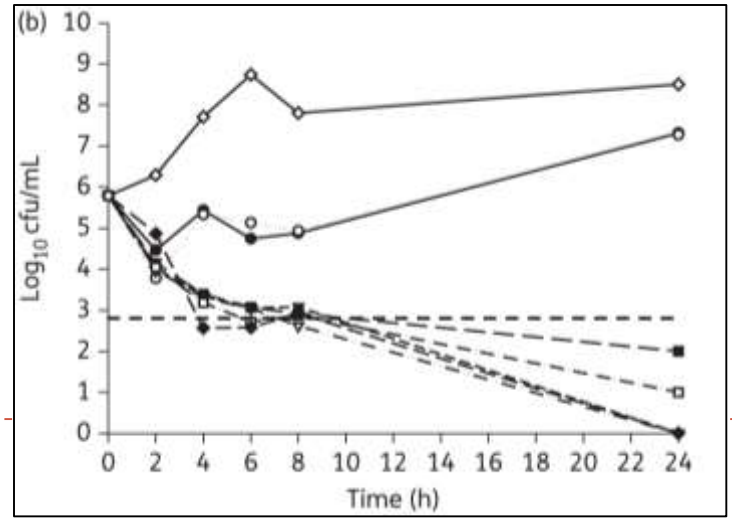
Synergistic activity and effectiveness of a double-carbapenem regimen in pandrug-resistant *Klebsiella pneumoniae* bloodstream infections

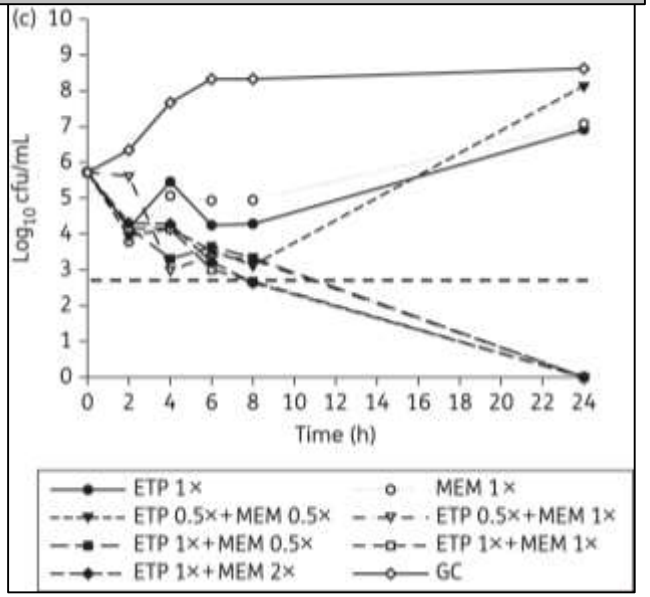
Alessandra Oliva, Alessandra D'Abramo, Claudia D'Agostino, Marco Iannetta, Maria T. Mascellino, Carmela Gallinelli, Claudio M. Mastroianni ✉, Vincenzo Vullo

Journal of Antimicrobial Chemotherapy, Volume 69, Issue 6, June 2014, Pages 1718–1720,
https://doi.org/10.1093/jac/dku027
Published: 11 February 2014

meropenem plus ertapenem







Antagonism

- Bactericidal agents with bacteriostatic
- More prominent in
  - Imunocompromised patients
  - In infections where localized host defenses may be inadequate such as meningitis and endocarditis

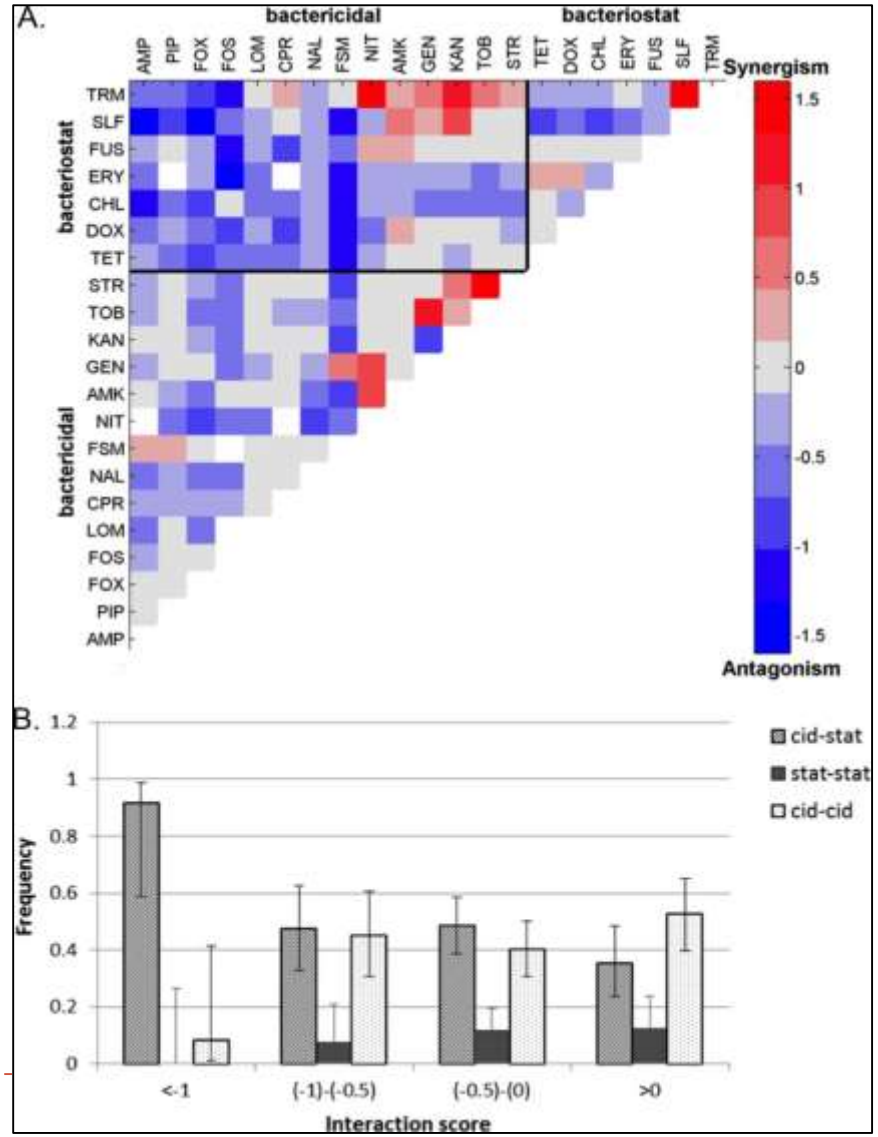
Antagonism between Bacteriostatic and Bactericidal Antibiotics Is Prevalent

Paolo S. Ocampo, Viktória Lázár, Balázs Papp, Markus Arnoldini, Pia Abel zur Wiesch, Róbert Busa-Fekete, Gergely Fekete, Csaba Pál, Martin Ackermann, Sebastian Bonhoeffer

DOI: 10.1128/AAC.02463-14

AAC JournalsASM.org

- Combinations of 21 different antibiotics
- Strong antagonistic interactions among combinations of bacteriostatic and bactericidal drugs



Antagonism between Bacteriostatic and Bactericidal Antibiotics Is Prevalent

Paolo S. Ocampo, Viktória Lázár, Balázs Papp, Markus Arnoldini, Pia Abel zur Wiesch, Róbert Busa-Fekete, Gergely Fekete, Csaba Pál, Martin Ackermann, Sebastian Bonhoeffer

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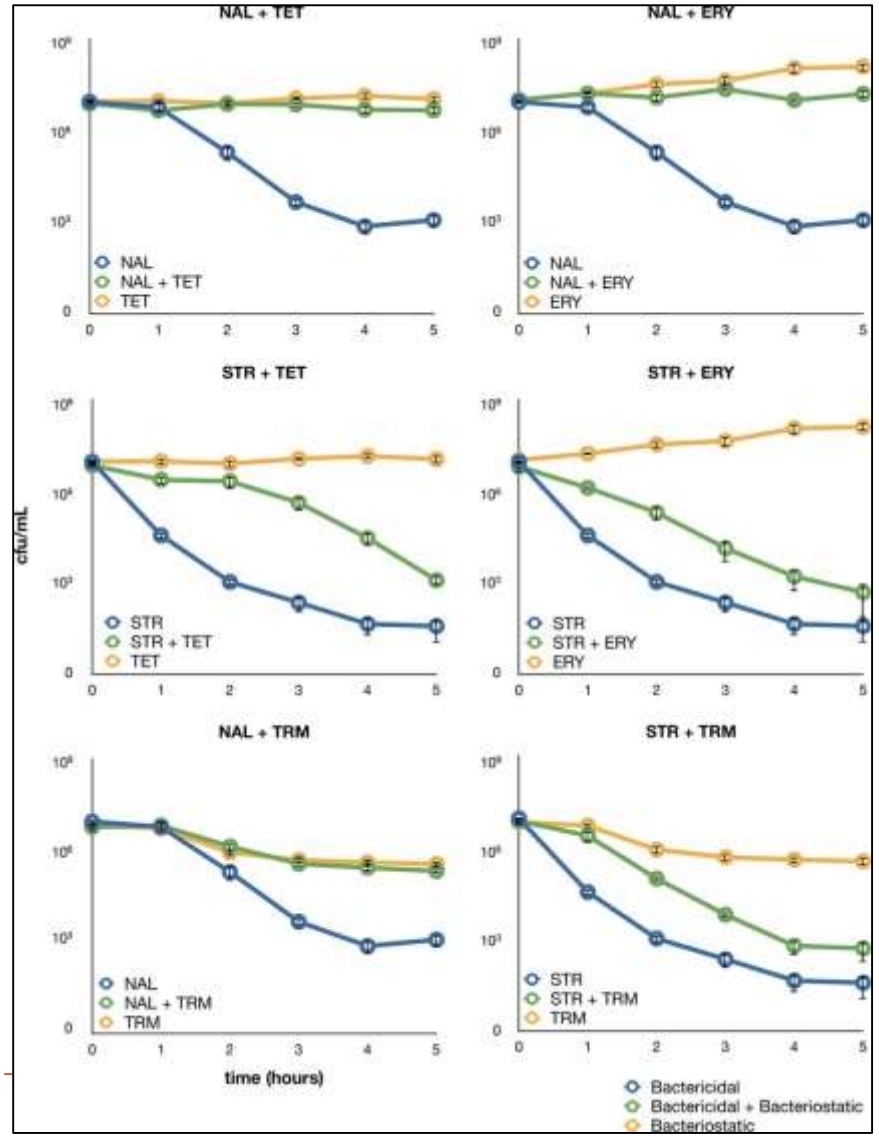


Combination Prevent Resistance

- Decreased resistant mycobacterium **tuberculosis** with combination treatment of
- **Reduction of β-lactamase induction** with combination β-lactam agents and aminoglycosides
- **HIV treatment**

Serial Antibiotic Therapy

- Serial use of antibiotics is indicated for a combination of antibiotics, in which one has bactericidal and the other bacteriostatic activity
- Combined administration would lead to antagonistic effects and therapeutical failure

Polymicrobial Infection

- Intraabdominal infection – ciprofloxacin & metronidazole
- Pelvic infection
- Mixed aerobic and anaerobic infection

The availability of broad spectrum antibiotics such as carbapenems and β-lactam- β-lactamase inhibitors restrict the use of combination antibiotics

Double β-lactams

Overview of Synergy with Reference to Double β-lactam combination

- Mostly additive effects
- Rarely synergic effect
- Sometimes antagonistic effect
  - Antagonism was seen mainly when treating enterobacter or pseudomonas infections

DICP 1991 Sep;25(9):972-7

Double β-lactam Combinations

- Generally a combination of an antipseudomonal penicillin and an extended-spectrum cephalosporin
  - piperacillin/tazobactam + ceftazidime
  - aztreonam + ceftazidime
  - nafcillin + ceftazidime

What's new in the treatment of serious MRSA infection?

Curr Opin Infect Dis. 2014 Dec;27(6):471-8. doi: 10.1097/QCO.0000000000000101.

Natasha E. Holmes[a] *and Benjamin P. Howden*[a,b,c]

KEY POINTS

- Ceftaroline and ceftobiprole are anti-MRSA cephalosporins approved for ABSSSI and pneumonia, and case reports have emerged of eosinophilic pneumonia associated with ceftaroline.
- Tedizolid offers once-daily oxazolidinone dosing with greater potency and reduced toxicity.
- Dalbavancin and oritavancin are lipoglycopeptides administered once weekly and may be convenient and cost-effective treatments for ABSSSI.
- β-Lactams may be combined with vancomycin or daptomycin to improve access to the cell wall or antibiotic binding in the treatment of MRSA infections.
- Resistance continues to emerge in anti-MRSA antimicrobials, although there are no data for new agents, such as tedizolid, dalbavancin and oritavancin.

COMBINATION THERAPY

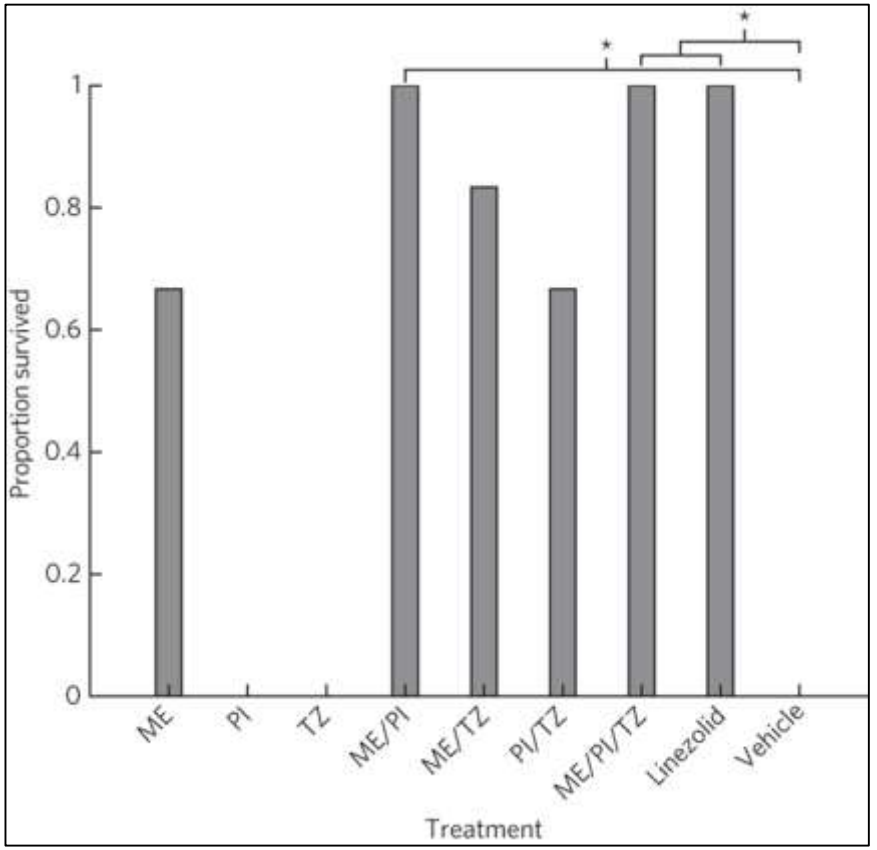
Combinations of vancomycin or daptomycin with β-lactams are being increasingly used to treat serious and invasive MRSA infections. Many in-vitro studies have demonstrated synergy with these combinations, even if the β-lactam itself does not possess anti-MRSA activity [46▪▪]. The proposed mechanism is the 'seesaw effect', in which β-lactams thin the cell wall to allow vancomycin to bind to target sites during cell wall synthesis, or in which β-lactams increase the negative cell surface charge to allow improved daptomycin binding and bactericidal activity [98▪▪]. Recent laboratory and animal studies have also demonstrated an impact of β-lactams on susceptibility to host immune factors [99▪▪]. Further studies to systematically evaluate the impact of combination therapy are warranted.

Nat Chem Biol. 2015 Nov;11(11):855-61. doi: 10.1038/nchembio.1911. Epub 2015 Sep 14.

Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA.

Gonzales PR[1], Pesesky MW[1], Bouley R[2], Ballard A[1], Biddy BA[1], Suckow MA[3,4], Wolter WR[3,4], Schroeder VA[3,4], Burnham CA[5,6], Mobashery S[2], Chang M[2], Dantas G[1,5,7].

"..triple β-lactam combination meropenem-piperacillin-tazobactam (ME/PI/TZ) acts synergistcally and is bactericidal against MRSA subspecies."



Efficacy of ME/PI/TZ treatment in a neutropenic mouse peritonitis model of MRSA N315. Survival in neutropenic (cyclophosphamide-treated) mice infected with MRSA N315, assessed after 6 d of treatment with antibiotics or vehicle. n = 6 mice per group; *P = 0.02, Fisher's exact test with Bonferroni correction.

CMI CLINICAL MICROBIOLOGY AND INFECTION
ESCMID OFFICIAL PUBLICATION OF EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Guidelines for the management of adult lower respiratory tract infections - Summary

November 2011 Volume 17, Supplement 6, Pages 1–24

M. Woodhead[a,*], F. Blasi[b], S. Ewig[c], J. Garau[d], G. Huchon[e], M. Ieven[f], A. Ortqvist[g], T. Schaberg[h], A. Torres[i], G. van der Heijden[j], R. Read[k], T.J.M. Verheij[l] Joint Taskforce of the European Respiratory Society and European Society for Clinical Microbiology and Infectious Diseases

Treatment options for patients with severe community-acquired pneumonia [C4] (ICU or intermediate care).

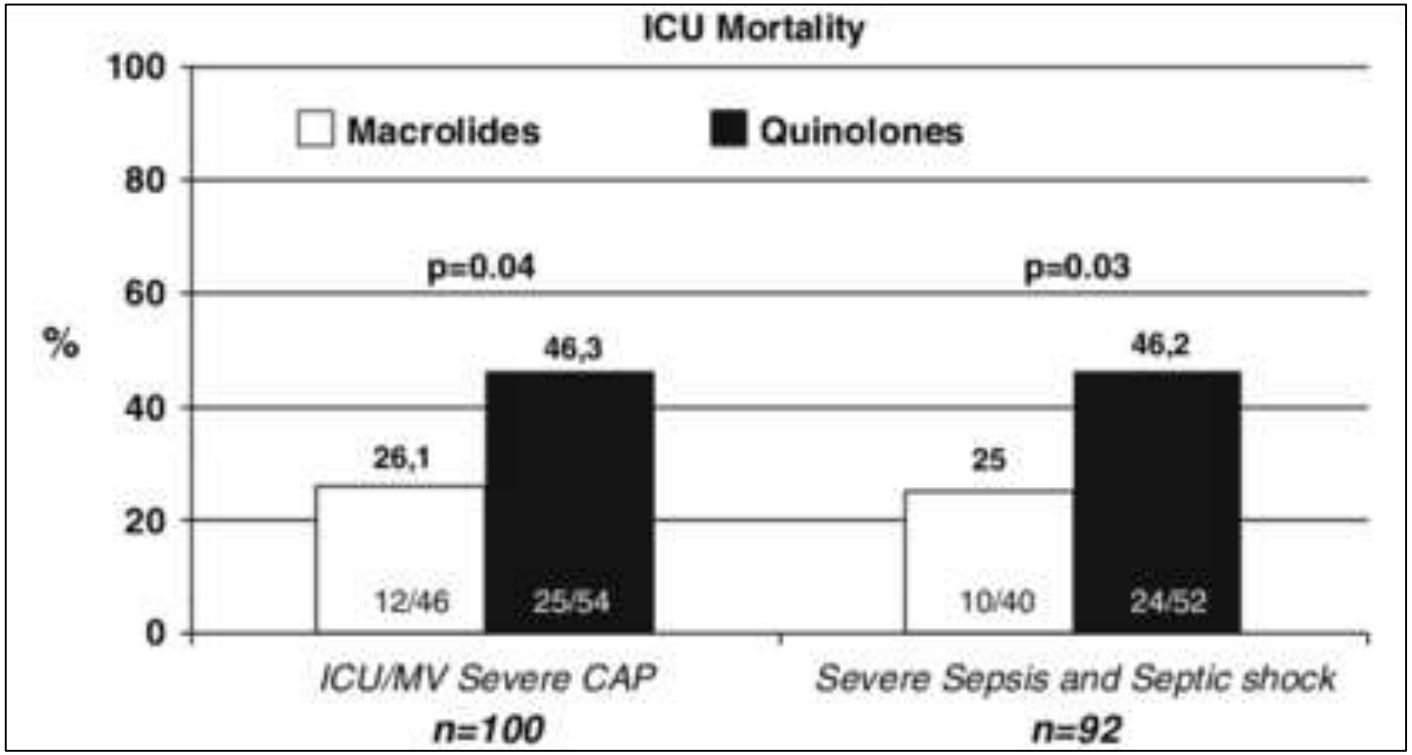
No risk factors for *P. aeruginosa*
Non-antipseudomonal cephalosporin III + macrolide[a]
or
moxifloxacin or levofloxacin ± non-antipseudomonal cephalosporin III
Risk factors for *P. aeruginosa*
Antipseudomonal cephalosporin[b] or acylureidopenicillin/β-lactamaseinhibitor or carbapenem (meropenem preferred, up to 6 g possible, 3 × 2 in 3-h infusion)
PLUS
ciprofloxacin[c] OR
PLUS
macrolide[a] + aminoglycoside (gentamicin, tobramycin or amikacin)

[a]New macrolides preferred to erythromycin.
[b]Ceftazidime has to be combined with penicillin G for coverage of *S. pneumoniae*.
[c]Levofloxacin 750 mg/24 h or 500 mg twice daily is an alternative and also covers Gram-positive bacteria if treatment is empirical [301,305–315].

Intensive Care Medicine
April 2010, Volume 36, Issue 4, pp 612–620 | Cite as

Combination antibiotic therapy with macrolides improves survival in intubated patients with community-acquired pneumonia

218 pts CAP intubated



Aminoglycoside and β-lactam Regimens*

Amikacin (or other aminoglycoside)	+	piperacillin/tazobactam ticarcillin/clavulanate (?) ceftazidime ceftriaxone (?) cefepime imipenem meropenem

* Choice of specific agents must be based on local pathogens and local susceptibility/resistance patterns

Aminoglycoside and β-lactam Regimens

- Rationale:
  - Synergy *in vitro*
  - Improved survival
  - Prevent emergence resistance

β lactam monotherapy versus β lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: systematic review and meta-analysis of randomised trials

Mical Paul, Ishay Benuri-Silbiger, Karla Soares-Weiser, Leonard Leibovici

BMJ 2004 ; 328 doi: https://doi.org/10.1136/bmj.38028.520995.63 (Published 18 March 2004)
Cite this as: *BMJ* 2004;328:668

64 trials with 7586 patients

- No difference in all-cause mortality (0,90, 95%CI 0,77-1,06)

Subset of Pseudomonas infections (426 pts)

- No difference in all-cause mortality (1,50, 95%CI 0,07-32,84)

β-lactams and Quinolones

- Antipseudomonal penicillin (piperacillin ± tazobactam) or extended-spectrum cephalosporin (ceftazidime, cefepime) + a quinolone (ciprofloxacin)
- Might be for tissue-based infections (pneumonia, enterocolitis, perirectal)
- Should not be used initially in patients receiving quinolone prophylaxis
- Less experience with these regimens – role of newer agents (gatifloxacin, moxifloxacin, gemifloxacin)?

De Pascale *et al. Critical Care* 2014, **18**:R90
http://ccforum.com/content/18/3/R90

CRITICAL CARE

RESEARCH Open Access

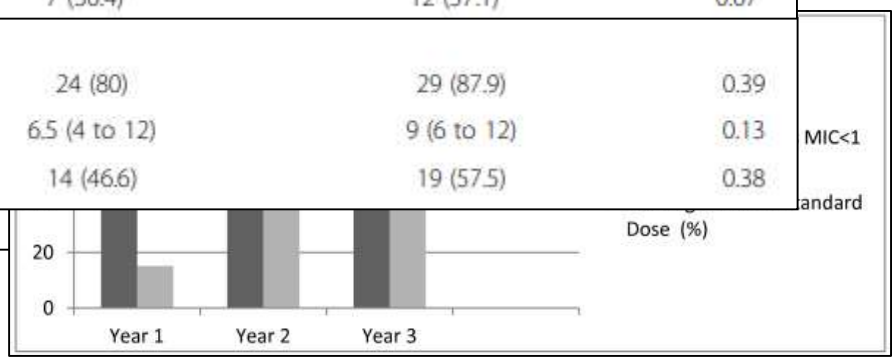
High dose tigecycline in critically ill patients with severe infections due to multidrug-resistant bacteria

Gennaro De Pascale[1*], Luca Montini[1], Mariano Alberto Pennisi[1], Valentina Bernini[1], Riccardo Maviglia[1], Giuseppe Bello[1], Teresa Spanu[3], Mario Tumbarello[2] and Massimo Antonelli[1]

Table 1 Clinical characteristics of the 63 patients with VAP in the standard-dose (SD) and high-dose (HD) tigecycline (TGC) groups

Variable	SD TGC group (n = 30)	HD TGC group (n = 33)	*P*-value
Clinical and microbiological outcome, n (%)			
ICU mortality	20 (66.6)	16 (48.4)	0.14
Clinical cure	10 (33.3)	19 (57.5)	0.05
Microbiological eradication	7 (30.4)	12 (57.1)	0.07
Microbiological and therapeutically aspects			
Concomitant use of other active antibiotics, n (%)	24 (80)	29 (87.9)	0.39
Duration of TGC treatment, days, median (IQR)	6.5 (4 to 12)	9 (6 to 12)	0.13
Initial inadequate treatment, n (%)	14 (46.6)	19 (57.5)	0.38

administration due to Gram-negative MDR bacteria.



Anti *P. aeruginosa* Combination

- Empirical therapy
- Combine 2 active drugs:
  - β-lactam + aminoglycoside
  - β-lactam + quinolone

Anti *P. aeruginosa* Combination

Antipseudomonal β-lactam

- Piperacillin
- Ceftazidime
- Cefepime
- Imipenem
- Aztreonam

+

Aminoglycoside

- Tobramycin
- Gentamycin
- Amikacin

OR

Fluoroquinolones

- Ciprofloxacin
- Levofloxain

HAP Due to *P. aeruginosa*

- Mortality high (>50%)
- Monotherapy inadequate
  - High rate of failure or relapse
  - Emergence of resistance

Bacteremia Due to *P. aeruginosa*

	Mortality rates	
	Monotherapy	Combined therapy
Pneumonia	7/8 (88%)	7/20 (35%)
Critically ill	11/12 (92%)	18/37 (47%)
All patients	20/43 (47%)	38/143 (27%)

Hilf, Am J Med 1989:87;540

Pathol Biol (Paris). 1997 May;45(5):420-3.

[Evaluation of bactericidal activity of cefpirome-aminoglycoside combination against Pseudomonas aeruginosa strains with intermediate sensitivity to cefpirome and in various phenotypes of beta-lactam resistance].

[Article in French]

Canis F[1], Cavallo JD, Husson MO.

Combination of cefpirome and aminoglycosides is bactericidal and showed synergic effect

Vancomycin-Based Combination Regiments

Vancomycin +

- Ceftazidime
- Cefepime
- Aztreonam
- Piperacilin/tazobactam
- Imipenem or meropenem
- Quinolone (ciprofloxacin)

Vancomycin-Based Combination Regiments

- Broad spectrum (including β-lactam-resistant gram-positives)
- Established efficacy (80-90%)

- Some added toxicity (need to monitor levels in some patients)
- Selection of tolerant/resistant organisms (GISA or VISA; VRSA; VRE, Leuconostoc, Pediococcus, Lactobacillus, Rhodococcus)
  - Should not be used on a routine basis

Options for treating carbapenem-resistant Enterobacteriaceae

Rafailidis, Petros I.[a,b]; Falagas, Matthew E.[a,c,d]

Current Opinion in Infectious Diseases: December 2014 - Volume 27 - Issue 6 - p 479–483
doi: 10.1097/QCO.0000000000000109
ANTIMICROBIALS: Edited by Monica A. Slavin and William Irving

KEY POINTS

- Combination regimens seem to offer therapeutic advantage over monotherapy.
- Carbapenem-containing regimens seem to offer therapeutic advantage over noncarbapenem-containing regimens, provided the resistance level for meropenem or imipenem is up to 8 mg/l.
- Combination regimens, including antibiotics among colistin, high-dose tigecycline, fosfomycin, and aminoglycoside, seem to offer therapeutic advantage when resistance to meropenem and imipenem exceeds the previously mentioned threshold.
- Definitive conclusions cannot be drawn, as we are still waiting for the results of randomized controlled trials on the treatment of infections due to carbapenem-resistant Enterobacteriaceae.

Colistin

Fosfomycin

Tigecycline

The Characteristics of Patients who are Likely and Unlikely to Colonized Pseudomonas

Colonization unlikely	Colonization likely
Admitted less than 5 days ago	Admitted more than 5 days ago
Admitted from home	Admitted from a Nursing Health care
No other admissions in past 3 months	Other admissions in the past 3 months
Completely healthy before	COPD or bronchiectasis
	A frequent antimicrobial or glucosteroid use dialysis patient

NEW guidelines for patient without risk for pseudomonas or MRSA

Regime	Drug	Antibiotic
	Potent antipneumococcal β-lactam (ceftriaxone or cefotaxime) or ampicillin-sulbactam plus	1 to 2 g daily 1-2 g every 8h 1,5-3g every 6h
plus	Either advanced macrolide azithromycin	500 mg daily
	or a respiratory fluoroquinolone levofloxacin, moxifloxacin	750 mg daily or 400 mg daily

NEW guidelines for patient with risk for pseudomonas and other resist pathogen but not MRSA

Regime	Drug	Antibiotic
	Piperacillin-tazobactam	4-5 g every 6h
or	Imipenem or meropenem	500 mg every 6h 1g every 8h
or	Cefepime, ceftazidime	2 g every 8h
plus	Fluoroquinolone (ciprofloxacin or levofloxacin)	750 mg every day 400 mg every 8h

Antimicrobial Therapy in the Intensive Care Unit

MS KRISHNA SARIN*, M VADIVELAN**, CHANAVEERAPPA BAMMIGATTI**

Table 2. Antibiotic Therapy Depending on the Site of Infection

Site of infection	Bacteria	Suggested treatment
UTI	*E. coli*	Ceftriaxone or Ceftazidime ± Aminoglycoside
Severe acute pyelonephritis	*P. aeruginosa*	
	Enterococcus species	
	Staphylococcus species	
Intra-abdominal sepsis	*E. coli*	Ertapenem
	P. aeruginosa	Piperacillin-Tazobactam
	Enterococcus species	Third- or fourth-generation cephalosporin (active against *P. aeruginosa*) + Metronidazole
	Bacteroides species	
Nosocomial pneumonia	Enterobacteriaceae	β-lactam (active against *P. aeruginosa*) ± Aminoglycoside ± Glycopeptide (vancomycin)
	P. aeruginosa	
	S. aureus	
	S. pneumoniae	
	H. influenzae	
Pneumonia without risk factors for MDR Pseudomonas	*S. aureus*	Third-generation Cephalosporin ± Macrolide
	S. pneumoniae	
	H. influenzae	
	Other gram-negative bacilli	
Skin infections	Streptococcus species	β-lactam + β-lactamase inhibitor
	Staphylococcus species	Piperacillin-Tazobactam
	Gram-negative bacilli	Carbapenem
CRBSI	Staphylococcus species	Vancomycin + β-lactam with activity against *P. aeruginosa*
	Enterobacteriaceae	
	P. aeruginosa	

CRITICAL CARE

Antimicrobial Therapy in the Intensive Care Unit

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Table 3. Treatment Options for Resistant Gram-positive Bacteria

Drug	Route of administration	Activity against MRSA	Activity against resistant *S. pneumoniae*	Activity against vancomycin-resistant Enterococci
Vancomycin	IV only	Yes	Yes	No
Daptomycin	IV only	Skin infection/Bloodstream infection	No	Yes
Linezolid	IV or oral	Pneumonia/Skin infection	No	Yes
Quinupristin-Dalfupristin	IV only	Yes	No	Yes, against *E. faecium*
Telavancin	IV only	Skin infection/Pneumonia	Yes	Yes
Tigecycline	IV only	Pneumonia/Skin infection	Yes	Yes
Ceftaroline	IV only	Pneumonia/Skin infection	Yes	No

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Antimicrobial Therapy in the Intensive Care Unit

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Table 4. Treatment Options for Resistant Gram-negative Bacteria

Organism	First-line therapy	Second-line therapy
Empirical therapy		
Monomicrobial infection	Carbapenem	Piperacillin-Tazobactam
	Tigecycline (not in UTIs) ± Antipseudomonal agent	Colistin
Polymicrobial infection	Carbapenem + Vancomycin	Piperacillin-Tazobactam + Vancomycin
	Tigecycline (not in UTIs) ± Antipseudomonal agent	Colistin + Vancomycin
Directed therapy		
ESBL-producing Enterobacteriaceae	Carbapenem	Tigecycline (not in UTIs)
	Piperacillin-Tazobactam	Fluoroquinolone
		Colistin
Carbapenemase-producing Enterobacteriaceae	Tigecycline	Fosfomycin (parenteral formulation)
	Colistin	
MDR *P. aeruginosa*	Meropenem	Colistin

Antimicrobial Therapy in the Intensive Care Unit

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Table 5. Practices Promoting the Optimization of Antimicrobial Use in the ICU Setting

Provide adequate initial treatment of serious infections (e.g., pneumonia, bloodstream infections)
Awareness of predominant disease causing pathogens
Up-to-date ICU-specific pathogen antibiograms
Drainage of abscesses, empyema cavities, other infected fluid collections
Removal of infected foreign bodies (e.g. central venous catheters)
Monitor serum drug concentrations when appropriate to achieve therapeutic levels
Avoid prolonged courses of empiric antibiotic therapy
Consider de-escalation of antibiotics based on available microbiologic data and clinical course
Use narrow-spectrum antibiotics when supported by clinical situation and culture data
Establish appropriate thresholds for prescribing antibiotics
Develop predetermined criteria for the discontinuation of antimicrobial therapy

Antimicrobial Therapy in the Intensive Care Unit

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Table 6. Predetermined duration of Antibiotic Therapy based on the IDSA Guidelines

Site of infection	Duration of antibiotic therapy (days)
Lung infection	
CAP due to *S. pneumoniae*	8
VAP	8
VAP and immunodepression	14
Pneumonia due to *Legionella pneumophila*	21
Pneumonia with lung necrosis	≥28
Intra-abdominal infections	
Community peritonitis	<8
Postoperative peritonitis	14
CNS infections	
Meningococcemia	5-8
Meningitis due to *S. pneumoniae*	10-14
Postoperative meningitis due to *S. epidermidis* or Enterobacteriaceae	14
Meningitis due to *Listeria monocytogenes*	21
Postoperative meningitis due to *S. aureus* or *P. aeruginosa*	21
Brain abscess	≥28
Catheter-related bacteremia	
S. epidermidis or Enterobacteriaceae	<8
S. aureus/Candida species (uncomplicated)	14
S. aureus (complicated)	≥28

Antimicrobial Therapy in the Intensive Care Unit

MS KRISHNA SARIN*, M VADIVELAN**, CHANAVEERAPPA BAMMIGATTI**

- **Prevention of infections in the ICU. A summary of the recommendations made by the Centers for Disease Control is given below:**
- Prevention of Central Venous Catheter Infections
  - Educate personnel about catheter insertion and care.
  - Use chlorhexidine to prepare the insertion site.
  - Use maximal barrier precautions during catheter insertion.
  - Consolidate insertion supplies (e.g., in an insertion kit or cart)
  - Use a checklist to enhance adherence to the bundle.
  - Empower nurses to halt insertion if asepsis is breached.
  - Cleanse patients daily with chlorhexidine.
  - Ask daily: Is the catheter needed? Remove catheter if not needed or used.
- Prevention of VAP
  - Elevate head end of bed to 30-45°.
  - Decontaminate oropharynx regularly with chlorhexidine.
  - Give ‘sedation vacation’ and assess readiness to extubate daily.
  - Use peptic ulcer disease prophylaxis.
  - Use deep-vein thrombosis prophylaxis (unless contraindicated).

Antimicrobial Therapy in the Intensive Care Unit

MS KRISHNA SARIN*, M VADIVELAN**, CHANAVEERAPPA BAMMIGATTI**

- **Prevention of infections in the ICU. A summary of the recommendations made by the Centers for Disease Control is given below:**
- Prevention of UTIs
  - Place bladder catheters only when absolutely needed (e.g. to relieve obstruction), not solely for the provider's convenience.
  - Use aseptic technique for catheter insertion and urinary tract instrumentation.
  - Minimize manipulation or opening of drainage systems.
  - Ask daily: Is the catheter needed? Remove catheter if not needed.
- Prevention of Surgical-site Infections
  - Choose a surgeon wisely.
  - Administer prophylactic antibiotics within one hour before surgery; discontinue within 24 hours.
  - Limit any hair removal to the time of surgery; use clippers or do not remove hair at all.
  - Prepare surgical site with chlorhexidine-alcohol.
  - Maintain normal perioperative blood glucose levels (cardiac surgery patients).
  - Maintain perioperative normothermia (colorectal surgery patients).
- Prevention of Pathogen Cross-transmission
  - Cleanse hands with alcohol hand rub before and after all contacts with patients or their environments.

Criteria for the Evaluation of Quality of Antibiotic Drug Use

- Sufficient **data** in the records evaluation?
- **Indication** for antibiotic therapy/prophylaxis?
- Appropriate **choice** of antibiotic? Cite an alternative agent considering:
  - Efficacy (susceptibility, antimicrobial activity)
  - Toxicity, allergic reactions
  - Cost
  - Spectrum (too broad?)
- Appropriate **duration**:
  - Too long
  - Too short
- Appropriate **pharmacokinetics**? Considering:
  - Dose
  - Interval
  - Route
- Appropriate **timing**:
  - To early (before cultures are taken)
  - Too late (e.g. surgical prophylaxis after incision)

RISK FACTORS

MULTIDRUG-RESISTANT PATHOGENS CAUSING HOSPITAL-ACQUIRED PNEUMONIA, HEALTHCARE-ASSOCIATED PNEUMONIA, AND VENTILATOR-ASSOCIATED PNEUMONIA

- Antimicrobial therapy in preceding 90 days
- Current hospitalization of 5 days or more
- High frequency of antibiotic resistance in the community or in the specific hospital unit
- Hospitalization for 2 days or more in the preceding 90 d
- Residence in a nursing home or extended care facility
- Home infusion therapy (including antibiotics)
- Chronic dialysis within 30 days
- Home wound care
- Family member with multidrug-resistant pathogen
- Immunosuppressive disease and/or therapy

Conclusion

- Combination antibiotics has clear cut (as well as borderline) indications
- Inappropriate use of antimicrobial combinations may have deleterious effect

What is De-escalation and Why?

- The practice of changing antibiotics from initial broad spectrum agent to a narrower, more focused spectrum when the pathogen identified
- Why?
- De-escalation is one of the most important strategies in reducing antibiotic resistance and has shown to improve patient outcome

General principles of de-escalation

- Include assessing the need for antibiotics everyday based on:
  - Clinical improvement
  - Adequate source control
  - Appropriate culture and sensitivity results

De-escalation success

- De-escalation is feasible in ~50% of the patients
- It is influenced by initial microbiologic results and type of initial antibiotherapy

Antibiotic De-escalation in the ICU: A Five-Step Policy

1. Stopping antibiotics in patients without documented infection
2. Stopping vancomycine or linezolid if no MRSA is identified
3. Broad-spectrum β-lactams restricted to infection caused by pathogens only susceptible to these agents
4. Switching to monotherapy after 3-5 days
5. Antibiotics stopped ASAP (after maximum of 8 days in most cases)

Step 1

Stopping Antibiotic in ICU Patients Without Documented Infection

- Obtaining specimens for cultures before antimicrobial administration is essential and enable therapy de-escalation
- All antibiotic therapy in the ICU should be re-evaluated on days 2 or 3, based on the clinical course of the disease and the microbiological culture results

Step 2

Stopping Vancomycine or Linezolid if no MRSA is Identified

- Vancomycin and linezolid should be stopped if no MRSA is identified
- Infections caused by MSSA should be treated with oxacillin except in case of allergy

Step 3

Streaming Antibiotic Therapy Once Culture Results are Available

- Restrict use of very broad spectrum agents, such as cabapenems, pip./taz., cefepime, to infections caused by pathogens only susceptible to these agents:
  - Treat infections caused by *Enterobacteriaceae* with 3rd gen. cephalosporin (except ESBL-producing strains and Group 3 GNB)
  - Treat *P. aeruginosa* infections by piperacillin-S strains with this specific antibiotic
  - Restrict use of ciprofloxacin to pts allergic to β-lactams

Step 4
Switching to monotherapy after 3-5 days

- Therapy can and should be switched to monotherapy in most pts after 3-5 days provided that:
  - Initial therapy was appropriate
  - Clinical course appears favorable
  - And that microbiological data exclude a very difficult-to-treat microorganism, with a very high *in vitro* MIC, as it can be observed with some nonfermenting-GNB and/or carbapenemase-producing GNB

Step 5
Shortening Duration of Therapy

- A too long duration of treatment may favor the emergence of MDR or pandrug-resistant strains, exposes to antibiotic toxicity, and increases costs, and NOT necessarily improves outcome

How to Carry Out De-escalation

- Target broad spectrum antibiotics that are used empirically
- Review at:
  - 72 hours after antimicrobial initiation or
  - Once a week review of a specific ward, unit, hospital
- Identify de-escalation opportunities
  - Were appropriate cultures taken initially?
  - Has there been any growth from the cultures?
  - If there is no growth, can the antibiotic be stopped?
  - If there is growth, can the antibiotic be de-escalated?

Morel *et al. Critical Care* 2010, **14**:R225
http://ccforum.com/content/14/6/R225

CRITICAL CARE

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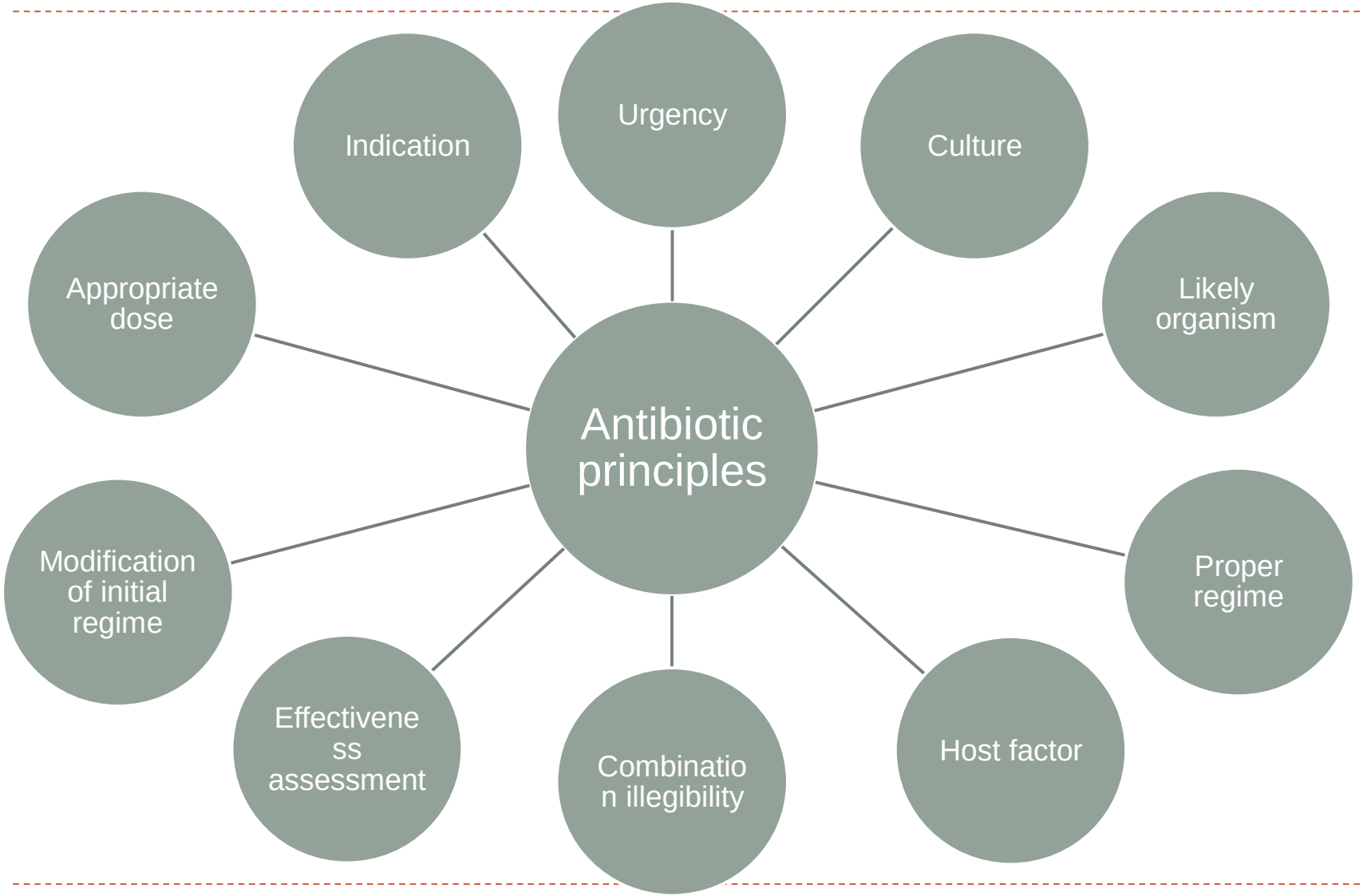
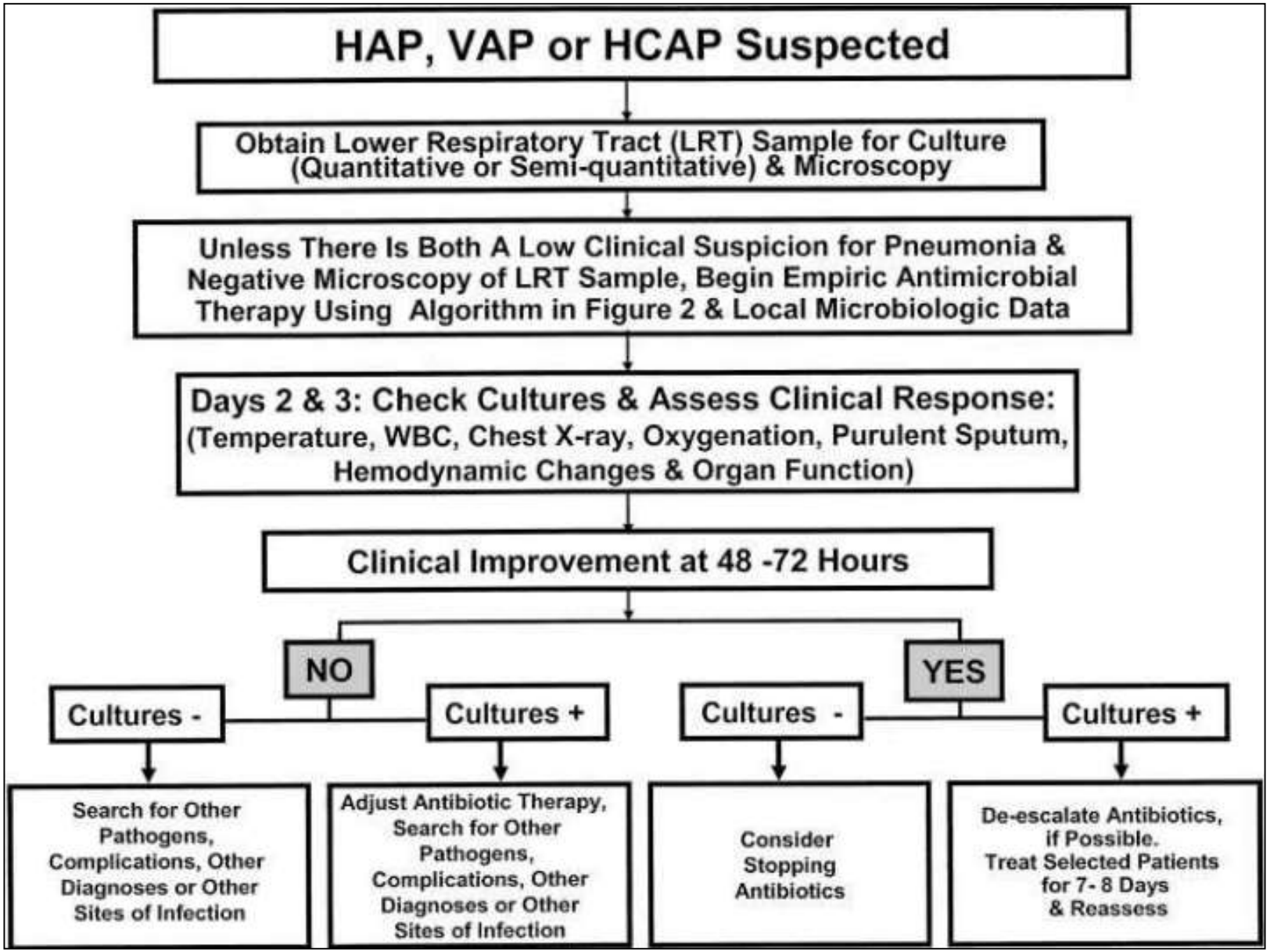
De-escalation as part of a global strategy of empiric antibiotherapy management. A retrospective study in a medico-surgical intensive care unit

Jérôme Morel[1*], Julie Casoetto[1], Richard Jospé[1], Gérald Aubert[2], Raphael Terrana[1], Alain Dumont[1], Serge Molliex[1], Christian Auboyer[1]

Conclusions: As part of a global management of empiric antibiotherapy in an intensive care unit, de-escalation might be safe and feasible in a large proportion of patients.

10 important questions should be routinely addressed




HAP, VAP or HCAP Suspected
Obtain Lower Respiratory Tract (LRT) Sample for Culture (Quantitative or Semi-quantitative) & Microscopy
Unless There Is Both A Low Clinical Suspicion for Pneumonia & Negative Microscopy of LRT Sample, Begin Empiric Antimicrobial Therapy Using Algorithm in Figure 2 & Local Microbiologic Data
Days 2 & 3: Check Cultures & Assess Clinical Response: (Temperature, WBC, Chest X-ray, Oxygenation, Purulent Sputum, Hemodynamic Changes & Organ Function)
Clinical Improvement at 48 -72 Hours
NO
YES
Cultures -
Cultures +
Cultures -
Cultures +
Search for Other Pathogens, Complications, Other Diagnoses or Other Sites of Infection
Adjust Antibiotic Therapy, Search for Other Pathogens, Complications, Other Diagnoses or Other Sites of Infection
Consider Stopping Antibiotics
De-escalate Antibiotics, if Possible. Treat Selected Patients for 7- 8 Days & Reassess

WHO priority pathogens list for R&D of new antibiotics

- The WHO list is divided into three categories according to the urgency of need for new antibiotics: critical, high and medium priority.
  - **Priority 1: CRITICAL**
    - *Acinetobacter baumannii*, carbapenem-resistant
    - *Pseudomonas aeruginosa*, carbapenem-resistant
    - *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing
  - **Priority 2: HIGH**
    - *Enterococcus faecium*, vancomycin-resistant
    - *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
    - *Helicobacter pylori*, clarithromycin-resistant
    - *Campylobacter* spp., fluoroquinolone-resistant
    - *Salmonellae*, fluoroquinolone-resistant
    - *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant
  - **Priority 3: MEDIUM**
    - *Streptococcus pneumoniae*, penicillin-non-susceptible
    - *Haemophilus influenzae*, ampicillin-resistant
    - *Shigella* spp., fluoroquinolone-resistant

The Top Ten Rule

1. All cell wall inhibitors are β-lactams **except** vancomycin
2. All penicillins are water soluble **except** nafcillin
3. All protein synthesis inhibitors are bacteriostatic **except** for the aminoglycosides
4. All cocci are gram-positive **except** *Neisseria spp.*
5. All bacilli are gram-negative **except** anthrax, tetanus, botulism, diphteria bacilli
6. All spirochaetes are gram-negative

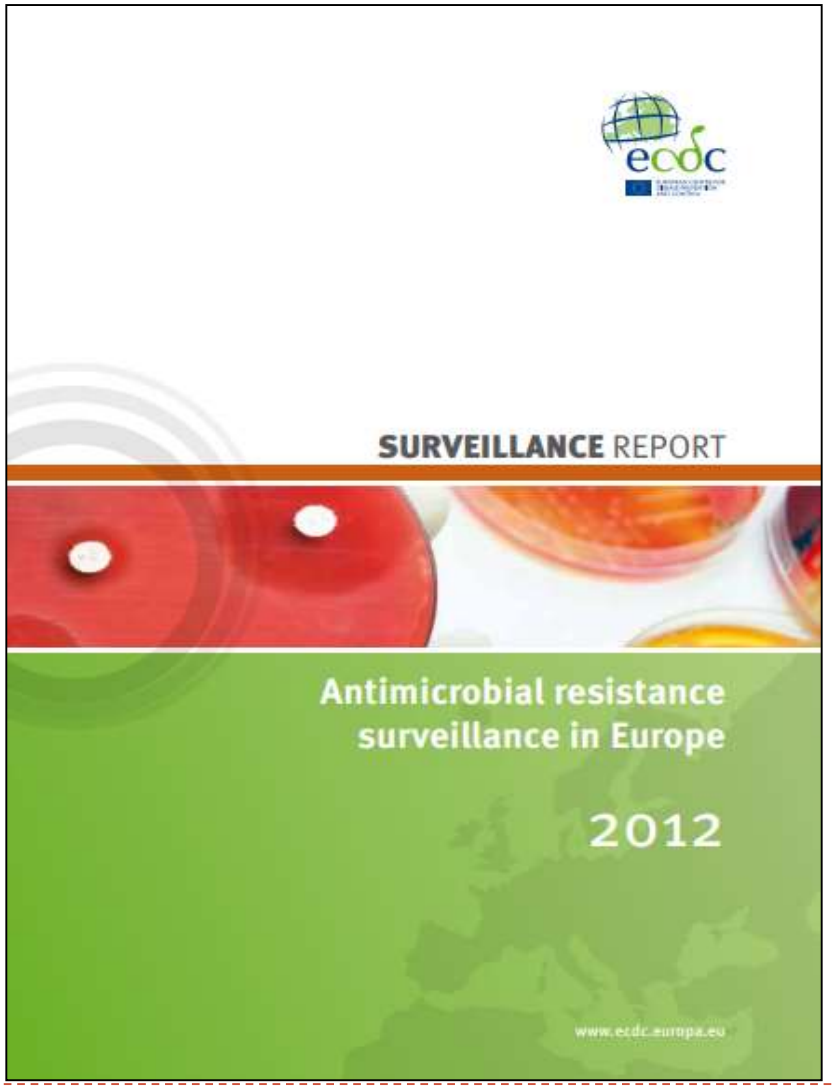
The Top Ten Rule

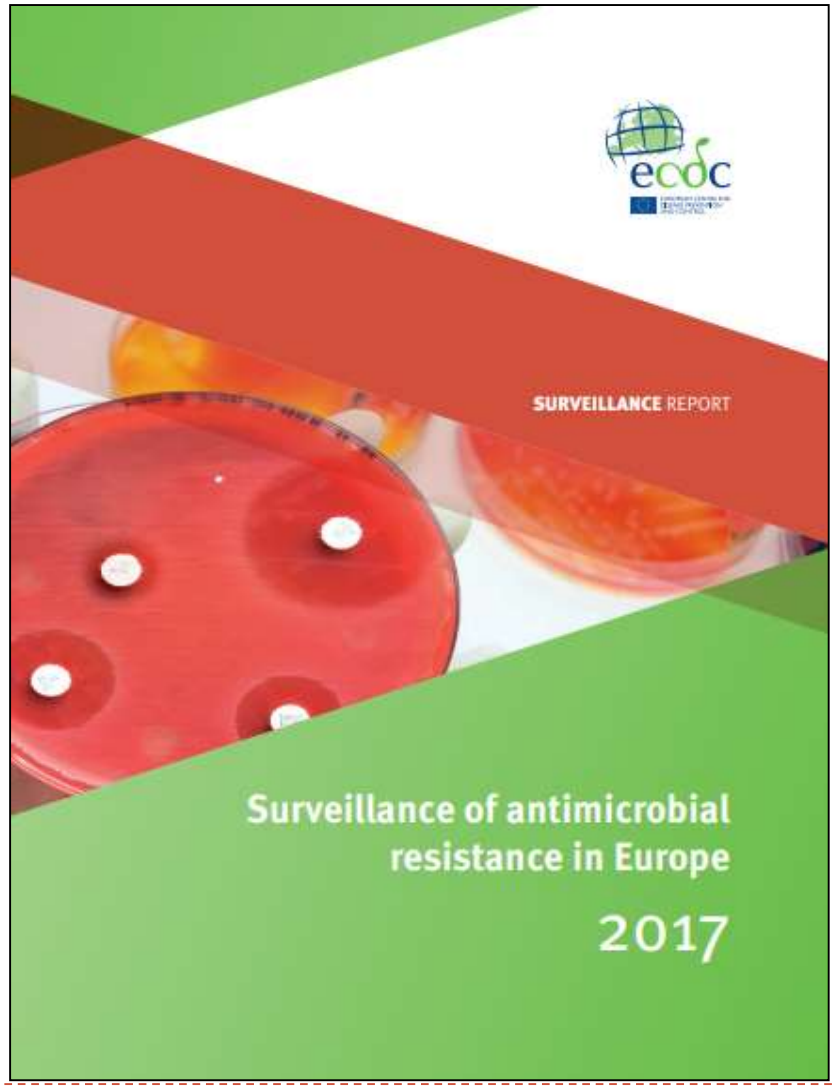
7. Tetracyclines and macrolides are used for intracellular bacteria
8. Beware pregnant women and tetracyclines, aminoglycosides, quinolones, sulfonamides
9. Antibiotics beginning with “C” are particularly associated with pseudomembranous colitis (e.g. Cephalosporins, Clindamycin, Ciprofloxacin)
10. While the penicillins are the most famous for causing allergies, a significant proportion of people with penicillin allergies may also react to cephalosporins. These should therefore also be avoided

Take Home Messages

- The FIRST dose of antibiotic is THE MOST IMPORTANT
  - Broad enough to cover likely pathogens
  - Big enough to achieve appropriate PK-PD targets
  - Volume of distribution is important
  - Clearance is NOT important
- The subsequent doses of antibiotics needs a bit more thought
  - Narrow enough to not cause antimicrobial resistance
  - Specific cover to optimise kill
  - Individualised dosing based on patient parameters
  - Clearance and Volume of distribution changes

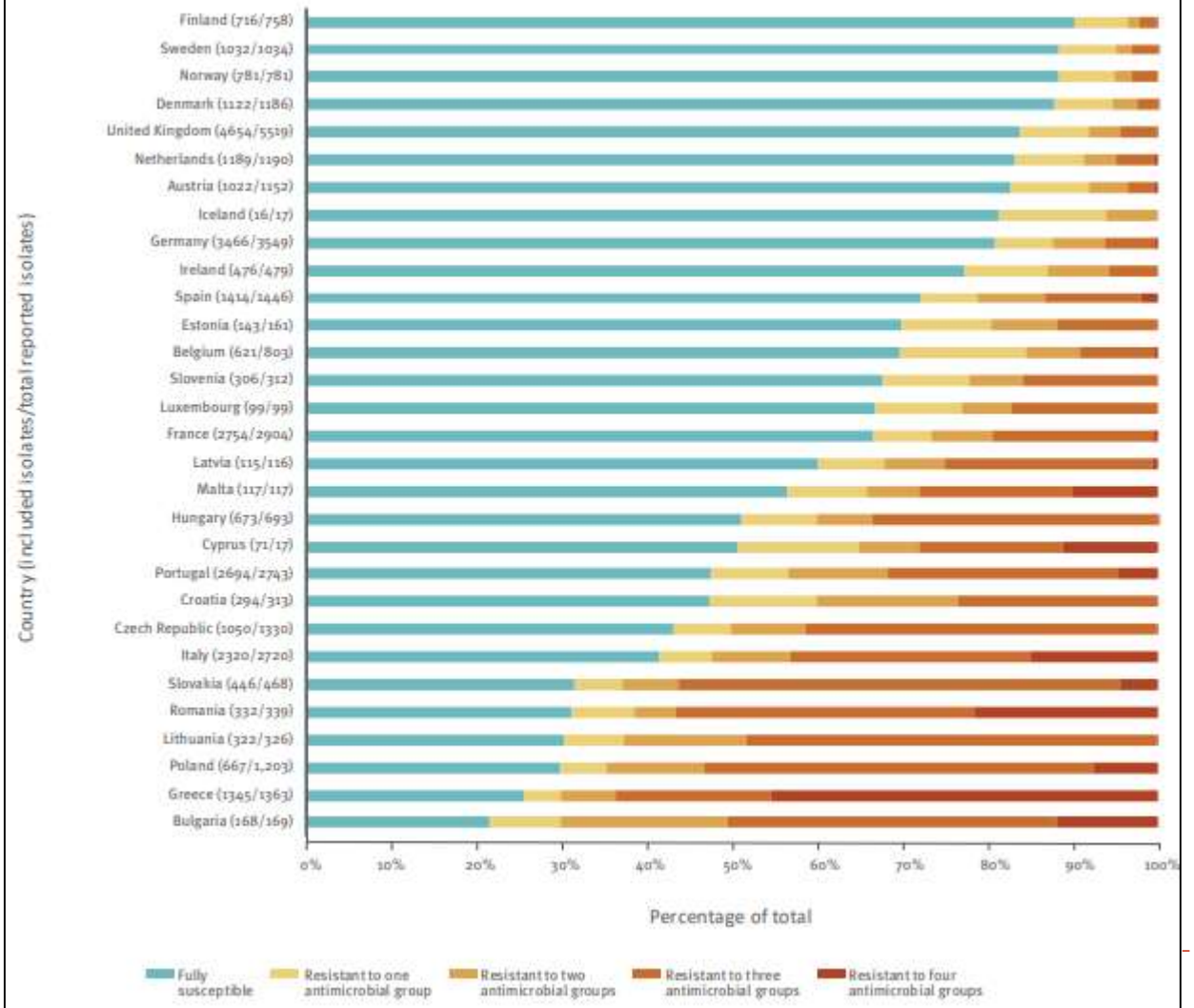
Surveillance of antimicrobial resistance in Europe, 2017





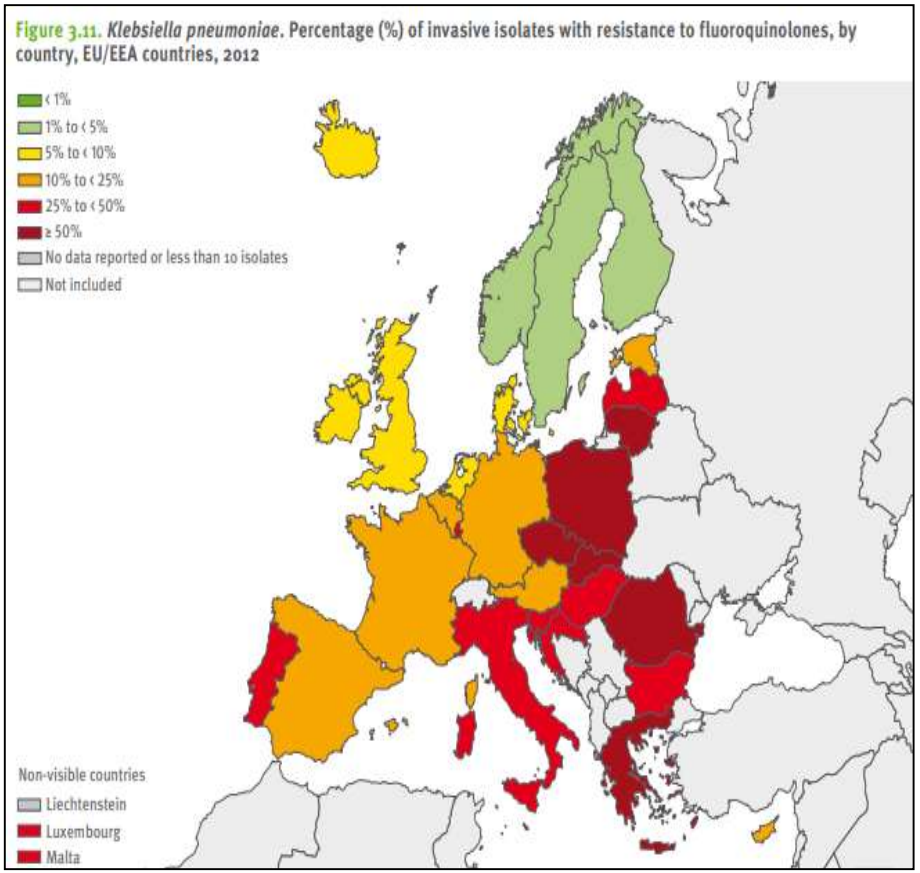
Klebsiella pneumoniae

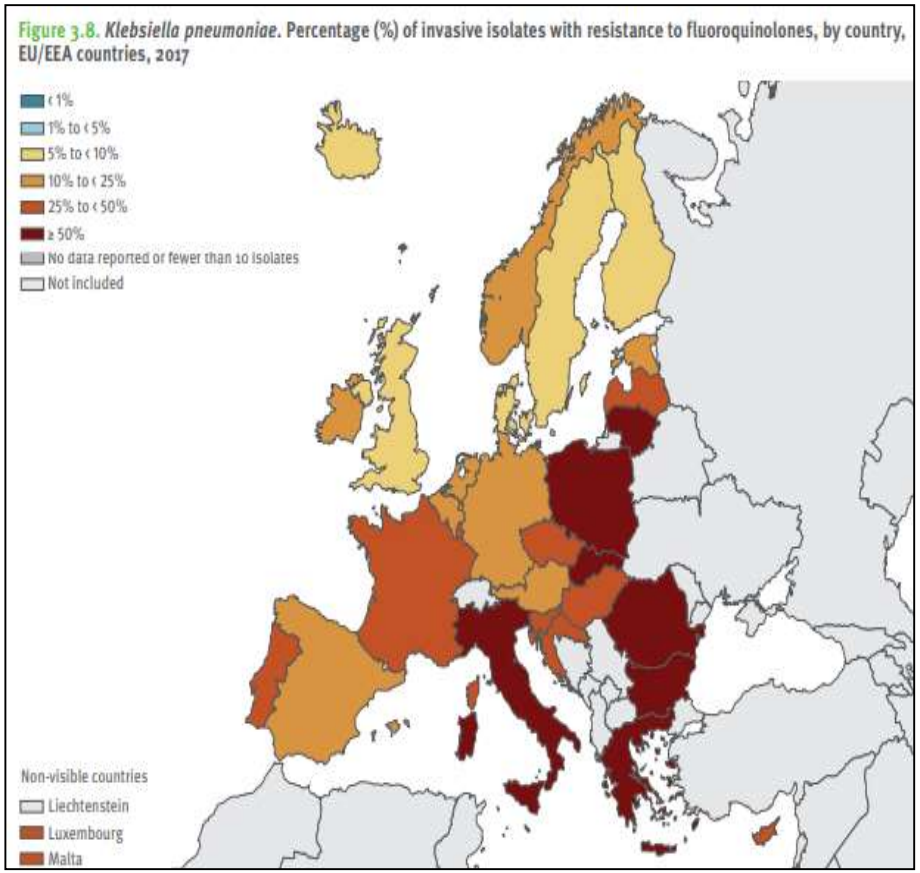
Figure 3.7. *Klebsiella pneumoniae*. Distribution of isolates: fully susceptible and resistant to one, two, three and four antimicrobial groups (among isolates tested against fluoroquinolones, third-generation cephalosporins, aminoglycosides and carbapenems), EU/EEA countries, 2017



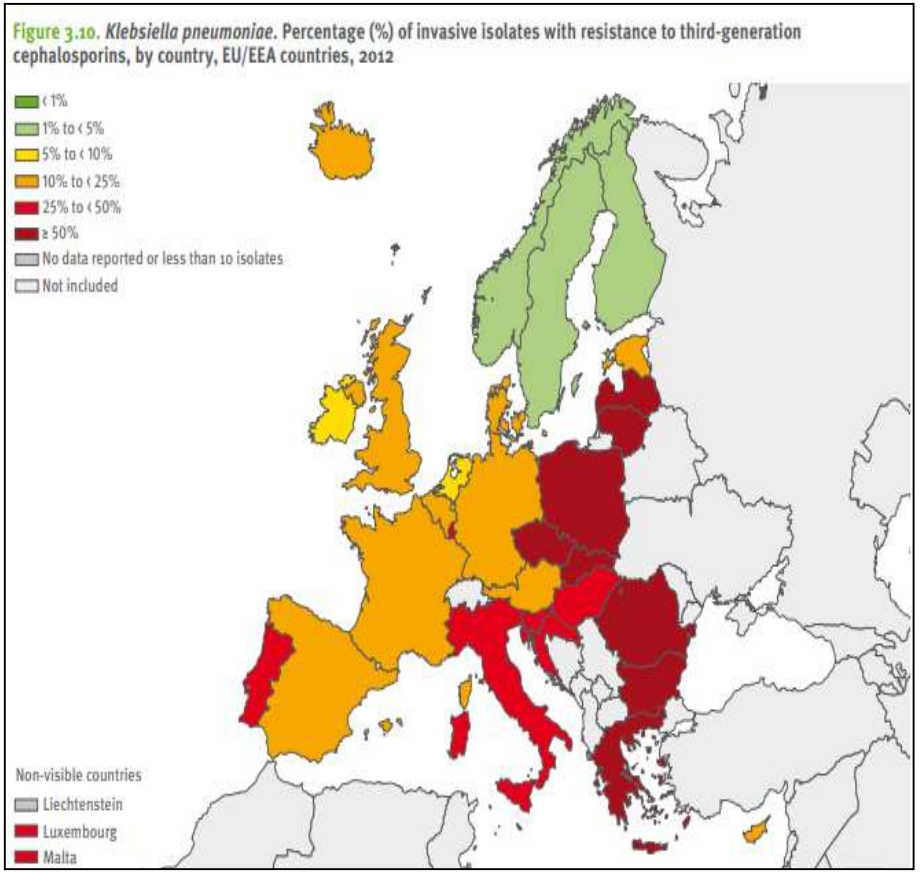
Only data from isolates tested against all included antimicrobial groups included in analysis.

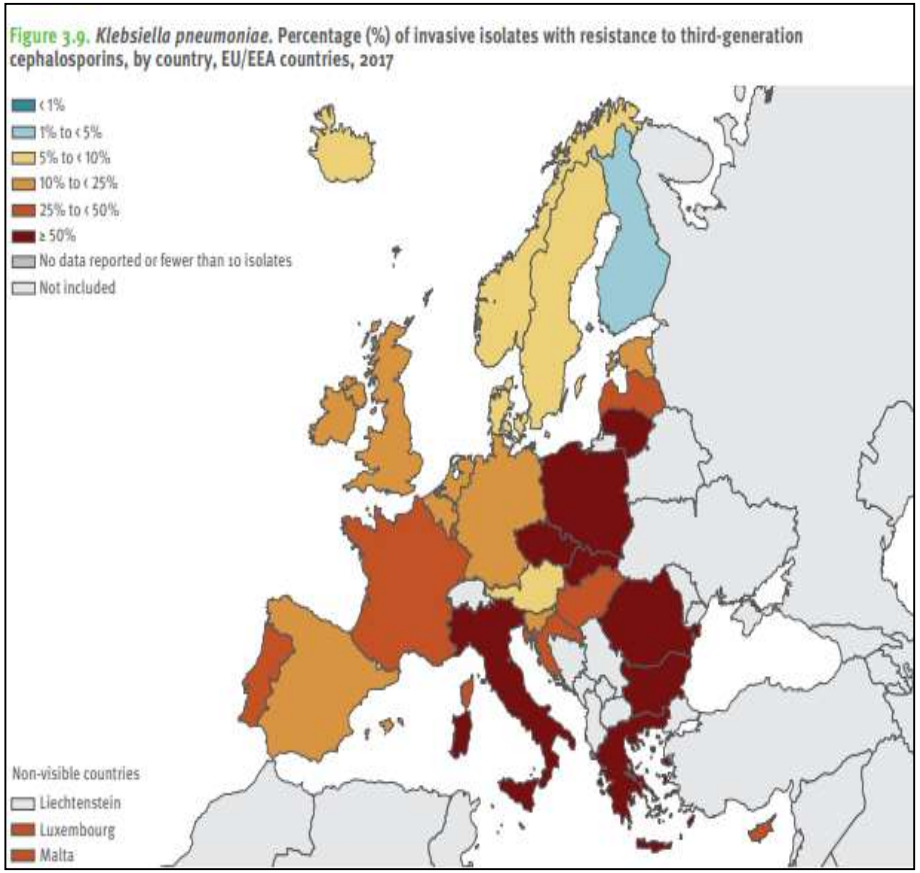
Klebsiella pneumoniae



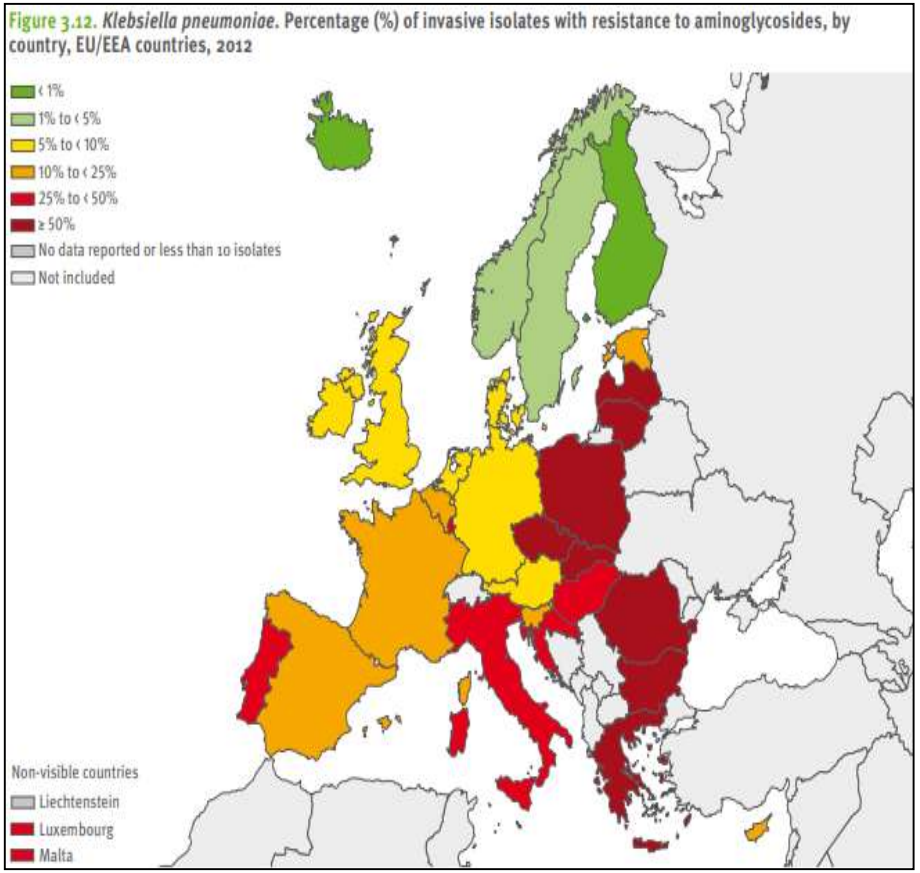


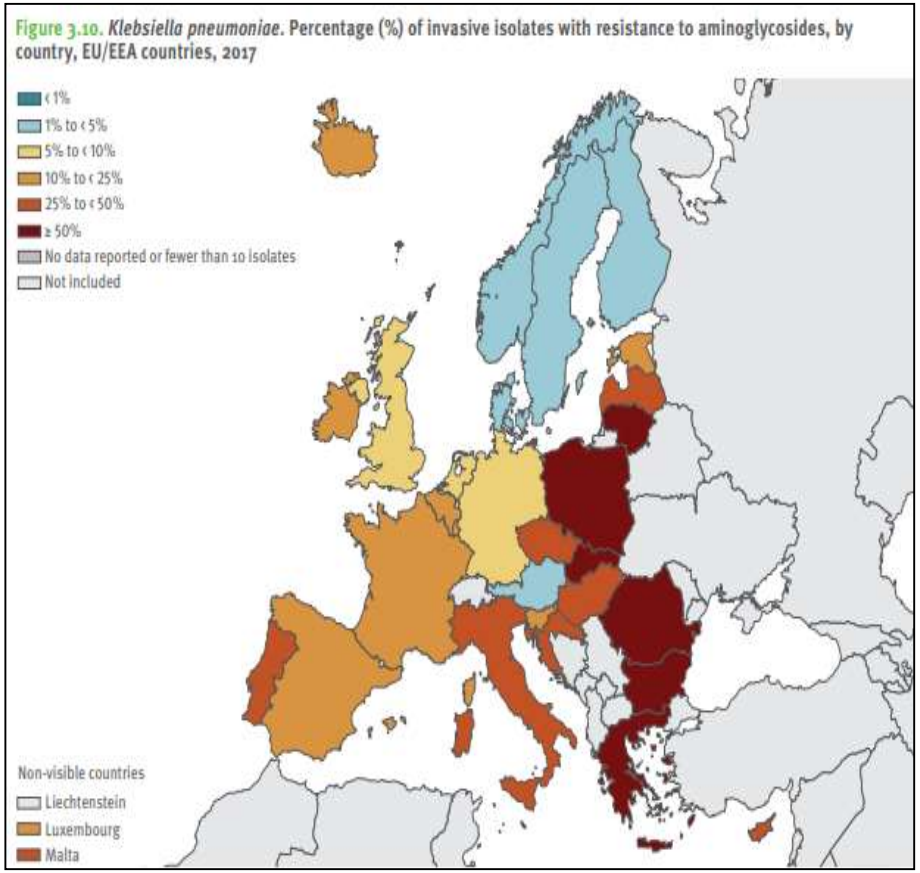
Klebsiella pneumoniae



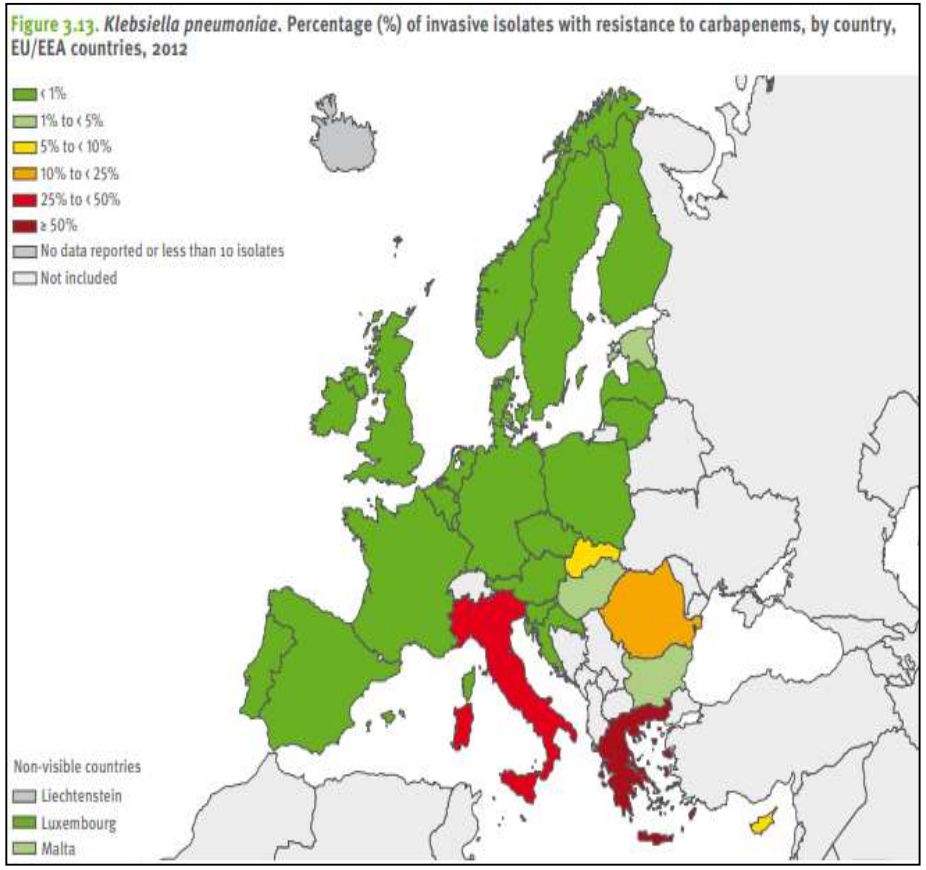


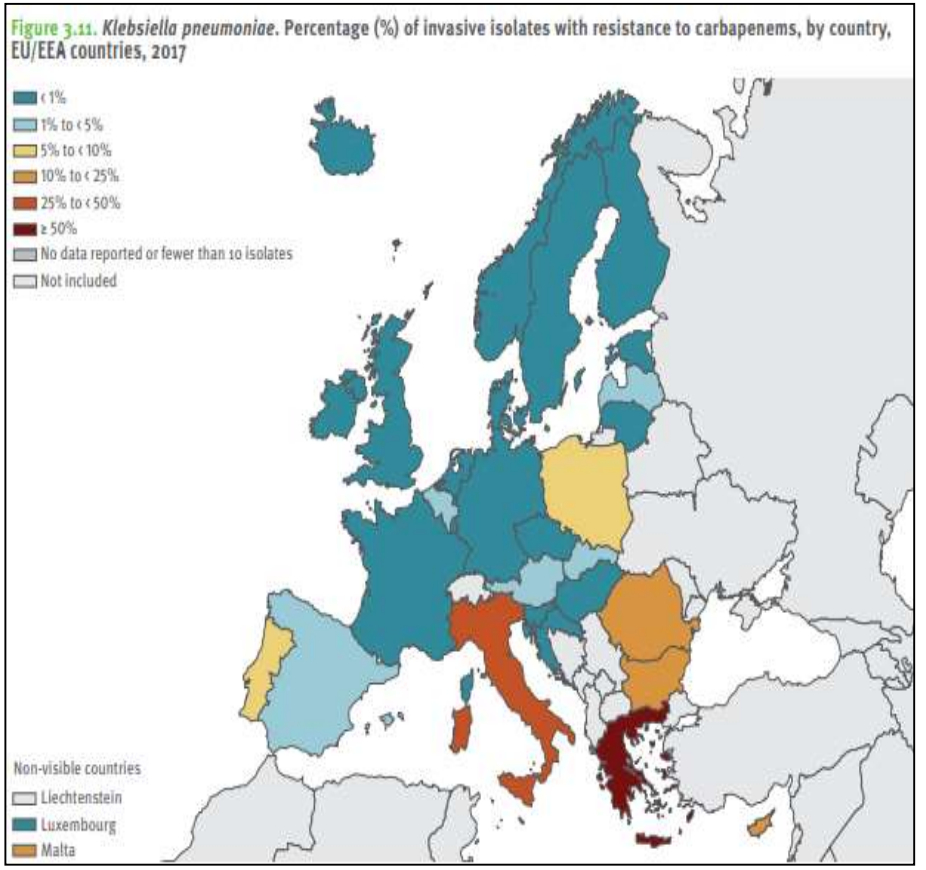
Klebsiella pneumoniae



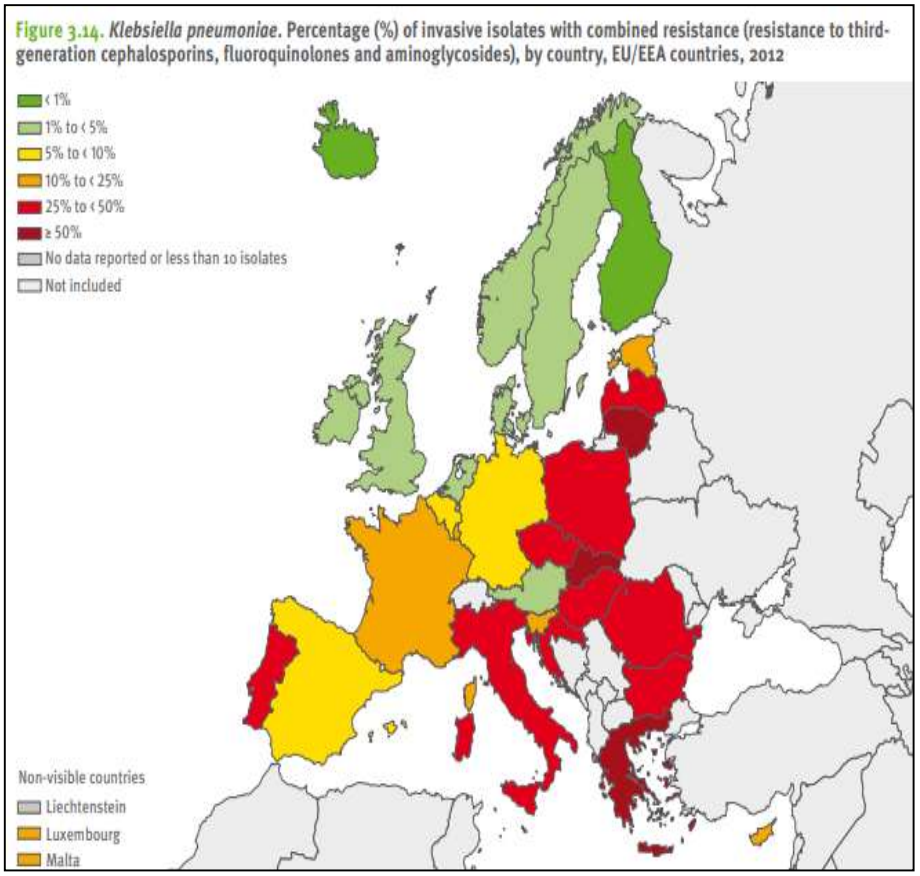


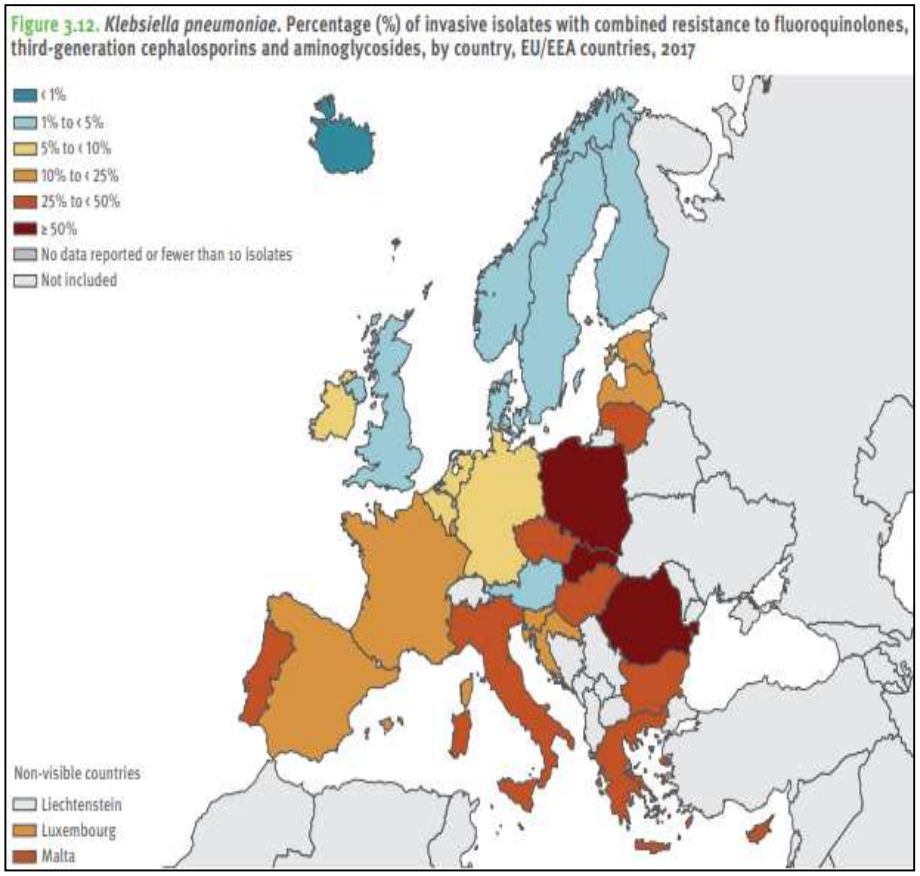
Klebsiella pneumoniae



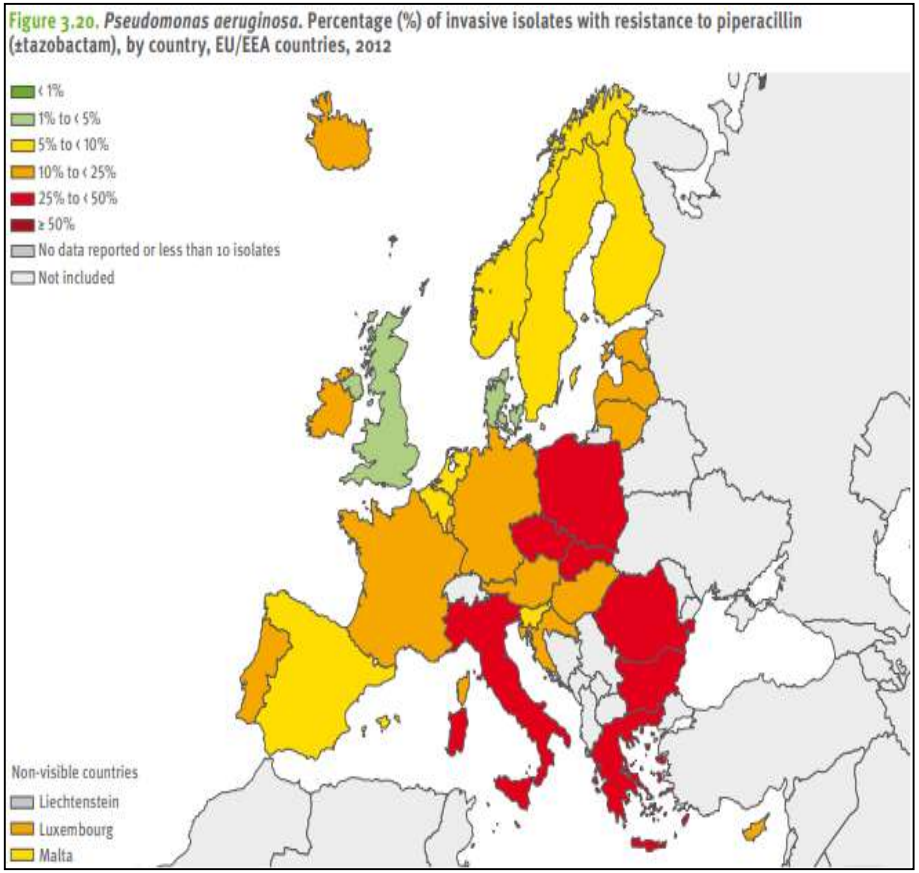


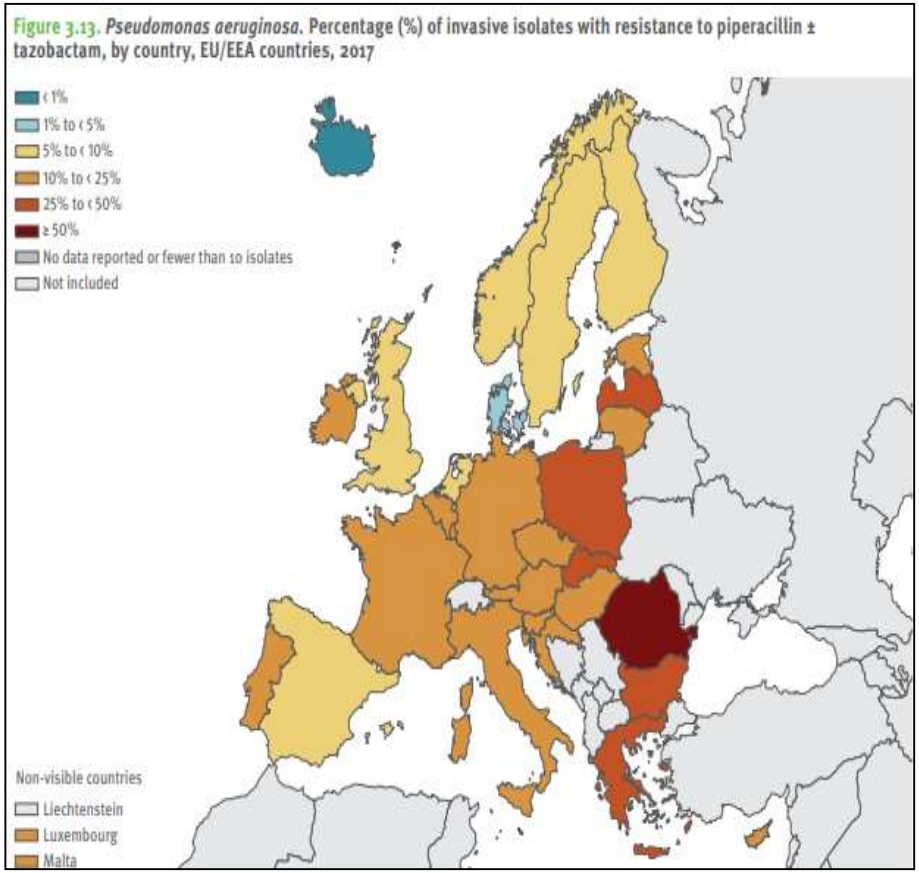
Klebsiella pneumoniae



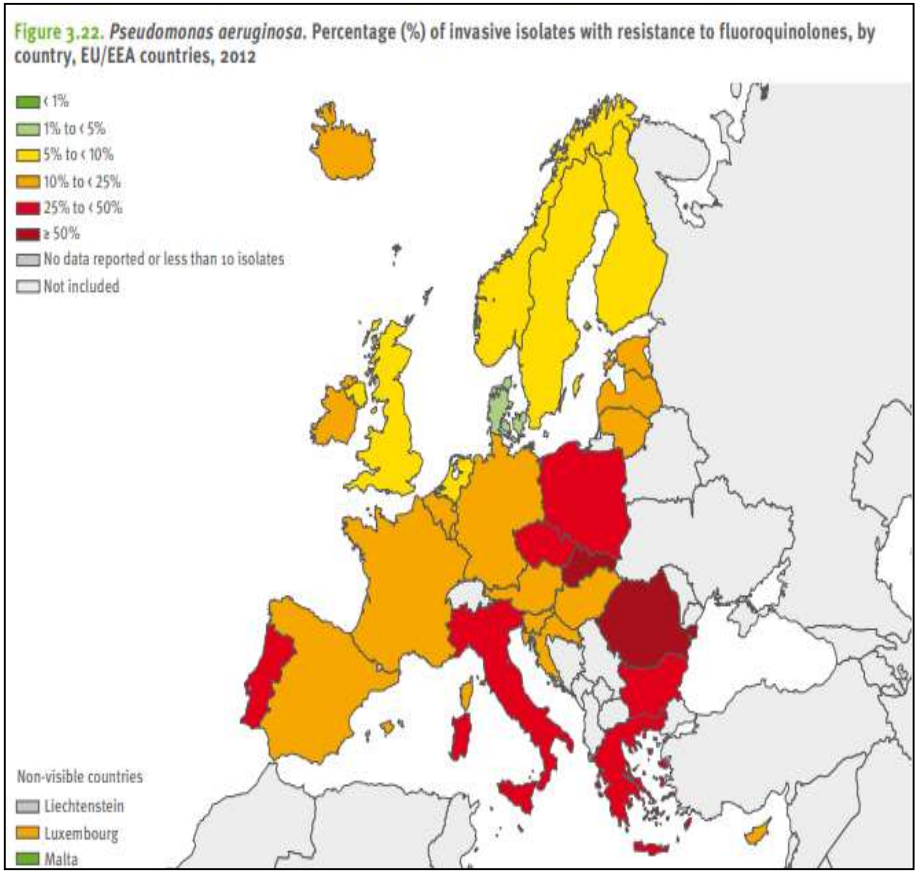


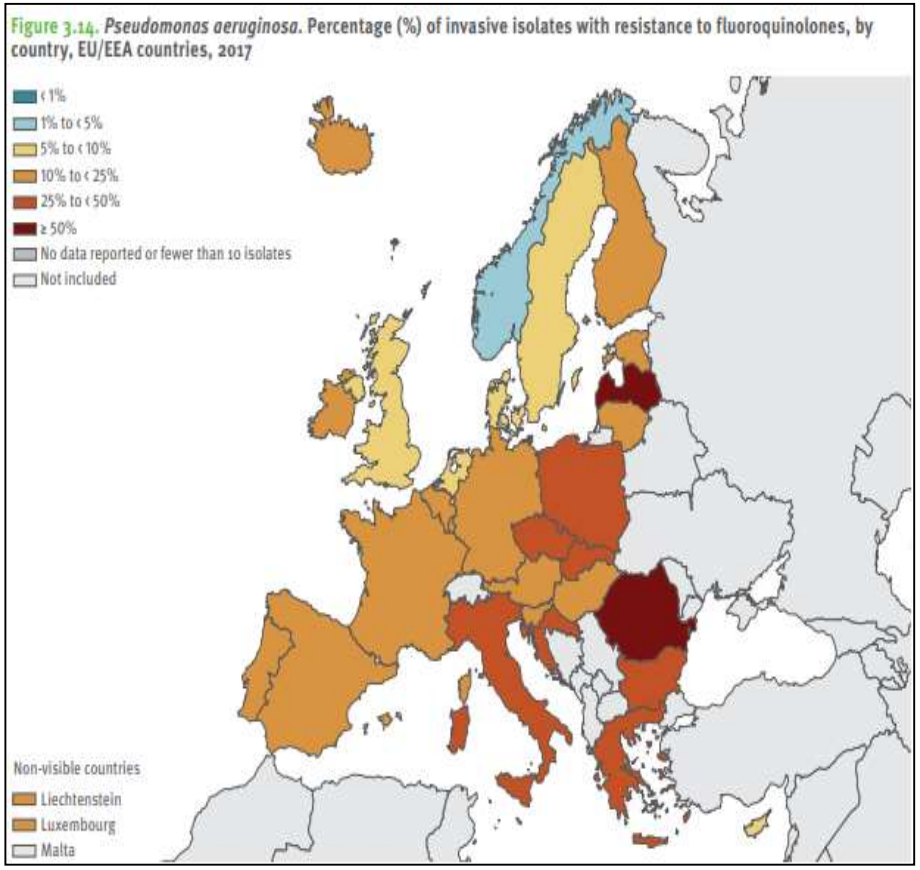
Pseudomonas aeruginosa



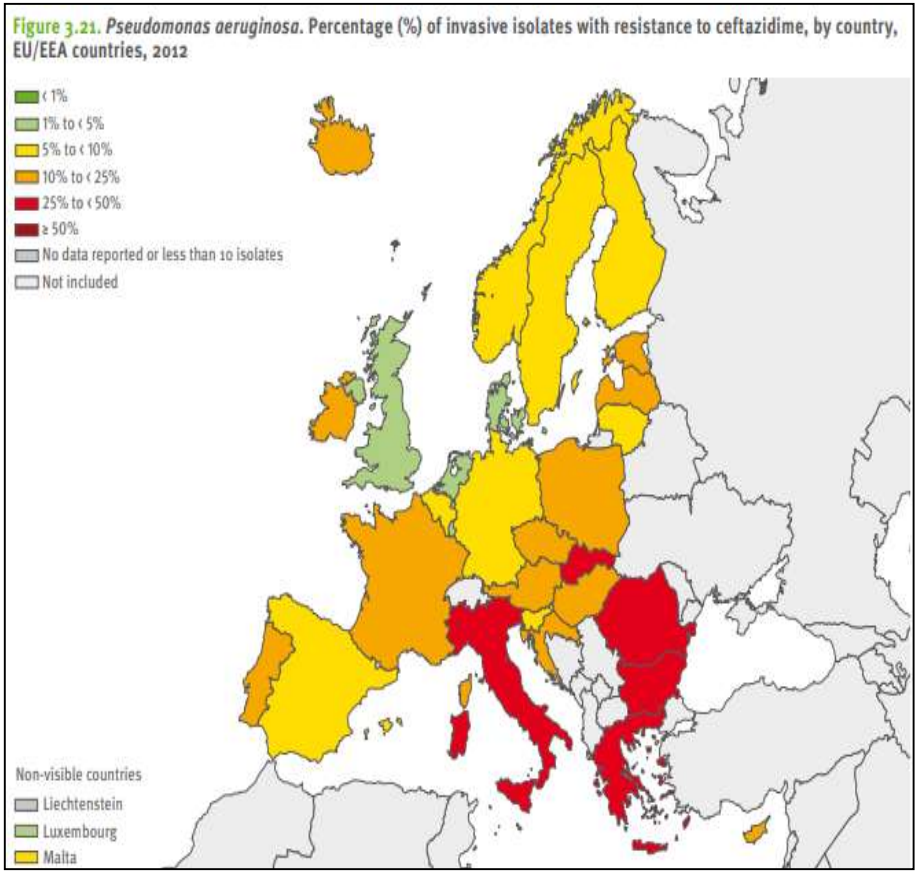


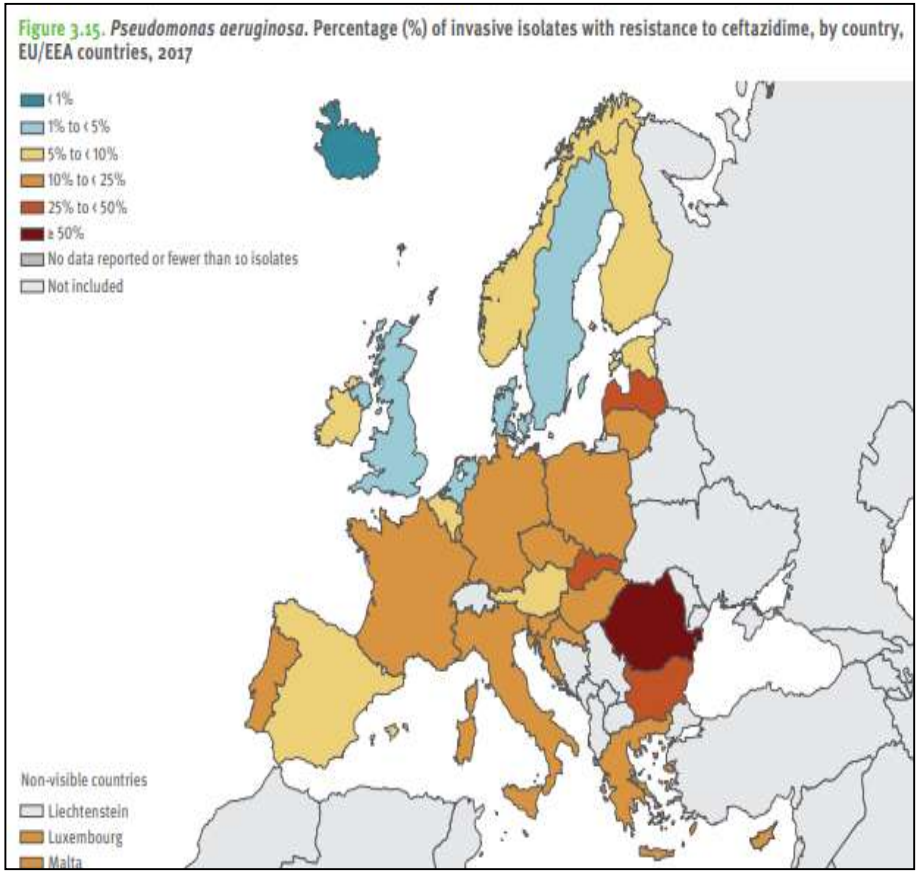
Pseudomonas aeruginosa



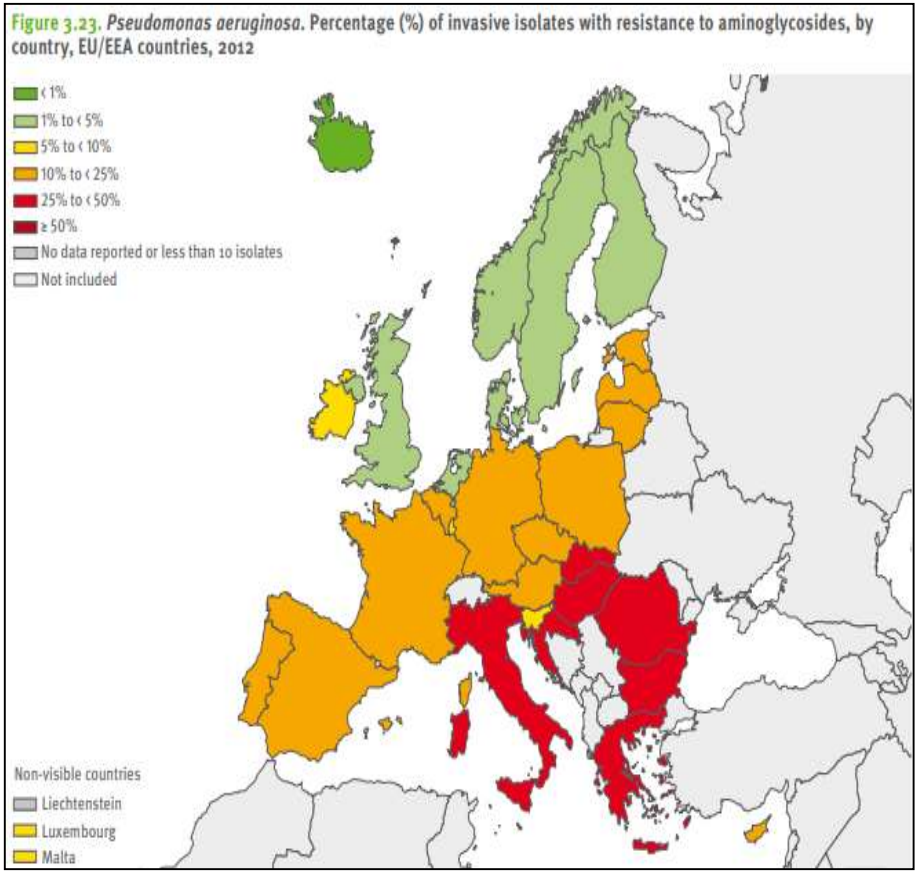


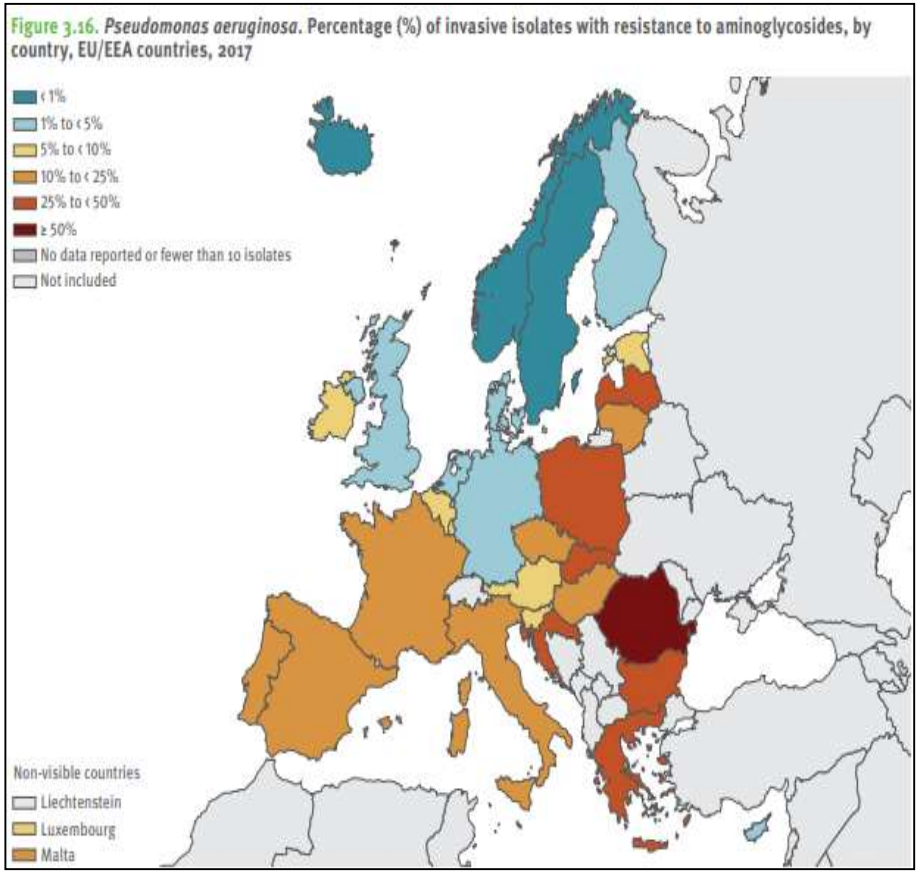
Pseudomonas aeruginosa



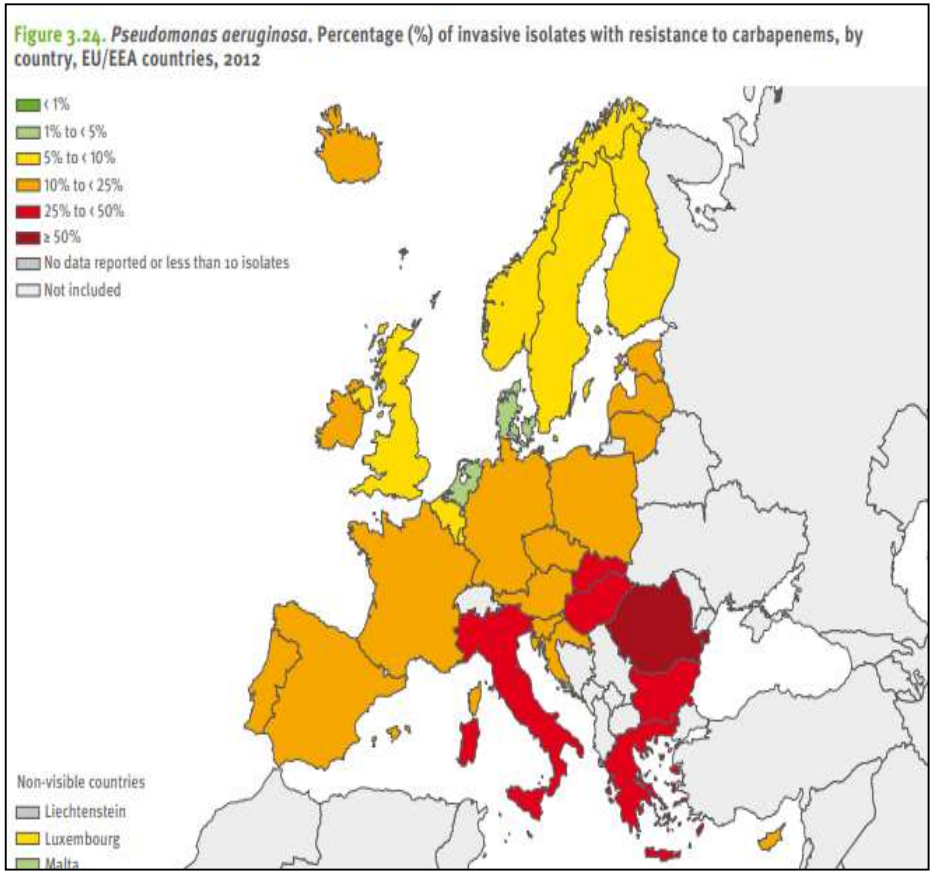


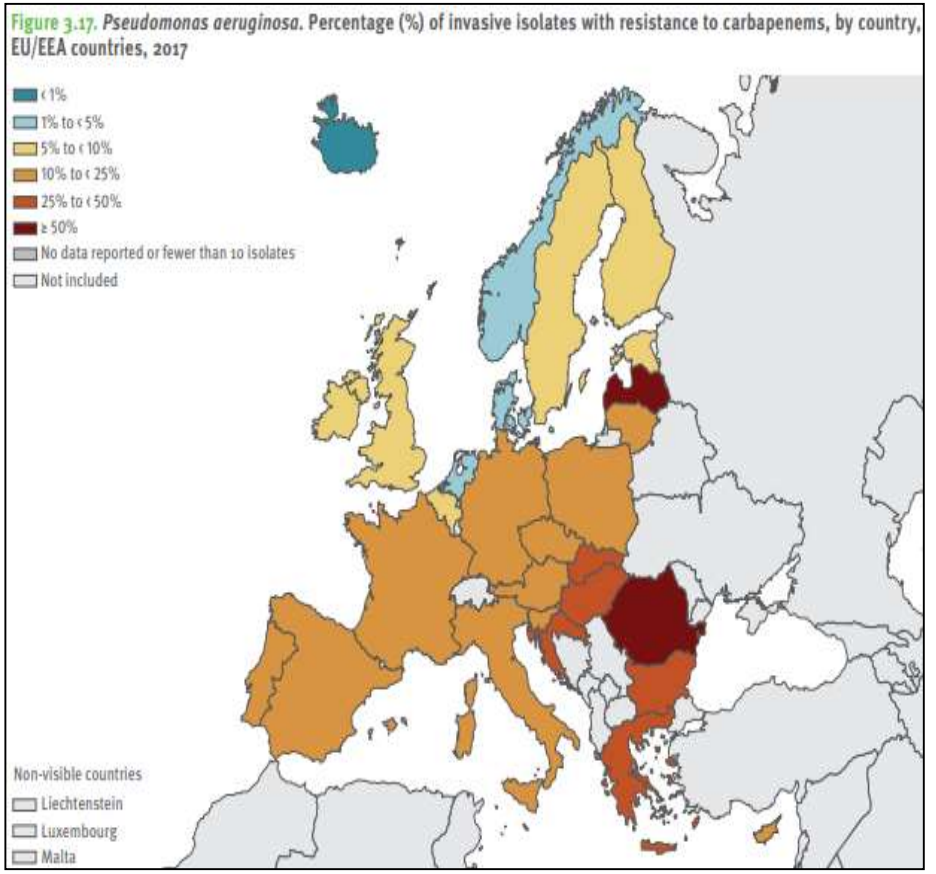
Pseudomonas aeruginosa



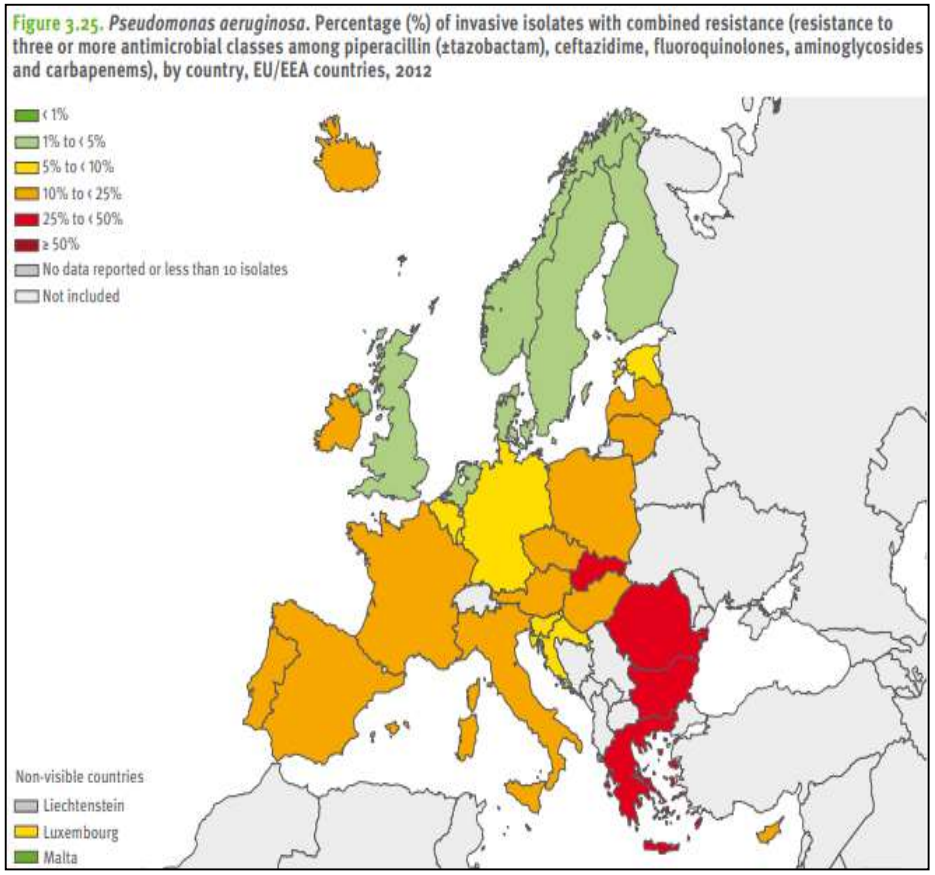


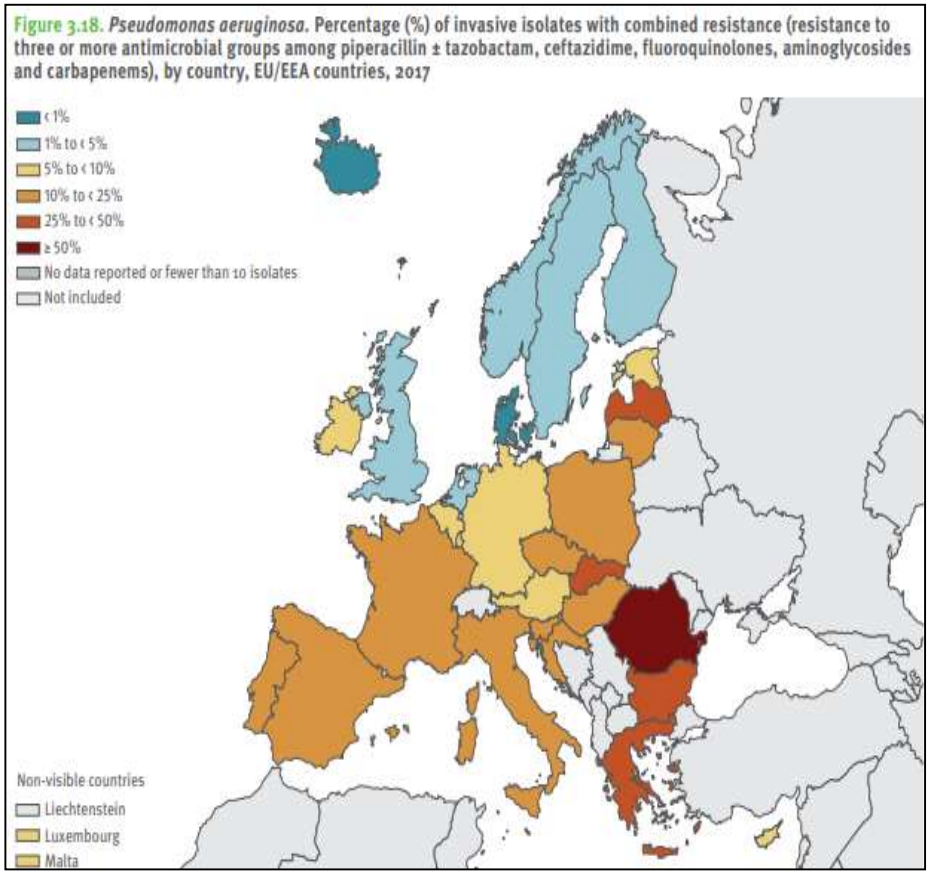
Pseudomonas aeruginosa





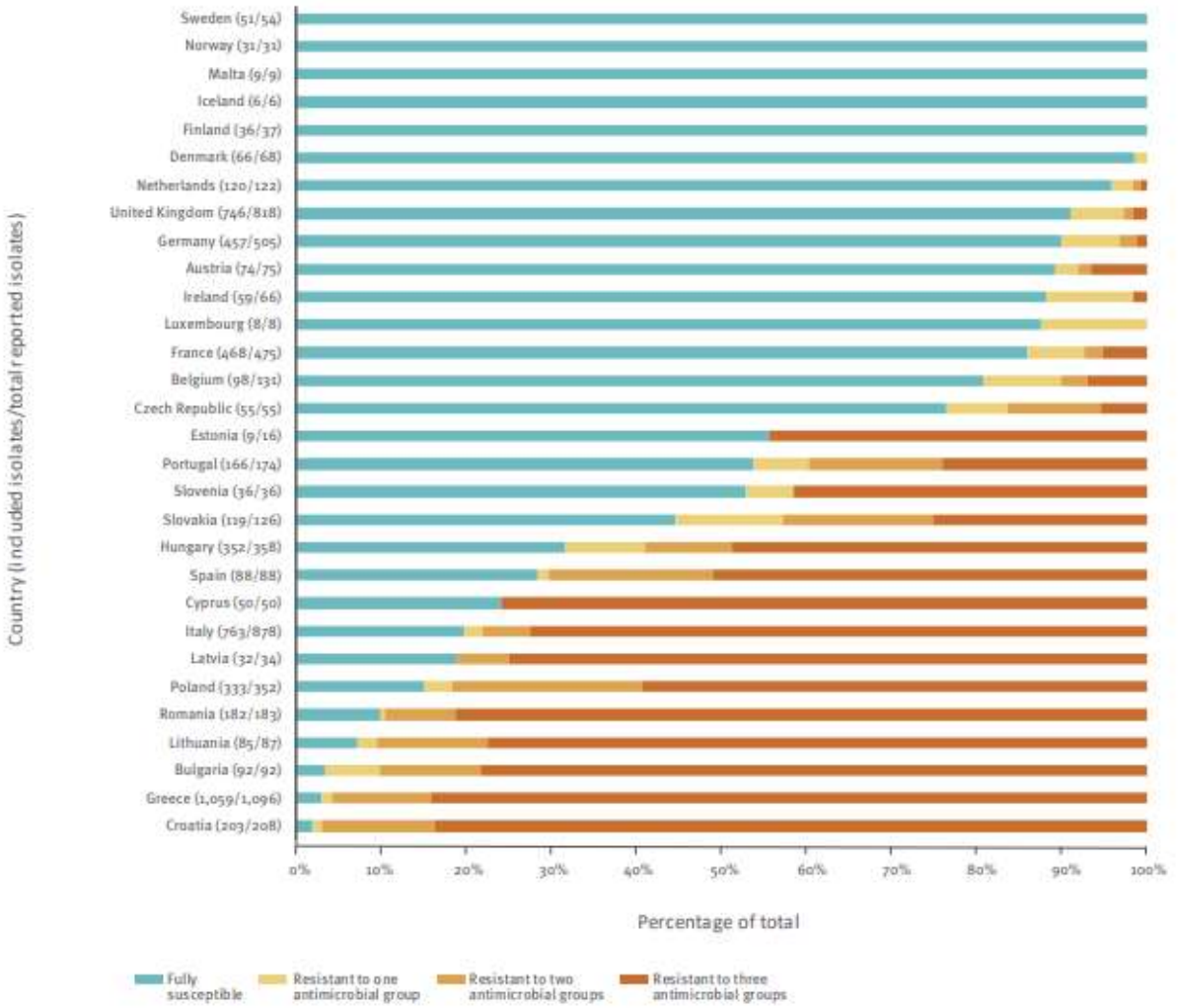
Pseudomonas aeruginosa





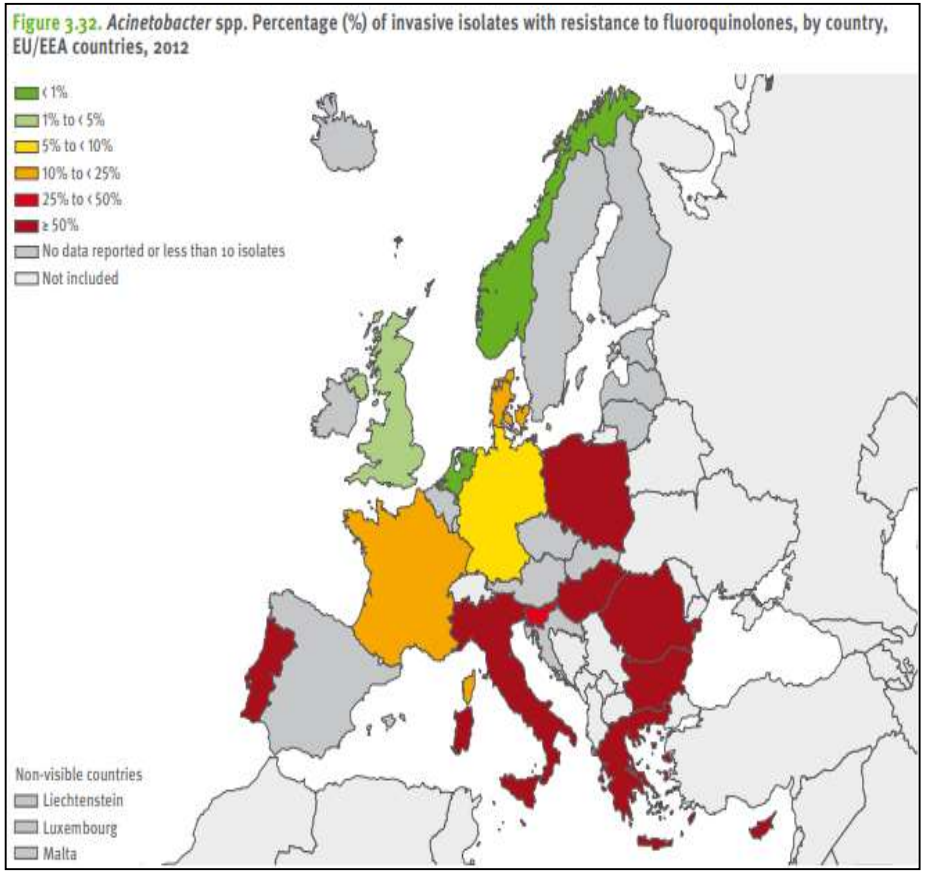
Acinetobacter spp.

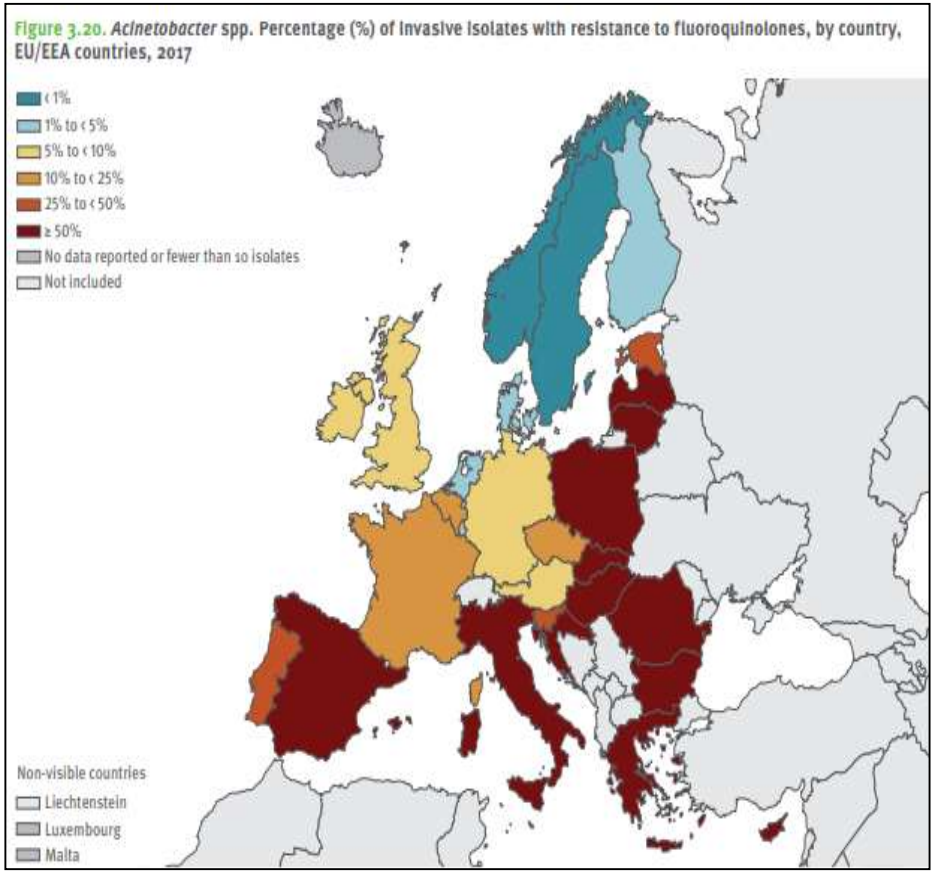
Figure 3.19. *Acinetobacter* spp. Distribution of isolates: fully susceptible and resistant to one, two and three antimicrobial groups (among isolates tested against fluoroquinolones, aminoglycosides and carbapenems), EU/EEA countries, 2017



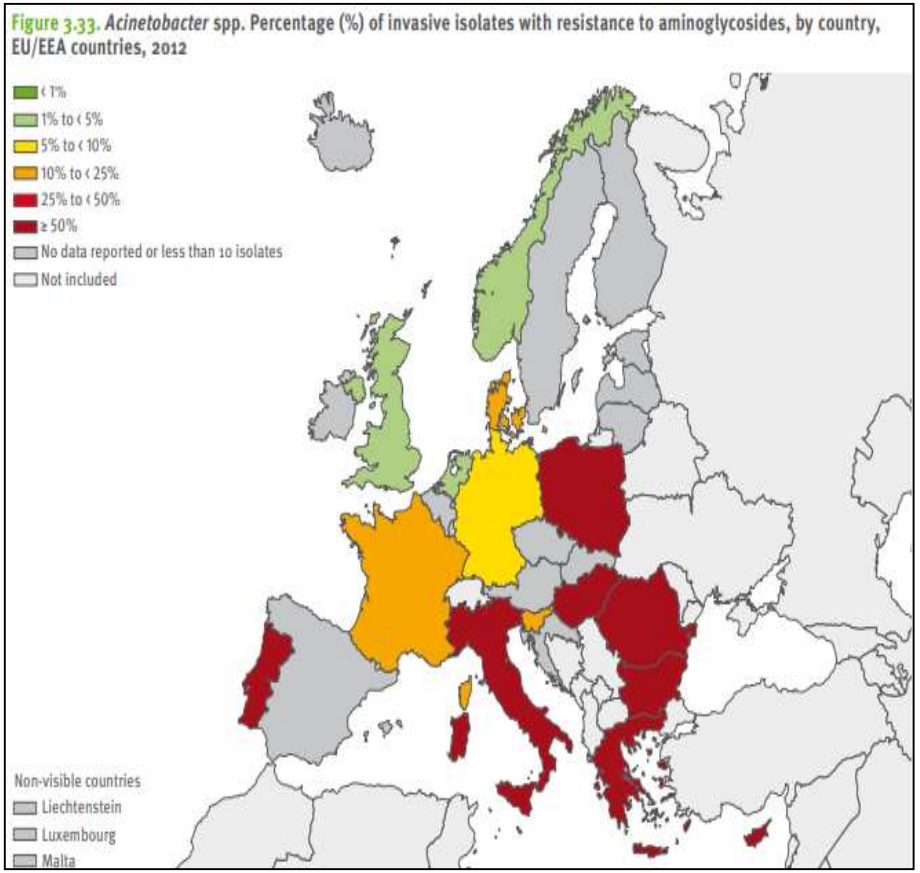
Only data from isolates tested against all included antimicrobial groups included in analysis.

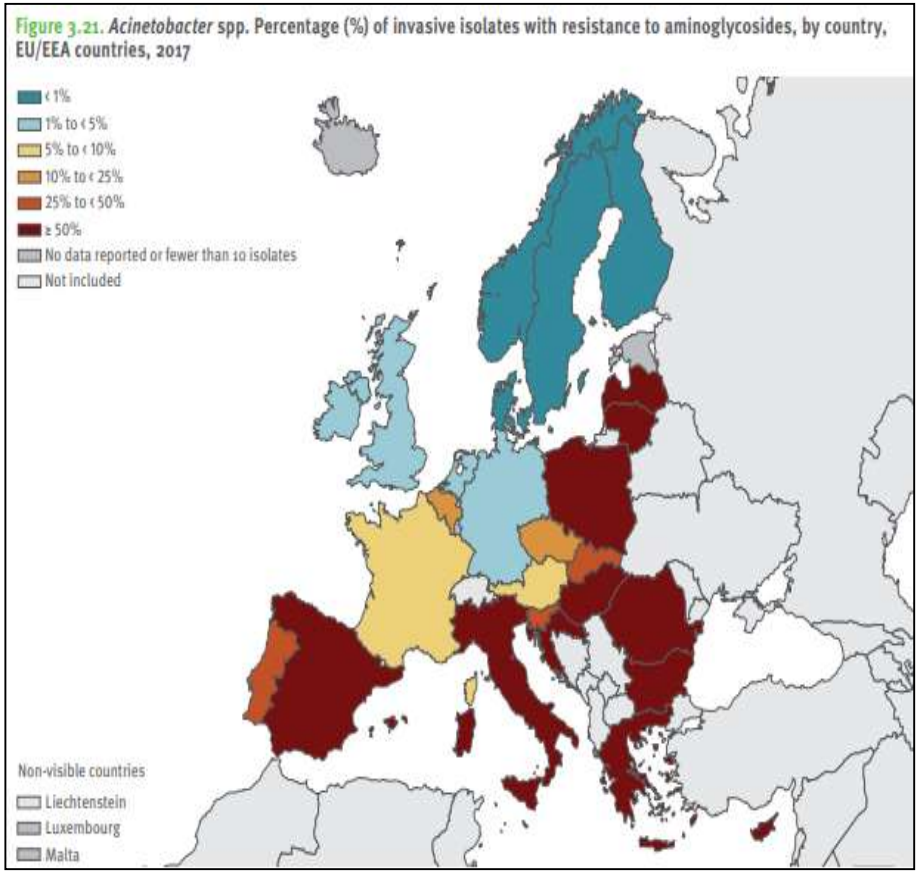
Acinetobacter spp.



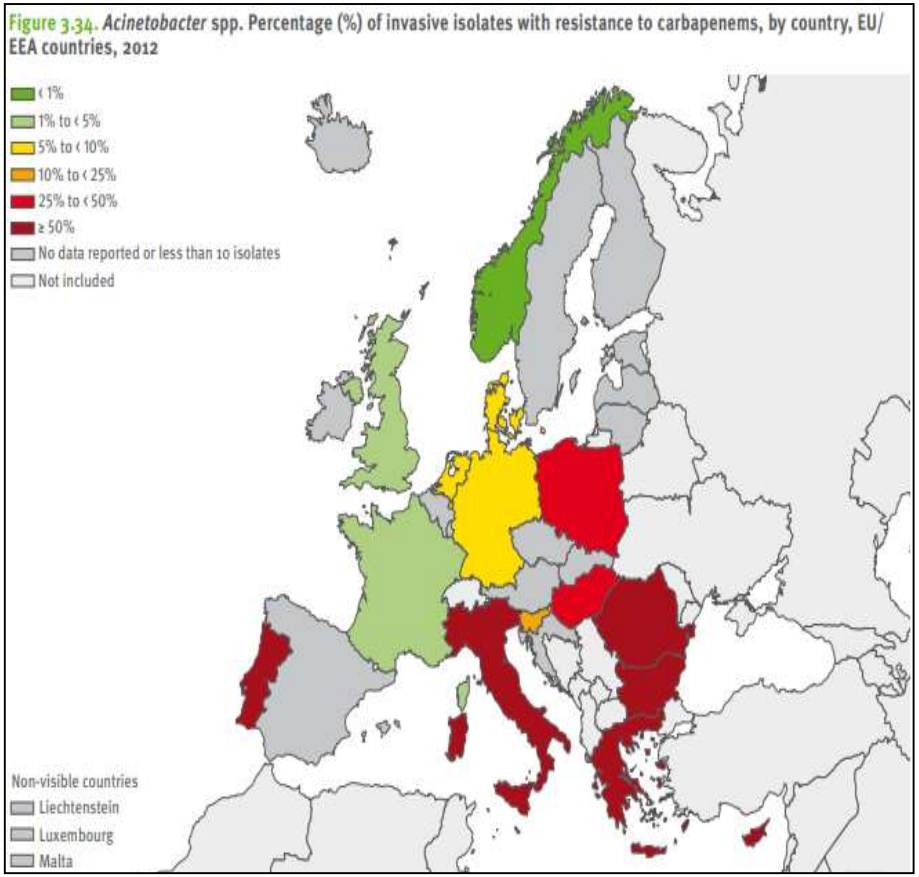


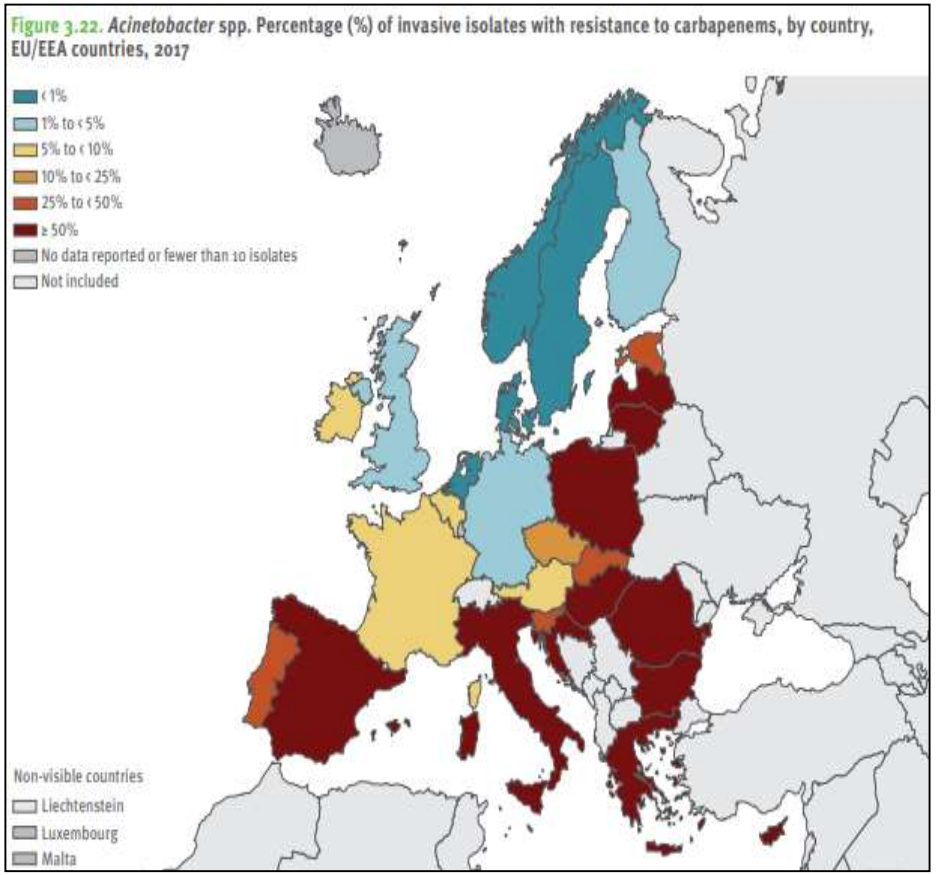
Acinetobacter spp.



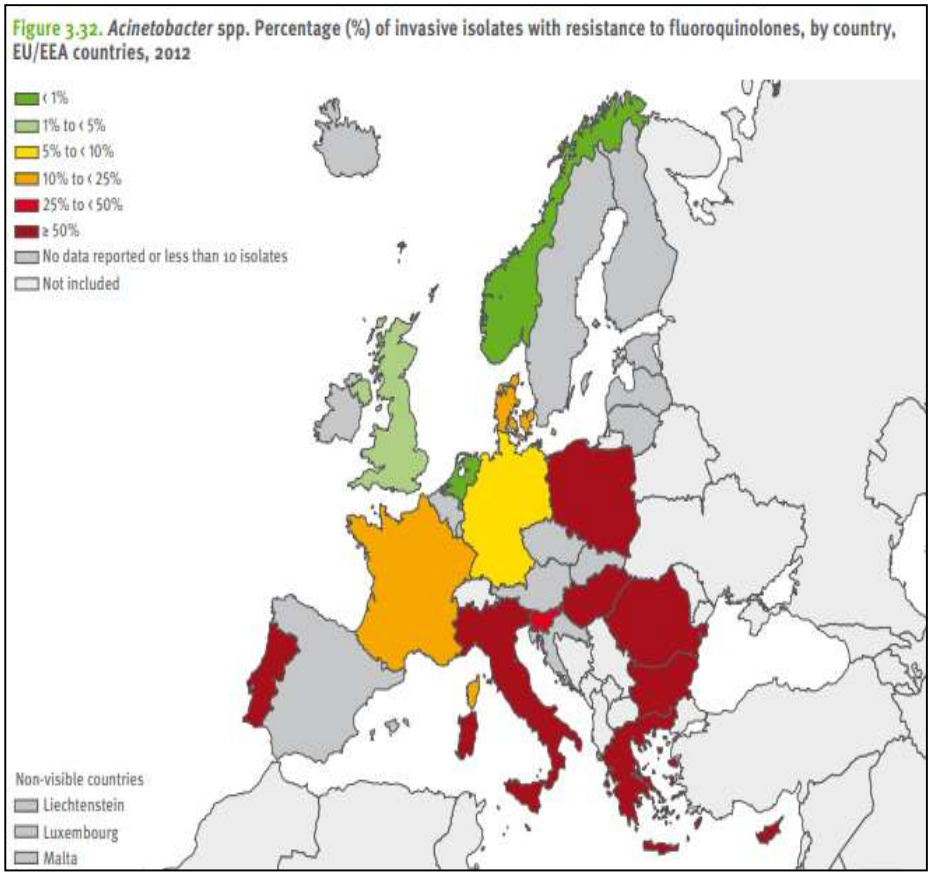


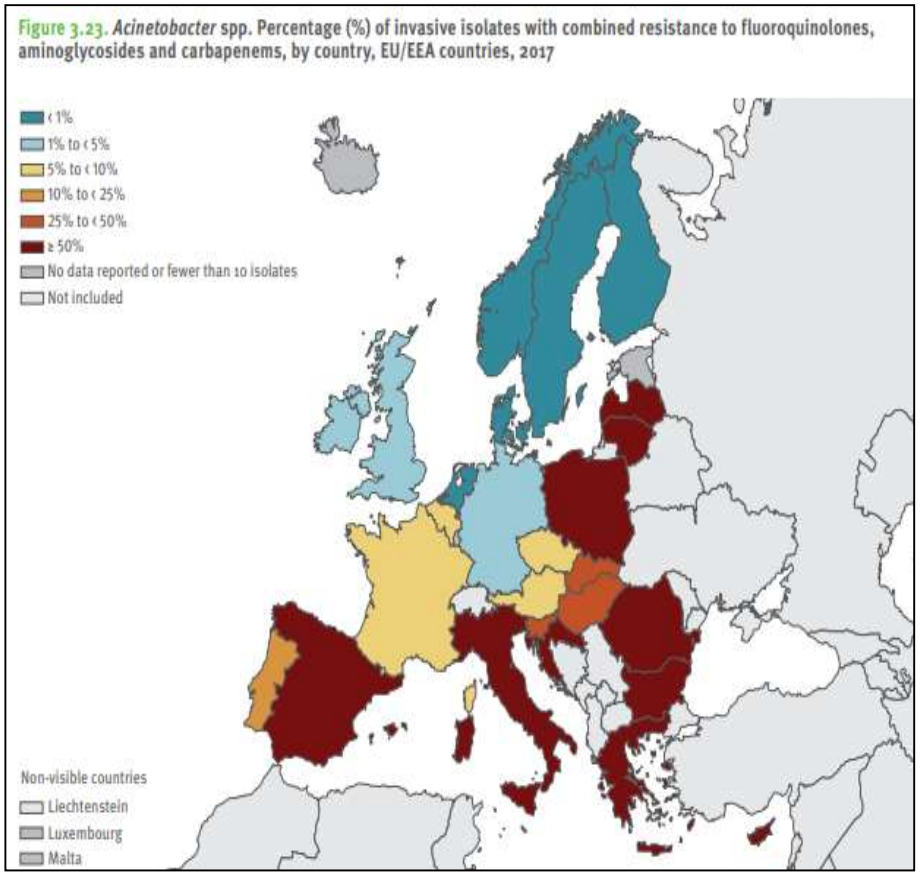
Acinetobacter spp.



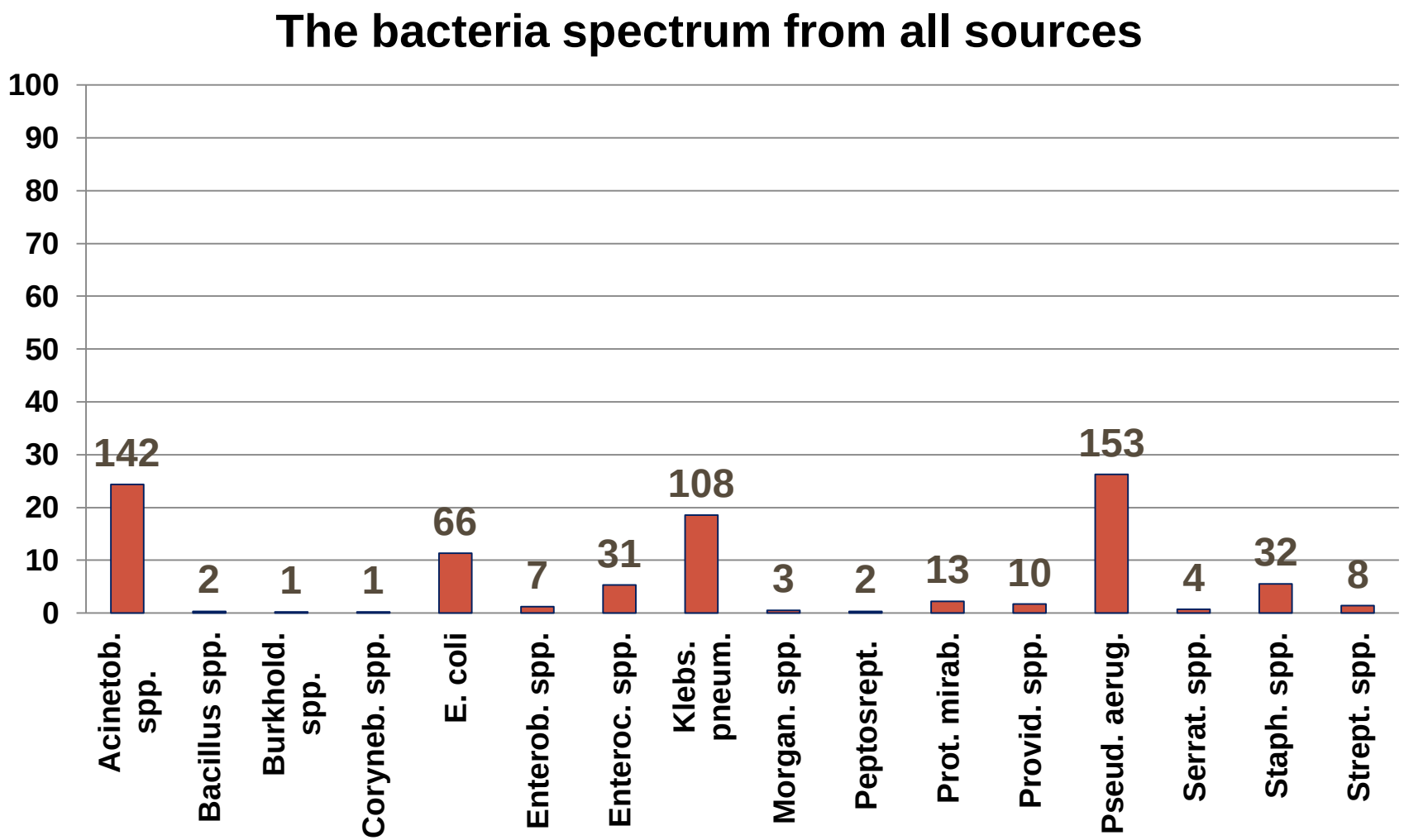


Acinetobacter spp.

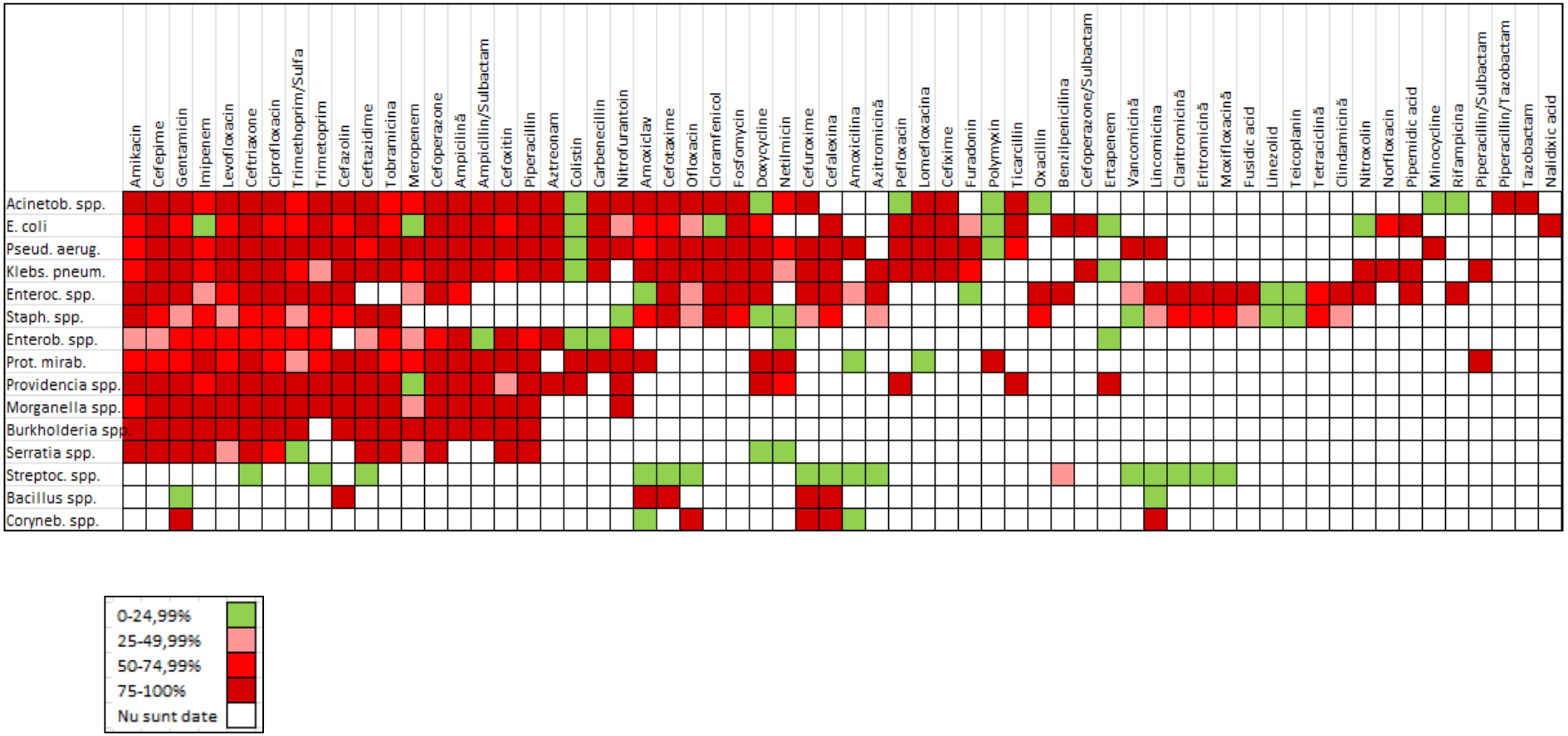




Antibiotic Resistance in ICU EI, 2016



Antibiotic Resistance in ICU EI, 2016



Antibiotic Resistance in ICU EI, 2016

The spectrum of activity of antibiotics

Gram pozitive	Gram negative	Pseudomonas	Klebsiella
Amoxiclav			
Teicoplanin			
	Colistin		
	Polimixină		
	Amoxicilină		
			Ertapenem

Potential Determinants Influencing Future Dissemination and Control of Antibiotic Resistance

Pathogen and microbial ecology

Determinant	Potential control measures and interventions
Evolution	Evolutionary engineering
Survival fitness	Inhibition of gene expression
Virulence	Anti- virulence strategies - Targeting toxins and secretion systems - Targeting signalling and regulation - Targeting biofilms and adherence - Biological response modifiers
Constitution of microbiome	Prebiotics Probiotics Faecal microbiota transplantation
Laboratory detection, identification and antimicrobial susceptibility testing	Improved rapid microbial diagnostic tests Real-time whole (meta)genome sequencing

Clinician prescribing practices

Determinant	Potential control measures and interventions
Training and knowledge	Under-and post graduate training Targeted educational interventions
Antimicrobial prescribing patterns	Multi-modal stewardship interventions
Prescribing heterogeneity	Decision support tools
Accountability	Individualized audit and feedback with comparative benchmarking Institutional regulations
Behaviour change	Targeted behaviour change techniques and interventions
Diagnostic uncertainty	Novel biomarkers and diagnostic tools Rapid, bedside molecular diagnostics

Population characteristics

Determinant	Potential control measures and interventions
Migration, travel and globalization	Screening and improved global surveillance from a "One health" perspective
Case mix and host susceptibility	Improved management of chronic comorbid diseases Vaccination
Antimicrobial demand and beliefs	School education and public information campaigns
Transmission and infection rates	Sustainable hand and food hygiene behaviour change Improved environmental cleaning and auditing

Politics and health-care policy

Determinant	Potential control measures and interventions
Healthcare policy	Change in reimbursement practices
Promotional industry activities	Regulation Novel antimicrobial discovery, development and marketing models
Antimicrobial use in food production	Novel agents for growth promotion and meta-prophylaxis
Technological research and development	Novel treatment and prevention approaches

Bassetti M, Carnelutti A, Peghin M. Patient specific risk stratification for antimicrobial resistance and possible treatment strategies in gram-negative bacterial infections Expert Rev Anti Infect Ther. 2017 Jan;15(1):55-65. Epub 2016 Nov 7 *Fig. 2 Potential determinants influencing future dissemination and control of antibiotic resistance. Reproduced and adapted with permission from Harbarth and Samore [12]*

Investigation Strategies and Methods

Developed by the Department of Epidemic and Pandemic Alert and Response of the World Health Organization with assistance from:

European Program for Intervention Epidemiology Training

Canadian Field Epidemiology Program

Thailand Ministry of Health

Institut Pasteur

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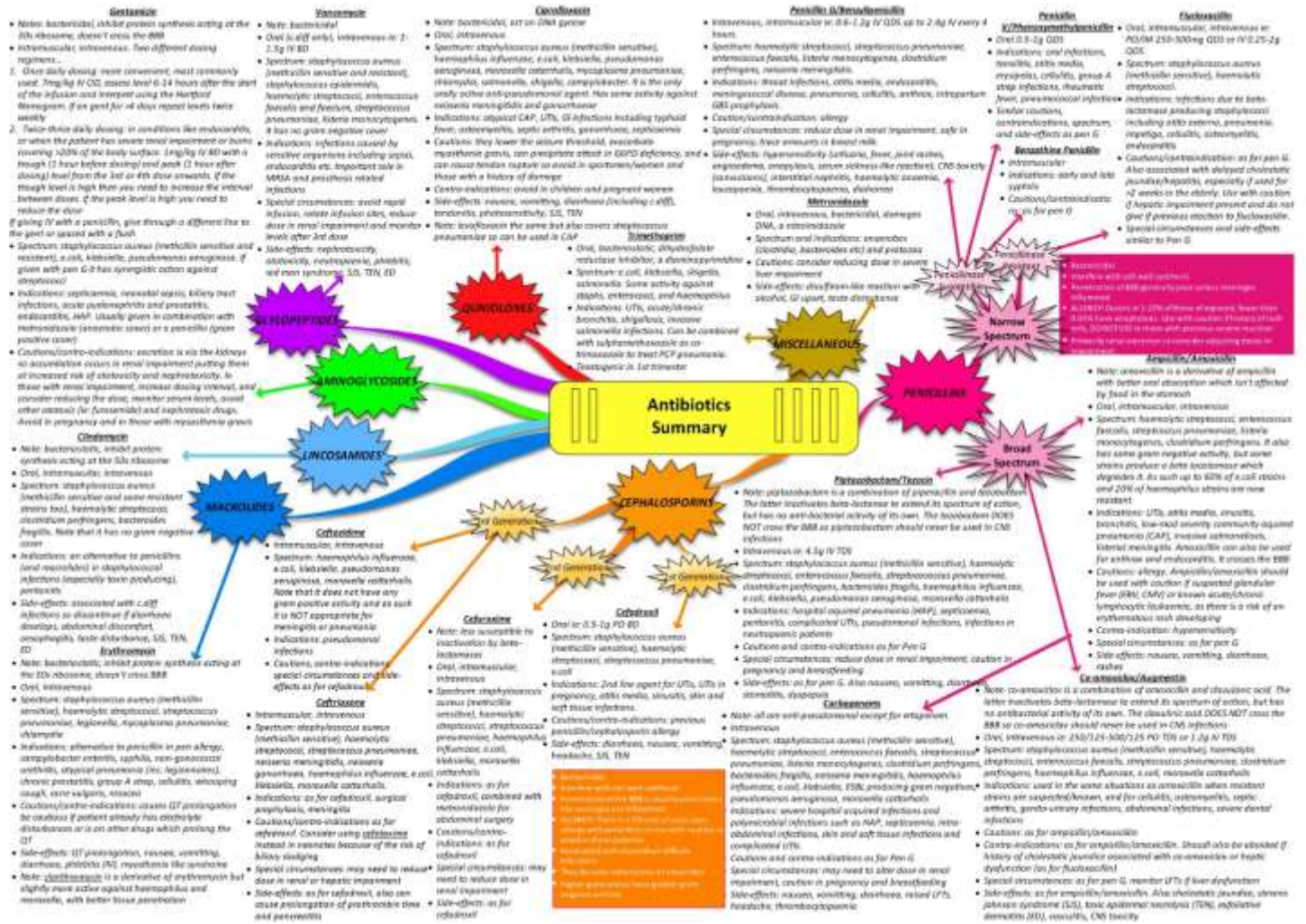
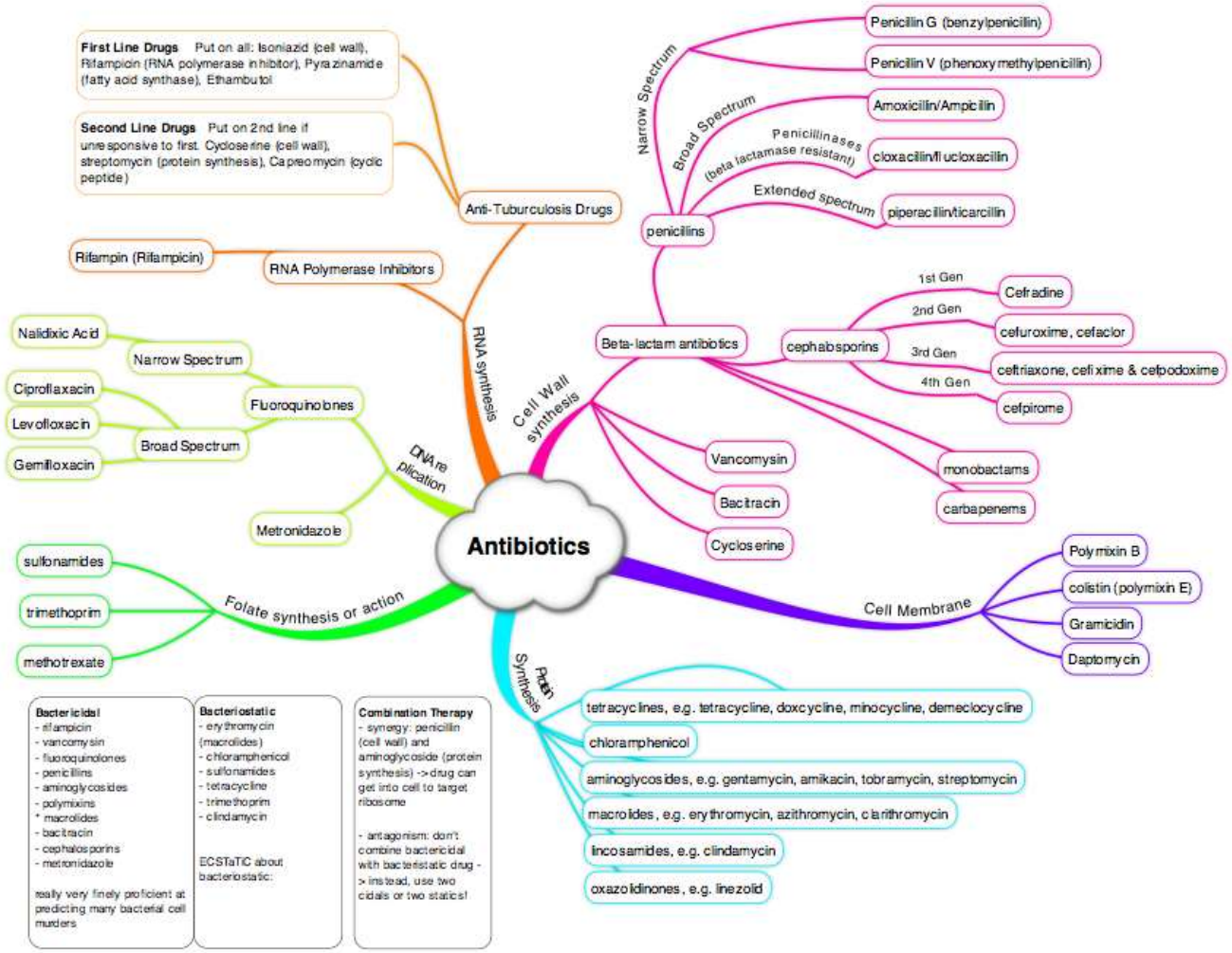
Antibiotic class	Resistance type	Resistance mechanism	Common example
Aminoglycoside	Decreased uptake	Changes in outer membrane	*P. aeruginosa*
	Enzymatic modification	AGE's	Gram-negative bacteria
Beta-lactams	Altered PBP	PBP 2a	Mec A in *S. aureus*, CONS, *S. pneumoniae*
	Enzymatic degradation	Penicillinase which are classified as per ambler classification	Gram-negative bacteria
Glycopeptides	Altered target	D-alanyl-alanine is changed to D-alanyl-D-lactate	Vancomycin resistance in *E. faecium* and *E. faecalis*
Macrolides	Altered target	Methylation of ribosomal active site with reduced binding	*erm*-encoded methylases *in S. aureus*, *S. pneumoniae*, and *S. pyogenes*
	Efflux pumps	Mef type pump	*S. pneumoniae* and *S. pyogenes*
Oxazolidinones	Altered target	Mutation leading to reduced binding to active site	*E. faecium* and *S. aureus*
Quinolones	Altered target	Mutation leading to reduced binding to active site(s)	Mutations in gyr A in enteric Gram-negative bacteria and *S. aureus*
	Efflux	Membrane transporters	Mutations in gyr A and par C in *S. pneumoniae*. Nor-A in *S. aureus*
Tetracyclines	Efflux	New membrane transporters	*tet* genes encoding efflux proteins in Gram-positive and Gram-negative bacteria
	Altered target	Production of proteins that bind to the ribosome and alter the conformation of the active site	*tet* (M) and *tet* (O) in Gram-positive and Gram-negative bacteria species
Chloramphenicol	Antibiotic inactivation	Chloramphenicol acetyl transferase	CAT in *S. pneumonia*
	Efflux pump	New membrane transporters	cml A gene and flo gene efflux in *E. coli*
Sulfa drugs	Altered target	Mutation of genes encoding DHPS	*E. coli*, *S. aureus*, *S. pneumoniae*

DHPS = Dihydropteroate synthase, P. aeruginosa = Pseudomonas aeruginosa, S. aureus = Staphylococcus aureus, S. pneumoniae = Streptococcus pneumoniae, E. faecium = Enterococcus faecium, E. faecalis = Enterococcus faecalis, S. pyogenes = Streptococcus pyogenes, E. coli = Escherichia coli, PBP = Penicillin binding protein, AGE's = Aminoglycoside modifying enzymes, CAT = Chloramphinecol acetyl transferases


Antibiotics Summary
QUINOLONES
AMINOGLYCOSIDES
LINCOSAMIDES
MACROLIDES
CEPHALOSPORINS
PENICILLINS
MISCELLANEOUS
Narrow Spectrum
Broad Spectrum
Gentamicin
Vancomycin
Ciprofloxacin
Penicillin G/Benzylpenicillin
Penicillin V/Phenoxymethylpenicillin
Flucloxacillin
Benzathine Penicillin
Amoxicillin/Amoxicillin
Co-amoxiclav/Augmentin
Clindamycin
Erythromycin
Cefuroxime
Ceftriaxone
Cefaclor
Cefadroxil
Cefalexin
Piperacillin/Tazobam/Tazocin
Carbapenems
Trimethoprim
Metronidazole


Antibiotics
RNA synthesis
Anti-Tuburculosis Drugs
First Line Drugs Put on all: Isoniazid (cell wall), Rifampicin (RNA polymerase inhibitor), Pyrazinamide (fatty acid synthase), Ethambutol
Second Line Drugs Put on 2nd line if unresponsive to first. Cycloserine (cell wall), streptomycin (protein synthesis), Capreomycin (cyclic peptide)
RNA Polymerase Inhibitors
Rifampin (Rifampicin)
DNA replication
Fluoroquinolones
Narrow Spectrum
Nalidixic Acid
Broad Spectrum
Ciprofloxacin
Levofloxacin
Gemifloxacin
Metronidazole
Cell Wall synthesis
Beta-lactam antibiotics
penicillins
Narrow Spectrum
Penicillin G (benzylpenicillin)
Penicillin V (phenoxymethylpenicillin)
Broad Spectrum
Amoxicillin/Ampicillin
Penicillinases (beta lactamase resistant)
cloxacillin/flucloxacillin
Extended spectrum
piperacillin/ticarcillin
cephalosporins
1st Gen
Cefradine
2nd Gen
cefuroxime, cefaclor
3rd Gen
ceftriaxone, cefixime & cefpodoxime
4th Gen
cefpirome
monobactams
carbapenems
Vancomysin
Bacitracin
Cycloserine
Cell Membrane
Polymixin B
colistin (polymixin E)
Gramicidin
Daptomycin
Protein Synthesis
tetracyclines, e.g. tetracycline, doxycycline, minocycline, demeclocycline
chloramphenicol
aminoglycosides, e.g. gentamycin, amikacin, tobramycin, streptomycin
macrolides, e.g. erythromycin, azithromycin, clarithromycin
lincosamides, e.g. clindamycin
oxazolidinones, e.g. linezolid
Folate synthesis or action
sulfonamides
trimethoprim
methotrexate
Bactericidal
- rifampicin
- vancomysin
- fluoroquinolones
- penicillins
- aminoglycosides
- polymixins
* macrolides
- bacitracin
- cephalosporins
- metronidazole
really very finely proficient at predicting many bacterial cell murders
Bacteriostatic
- erythromycin (macrolides)
- chloramphenicol
- sulfonamides
- tetracycline
- trimethoprim
- clindamycin
ECSTaTiC about bacteriostatic:
Combination Therapy
- synergy: penicillin (cell wall) and aminoglycoside (protein synthesis) -> drug can get into cell to target ribosome
- antagonism: don't combine bactericidal with bacteristatic drug -> instead, use two cidals or two statics!

Effect of Empirical Treatment With Moxifloxacin and Meropenem vs Meropenem on Sepsis-Related Organ Dysfunction in Patients With Severe Sepsis

A Randomized Trial

Frank M. Brunkhorst, MD; Michael Oppert, MD; Gernot Marx, MD; Frank Bloos, MD, PhD; Katrin Ludewig, MD; Christian Putensen, MD; Axel Nierhaus, MD; Ulrich Jaschinski, MD; Andreas Meier-Hellmann, MD; Andreas Weyland, MD; Matthias Gründling, MD; Onnen Moerer, MD; Reimer Riessen, MD; Armin Seibel, MD; Maximilian Ragaller, MD; Markus W. Bücher, MD; Stefan John, MD; Friedhelm Bach, MD; Claudia Spies, MD; Lorenz Reill, MD; Harald Fritz, MD; Michael Kiehntopf, MD ; Evelyn Kuhnt, MSc; Holger Bogatsch, MD; Christoph Engel, MD; Markus Loeffler, MD, PhD; Marin H. Kollef, MD; Konrad Reinhart, MD; Tobias Welte, MD; for the German Study Group Competence Network Sepsis (SepNet)

» Author Affiliations | Article Information

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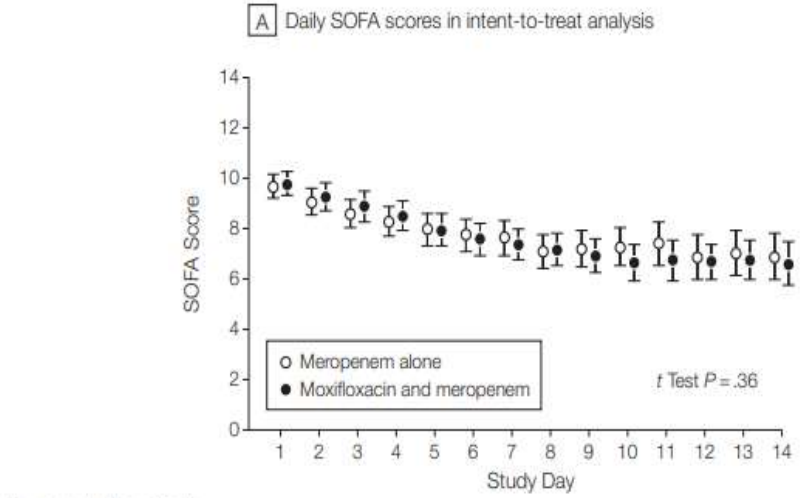
Figure 2. Daily Sequential Organ Failure Assessment (SOFA) Scores



The data markers indicate means and the error bars indicate 95% CIs.

Figure 3. Overall Survival