Correlação entre estrutura bacteriana e mecanismos de ação e resistência aos antimicrobianos e cenário atual da resistência bacteriana

Dr. Svetoslav Dimitrov Todorov

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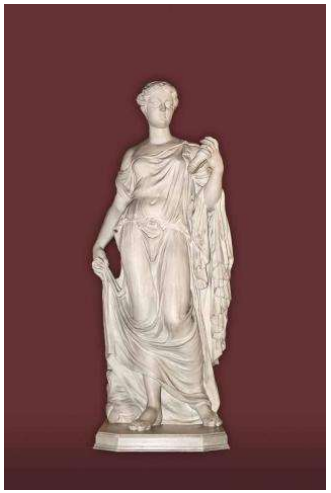
Apollo Healer

Asclepius

Hygieia



Panacea

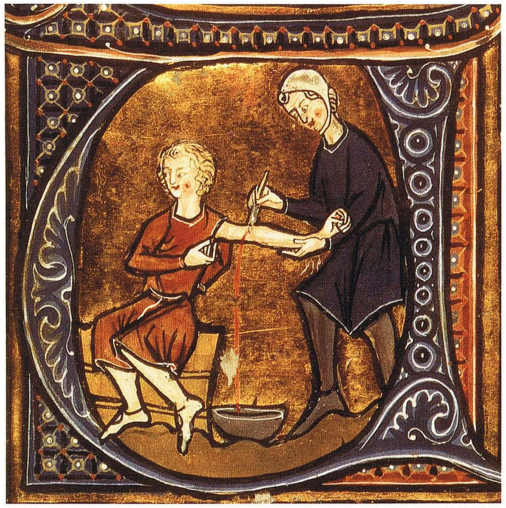
Juro por Apolo Curandeiro, por Asclépio, por Hígia, por Panaceia e por todos os deuses e deusas, tornando-os minhas testemunhas, que cumprirei, de acordo com minha capacidade e julgamento, este juramento e este contrato.

Manter meu professor nesta arte igual a meus próprios pais; torná-lo parceiro no meu sustento; quando ele precisa de dinheiro para compartilhar o meu com ele; considerar sua família como meus próprios irmãos, e ensinar-lhes esta arte, se quiserem aprendê-la, sem honorários ou contratos; transmitir preceitos, instruções orais e todas as outras instruções aos meus próprios filhos, aos filhos do meu professor e aos alunos contratados que fizeram o juramento do Curador, mas a mais ninguém.

Usarei os regimes dietéticos que beneficiarão meus pacientes de acordo com minha maior capacidade e julgamento, e não lhes farei nenhum dano ou injustiça. Nem administrarei veneno a ninguém quando solicitado, nem sugerirei tal procedimento. Da mesma forma, não darei a uma mulher um pessário para provocar um aborto. Mas manterei pura e santa tanto minha vida quanto minha arte. Não usarei a faca, nem mesmo, na verdade, em quem sofre de pedra, mas darei lugar aos que são artesãos nela.

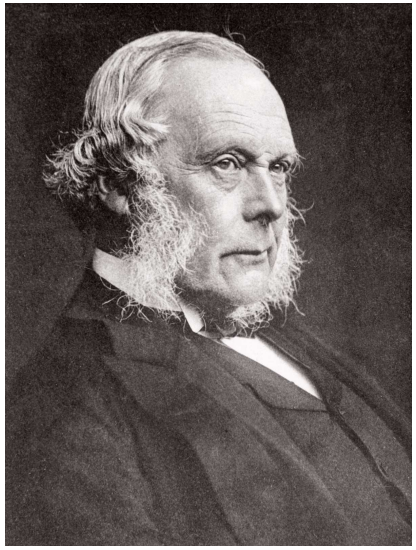
Em todas as casas em que eu entrar, entrarei para ajudar os enfermos e me absterei de todas as ações erradas e danos intencionais, especialmente de abusar dos corpos de homem ou mulher, vinculados ou livres. E tudo o que eu ver ou ouvir no decorrer da minha profissão, bem como fora da minha profissão, nas minhas relações com os homens, se for algo que não deva ser publicado no exterior, nunca divulgarei, considerando tais coisas como segredos sagrados.

Agora, se eu cumprir este juramento e não o quebrar, que eu possa ganhar para sempre reputação entre todos os homens por minha vida e por minha arte; mas se eu quebrá-lo e me renunciar, que o oposto aconteça comigo.



Edward Jenner (1749 – 1823), smallpox vaccine

Luis Pasteur (1822 – 1895), vaccine vs rabies

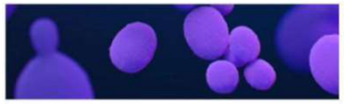


Joseph Lister (1827-1912), antiseptic techniques

CDC's Antibiotic Resistance Threats in the United States (2019 AR Threats Report)

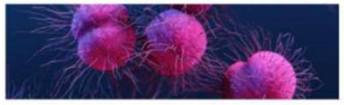
MICROORGANISMS WITH A THREAT LEVEL OF URGENT


CARBAPENEM-RESISTANT
ACINETOBACTER


CANDIDA AURIS


CLOSTRIDIOIDES DIFFICILE


CARBAPENEM-RESISTANT
ENTEROBACTERIACEAE


DRUG-RESISTANT
NEISSERIA GONORRHOEAE

MICROORGANISMS WITH A THREAT LEVEL OF SERIOUS

Serious Threats

These germs are public health threats that require prompt and sustained action:


DRUG-RESISTANT
CAMPYLOBACTER

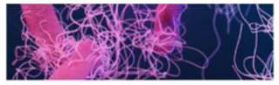

DRUG-RESISTANT
CANDIDA


ESBL-PRODUCING
ENTEROBACTERIACEAE


VANCOMYCIN-RESISTANT
ENTEROCOCCI


MULTIDRUG-RESISTANT
PSEUDOMONAS AERUGINOSA


DRUG-RESISTANT
NONTYPHOIDAL *SALMONELLA*


DRUG-RESISTANT
***SALMONELLA* SEROTYPE TYPHI**


DRUG-RESISTANT
SHIGELLA


METHICILLIN-RESISTANT
STAPHYLOCOCCUS AUREUS


DRUG-RESISTANT
STREPTOCOCCUS PNEUMONIAE


DRUG-RESISTANT
TUBERCULOSIS

CDC's Antibiotic Resistance Threats in the United States (2019 AR Threats Report)

- More than **2.8 million antibiotic-resistant infections** occur in the United States each year, and more than **35,000 people** die as a result.
- **223,900 people** in the United States required hospital care for ***Clastridioides difficile*** and **at least 12,800 people** died in 2017.
- Germs continue to spread and develop new types of resistance, and progress may be undermined by some community-associated infections that are on the rise.
- More action is needed to address antibiotic resistance.
  - Stop relying *only* on new antibiotics
  - Preventing infections in the first place
  - Slowing the development of resistance through better antibiotic use
  - Stopping the spread of resistance

The Top 10 Causes of Death (2008)

Low-income countries	Deaths in millions	% of deaths
Lower respiratory infections	1.05	11.3%
Diarrhoeal diseases	0.76	8.2%
HIV/AIDS	0.72	7.8%
Ischaemic heart disease	0.57	6.1%
Malaria	0.48	5.2%
Stroke and other cerebrovascular disease	0.45	4.9%
Tuberculosis	0.40	4.3%
Prematurity and low birth weight	0.30	3.2%
Birth asphyxia and birth trauma	0.27	2.9%
Neonatal infections	0.24	2.6%

World	Deaths in millions	% of deaths
Ischaemic heart disease	7.25	12.8%
Stroke and other cerebrovascular disease	6.15	10.8%
Lower respiratory infections	3.46	6.1%
Chronic obstructive pulmonary disease	3.28	5.8%
Diarrhoeal diseases	2.46	4.3%
HIV/AIDS	1.78	3.1%
Trachea, bronchus, lung cancers	1.39	2.4%
Tuberculosis	1.34	2.4%
Diabetes mellitus	1.26	2.2%

Infectious Diseases (39.4%)

WHO 2008

The dose makes the poison,

a principle of toxicology, was first expressed by Paracelsus.

It means that a substance can produce the harmful effect associated with its toxic properties only if it reaches a susceptible biological system within the body in a high enough concentration (dose).

Paracelsus

(**Philippus Aureolus Theophrastus Bombastus von Hohenheim**, 11 November or 17 December 1493 – 24 September 1541) was a German-Swiss Renaissance physician, botanist, alchemist, astrologer, and general occultist

Antibiotics

Antibiotics are medications that fight bacterial infections.

- They work by disrupting the processes necessary for bacterial cell growth and proliferation.
- It's important to take antibiotics exactly as prescribed. Failure to do so could make a bacterial infection worse.
- Antibiotics don't treat viruses, but they're sometimes prescribed in viral illnesses to help prevent a "secondary bacterial infection." Secondary infections occur when someone is in a weakened or compromised state due to an existing illness.

History of discovery and development.

Mode of action of antibiotics, MIC, spectrum of activity.

Methods for establish need of appropriate antibiotics.

Antibiotics as prevention? Where is the limit and what are associated risks?

Antibiotics

History of discovery and development

The term **antibiotics** literally means “against life” (Greek origin); in this case, against microbes.

There are many types of antibiotics

antibacterials: Will be given a focus

antivirals:

1. Pandavir, Nigericin and **other proteinase inhibitors**;
2. ε-aminocaproic acid, aprotonin and ambroxol have achieved a reduction of hyaluronic acid-cleavage and virus activation for influenza viruses

antifungals: clotrimazole (Canesten), econazole, miconazole, terbinafine (Lamisil), fluconazole (Diflucan), ketoconazole (Daktarin), nystatin (Nystan), amphotericin.

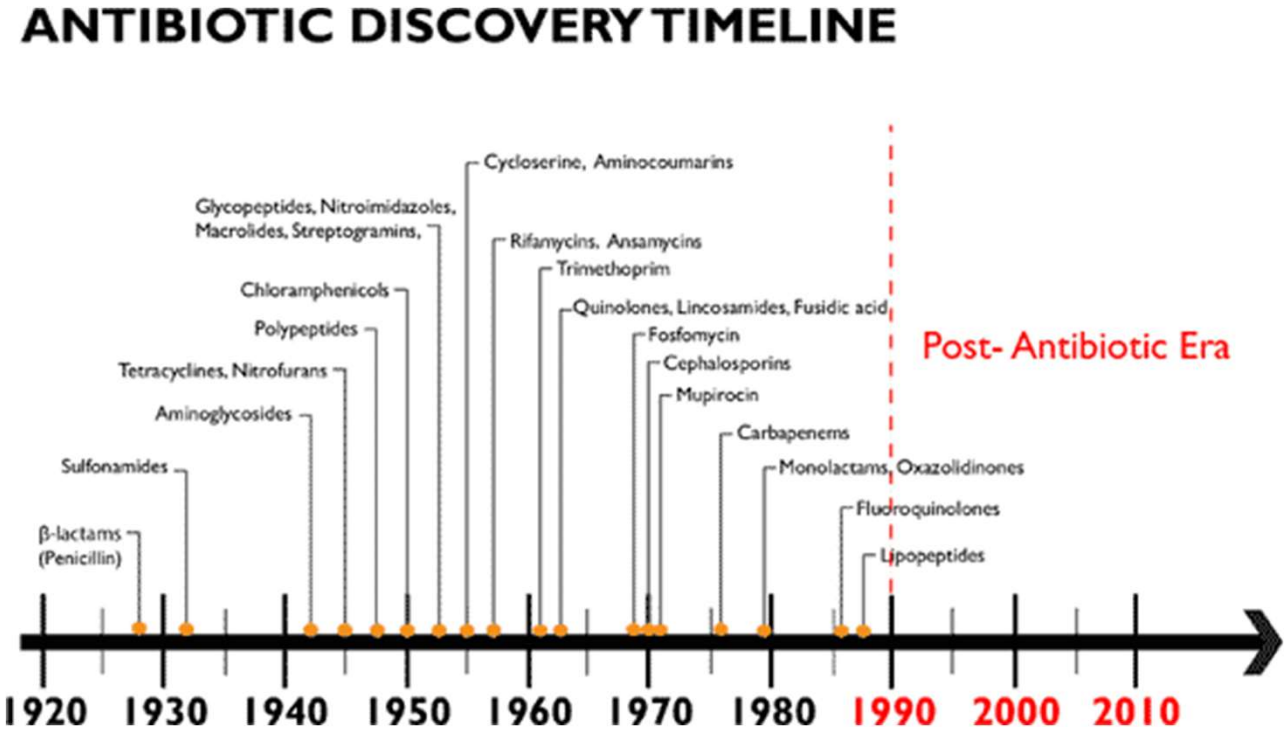
antiparasitics: Mebendazole (for most nematode infections); Pyrantel pamoate (for most nematode infections); Thiabendazole (for roundworm infections); Diethylcarbamazine (for treatment of Lymphatic filariasis); Ivermectin (for prevention of river blindness)

Some drugs are effective against many organisms; these are called **broad-spectrum antibiotics**. Others are effective against just a few organisms and are called **narrow spectrum antibiotics**. The most commonly used antibiotics are **antibacterials**.

Antibiotics

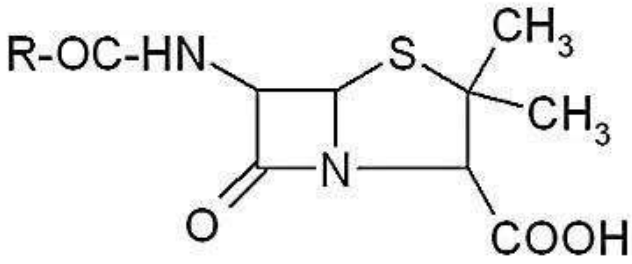
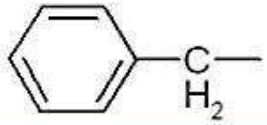
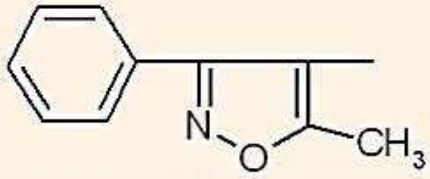
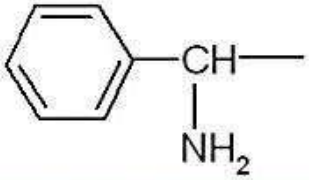
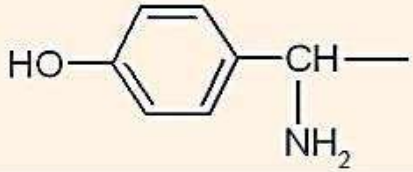
History of discovery and development

Sir Alexander Fleming (1881 –1955) was a Scottish physician and microbiologist, best known for discovering the world's first broadly effective antibiotic substance, which he named penicillin. His discovery in 1928 of what was later named benzylpenicillin (or penicillin G) from the mould *Penicillium rubens* is described as the "single greatest victory ever achieved over disease." For this discovery, he shared the Nobel Prize in Physiology or Medicine in 1945 with Howard Florey and Ernst Boris Chain.



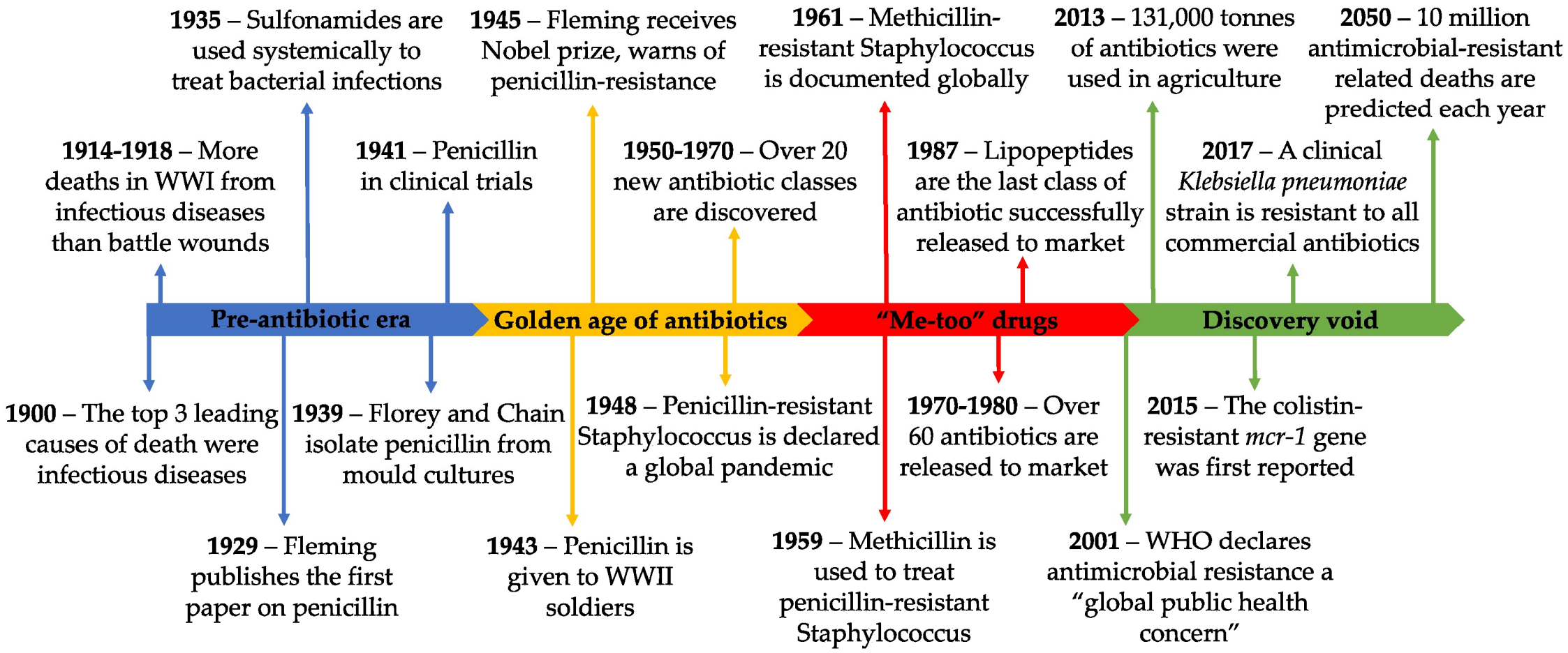
Antibiotics

History of discovery and development

Penicillin derivative	R
	
Benzylpenicillin	
Oxacillin	
Ampicillin	
Amoxicillin	

Antibiotics

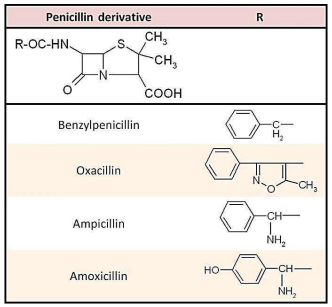
History of discovery and development



Antibiotics

History of discovery and development

Although there are well over 100 antibiotics, the majority come from only a few types of drugs.
These are the main classes of antibiotics:



1. Penicillins such as penicillin and amoxicillin

2. Cephalosporins such as cephalexin (Keflex)

3. Macrolides such as erythromycin (E-Mycin), clarithromycin (Biaxin), and azithromycin (Zithromax)

4. Fluoroquinolones such as ciprofolxacin (Cipro), levofloxacin (Levaquin), and ofloxacin (Floxin)

5. Sulfonamides such as co-trimoxazole (Bactrim) and trimethoprim (Proloprim)

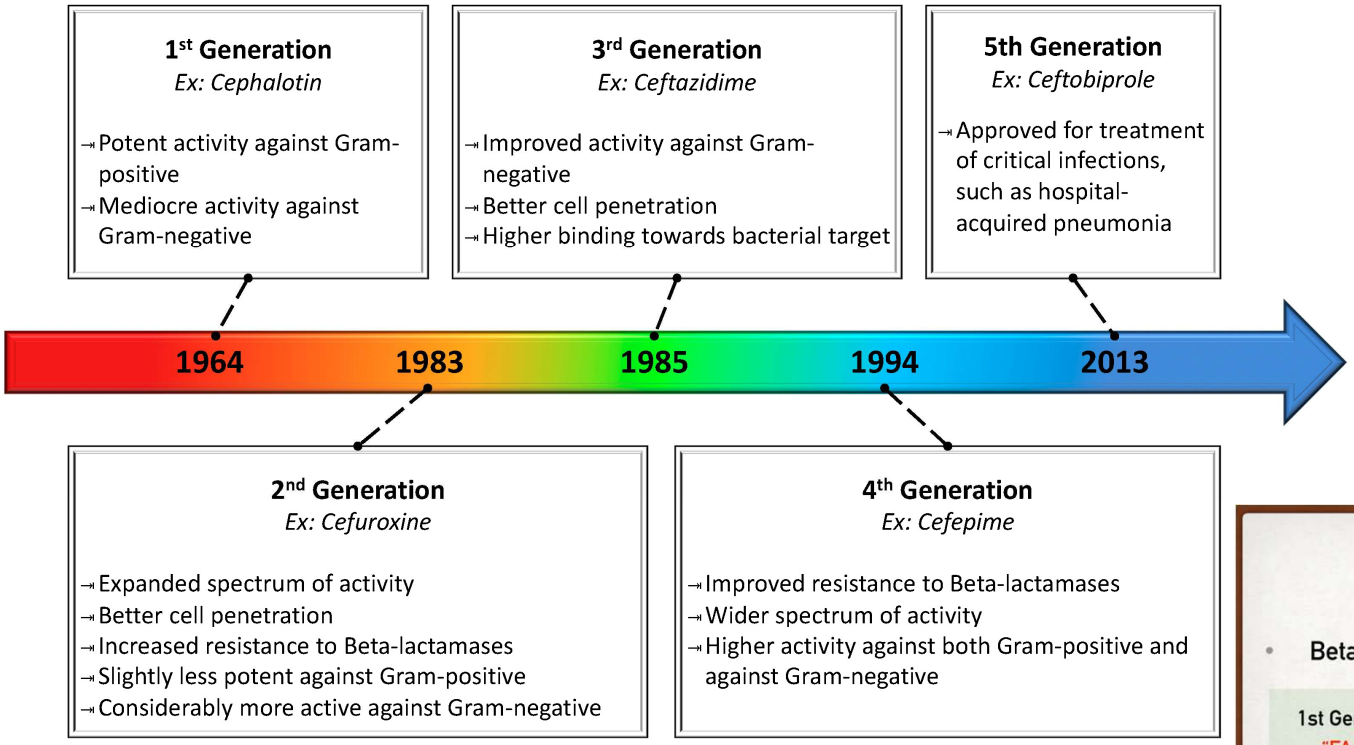
6. Tetracyclines such as tetracycline (Sumycin, Panmycin) and doxycycline (Vibramycin)

7. Aminoglycosides such as gentamicin (Garamycin) and tobramycin (Tobrex)

Most antibiotics have two names, **the trade or brand name**, created by the drug company that manufactures the drug, and a generic name, based on the antibiotic's chemical structure or chemical class. Trade names such as Keflex and Zithromax are capitalized. Generics such as cephalexin and azithromycin are not capitalized.

Antibiotics

History of discovery and development



Cephalosporin Antibiotics
Made Easy - Simple Trick!!

- Beta-lactam: Grouped into 5 generations

1st Generation "FA/PHA"	2nd Generation "Everything Else"	3rd Generation "ONE/TEN/IME"	4th Generation "PI"	5th Generation "ROL"
* CEFAZOLIN CEFADROXIL * CEPHALEXIN CEPHALOTHIN CEPHAPIRIN CEPHRADINE	CEFOXITIN CEFOTETAN CEFMETAZOLE CEFPROZIL CEFACLOR CEFUROXIME	* CEFTRIAXONE CEFTIBUTEN * CEFOTAXIME * CEFTAZIDIME CEFPODOXIME CEFIXIME CEFDINIR MOXALACTAM	* CEFEPIME CEFPIROME	* CEFTAROLINE CEFTOBIPROLE

Antibiotics

Mode of action of antibiotics, MIC, spectrum of activity

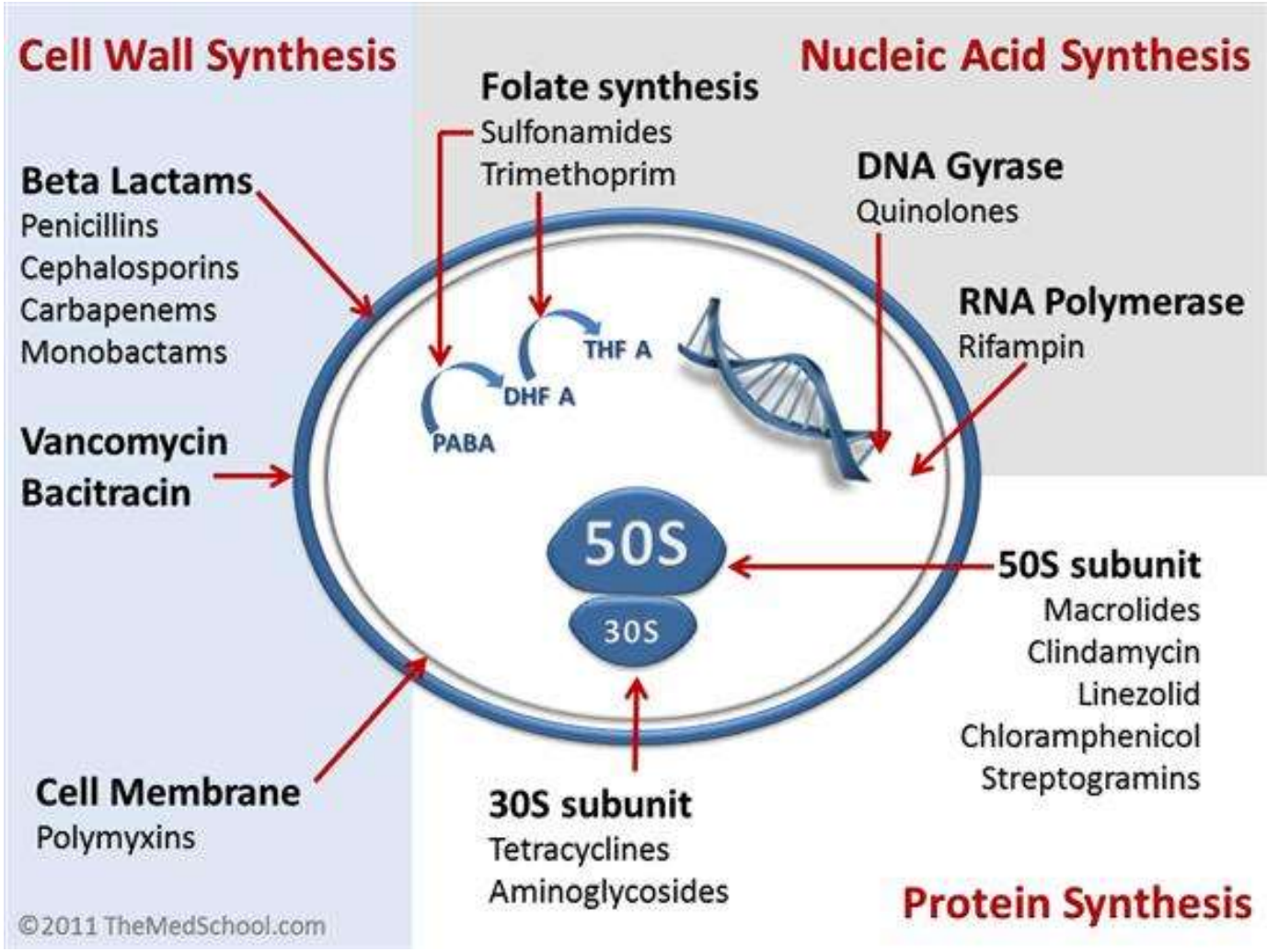
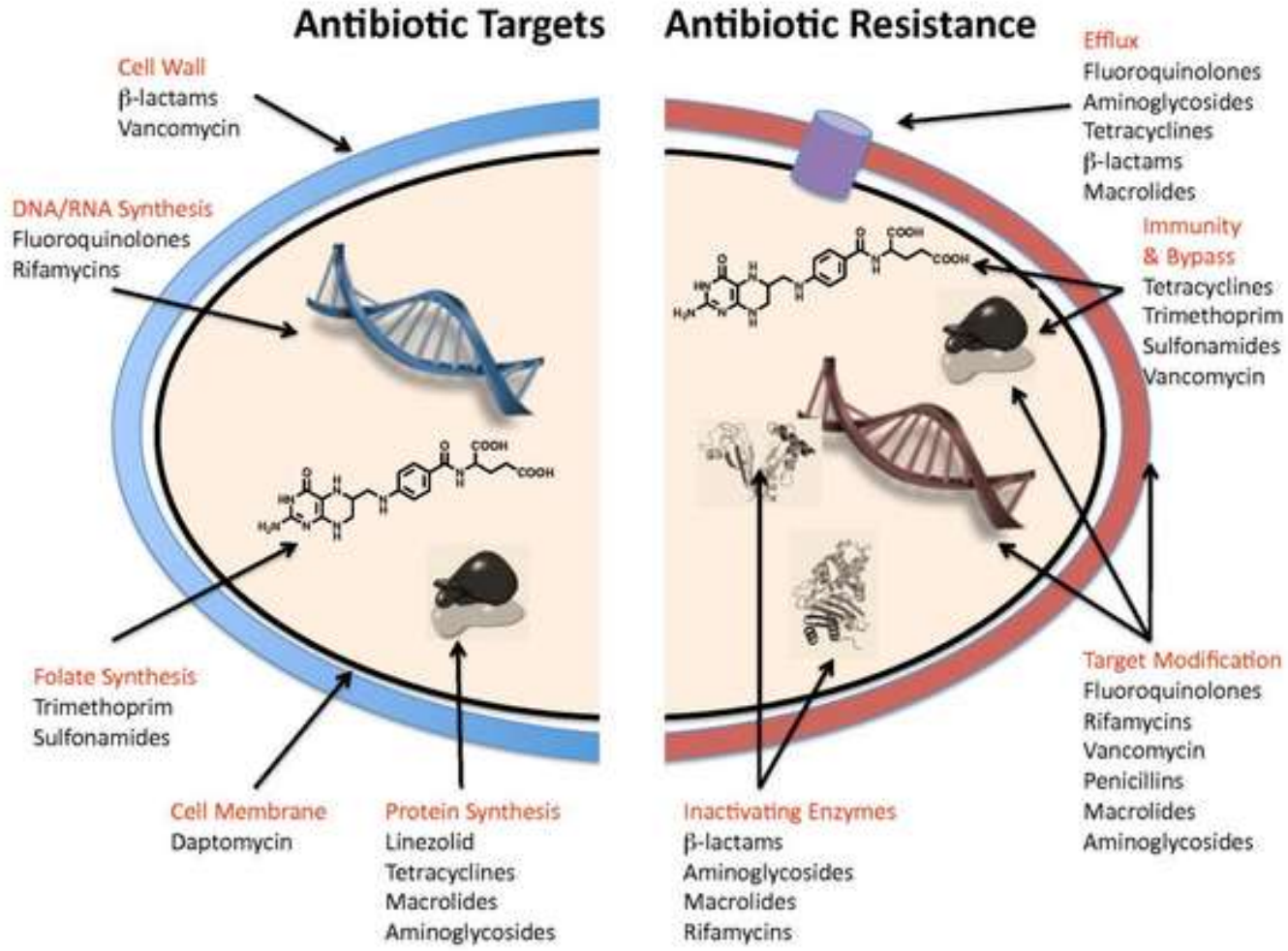


Table 1. Mode of action and resistance mechanisms of antibiotics.

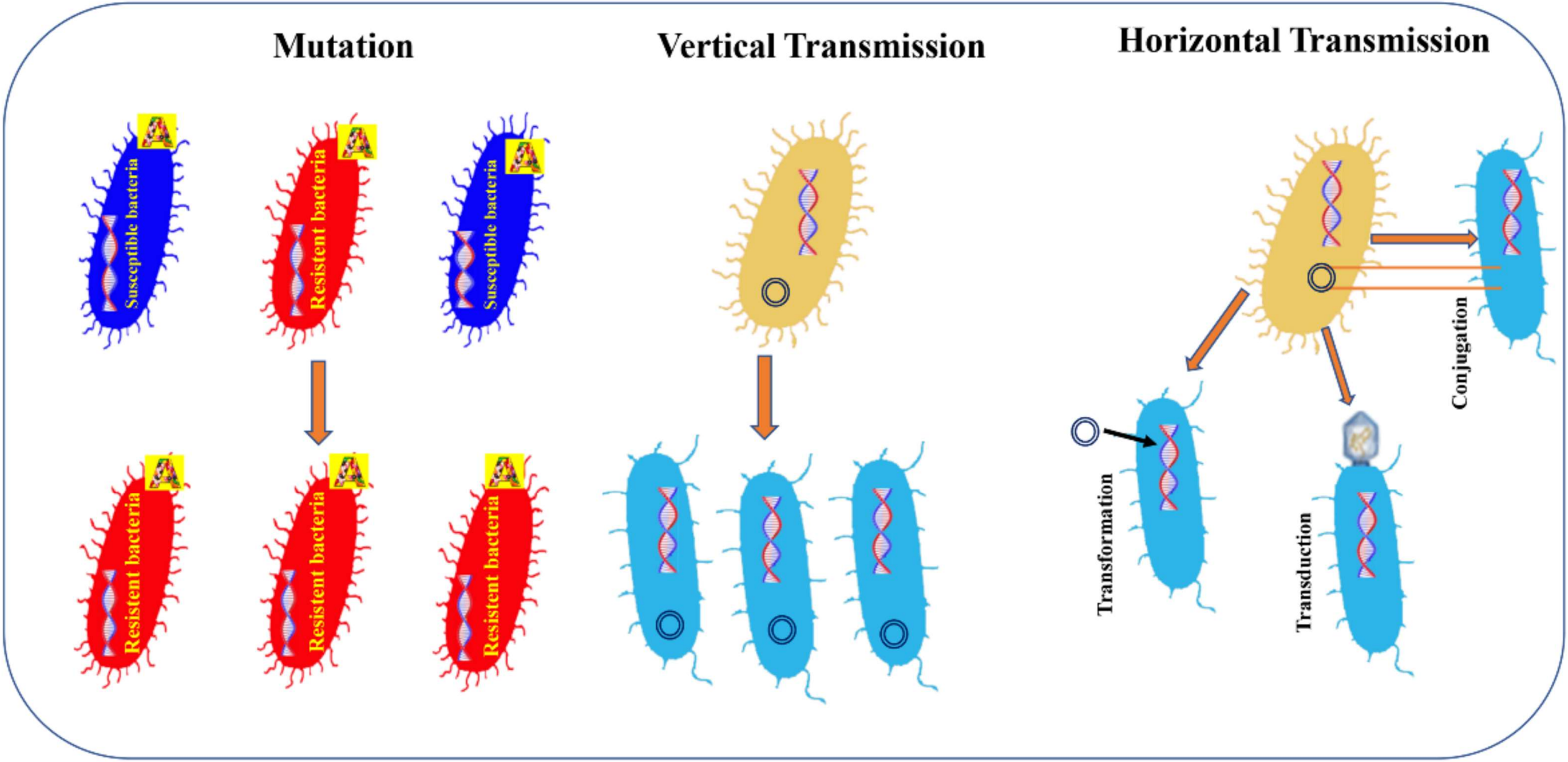
Antimicrobial Groups	Mechanism of Action	Resistance Mechanism
β-Lactams Penicillins	Inhibits cell wall production	Beta-lactamase production Penicillinase
Cephalosporins Carbapenems		Cephalosporinase Carbapenemase
β-Lactamase inhibitors	Block the activity of beta-lactamase enzymes	Extended-spectrum beta-lactamase (ESBL)
Aminoglycosides, Chloramphenicol Macrolides, Tetracyclines	Inhibit ribosome assembly by binding to the bacterial 30S or 50S (inhibit protein synthesis)	Multifactorial (enzymatic modification, target site modification and efflux pumps)
Fluoroquinolone	Inhibit DNA replication	Multifactorial (target-site gene mutations, efflux pumps and modifying enzyme)
Sulfonamides and trimethoprim	Inhibit folic acid metabolism	Horizontal spread of resistance genes, mediated by transposons and plasmids, expressing drug-insensitive variants of the target enzymes.

Antibiotics

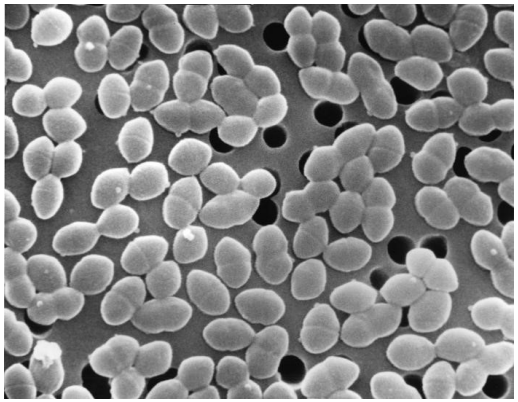
Mode of action of antibiotics, MIC, spectrum of activity



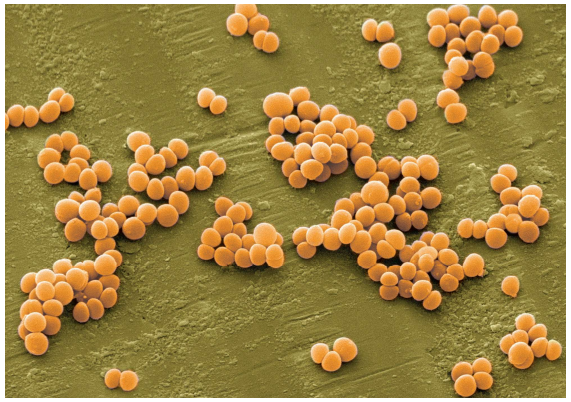
How antibiotic resistance spread



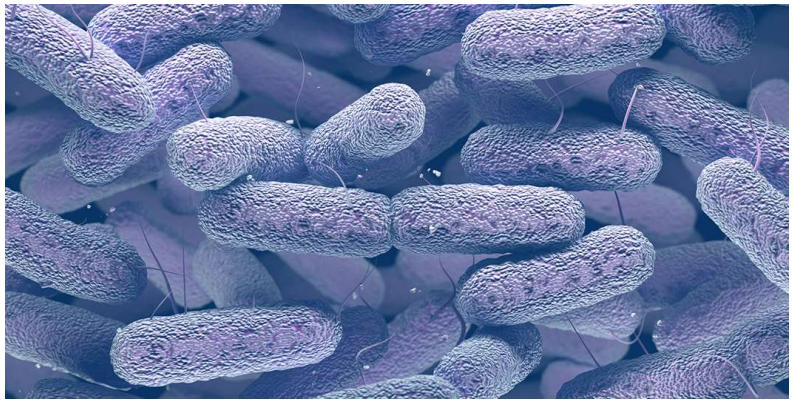
Gram-negative and Gram-positive bacterial species that belongs to the group of pathogens grouped under the acronym "ESKAPE":



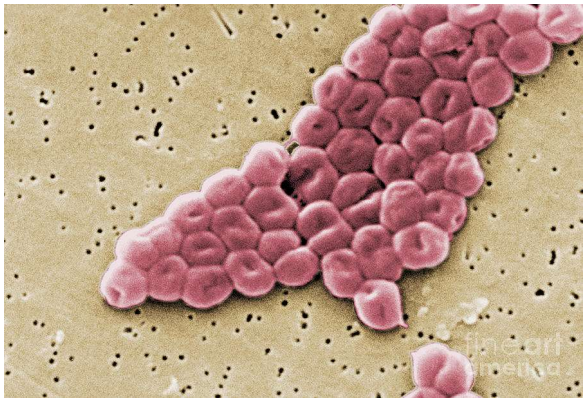
Enterococcus faecium,



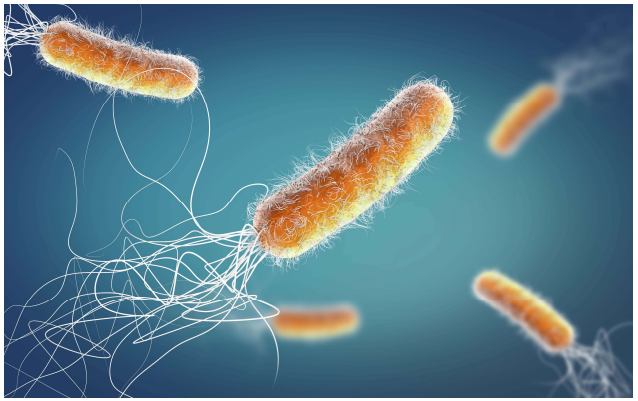
Staphylococcus aureus,



Klebsiella pneumonia,



Acinetobacter baumannii,



Pseudomonas aeruginosa,

and Enterobacter species),

which refers to the ability of these bacteria to escape the effect of bactericidal activity of antibiotics.

Acinetobacter baumannii (Gram-negative) is an opportunistic pathogen that causes hospital-acquired infections worldwide and can develop resistance to antibiotics by different mechanisms such as:

(1) the production of enzymes that **degrade beta-lactam antibiotics**.

The production of all four classes of β-lactamases (A, B, C, and D) **through the incorporation of exogenous DNA** into its genome would underlie the rapid evolution of this strain toward multi-resistance.

Class B β-lactamases are metallo-β-lactamases (MBLs) that have a broad substrate range, being able to inhibit almost all β-lactam antibiotics;

Class C β-lactamases are a group of broadly disseminated enzymes usually resistant to cephamycins (cefoxitin and cefotetan), penicillins and cephalosporins;

Class D β-lactamases that can hydrolyze extended-spectrum cephalosporins and carbapenems.

Moreover, *A. baumannii* has an intrinsic *ampC* cephalosporinase.

(**2**) the expression of efflux pumps.
In ***A. baumannii*** efflux pumps are involved in bacterial resistance to a number of antibiotics belonging to different chemical classes such as aminoglycosides, tetracyclines, erythromycin, chloramphenicol, trimethoprim, fluoroquinolones and different beta-lactams.

Different studies have shown that at least four classes of efflux pumps are associated with *A. baumannii* antimicrobial resistance.

(**3**) the enzymatic modification of aminoglycosides. Enzymatic modification is the most common type of aminoglycoside resistance.
Acetyltransferases, adenylyltransferases and phosphotransferases are three classes of enzymes that play a critical role in the resistance of *A. baumannii* to aminoglycosides. The genes encoding for aminoglycoside modifying enzymes can be **transferred through plasmids and transposons**.

(**4**) The production of **modified porins** that **decreases the permeability of the outer membrane**.
In *A. baumannii* the reduced expression of porins, proteins that allow the transport of molecules across the outer membrane, is associated with carbapenem resistance. Moreover, *A. baumannii* may acquire resistance to colistin, a polypeptide antibacterial agent that targets Lipopolisacharides (LPS), as a result of mutation of the genes involved in LPS biosynthesis.

(**5**) The modification of the antibiotic target.
In *A. baumannii* this mechanism of resistance is **mediated by overexpression of penicillin-binding proteins** that results in imipenem resistance or by **mutations of DNA gyrase** that prompts quinolone and tetracycline resistance.

Enterococci

- Enterococci are Gram-positive cocci, facultative anaerobes gastrointestinal commensals capable of persisting in a range of stressful and hostile environments.
- Although more than **50 different species** of enterococci have been described, only two species in human cause the majority of enterococcal infections: *E. faecalis* and *E. faecium.*
- *E. faecalis* is the most pathogenic species although *E. faecium* is more resistant to many antimicrobial agents and especially in immunocompromised hosts the latter can cause severe morbidity and mortality.
- In general, these microorganisms are typically harmless in healthy individuals while in immunocompromised patients are involved in hospital-acquired infections such as catheter-associated urinary tract infections, endocarditis and bacteremia.

Classification (based on phenotypic characters in clinical isolates)

Group 1	Group 3	Group 5
E.*avium* E.*gilvus* E.*malodartus* E.*pallens* E. *pseudoavium* E.*raffinosus* E.*sacchrolyticus*	E.*dispar* E.*durans* E.*hirae* E.*ratti* E.*villorum*	E.*columbae* E.*canis* E.*moraviensis*
Group 2	Group 4	
E.*fecalis* E.*fecium* E.*casseliflavus* E.*gallinarum* E.*mundtii* E.*hemoperoxidus* Enterococcus sp	E.*asini* E.*cecorum* E.*sulfures* E.*phoeniculicola* Enterococcus sp	

Koneman textbook of diagnostic microbiology

Enterococcus

- Gram positive cocci, non motile, non sporing
- Catalase Negative
- Previously classified as group D streptococci
- Natural inhabitants of GIT
- Distinct features
  - Ability to grow at 10°C and 45°C
  - Ability to grow in 6.5%NaCl
  - Ability to grow at 9.6pH
  - Ability to hydrolyze esculin in 40% bile
  - Ability to process pyrrolidonyl arylamidase (PYR)

Enterococci

- Enterococci are becoming increasingly resistant to antimicrobial agents, and this is mainly due to:

(1) the large use in hospitals of broad-spectrum antibiotics (penicillins and cephalosporins) promotes intestinal colonization of *E. faecium* by greatly increasing the normal Gram-negative intestinal microbiota (mutated and the overexpression of β-lactamase enzymes lead to high levels of resistance to β-lactam antibiotics);

(2) the intrinsic resistance of enterococci to several commonly used antibiotics;

(3) the capacity of these strains to acquire and disseminate determinants of antibiotic resistance.

Enterococci

- In *E. faecium,* at least three different pathways involved in cephalosporins resistance have been identified.
- In the 1970s, vancomycin was introduced to contrast the diffusion of enterococci resistant to third-generation cephalosporins.
- In the 1990s due to the heavy use of vancomycin, vancomycin-resistant enterococci (VRE) emerged as the second most common nosocomial pathogen.
- *E. faecium* can acquire genes through mobile genetic elements such as plasmids and transposons (i.e vancomycin resistance can be transferred by the *vanA* gene cluster on the transposon Tn*1546*).
- Vancomycin acts by targeting the D-alanyl-D-alanine terminus of peptidoglycan inhibiting cell wall synthesis.
- Vancomycin-resistance is mediated by several *van* gene clusters.
- *Van A* gene cluster is the most common type and was located on transposon on a 10,581-bp transposon (Tn*1546*) of *E. faecium*.

Enterococci

E. faecium is considered a MDR bacteria since it is intrinsically resistant to aminoglycoside like tobramycin, kanamycin, gentamicin being capable of producing aminoglycoside-modifying enzymes (AMEs) including aminoglycoside nucleotidyltransferases (ANTs) aminoglycoside acetyltransferases (AACs) and aminoglycoside phosphotransferases (APHs).

Moreover, mutations within the *rpsL* gene, which encodes the ribosomal protein S12, can result in high level resistance to streptomycin.

Moreover, high-level fluoroquinolones resistance in *E. faecium* is most frequently linked with point mutations in *gyrA* and *parC* genes that encode subunits A of DNA gyrase and topoisomerase IV or with efflux transporter *NorA* that pump out these drugs.

Enterobacter spp.
Enterobacter species are motile aerobic Gram-negative bacilli belonging to Enterobacteriaceae family.

The *Enterobacter cloacae complex* (ECC) includes different pathogens, capable of producing a wide variety of infections, the most frequent of which are *Enterobacter cloacae* and *Enterobacter aerogenes*.

In 2019, *Enterobacter aerogenes* was re-classified as *Klebsiella aerogenes* owing to its higher genotypic similarity with the genus *Klebsiella.*

Enterobacter species are non-fastidious Gram-negative rods that are sometimes encapsulated.

They can cause opportunistic infections in immunocompromised, usually hospitalized, patients having acquired a wide range of antibiotic resistance mechanisms.

Many *Enterobacter* strains **produce variety of enzymes (**ESBLs and carbapenemases, including VIM, OXA, metallo-β-lactamase-1, and KPC).

In this bacterial group, an important role in the development of antibiotic resistance is represented by the **permanent depression of ampC β-lactamases**, which can be expressed at high levels.

Enterobacter spp.

These MDR strains are resistant to almost all available antimicrobial drugs, **except tigecycline and colistin**.

Moreover, a recent report indicates that pan-drug-resistant *K. aerogenes* has also emerged, displaying resistance to the last-resort antibiotic colistin.

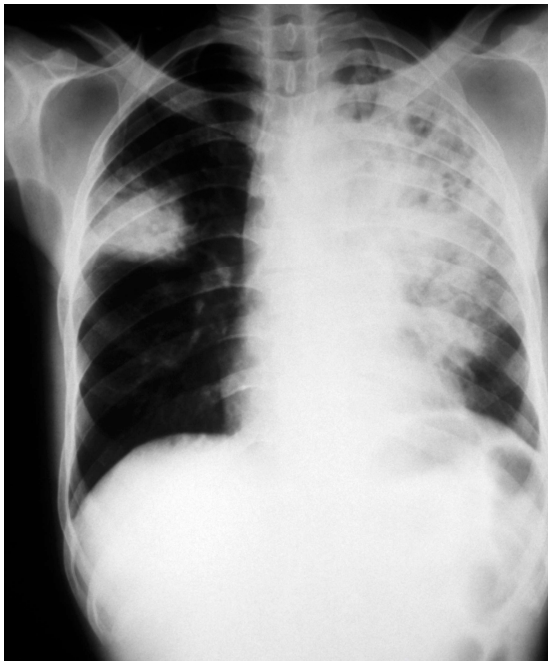
To further complicate the treatment of bacterial infections, *K. aerogenes* is capable of harboring subpopulations of colistin-resistant bacteria which are undetectable using current diagnostic testing strategies.

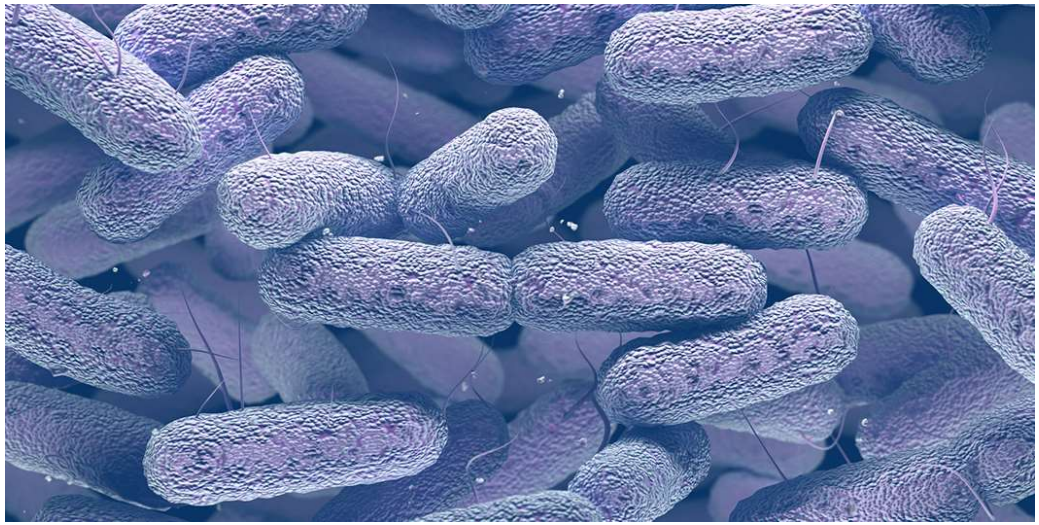
Klebsiella pneumonia

Klebsiella pneumoniae is a member of the family Enterobacterales, non-fastidious, commonly encapsulated Gram-negative bacillus.

K. pneumoniae can cause different types of nosocomial and community acquired infections, including urinary tract infections, pneumonia, liver abscess, surgical site infections and bloodstream infections especially in immunocompromised patients.

Since the bacteria **doesn't spread through the air**, to get a *Klebsiella* infection person-to-person contact is required.





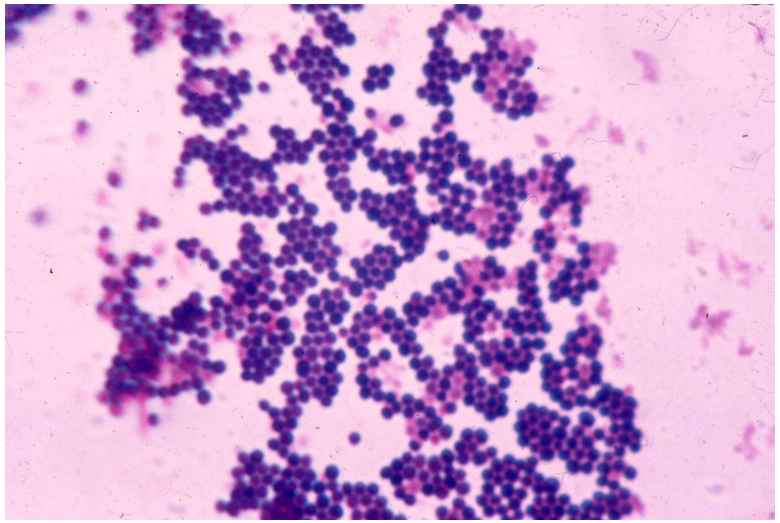
Klebsiella pneumonia

Klebsiella has become highly resistant to antibiotics by the widespread acquisition of genes encoding enzymes, such as ESBLs (Extended spectrum beta-lactamases) and carbapenemases.

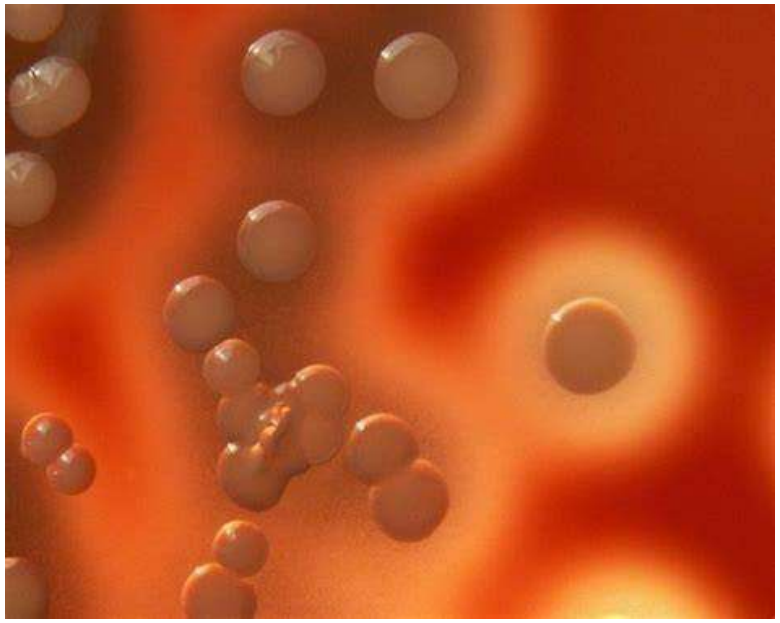
Carbapenem-resistant *K. pneumoniae* strains are the most clinically prominent carbapenem-resistant Enterobacteriaceae (CRE). **Carbapenems often are the last line of defense** against Gram-negative persistent infections, therefore the increasing prevalence of carbapenemase-producing *K. pneumoniae* (*KPC*) strains harboring the carbapenemase encoding *blaKPC-3* gene, is a major threat to public health.

Staphylococcus aureus

Staphylococcus. aureus is a **Gram-positive**, facultative anaerobe, catalase- and coagulase-positive coccus that tends to form irregular grape-like clusters.
S. aureus causes infections ranging from mild to life-threatening such as skin and soft tissue infections, bacterial endocarditis, pleuropulmonary and device-related infections.



This microorganism is associated with its great ability to develop resistance against old and new antibiotics. Only three years after the discovery of penicillin appeared penicillin-resistant *S. aureus* carrying a plasmid-encoded **β-lactamases** capable of hydrolyzing the β-lactam ring of penicillin.

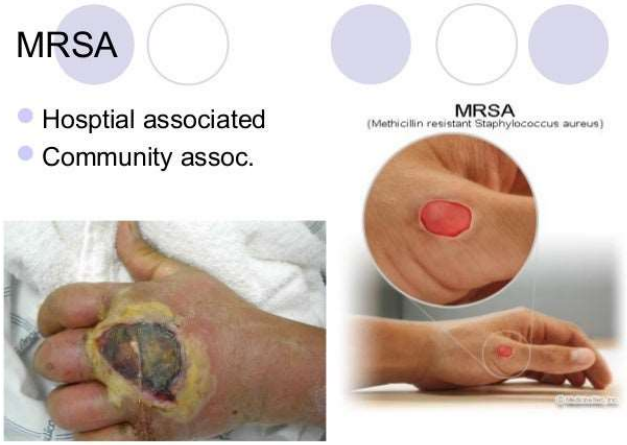


This gene is carried on transposable elements that have moved into plasmids which often also carried genes resistant to other antibiotics such as **erythromycin and gentamicin**.

Staphylococcus aureus

In 1959, methicillin, a semi-synthetic penicillin, was introduced to combat infections caused by penicillin-resistant bacteria; however, as early as **1961 the first methicillin-resistant *S. aureus* strain was identified**. Methicillin and other β-lactam antibiotics inhibit the growth of *S. aureus* by binding to the penicillin-binding proteins (PBPs).

S. aureus became resistant to methicillin (**MRSA**) by acquiring, via horizontal gene transfer, the genes *mecA* and *mecC* which inactivate methicillin by the synthesis of an alternative PBP (penicillin binding proteins), designated PBP2a, that has very low affinity for almost all β-lactam antibiotics.



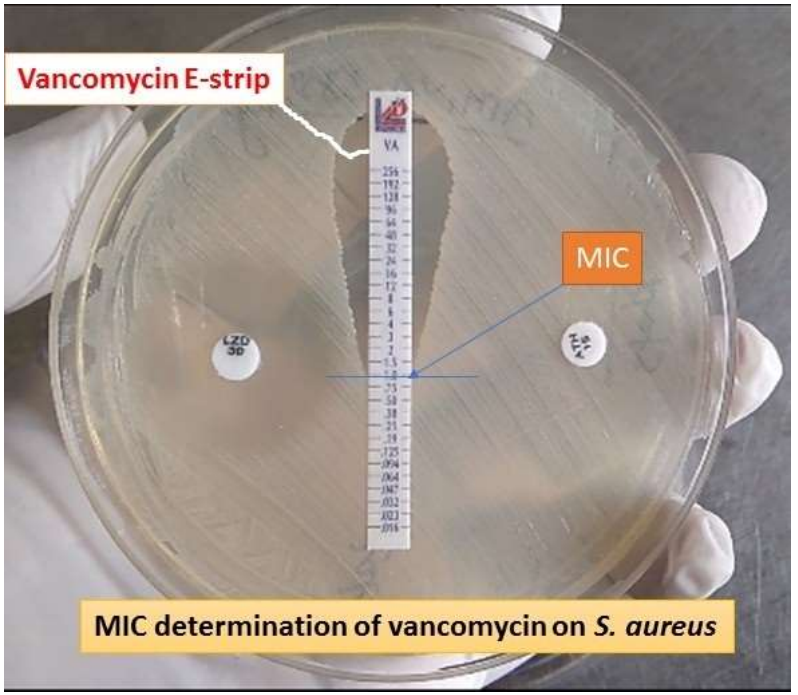
Staphylococcus aureus

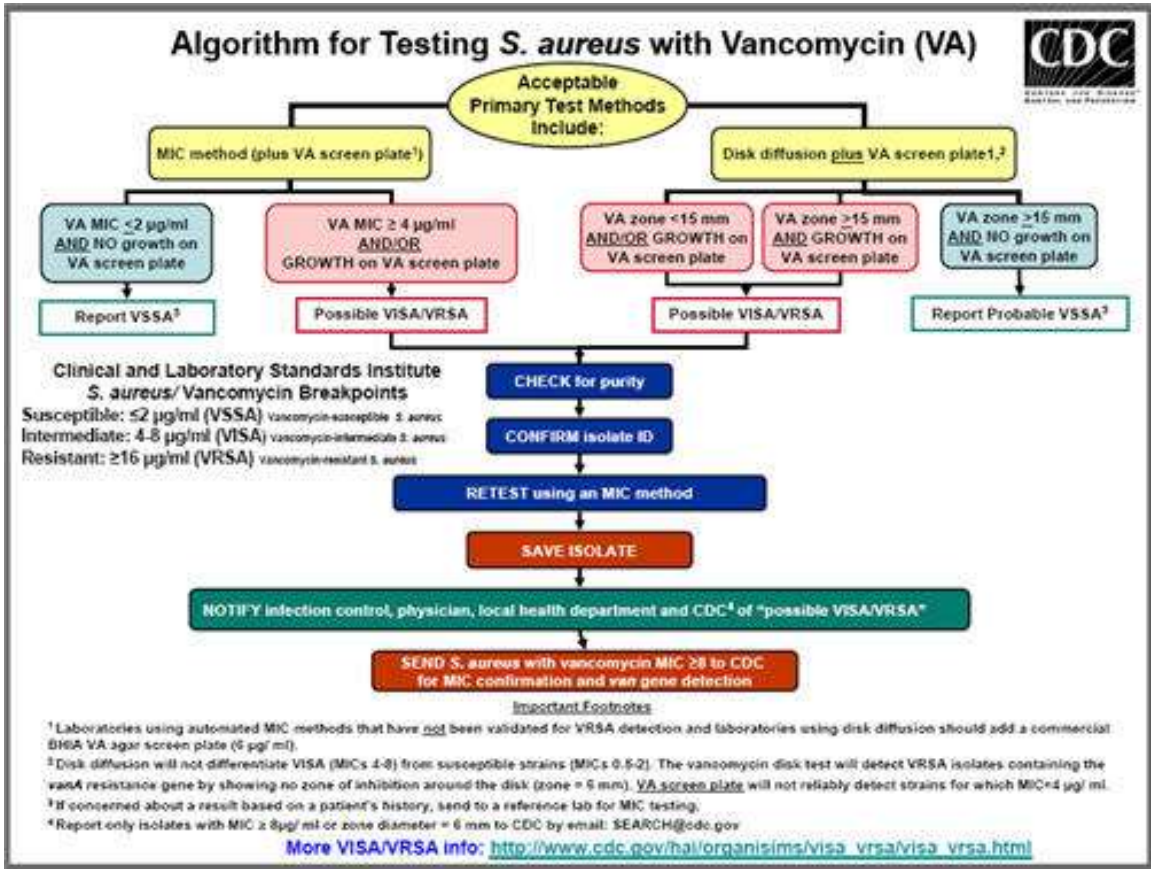
Vancomycin has been considered a last-resort antibiotic against severe MRSA and other resistant Gram-positive infections. By the late 1980s vancomycin resistance first appeared in enterococci (VRE) and in recent years in *S. aureus* (VRSA).

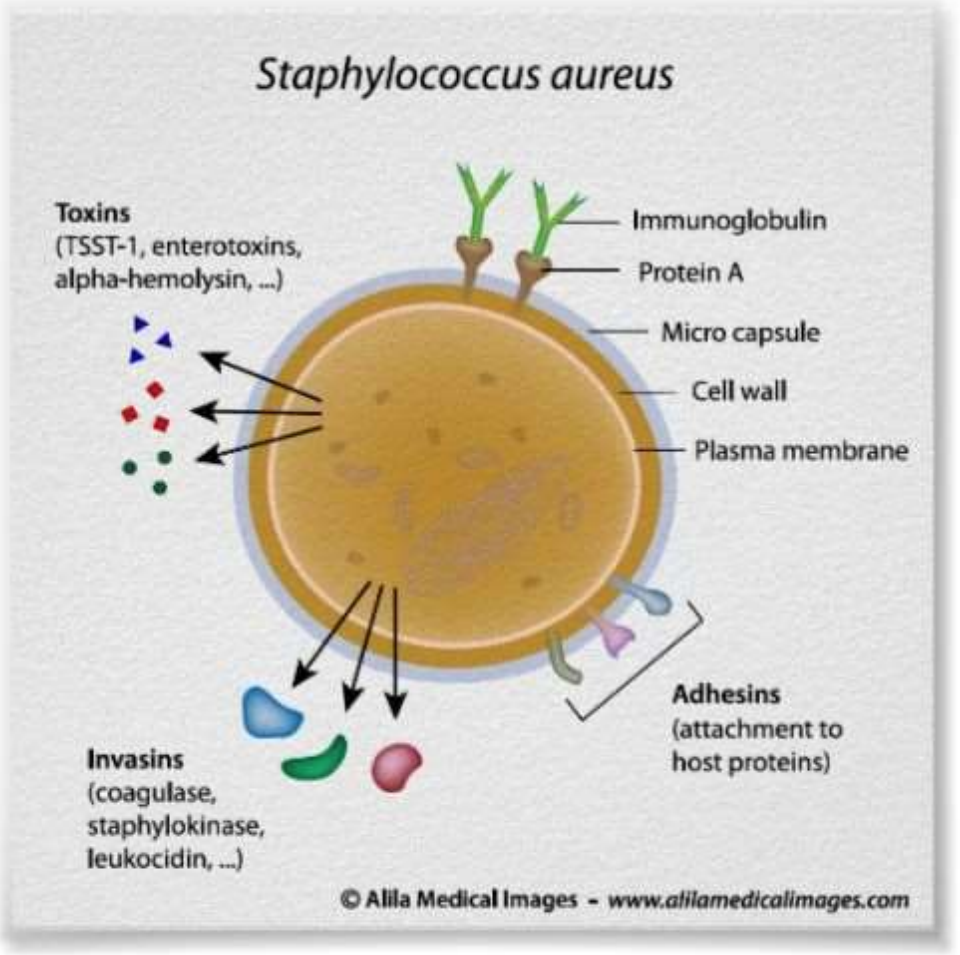
The resistance mechanism of VRSA is mediated by the *VanA* operon carried on the mobile genetic element Tn1546 acquired from vancomycin-resistant *Enterococcus*.

In 1997, reported for the first time was the first clinical isolate of **vancomycin-intermediate** *S. aureus* (V**I**SA) which is not inhibited *in vitro* at vancomycin concentration below 4–8 µg/mL.

Vancomycin-resistant *S. aureus* (V**R**SA) is inhibited only at concentrations of 16 µg/mL or more. VISA and VRSA have emerged from MRSA; however, VRSA does not progress from VISA because both have different resistance mechanisms.




Algorithm for Testing S. aureus with Vancomycin (VA)
CDC
Acceptable Primary Test Methods Include:
MIC method (plus VA screen plate[1])
Disk diffusion plus VA screen plate[1,2]
VA MIC ≤2 µg/ml AND NO growth on VA screen plate
VA MIC ≥ 4 µg/ml AND/OR GROWTH on VA screen plate
VA zone <15 mm AND/OR GROWTH on VA screen plate
VA zone ≥15 mm AND GROWTH on VA screen plate
VA zone ≥15 mm AND NO growth on VA screen plate
Report VSSA[3]
Possible VISA/VRSA
Possible VISA/VRSA
Report Probable VSSA[3]
Clinical and Laboratory Standards Institute
S. aureus/ Vancomycin Breakpoints
Susceptible: ≤2 µg/ml (VSSA) Vancomycin-susceptible S. aureus
Intermediate: 4-8 µg/ml (VISA) vancomycin-intermediate S. aureus
Resistant: ≥16 µg/ml (VRSA) Vancomycin-resistant S. aureus
CHECK for purity
CONFIRM isolate ID
RETEST using an MIC method
SAVE ISOLATE
NOTIFY infection control, physician, local health department and CDC[4] of "possible VISA/VRSA"
SEND S. aureus with vancomycin MIC ≥8 to CDC for MIC confirmation and van gene detection
Important Footnotes
[1] Laboratories using automated MIC methods that have not been validated for VRSA detection and laboratories using disk diffusion should add a commercial BHIA VA agar screen plate (6 µg/ ml).
[2] Disk diffusion will not differentiate VISA (MICs 4-8) from susceptible strains (MICs 0.5-2). The vancomycin disk test will detect VRSA isolates containing the vanA resistance gene by showing no zone of inhibition around the disk (zone = 6 mm). VA screen plate will not reliably detect strains for which MIC=4 µg/ ml.
[3] If concerned about a result based on a patient's history, send to a reference lab for MIC testing.
[4] Report only isolates with MIC ≥ 8µg/ ml or zone diameter = 6 mm to CDC by email: SEARCH@cdc.gov
More VISA/VRSA info: http://www.cdc.gov/hai/organisms/visa_vrsa/visa_vrsa.html


Staphylococcus aureus
Toxins
(TSST-1, enterotoxins, alpha-hemolysin, ...)
Immunoglobulin
Protein A
Micro capsule
Cell wall
Plasma membrane
Adhesins
(attachment to host proteins)
Invasins
(coagulase, staphylokinase, leukocidin, ...)
© Alila Medical Images - www.alilamedicalimages.com

Staphylococcus aureus

According to the World Health Organization (WHO), the pathogenicity and antibiotic resistance pattern of *S. aureus* poses a severe threat to human health worldwide. MRSA, VISA and VRSA are well-recognized as a major pathogen of **hospital acquired infections** and are considered to be high priority agents since, without effective containment and therapeutic solutions, they could cause serious infections that are impossible to control worldwide.

MRSA infections are usually difficult to treat, and thus several classes of antibiotics have been used over the past decade to treat these infections that have contributed to the emergence and spread of MDR strains. In MRSA the resistance to a single antimicrobial agent as well as to different classes of antibiotics occurs through the activation of several different mechanisms such as

(1) mutation in target genes (e.g. the resistance towards fluoroquinolones is due to mutation in *gyrA* and *gyrB* genes of topoisomerse II);
(2) target alterations;
(3) overexpression of efflux pump (NorA pump).

Staphylococcus aureus

Daptomycin, a cyclic peptide antibiotic with a fatty acid side chain that bind to the bacterial cytoplasmic membrane in the presence of calcium ions, is an important alternative to vancomycin for the treatment of patients with infections caused by MRSA. However, although daptomycin resistance in *S. aureus* is uncommon, resistance to this drug during therapy is increasing due to mutations of different proteins that result in a reduced drug binding to its target site.

In recent years, due to the increasing rate of MRSA infection, there is a renewed interest in the use of macrolide-lincosamide-streptogramin (MLS) agents to treat such infections.

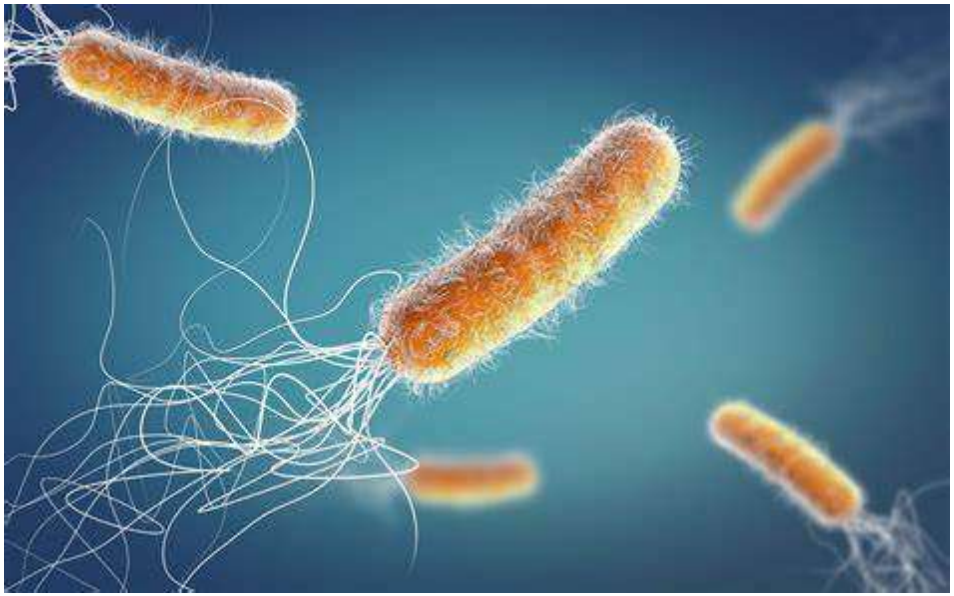
Other alternatives are bacteriocins and/or combinations of antibiotics with other antimicrobials.

Pseudomonas aeruginosa

P. aeruginosa is an aerobic Gram-negative bacterium commonly found in the environment and one of the most common pathogens responsible for a variety of acute and chronic **nosocomial infections** including severe **respiratory infections** in patients with compromised host defenses.

P. aeruginosa is the **third most common** Gram-negative bacteria causing nosocomial bloodstream infections.

P. aeruginosa has shown intrinsic resistance to many antibiotics that is due to different mechanisms of resistance that are both intrinsic and acquired from other microorganisms.



Pseudomonas aeruginosa
The main mechanisms of resistance are:

1. Over-expression of efflux pumps, decreasing outer membrane permeability and acquisition or mutation of resistance genes that encode for proteins that control the passive diffusion of antibiotics across the outer membrane. **Ceftazidime and cefepime** belonging, respectively, to the third and fourth generation of cephalosporins, are broad-spectrum antimicrobials that have *P. aeruginosa* coverage.

2. Like *A. baumanni*, also in *P. aeruginosa* **all four major classes of β-lactamases (A, B, C and D) have been identified**. Endogenous β-lactamase such as AmpC β-lactamase can be induced by several β-lactams such as benzylpenicillin and imipenem.

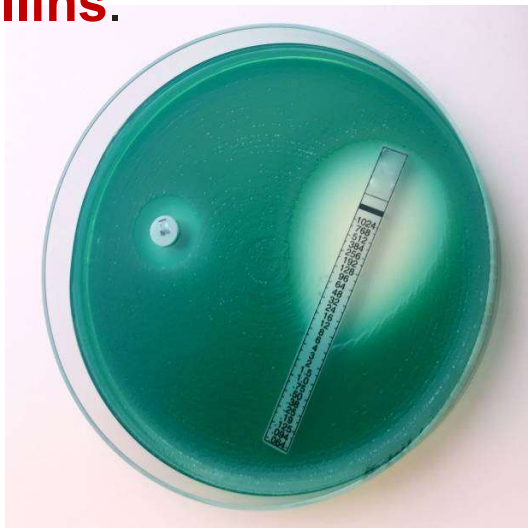
3. Moreover, *P. aeruginosa* can acquire resistance through a gene mutation which leads to overexpression of AmpC β-lactamases.

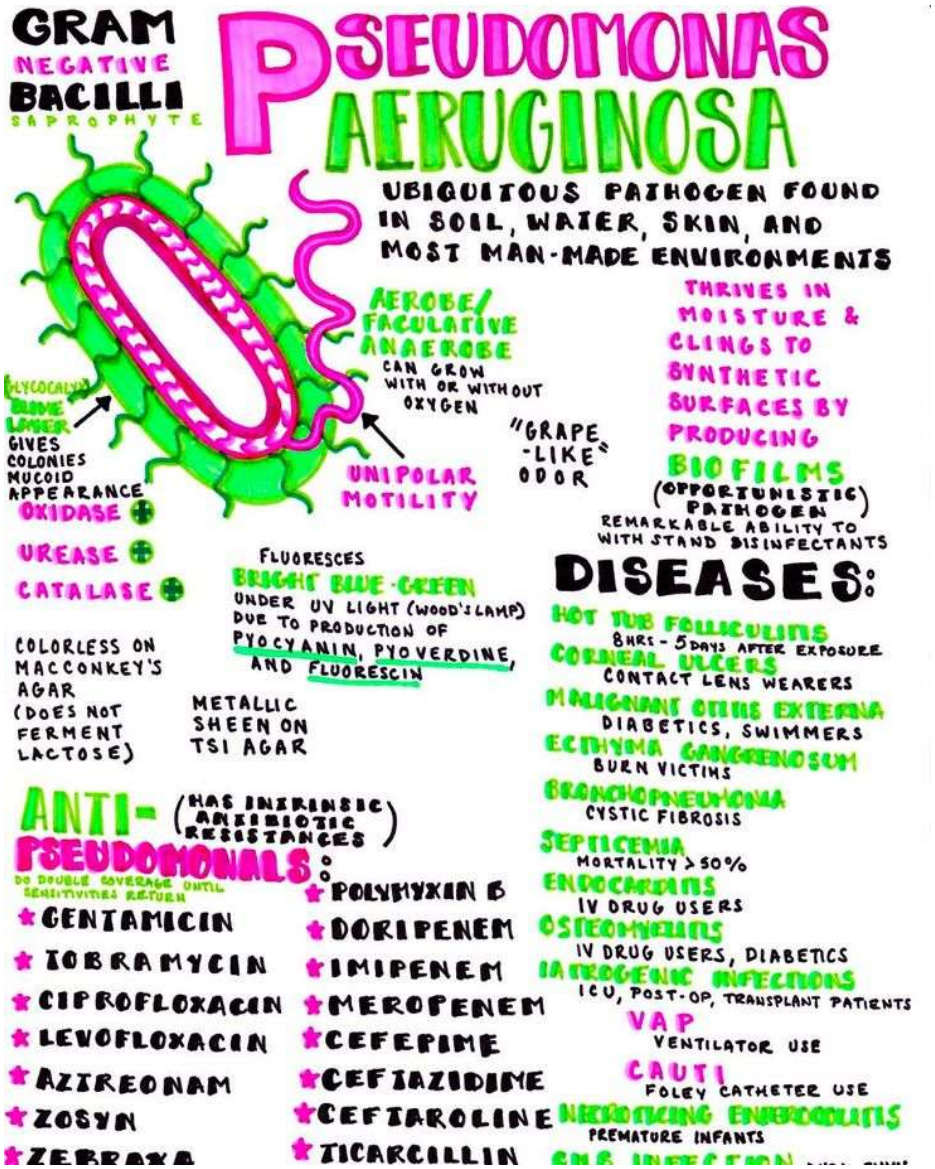
4. *Pseudomonas* **resistance to aminoglycosides is mediated** by transferable aminoglycoside modifying enzymes (AMEs) that decrease the binding affinity to their target in the bacterial cell.

Pseudomonas aeruginosa

The treatment of MDR *P. aeruginosa* involves **colistin** in combination with an anti-pseudomonas agent like **imipenem, piperacillin, aztreonam, ceftazidime or ciprofloxacin**.

Drug resistance in *P. aeruginosa* have been successfully treated with **fosfomycin** in combination with **aminoglycosides, cephalosporins and penicillins**.



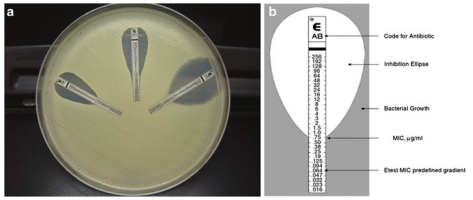


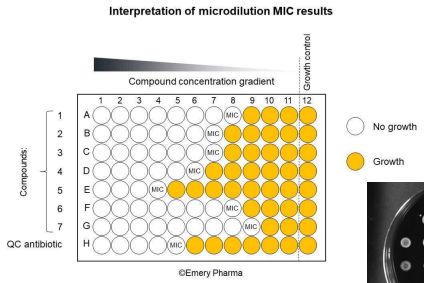
Antibiotics Mode of action of antibiotics, MIC, spectrum of activity

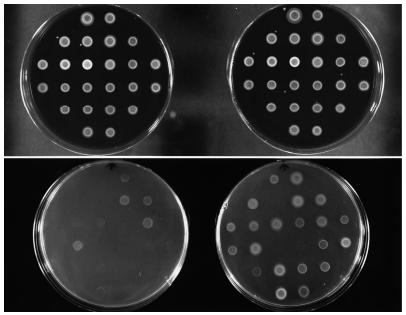
Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation; and **Minimum bactericidal concentrations** (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.

J Antimicrob Chemother 2001, 48, 5-16. doi: 10.1093/jac/48.suppl_1.5.

Minimum inhibitory concentration (MIC) can be determined **by culturing microorganisms in liquid media or on plates of solid growth medium**.







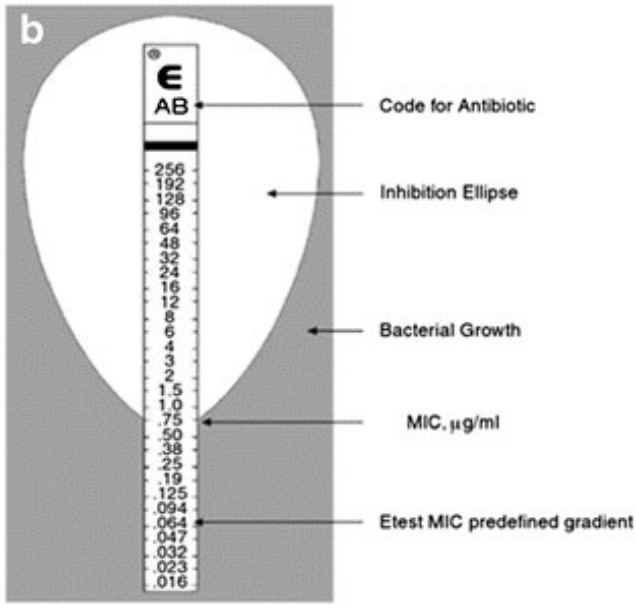
The Kirby-Bauer disk diffusion method is one of the most widely practiced antimicrobial susceptibility tests (AST). It is affected by many factors among which are the media used. **Mueller-Hinton agar (MHA)** is the standard medium recommended in guidelines.

Antibiotic susceptibility is defined as the sensitivity of a bacteria to antibiotics and can be measured using a **broth dilution test or an Epsilometer test, also called an E-test**.

Antibiotics

Mode of action of antibiotics, MIC, spectrum of activity

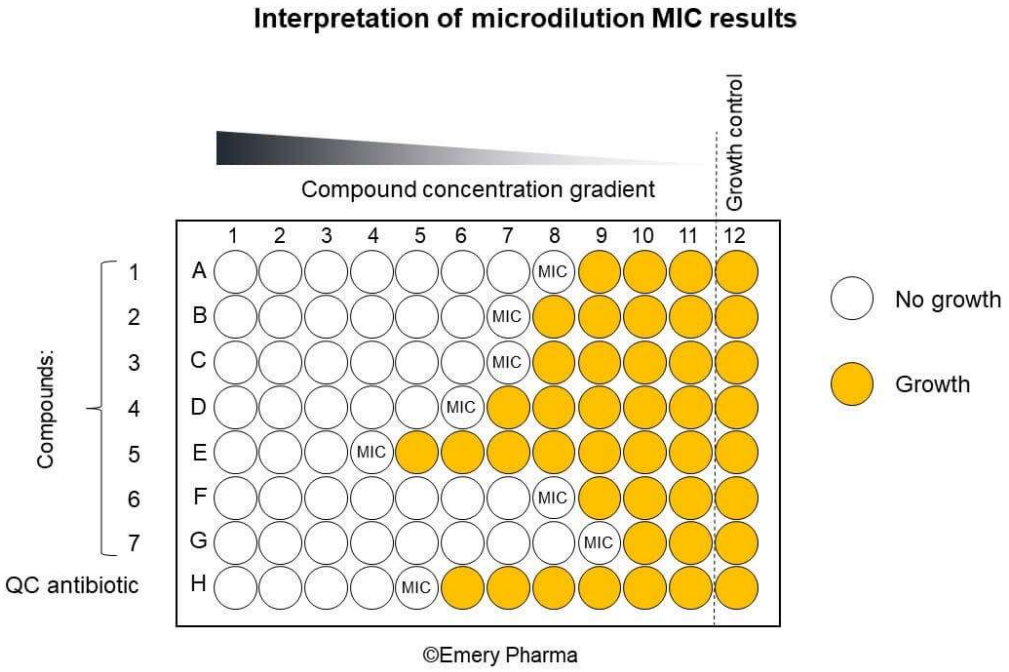
With fixed quantity of antibiotic or with gradient of the antibiotic (between specific amounts)



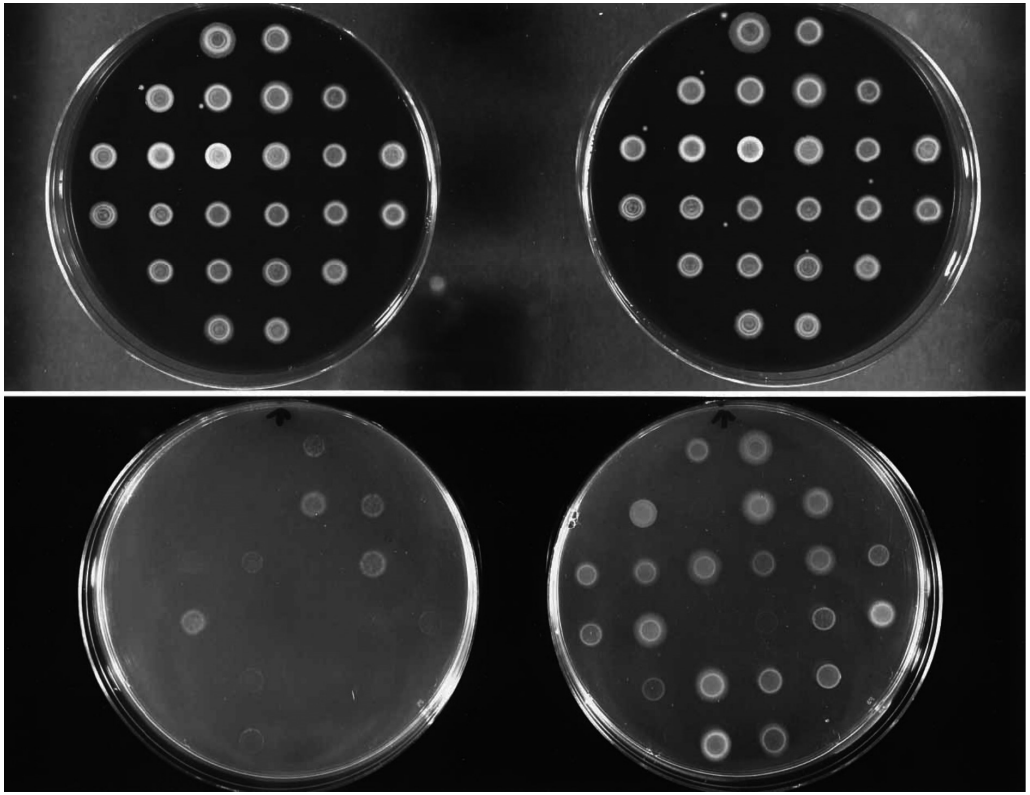
Antibiotics

Mode of action of antibiotics, MIC, spectrum of activity

Gradient of the antibiotic (between specific amounts) added to the liquid growth media and inoculated with studied microorganisms.



Gradient of the antibiotic (between specific amounts) added to the solid growth media and inoculated with studied microorganisms.



Antibiotics

Mode of action of antibiotics, MIC, spectrum of activity

efsa
European Food Safety Authority
EFSA Journal 20YY;volume(issue):NNNN

SCIENTIFIC OPINION[1]

Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance[2]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[3,4]

European Food Safety Authority (EFSA), Parma, Italy

Endorsed for public consultation on 1 February 2012

	ampicillin	vancomycin	gentamicin	kanamycin	streptomycin	erythromycin	clindamycin	tylosine	tetracycline	chloramphenicol
Enterococcus faecium	2	4	32	1024	128	4	4	4	4	16

	ampicillin	gentamicin[a]	kanamycin[a]	streptomycin[a]	tetracycline	chloramphenicol	nalidixic acid	sulfonamide	trimethoprim[a]	apramycin
Escherichia coli	8	2	8	16	8	16	16	256	2	8

[a] Possible interference of the growth medium

Table 1. Bacterial cut-off values (mg/L)

	ampicillin	vancomycin	gentamicin	kanamycin	streptomycin	erythromycin	clindamycin	tetracycline	chloramphenicol
Lactobacillus obligate homofermentative[a]	1	2[b]	16	16	16	1	1	4	4
Lactobacillus acidophilus group	1	2	16	64	16	1	1	4	4
Lactobacillus obligate heterofermentative[c]	2	n.r.	16	32	64	1	1	8	4
Lactobacillus reuteri	2	n.r.	8	64	64	1	1	16	4
Lactobacillus facultative heterofermentative	4	n.r.	16	64	64	1	1	8	4
Lactobacillus plantarum/pentosus	2	n.r.	16	64	n.r.	1	2	32	8
Lactobacillus rhamnosus	4	n.r.	16	64	32	1	1	8	4
Lactobacillus casei /paracasei	4	n.r.	32	64	64	1	1	4	4
Bifidobacterium	2	2	64	n.r.	128	1	1	8	4
Pediococcus	4	n.r.	16	64	64	1	1	8	4
Leuconostoc	2	n.r.	16	16	64	1	1	8	4
Lactococcus lactis	2	4	32	64	32	1	1	4	8
Streptococcus thermophilus	2	4	32	64	64	2	2	4	4
Bacillus spp	n.r.	4	4	8	8	4	4	8	8
Propionibacterium	2	4	64	64	64	0.5	0.25	2	2
Other Gram +	1	2	4	16	8	0.5	0.25	2	2

n.r. not required.
[a] including *L. delbrueckii, L. helveticus*
[b] not required for *L. salivarius*
[c] including *L. fermentum*

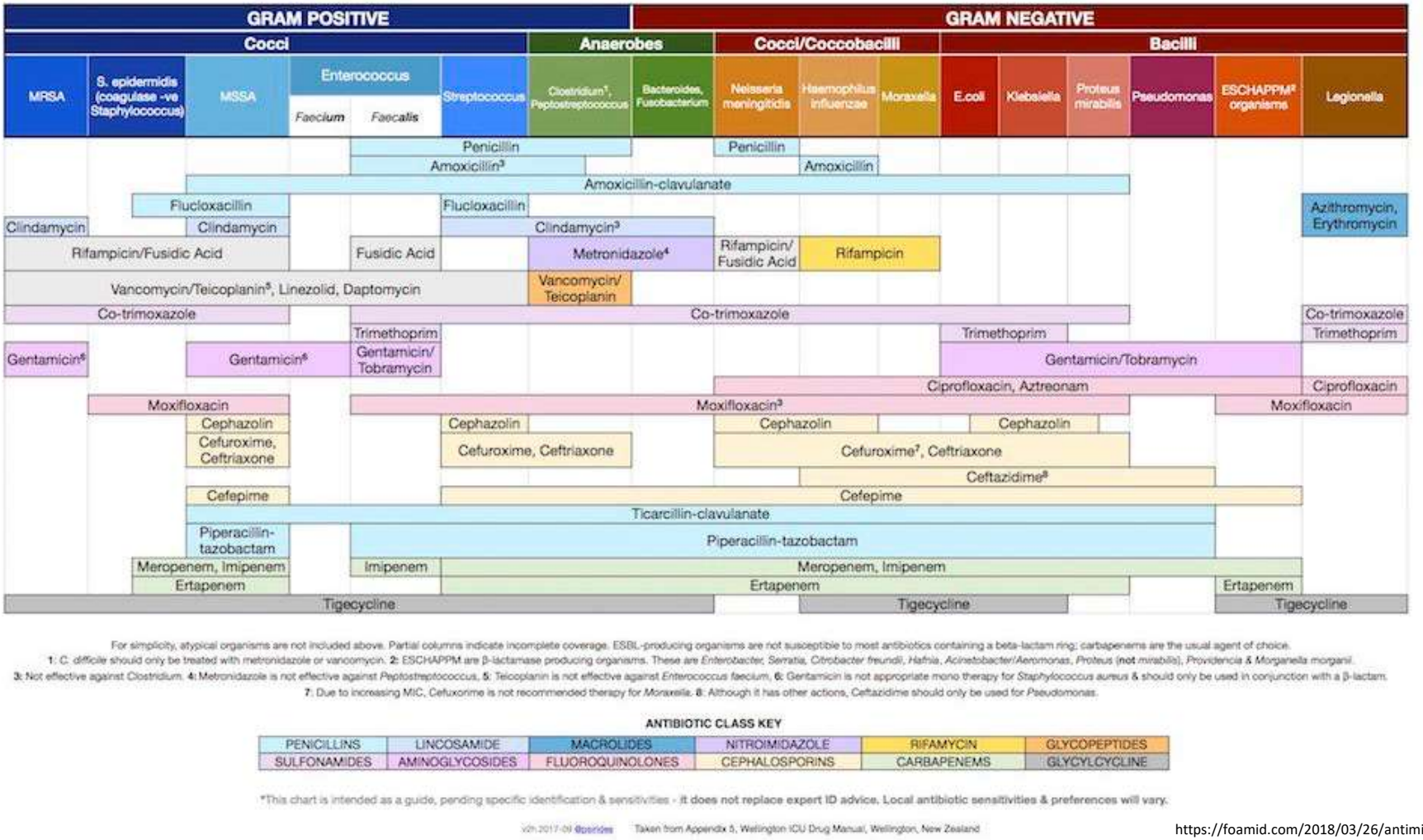
Antibiotics Mode of action of antibiotics, MIC, spectrum of activity

- Antibiotics can either have a **narrow or broad spectrum of activity**.
  - Narrow-spectrum antibiotics are more specific and only active against certain groups or strains of bacteria.
    - Advantages: Narrow-spectrum antibiotic **allow to kill or inhibit only those bacteria species that are unwanted** (i.e. causing disease). As such, it leaves most of the beneficial bacteria unaffected, hence minimizing the collateral damage on the microbiota. Low propensity for bacterial resistance development.
    - Disadvantages: **Often, the exact species of bacteria causing the illness is unknown**, in which case narrow-spectrum antibiotics can't be used, and broad-spectrum antibiotics are used instead.

  - Broad-spectrum antibiotics instead inhibit a wider range of bacteria.
    - Advantages: Narrow-spectrum antibiotics target a few types of bacteria. Broad-spectrum antibiotics **target many types of bacteria**. Both types work well to treat infections. But using broad-spectrum antibiotics when they're not needed can create antibiotic-resistant bacteria that are hard to treat
    - Disadvantages: Broad-spectrum antibiotics **can lead to a dangerous form of diarrhea, called "*Clostridium difficille*"** It can require removal of the bowel. It kills about 15,000 people in the U.S. each year. Antibiotics can also cause other side effects, such as vaginal infections, nausea, and vomiting.

Antibiotics

Mode of action of antibiotics, MIC, spectrum of activity



For simplicity, atypical organisms are not included above. Partial columns indicate incomplete coverage. ESBL-producing organisms are not susceptible to most antibiotics containing a beta-lactam ring; carbapenems are the usual agent of choice.
1: *C. difficile* should only be treated with metronidazole or vancomycin. **2**: ESCHAPPM are β-lactamase producing organisms. These are *Enterobacter, Serratia, Citrobacter freundii, Hafnia, Acinetobacter/Aeromonas, Proteus* (**not** *mirabilis*), *Providencia & Morganella morganii*.
3: Not effective against *Clostridium*. **4**: Metronidazole is not effective against *Peptostreptococcus*. **5**: Teicoplanin is not effective against *Enterococcus faecium*. **6**: Gentamicin is not appropriate mono therapy for *Staphylococcus aureus* & should only be used in conjunction with a β-lactam.
7: Due to increasing MIC, Cefuxorime is not recommended therapy for *Moraxella*. **8**: Although it has other actions, Ceftazidime should only be used for *Pseudomonas*.

ANTIBIOTIC CLASS KEY

PENICILLINS	LINCOSAMIDE	MACROLIDES	NITROIMIDAZOLE	RIFAMYCIN	GLYCOPEPTIDES
SULFONAMIDES	AMINOGLYCOSIDES	FLUOROQUINOLONES	CEPHALOSPORINS	CARBAPENEMS	GLYCYLCYCLINE

*This chart is intended as a guide, pending specific identification & sensitivities - **it does not replace expert ID advice. Local antibiotic sensitivities & preferences will vary.**

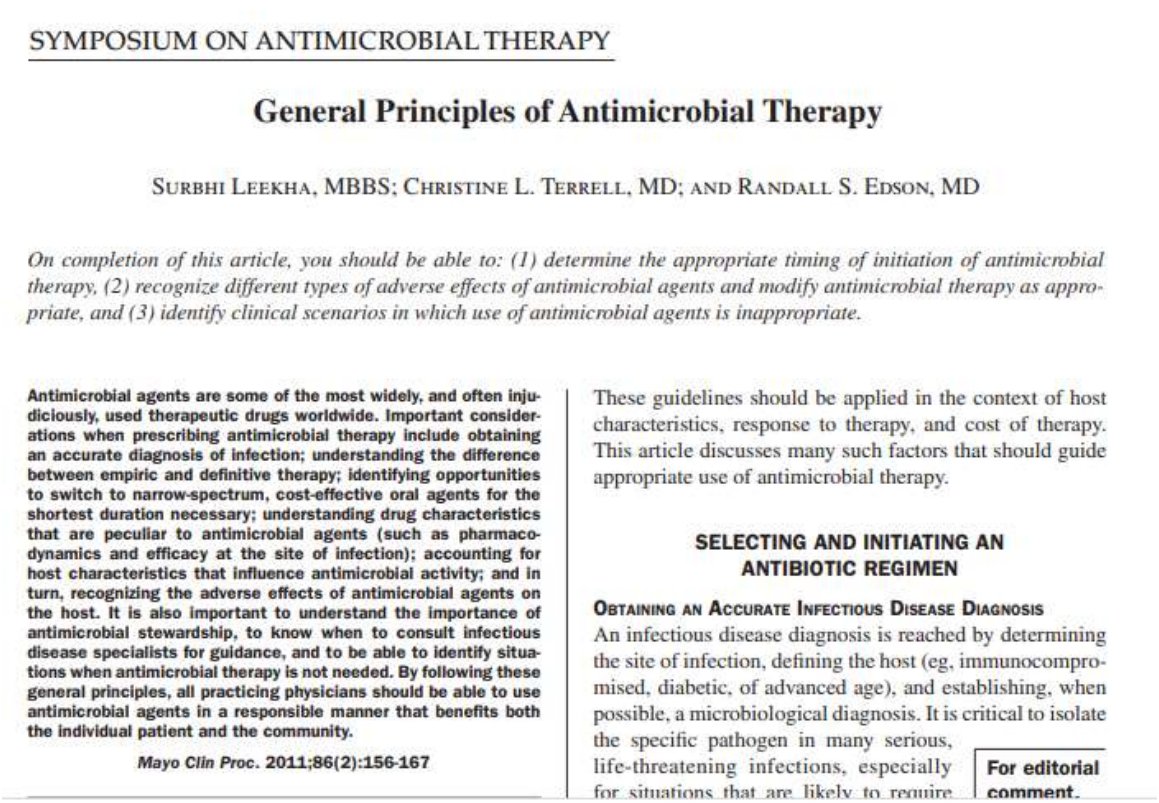
v2h 2017-09 @perides Taken from Appendix 5, Wellington ICU Drug Manual, Wellington, New Zealand

https://foamid.com/2018/03/26/antimicrobials-spectrum-of-activity/

Antibiotics Methods for establish need of appropriate antibiotics

A classic method for detecting the presence of antibiotics is the use of **microbiological assays** that employ antibiotic-sensitive species of bacteria to determine whether specific antibiotics are present in a given sample and, with limited sensitivity, their concentration.

Biomolecular approaches (including DNA, RNA tests, FlowCytometry combined with FISH, diagnostic kits) can be applied for rapid identification of the bacteria involve in the clinical case.



SYMPOSIUM ON ANTIMICROBIAL THERAPY

General Principles of Antimicrobial Therapy

SURBHI LEEKHA, MBBS; CHRISTINE L. TERRELL, MD; AND RANDALL S. EDSON, MD

On completion of this article, you should be able to: (1) determine the appropriate timing of initiation of antimicrobial therapy, (2) recognize different types of adverse effects of antimicrobial agents and modify antimicrobial therapy as appropriate, and (3) identify clinical scenarios in which use of antimicrobial agents is inappropriate.

Antimicrobial agents are some of the most widely, and often injudiciously, used therapeutic drugs worldwide. Important considerations when prescribing antimicrobial therapy include obtaining an accurate diagnosis of infection; understanding the difference between empiric and definitive therapy; identifying opportunities to switch to narrow-spectrum, cost-effective oral agents for the shortest duration necessary; understanding drug characteristics that are peculiar to antimicrobial agents (such as pharmacodynamics and efficacy at the site of infection); accounting for host characteristics that influence antimicrobial activity; and in turn, recognizing the adverse effects of antimicrobial agents on the host. It is also important to understand the importance of antimicrobial stewardship, to know when to consult infectious disease specialists for guidance, and to be able to identify situations when antimicrobial therapy is not needed. By following these general principles, all practicing physicians should be able to use antimicrobial agents in a responsible manner that benefits both the individual patient and the community.

Mayo Clin Proc. 2011;86(2):156-167

These guidelines should be applied in the context of host characteristics, response to therapy, and cost of therapy. This article discusses many such factors that should guide appropriate use of antimicrobial therapy.

SELECTING AND INITIATING AN ANTIBIOTIC REGIMEN

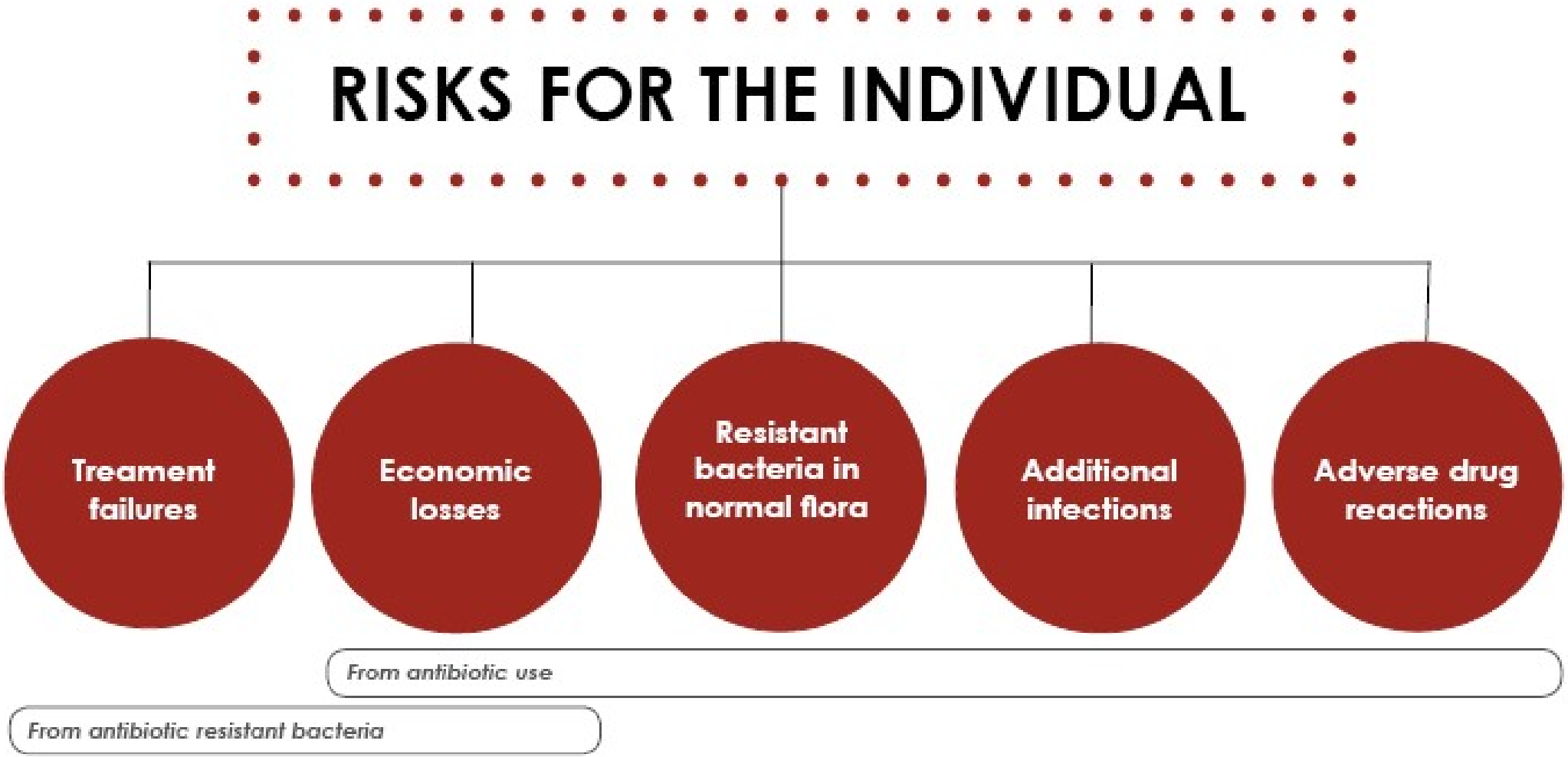
OBTAINING AN ACCURATE INFECTIOUS DISEASE DIAGNOSIS

An infectious disease diagnosis is reached by determining the site of infection, defining the host (eg, immunocompromised, diabetic, of advanced age), and establishing, when possible, a microbiological diagnosis. It is critical to isolate the specific pathogen in many serious, life-threatening infections, especially for situations that are likely to require

For editorial comment.

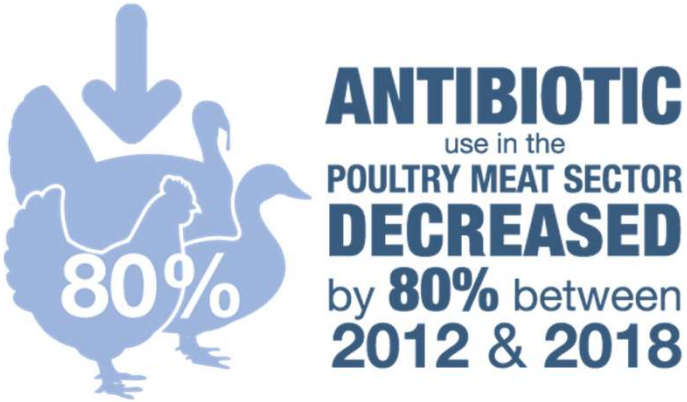
Antibiotics

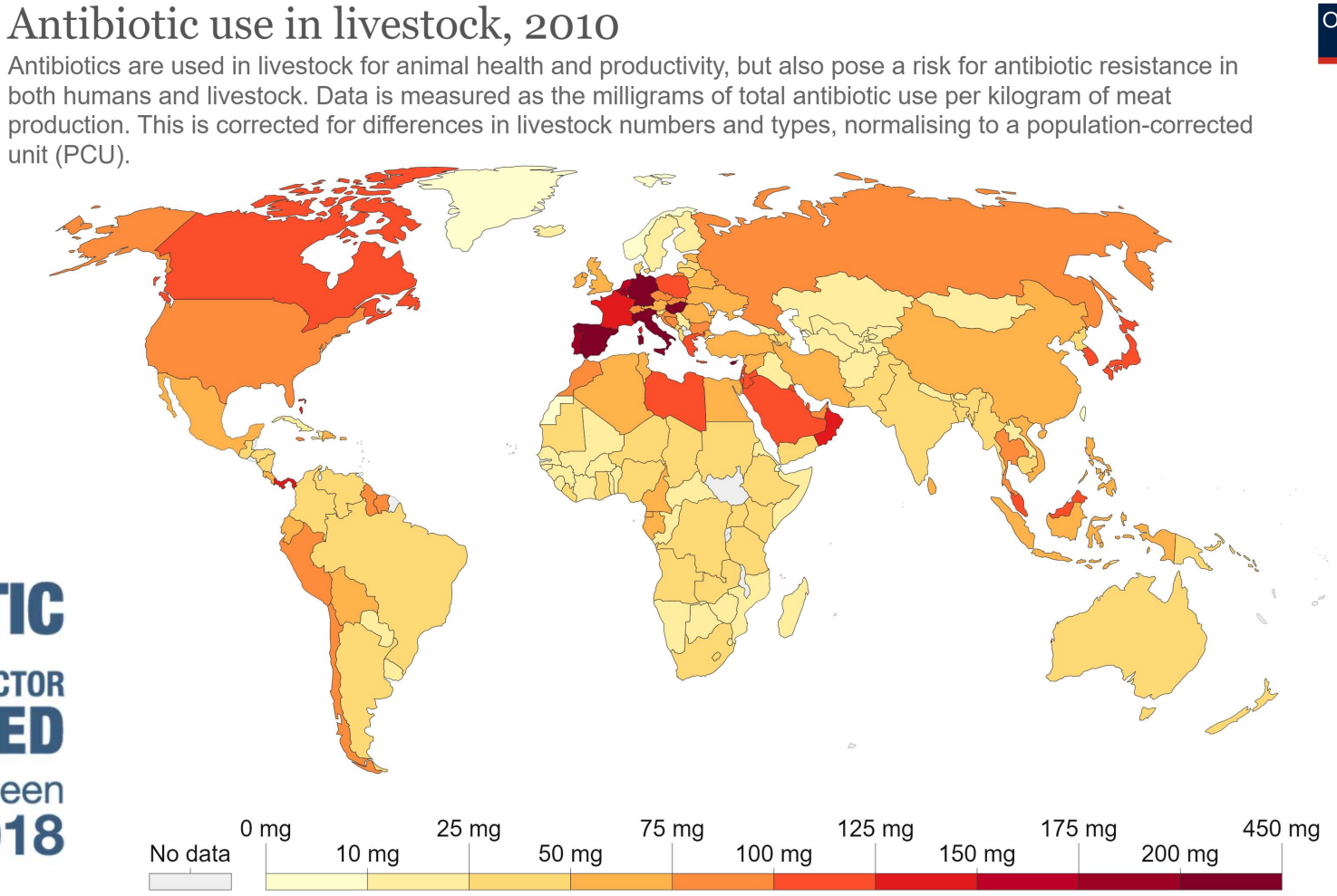
Prevention? Limits? Associated risks?



Antibiotics

Prevention? Limits? Associated risks?



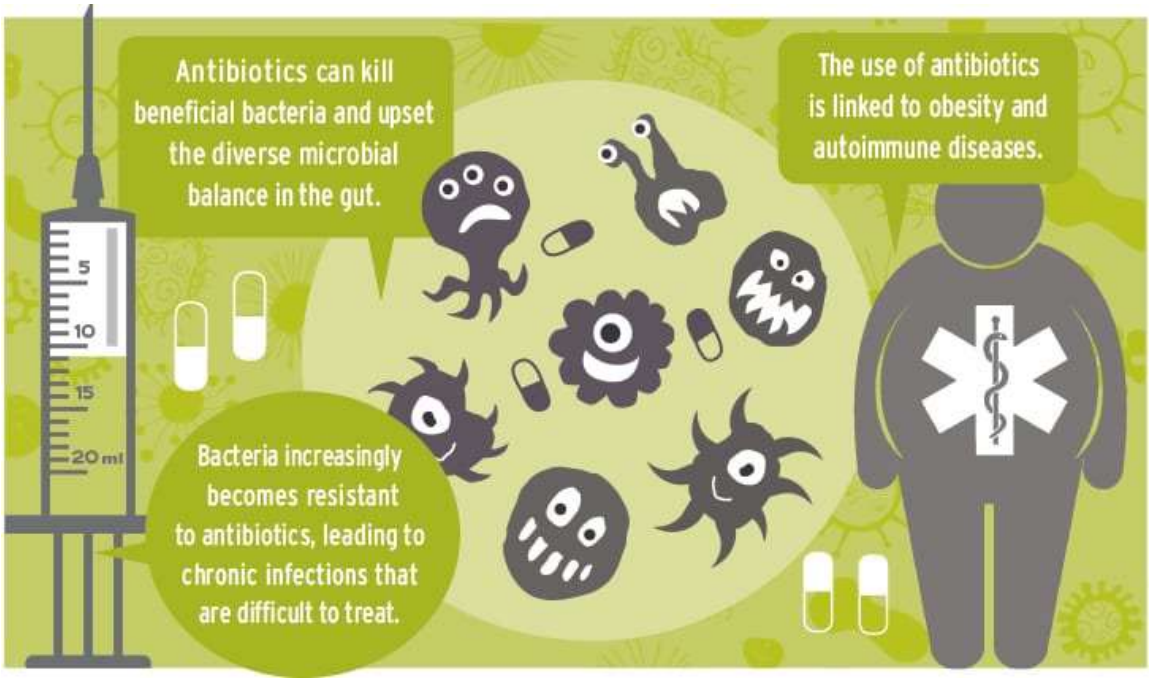


Antibiotics

Prevention? Limits? Associated risks?

Where antibiotics can be bought for human or animal use without a prescription, the emergence and spread of resistance is made **worse**.
Examples of **misuse** include taking antibiotics for viral infections such as colds and flu, and using them as **animal growth promoters** on farms or in aquaculture.

Anytime antibiotics are used, they can contribute to antibiotic resistance. This is because **increases in antibiotic resistance are driven by a combination of germs exposed to antibiotics**, and the spread of those germs and their mechanisms of resistance.

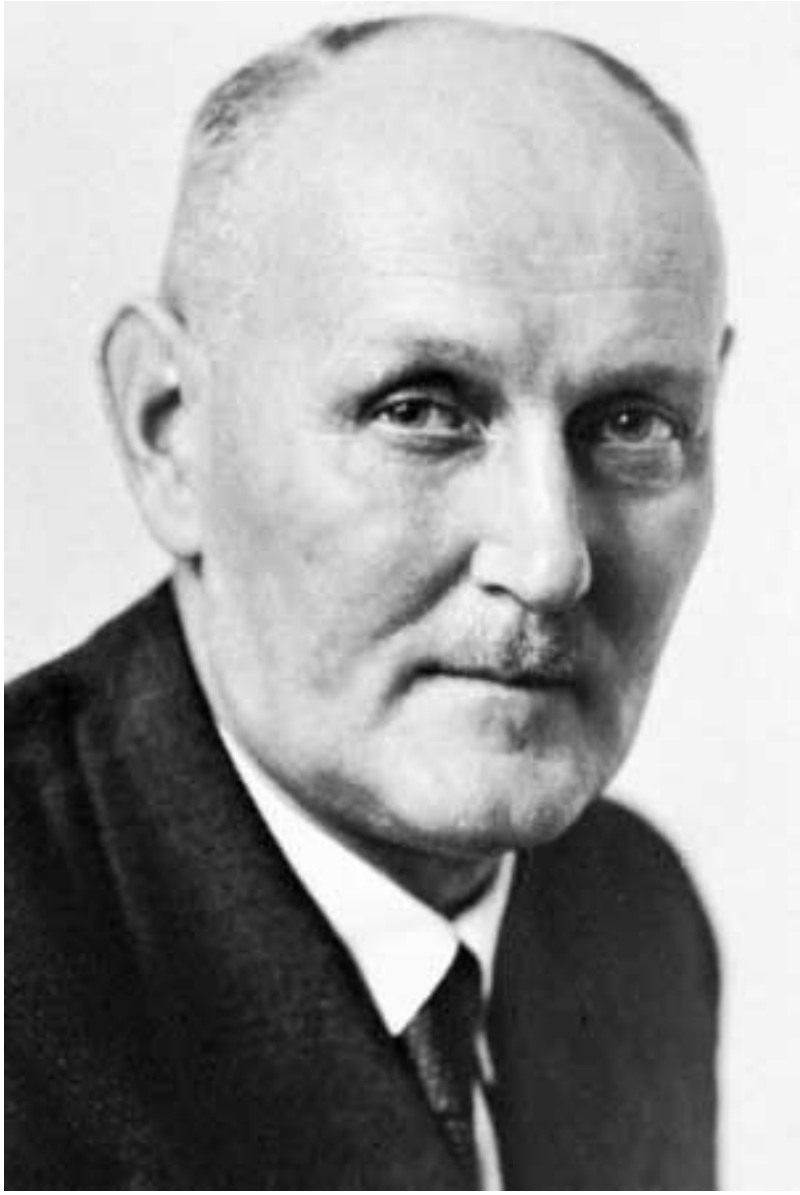


The overuse of antibiotics has been an important clinical issue, and antibiotic exposure is linked to alterations in gut microbiota, which has been related to risks of various chronic diseases such as cardiovascular disease and cancer. Also, **duration of antibiotic exposure may be a risk factor of premature death**.

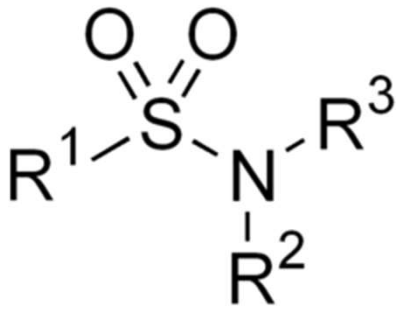
Sulfonamides

1930s: The first commercially available antibacterial was Prontosil, a sulfonamide developed by the German biochemist Gerhard Domagk (1895 – 1964).

In 1939, Domagk received the Nobel Prize in Medicine for this discovery, the first drug effective against bacterial infections. He was forced by the Nazi regime to refuse the prize and was arrested by the Gestapo and detained for a week



Sulfonamides



Sulfonamide functional group

Sulfonamide is a functional group that is the active basis of several groups of drugs, which are called **sulphonamides**, **sulfa drugs** or **sulpha drugs**. The original antibacterial sulfonamides are synthetic (nonantibiotic) antimicrobial agents that contain the sulfonamide group.
Some sulfonamides are also devoid of antibacterial activity, e.g., the anticonvulsant sultiame. The sulfonylureas and thiazide diuretics are newer drug groups based upon the antibacterial sulfonamides.

In bacteria, antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthase (DHPS), an enzyme involved in folate synthesis. Sulfonamides are therefore bacteriostatic and inhibit growth and multiplication of bacteria, but do not kill them.

Sulfonamides

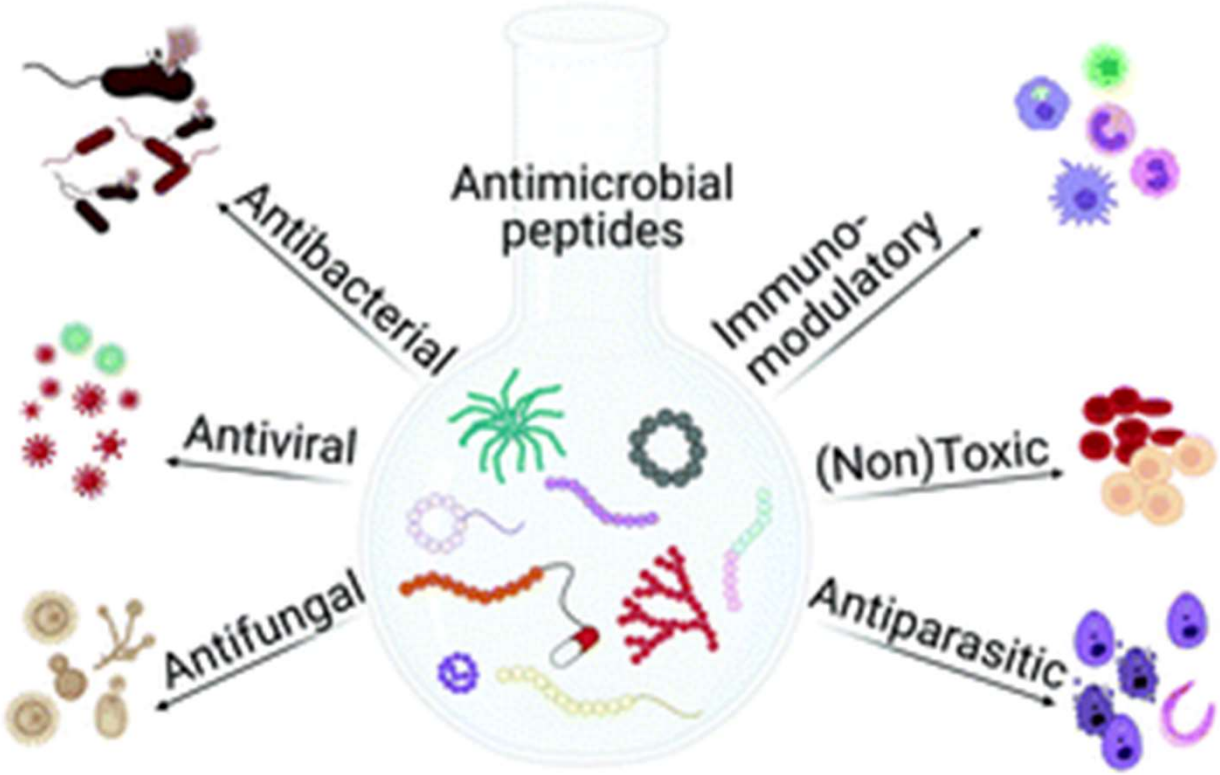
Sulfonamides are used to treat allergies and coughs, as well as having antifungal and antimalarial functions.

The moiety is also present in other medications that are not antimicrobials, including thiazide diuretics (including hydrochlorothiazide, metolazone, and indapamide, among others), loop diuretics (including furosemide, bumetanide, and torsemide), acetazolamide, sulfonylureas (including glipizide, glyburide, among others), and some COX-2 inhibitors (e.g., celecoxib).

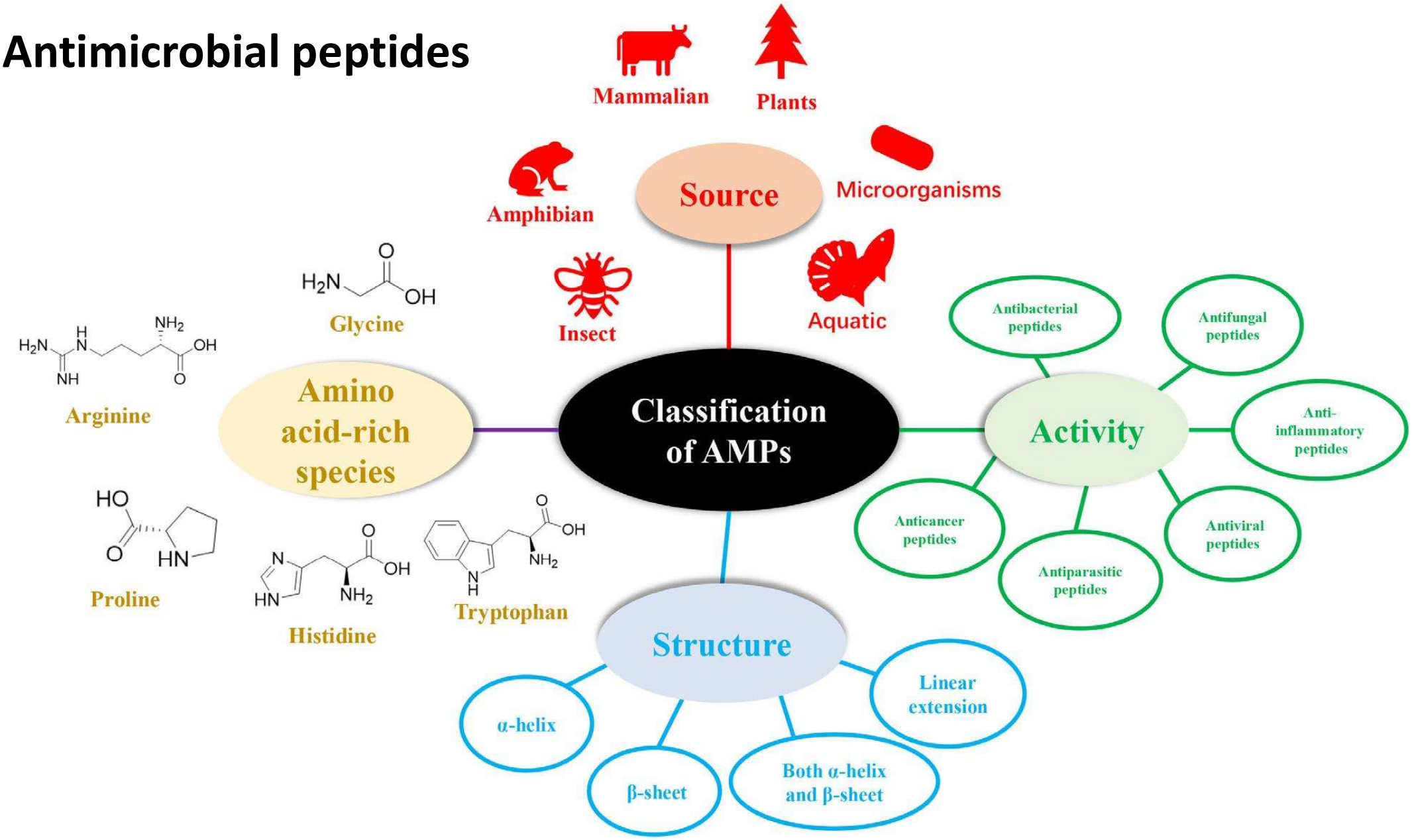
Sulfasalazine, in addition to its use as an antibiotic, is also used in the treatment of inflammatory bowel disease.

Antimicrobial peptides

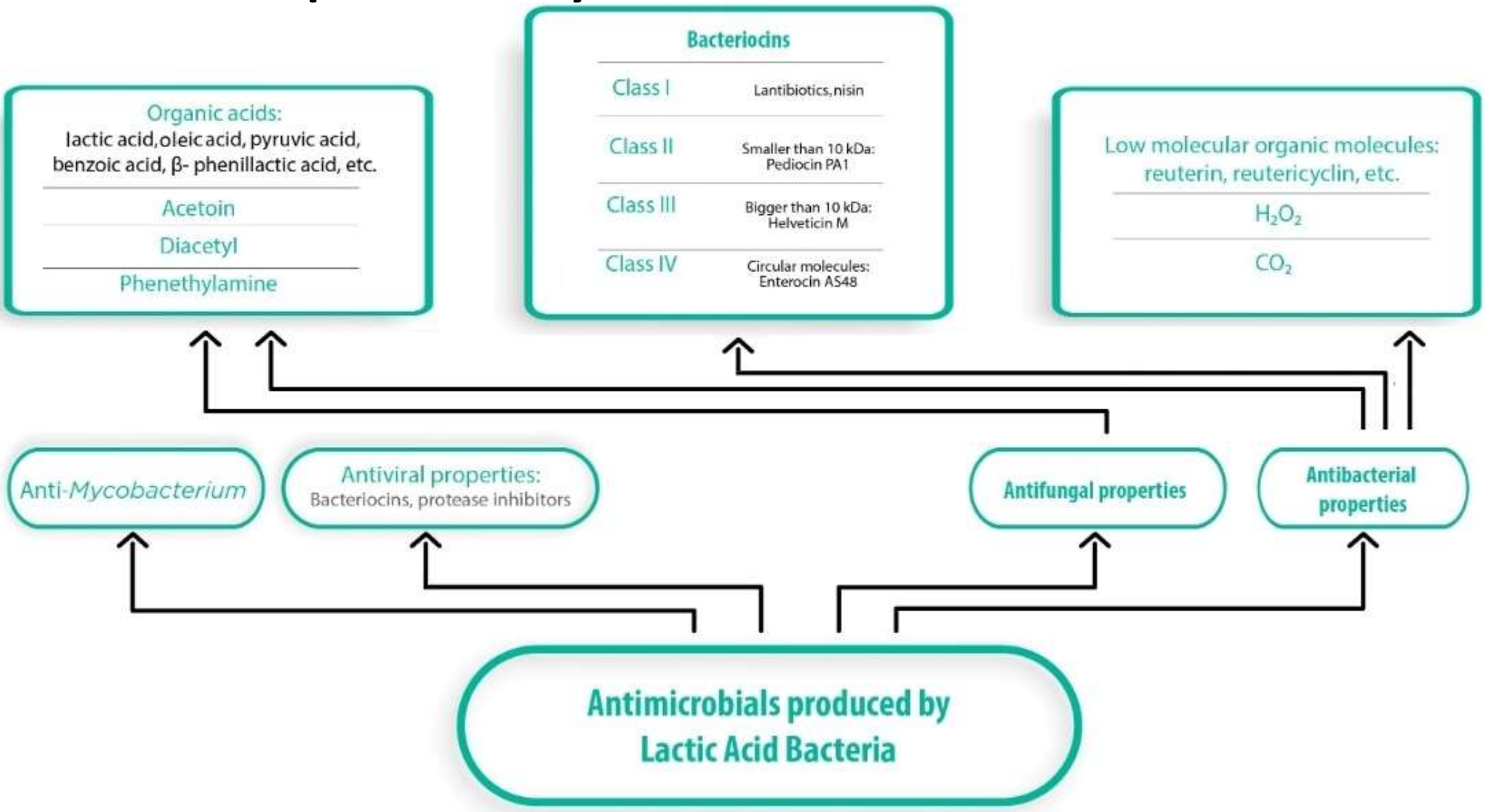
Antimicrobial peptides (AMPs) occur naturally in all living organisms such as bacteria, fungi, plants, and animals. They compose the first line of defense innate immunity from animals and plants [54]. AMPs share some characteristics as **amino acids, size, charge, secondary structure and hydrophobicity.**



Antimicrobial peptides

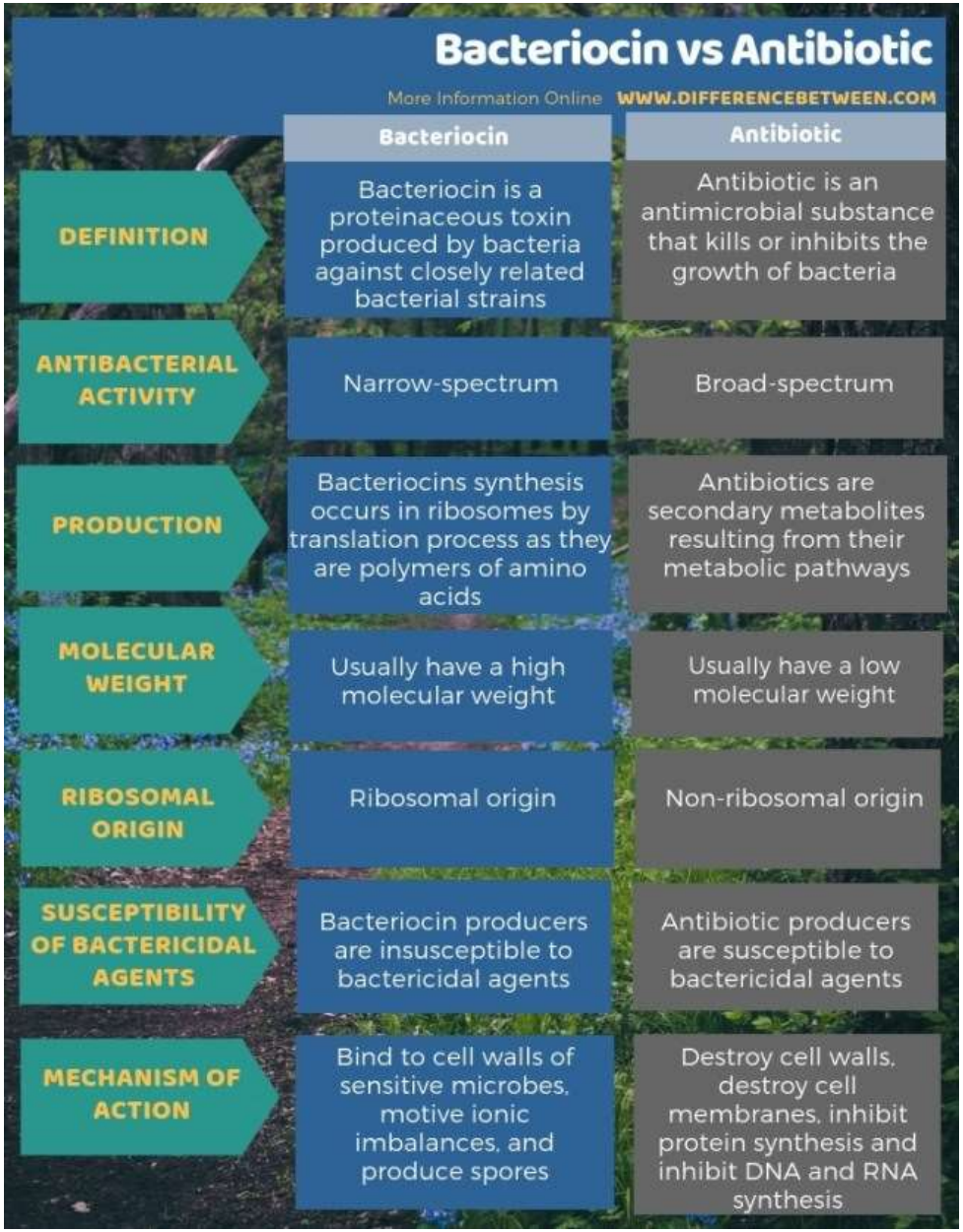


Antimicrobials produced by LAB

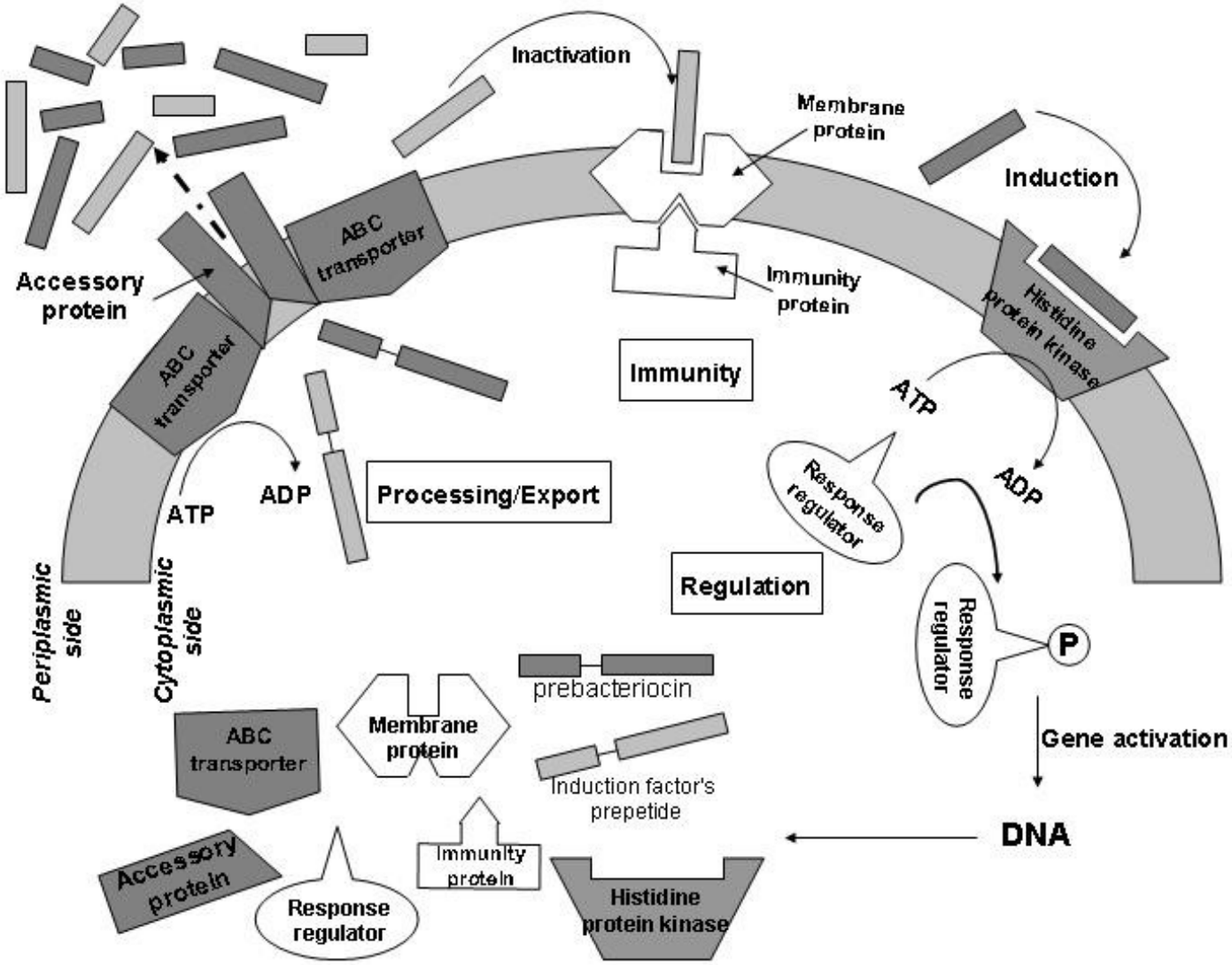


Bacteriocins

By definition, bacteriocins produced by lactic acid bacteria are ribosomal synthesized polypeptides that exhibit bactericidal or bacteriostatic activity against genetically closely related bacteria (Chikindas et al., 2018).

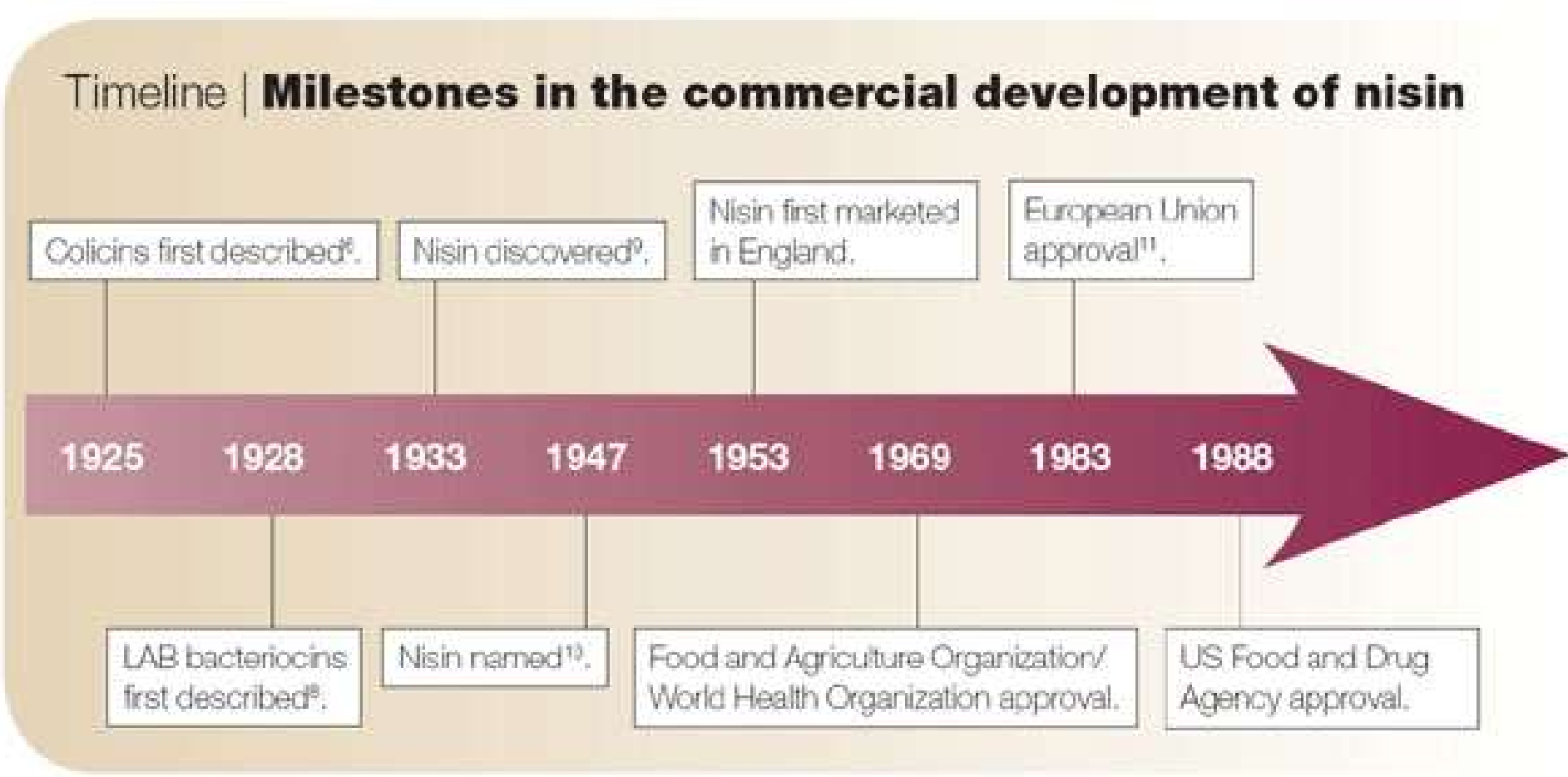


Bacteriocins



Schematic diagram of the biosynthesis machinery for production of Class IIa bacteriocins: three component regulatory system, synthesis, processing, excretion and immunity (Havarstein et al., 1995; Venema et al., 1995; Nes et al., 1996; Ennahar et al., 2000.)

Bacteriocins



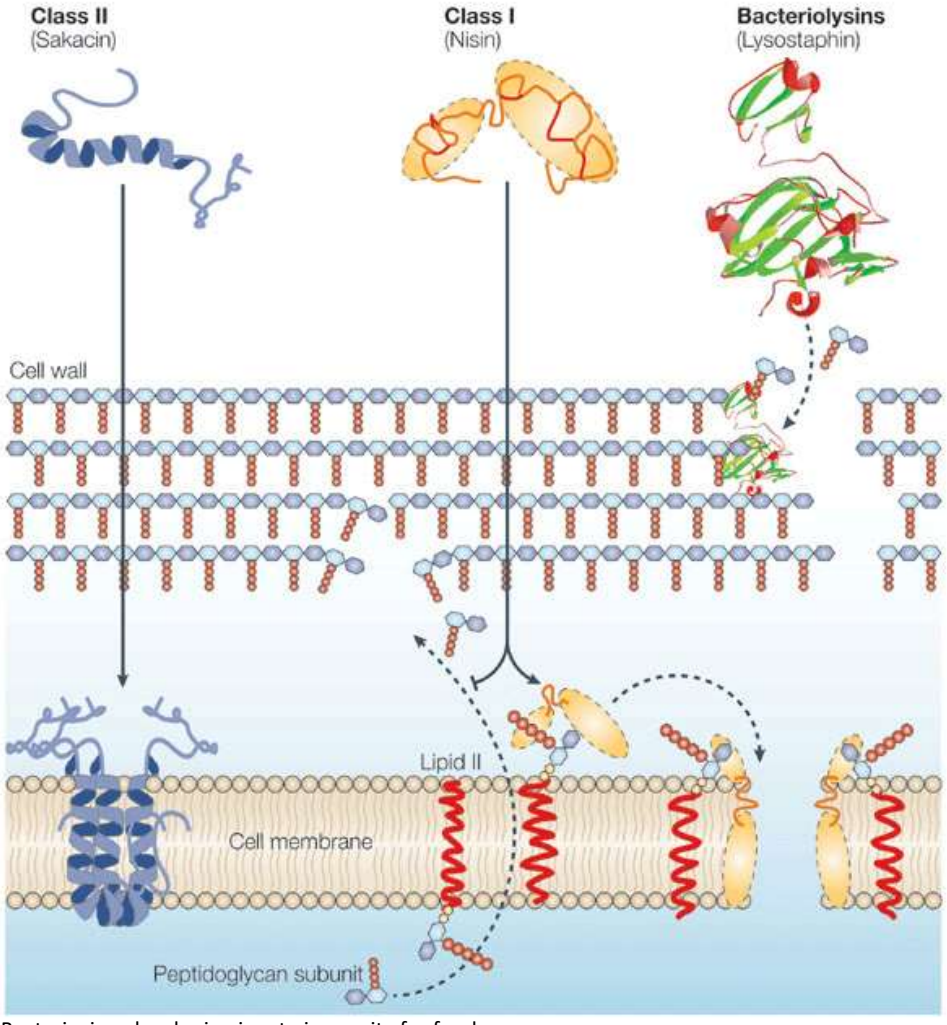
Last 20 years

Application of bacteriocins in human and veterinary medicine

Bacteriocins: developing innate immunity for food
Paul D. Cotter, Colin Hill & R. Paul Ross
Nature Reviews Microbiology 3, 777-788 (2005)

Bacteriocins of Gram-positive bacteria having activity spectra extending beyond closely-related species. Todorov SD, Franco BDGM & Tagg JR. Beneficial Microbes. 10 (3), 315-328 (2019)

Bacteriocins



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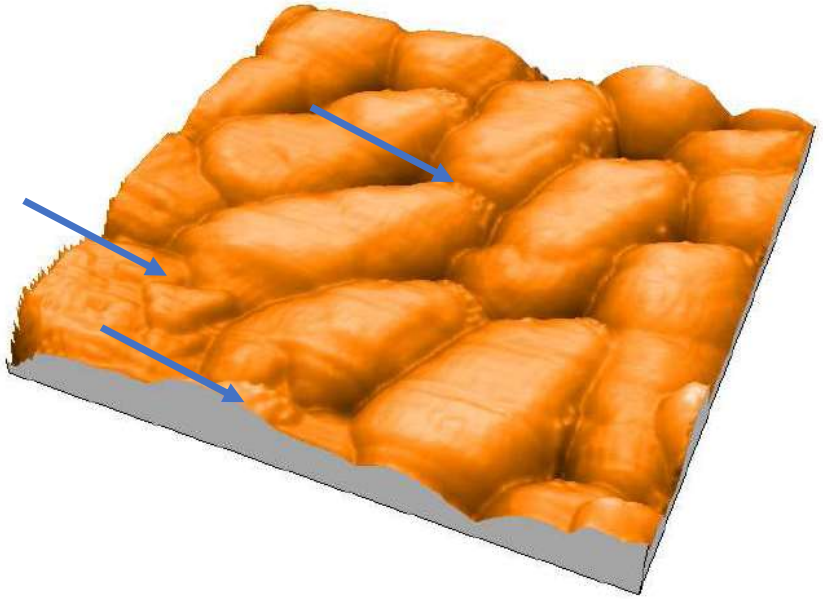
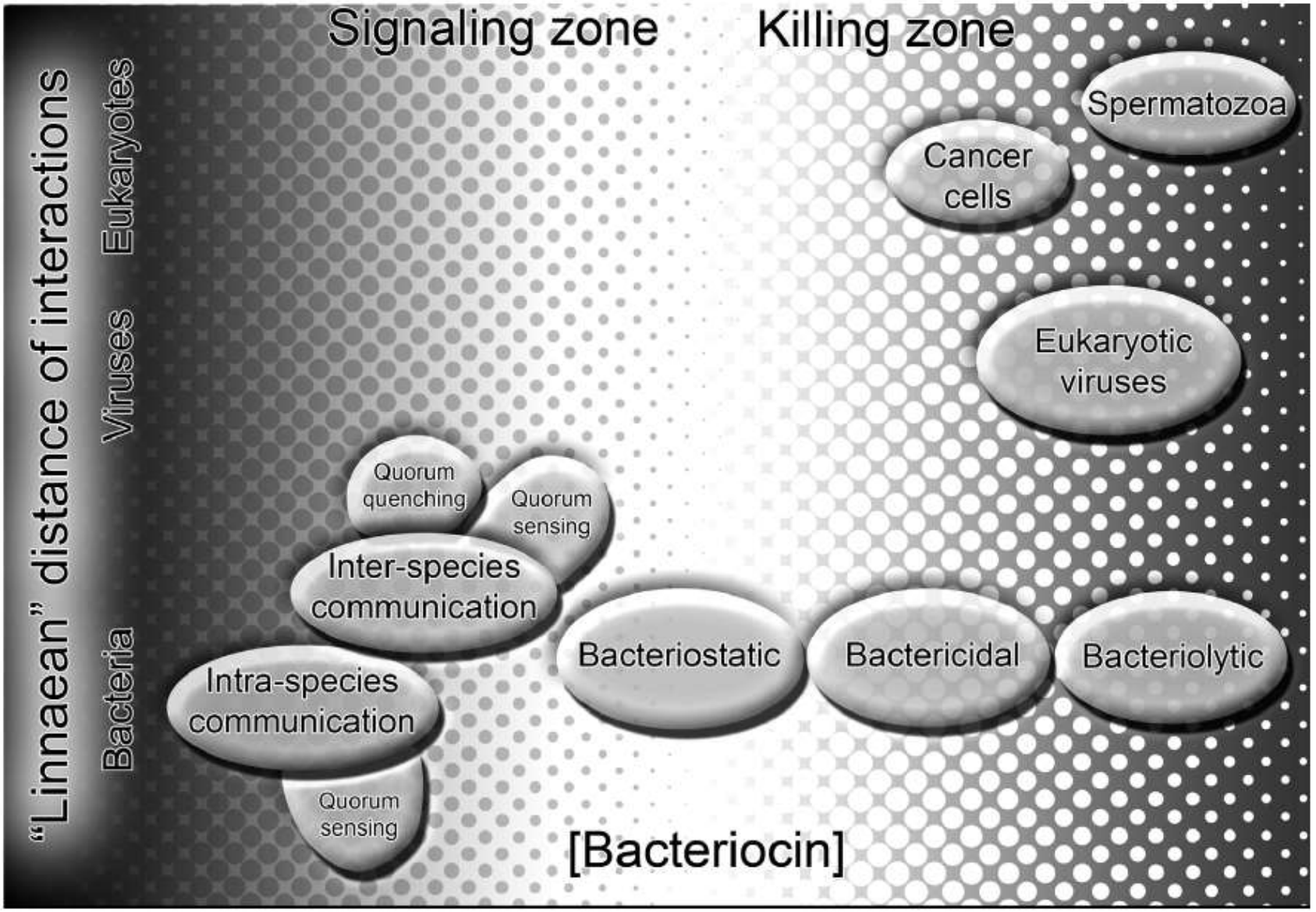


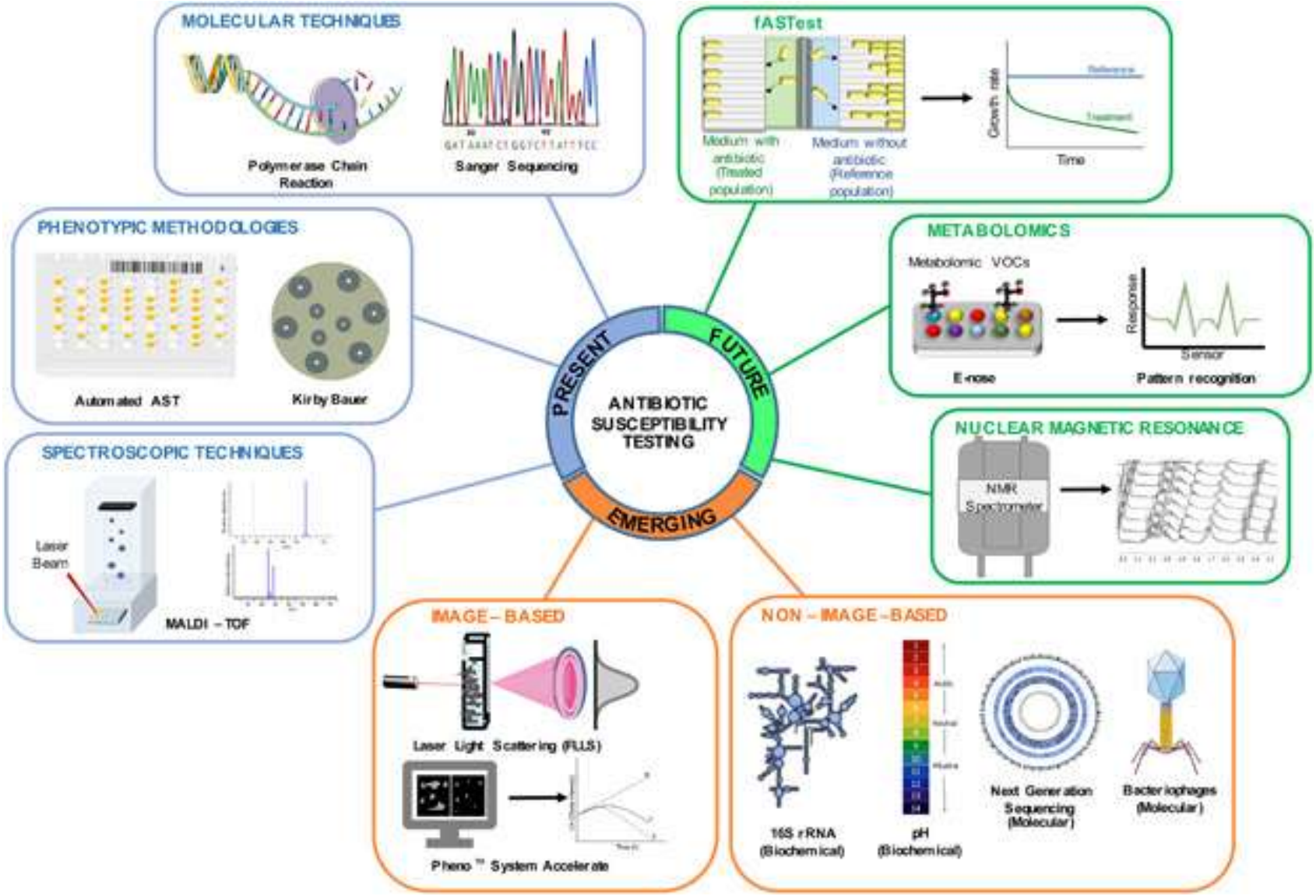
Listeria ivanovii subsp. *ivanovii* ATCC 19119
treated with bacteriocin ST5HA

Bacteriocins



Chikindas et al. 2018 Current Opinion in Biotechnology

Antibiotic susceptibility testing



Antibiotic presence testing

ncbi.nlm.nih.gov/pmc/articles/PMC2758187/table/Tab1/?report=objectonly

Overview of EU maximum residue limits (MRLs) (μg kg^{-1}), established until 1 January 2009

Pharmacologically active substance	Animal species	Commission regulation	Target tissues				Milk	Egg	Honey
			Muscle	Fat	Liver	Kidney			
β-Lactam antibiotics									
Amoxicillin	All food-producing species	508/1999	50	50	50	50	4		
Ampicillin	All food-producing species	508/1999	50	50	50	50	4		
Benzylpenicillin	All food-producing species	508/1999	50	50	50	50	4		
Cefacetril	Bovine	2162/2001					125		
Cefalexin	Bovine	2728/1999	200	200	200	1,000	100		
Cefapirin	Bovine	1553/2001	50	50		100	60		
Cefazolin	Bovine, ovine, caprine	508/1999					50		
Cefoperazon	Bovine	807/2001					50		
Cefquinome	Bovine, porcine, Equidae	508/1999, 1931/1999, 2145/2003	50	50	100	200	20		
Ceftiofur	All mammalian food-producing species	1231/2006	1,000	2,000	2,000	6,000	100		
Cloxacillin	All food-producing species	508/1999	300	300	300	300	30		
Dicloxacillin	All food-producing species	508/1999	300	300	300	300	30		
Fenoxymethylpenicillin	Porcine	1286/2000	25		25	25			
Fenoxymethylpenicillin	Poultry	1299/2005	25	25	25	25			
Nafcillin	All ruminants	546/2004	300	300	300	300	30		
Oxacillin	All food-producing species	508/1999	300	300	300	300	30		

If no MRL for egg or milk has been established, the substance is not to be used in animals from which eggs or milk are produced for human consumption.

Anal Bioanal Chem (2009) 395:893–905
DOI 10.1007/s00216-009-2841-6

REVIEW

Microbial screening methods for detection of antibiotic residues in slaughter animals

Mariël G. Pikkemaat

Antibiotic presence testing

Anal Bioanal Chem (2009) 395:893–905
DOI 10.1007/s00216-009-2841-6

REVIEW

Microbial screening methods for detection of antibiotic residues in slaughter animals

Mariël G. Pikkemaat

Type of assay: name and/or indicator organism	Intended matrix	Reference
Tube test		
Bacillus stearothermophilus based tube test	Milk	[4]
Premi®Test (*B. stearothermophilus*) and solvent extraction	Multiple matrices	[5]
Premi®Test (*B. stearothermophilus*)	Poultry tissue fluid	[51]
Delvotest® SP-NT and Copan (*B. stearothermophilus*)	Milk	[59]
Delvotest® SP-NT (*B. stearothermophilus*)	Milk	[60]
Single-plate assay		
New Dutch kidney test (*B. subtilis*)	Renal pelvis fluid absorbed on paper disk	[12]
Belgian kidney test (*B. subtilis*)	Renal pelvis fluid/kidney cortex	[13]
B. subtilis plate test	Kidney tissue	[14]
STOP (*B. subtilis*)	Muscle fluid absorbed with swab	[27]
CAST (*B. megaterium*)	Kidney fluid absorbed with swab	[28]
FAST (*B. megaterium*) bromocresol purple indicator for reduced assay time	Kidney fluid absorbed with swab	[29]
Escherichia coli plate test (specific detection of quinolones)	Muscle	[39]
Yersinia ruckeri plate test (specific detection of quinolones)	Fish	[44]
E. coli plate test (specific detection of quinolones)	Not specified	[40]
Klebsiella pneumoniae plate test (specific detection of quinolones)	Egg	[42]
K. pneumoniae plate test (specific detection of quinolones)	Poultry tissue	[43]

In the *first column* the type of assay is mentioned, including the indicator organism(s) and (if applicable) the name of the test. The *second column* mentions the type of matrix for which the test is intended

Antibiotic presence testing

Microbiological assays. A classic method for detecting the presence of antibiotics is the use of microbiological assays that employ **antibiotic-sensitive species of bacteria** to determine whether specific antibiotics are present in a given sample and, with limited sensitivity, their concentration.

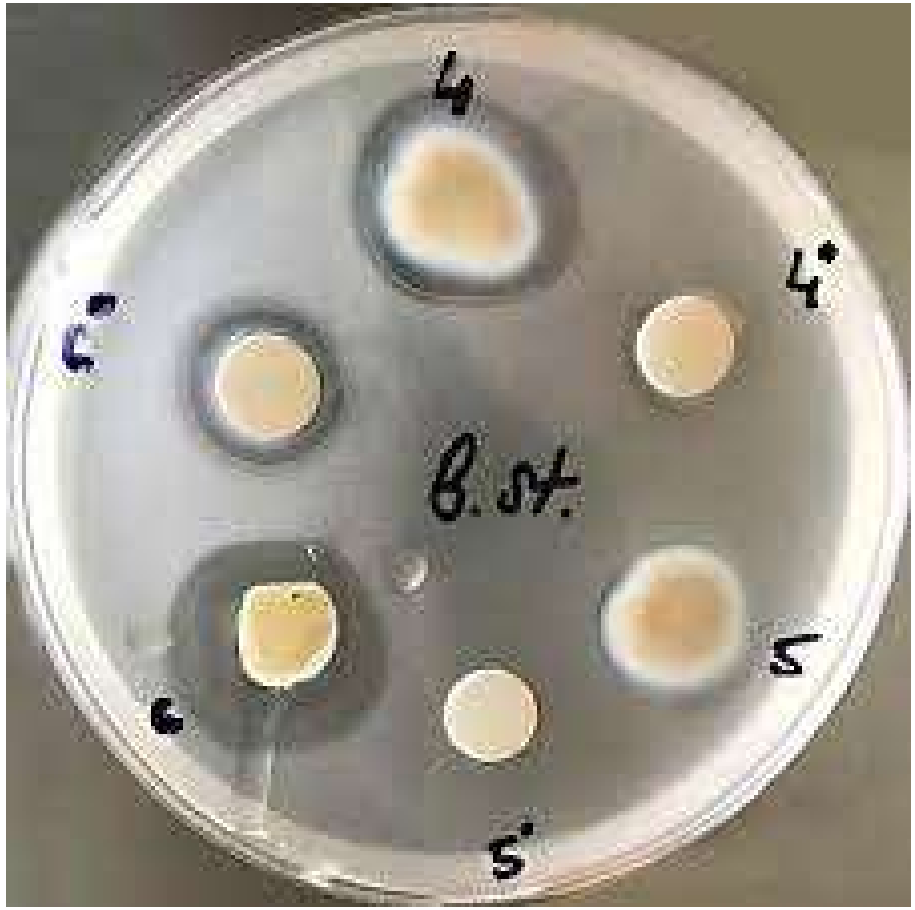
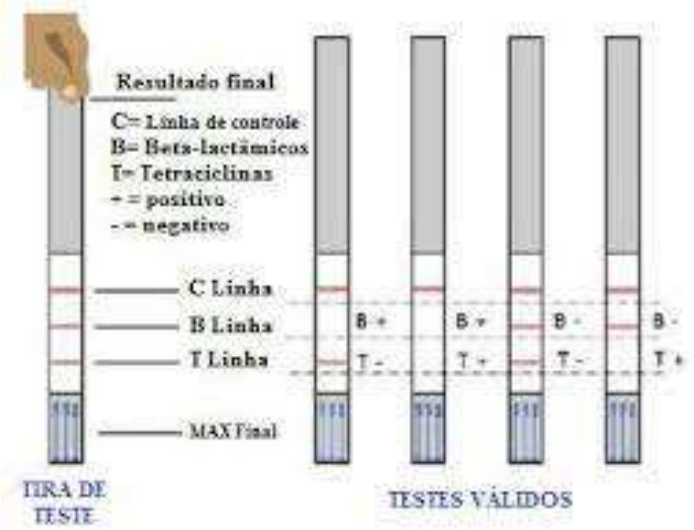


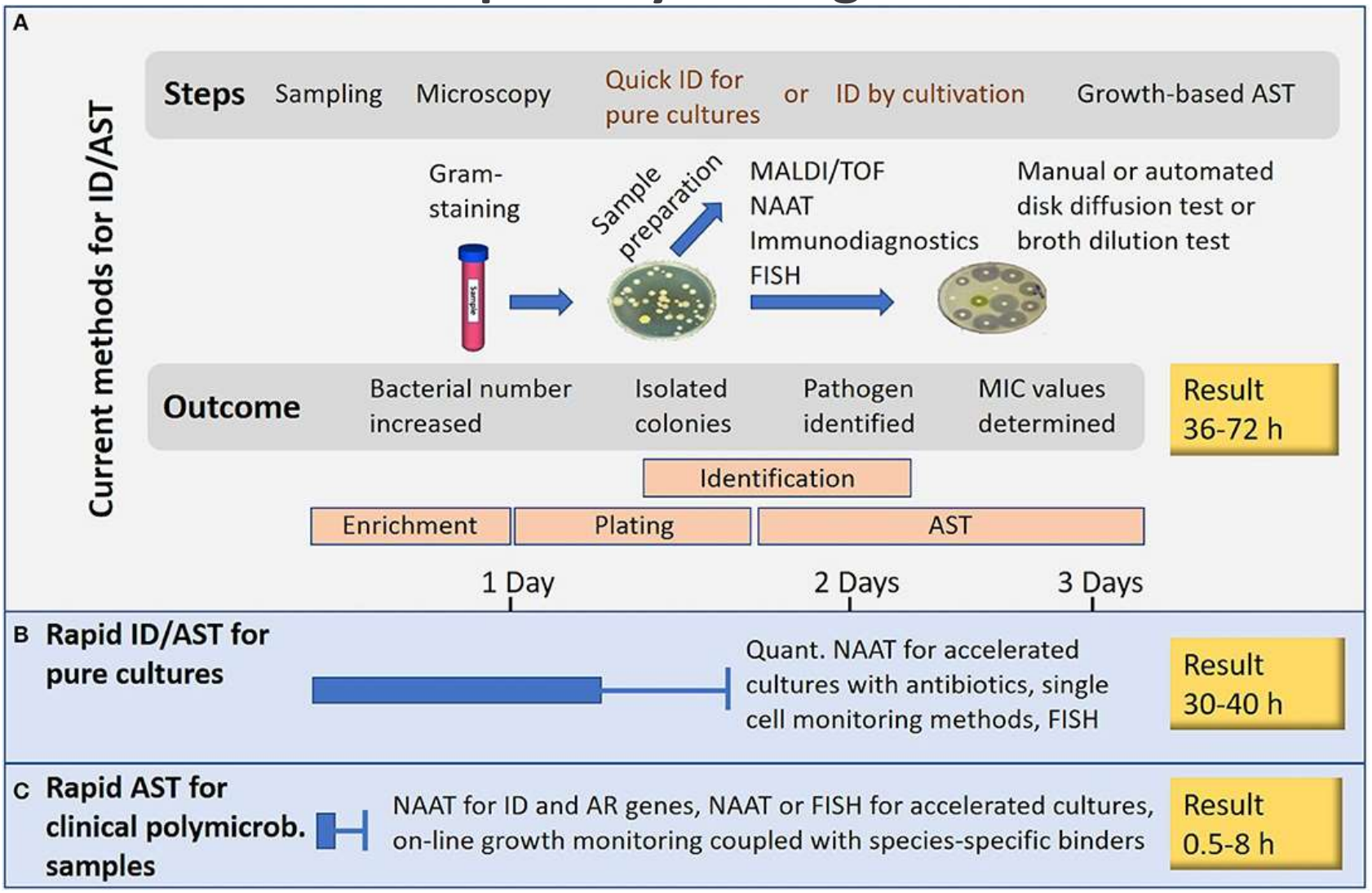
Plate with *Bacillus stearothermophilus* test organisms

ALTERNATIVE MILK (AND OTHER APPLICATIONS) ANTIBIOTIC TESTING

The MRLBL3 Beta-Lactam test provides results in 8 minutes using the ROSA lateral flow technology. The test system is designed for use by dairy, intake, laboratory, field, and regulatory personnel, and the Charm system is used by the UK major Dairy processors.



AST : antimicrobial susceptibility testing



Nucleic Acid Amplification Technology (NAAT)

Vasala et al. 2020. Front. Cell. Infect. Microbiol., 15 July 2020 | https://doi.org/10.3389/fcimb.2020.00308

AST : antimicrobial susceptibility testing

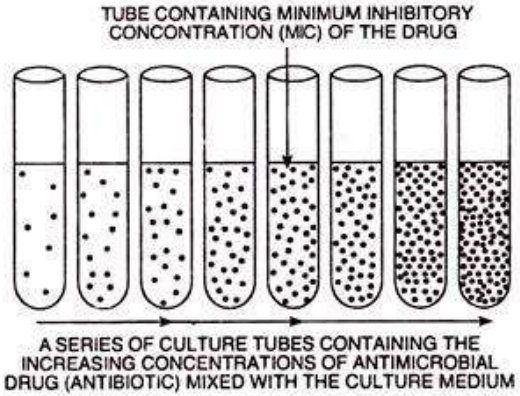


FIG. 46.10. Antimicrobial susceptibility test by broth dilution technique. Growth of microbial pathogen takes place only in those tubes that contain antimicrobial drug concentrations below the MIC.

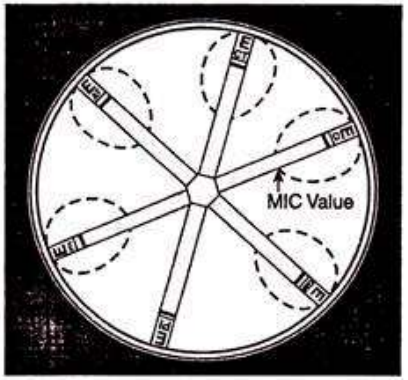


FIG. 46.14. Antimicrobial drug susceptibility determined by Etest. Different drugs used from 8 O'clock are PTc = piperacillin/tazobactam, AT = aztreonam, CT = cefotaxime, (CI = ciprofloxacin, GM = gentamicin, and IP = imipenem. The strips are arranged so that the lowest concentration of drug in each strip is at the centre of the plate. The lowest concentration of drug that inhibits microbial growth is the MIC value for that particular drug.

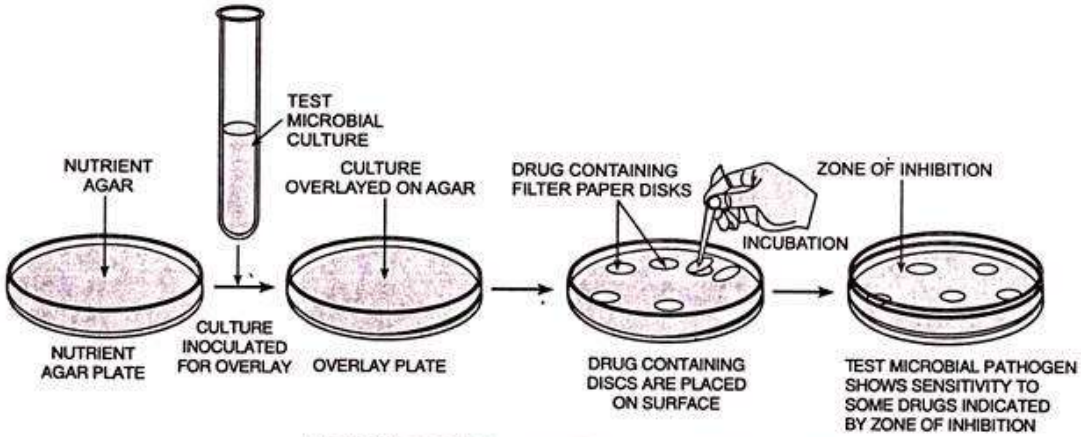


FIG. 46.11. Antimicrobial drug susceptibility test by agar diffusion technique.

TABLE 46.5. Inhibition zone diameter of some antimicrobial drugs (antibiotics)

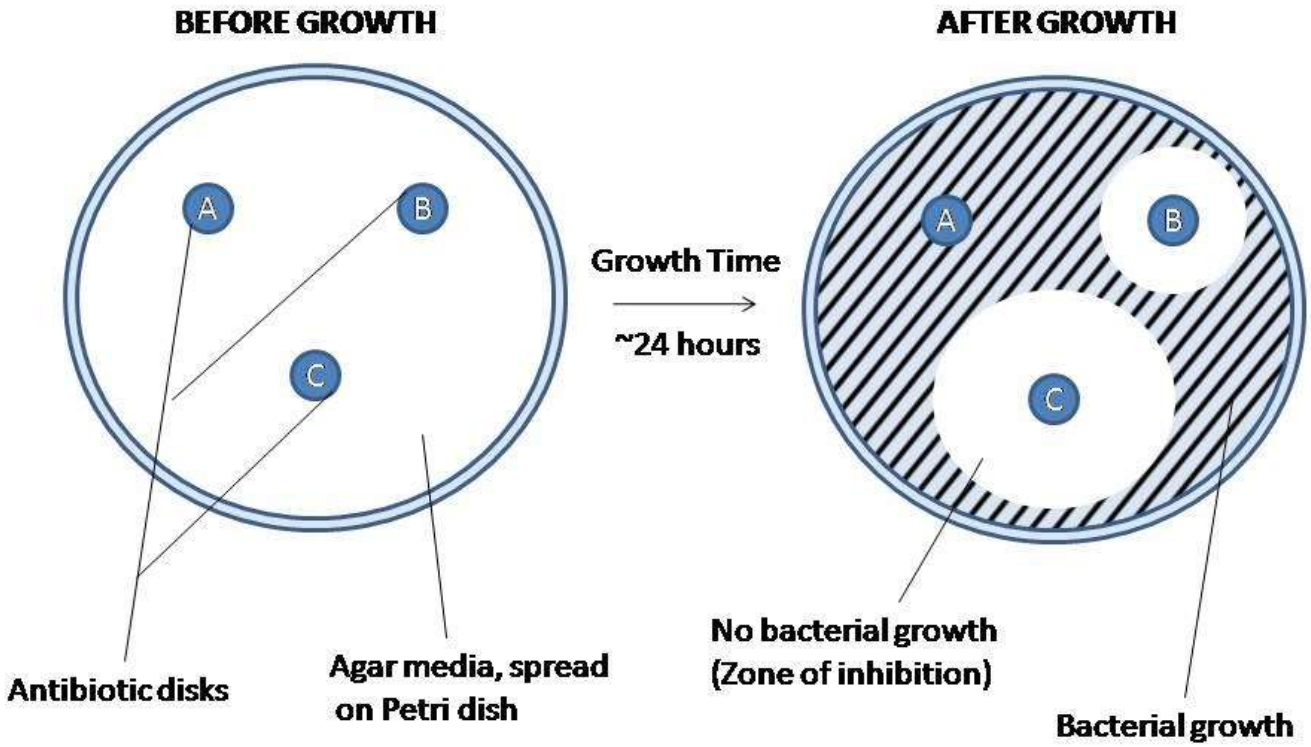
Antimicrobial drug (antibiotic)	*Inhibition zone diameter (nearest mm)*			
	Disc content	*Resistant*	*Intermediate*	*Susceptible*
Ampicillin (for Enterobacteriaceae)	10 µg	13 or less	14-16	17 or more
Ampicillin (for Staphylococci)	10 µg	28 or less	—	29 or more
Ceftriazone	30 µg	13 or less	14-20	21 or more
Chloramphenicol	30 µg	12 or less	13-17	18 or more
Erythromycin	15 µg	13 or less	14-22	23 or more
Gentamicin	10 µg	12 or less	13-14	15 or more
Methicillin (for Enterobacteriaceae)	5 µg	9 or less	10-13	14 or more
Penicillin G (for Staphylococci)	10 units*	28 or less	—	29 or more
Penicillin G (for other microbes)	10 units*	14 or less	—	15 or more
Streptomycin	10 µg	6 or less	7-9	10 or more
Sulfonamide	10 µg	12 or less	13-16	17 or more
Tetracycline	30 µg	14 or less	15-18	19 or more
Vancomycin (for Enterococci)	10 µg	14 or less	15-16	17 or more
Vancomycin (for Staphylococci)	10 µg	14 or less (test for MIC)	—	15 or more

*One milligram of penicillin G sodium = 1600 units.

[Tests for Antimicrobial Drug Susceptibility (biologydiscussion.com)]

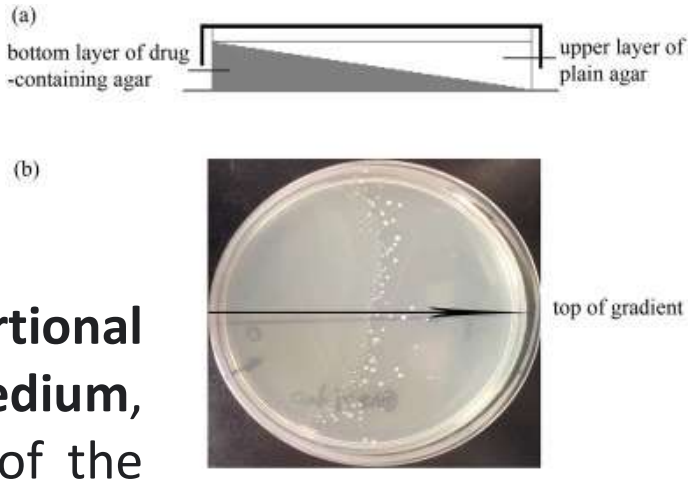
Antibiotic susceptibility testing

What are the different sensitivity testing methods? In-vitro antimicrobial susceptibility testing can be performed using a variety of formats, the most common being **disk diffusion, agar dilution, broth macrodilution, broth microdilution, and a concentration gradient test**.



Antibiotic susceptibility testing

MIC is the minimum inhibitory concentration of antibiotic (drug) that prevent the bacteria to grow any more, while **MBC** is the minimum bacteriocidal concentration of antimicrobial which kill all bacteria.

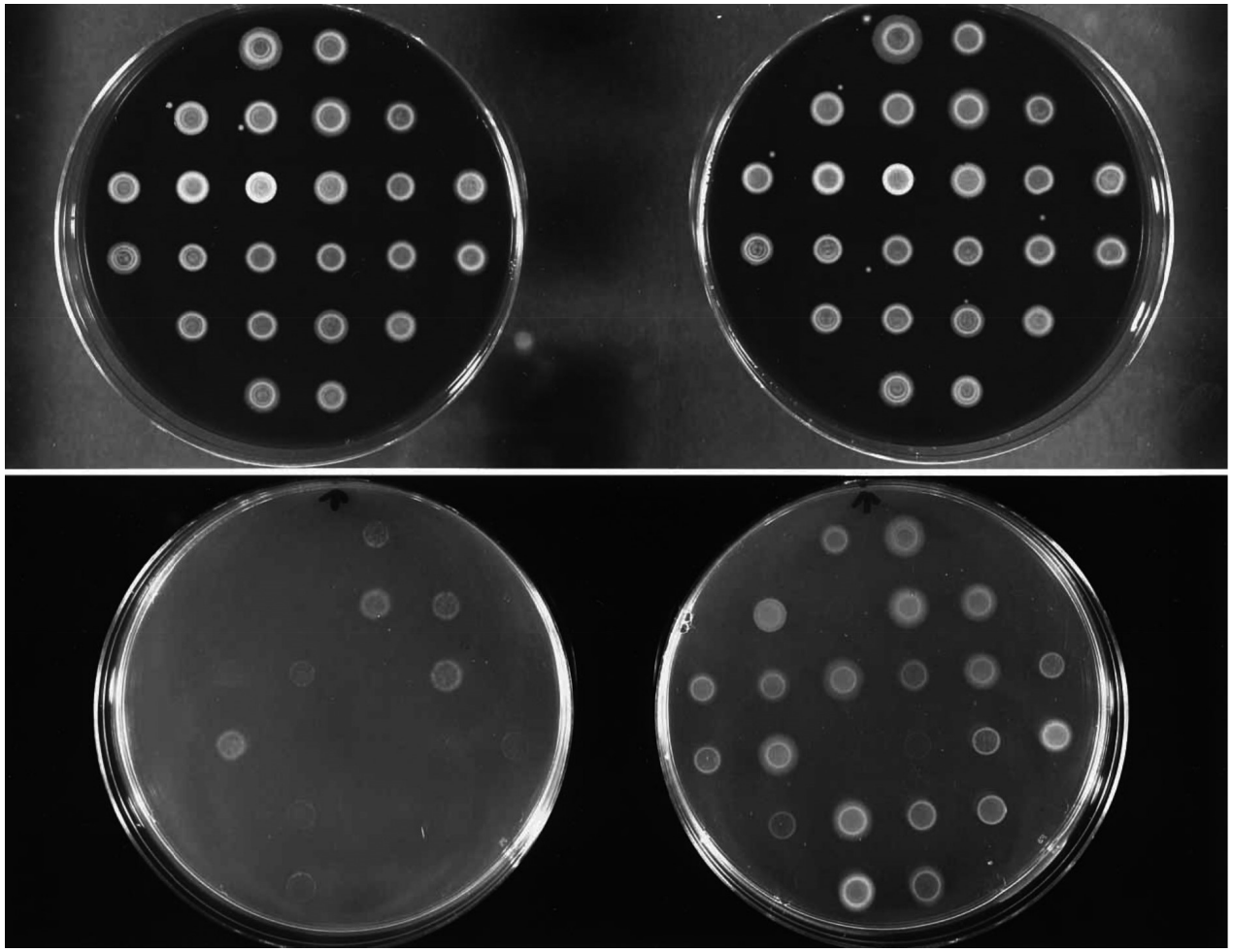


The gradient plate technique allows **a gradual, proportional increase of drug concentration in the agar medium**, extending over the entire cell monolayer. The slope of the gradient and the length of incubation do not seem to alter the results appreciably

ETEST is a well-established method for Minimum Inhibitory Concentration (MIC) determinations in microbiology laboratories around the world. ETEST **consists of a predefined gradient of antibiotic concentrations immobilized on a plastic strip and is used to determine the MIC of antibiotics and antifungal agents**.

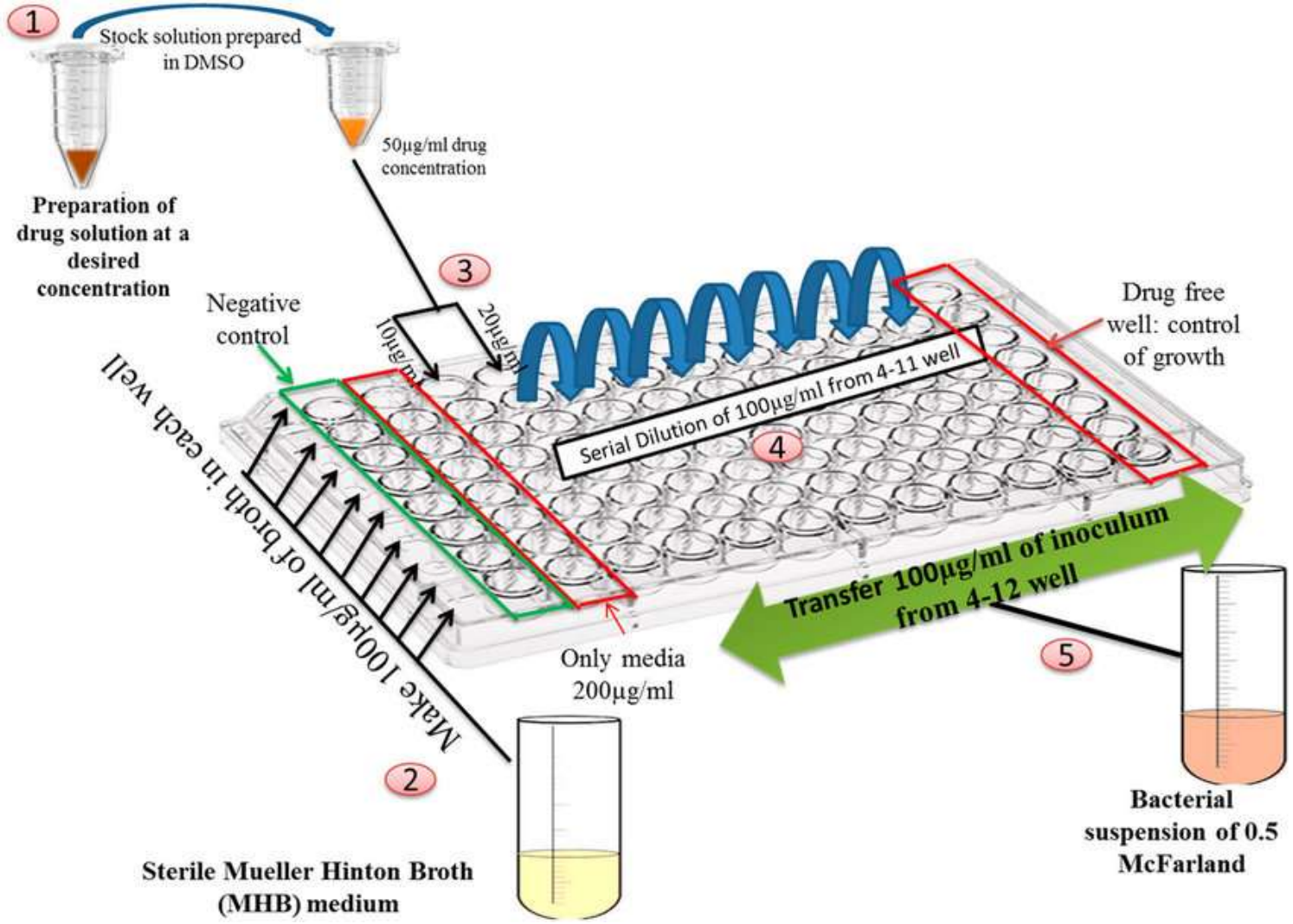
Antibiotic susceptibility testing

Agar dilution involves the incorporation of different concentrations of the antimicrobial substance into a nutrient agar medium followed by the application of a standardized number of cells to the surface of the agar plate.



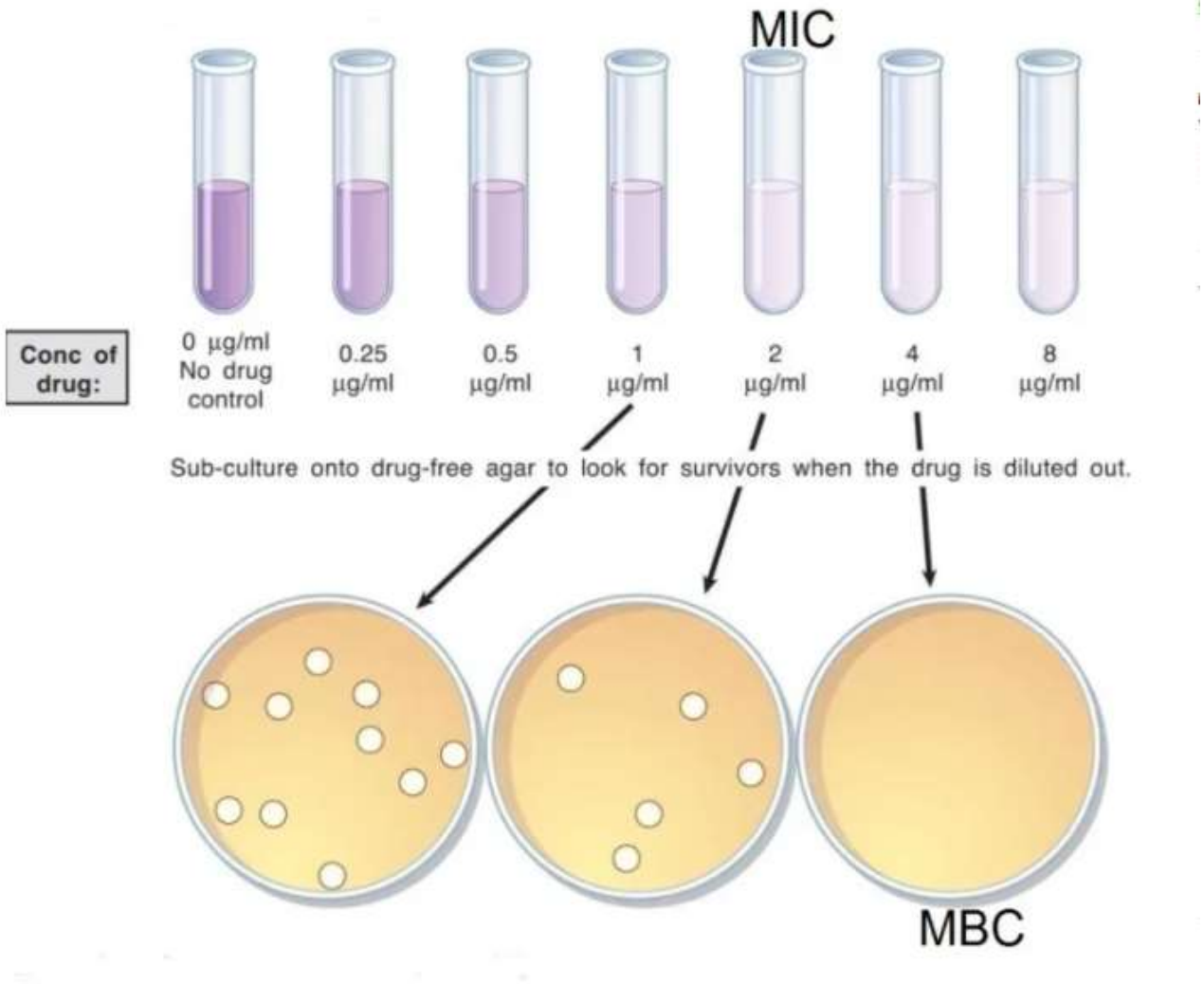
Antibiotic susceptibility testing

Broth microdilution is the standard method used in most reference laboratories. The method **typically tests twofold dilutions of multiple antimicrobial agents in 96-well disposable plastic trays.**

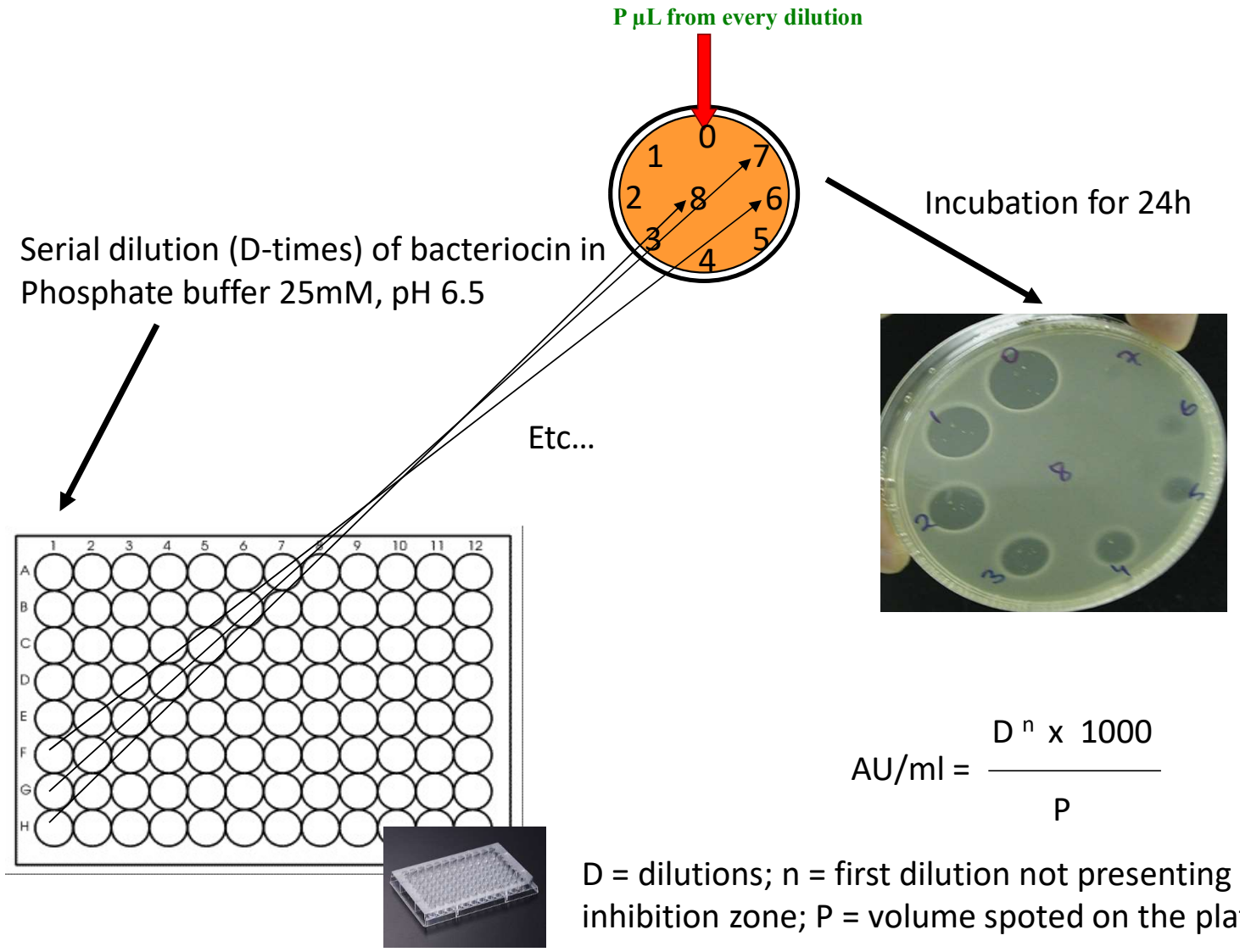


Antibiotic susceptibility testing

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation; and **Minimum bactericidal concentrations** (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.



Determination of the AU/mL for bacteriocins and antibiotics



$$AU/ml = \frac{D^{n} \times 1000}{P}$$

D = dilutions; n = first dilution not presenting inhibition zone; P = volume spoted on the plate