DTU Food
National Food Institute

Antimicrobials, antimicrobial resistance and antimicrobial susceptibility testing

The basics

CRL Training course
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What is an antibiotic?

Antibiotic is a substance **produced by a microorganism**, that have the capacity, in dilute solution, to **selectively inhibit or kill** other microorganisms (Paul Vuillemin 1941).

Some of the most toxic substances in the world
0.001 – 4 mg/L

Note the minimum effects on eucaryotic cells

Definition of an antimicrobial agent

Antimicrobial agents is a broader term - Referring to any substance that can affect microbial life, including synthetic and semi-synthetic compounds and substances without selective toxicity (e.g. disinfectants)

Origin of antimicrobial classes

Class	Antimicrobial agent(s)	Producing organism(s)	Yr of isolation
β-Lactam antibiotics	Natural penicillins	*Penicillium notatum*, *Penicillium chrysogenum*	1929, 1940
	Cephalosporin C	*Cephalosporium acremonium*	1945, 1953
	Imipenem	*Streptomyces cattleya*	1976
	Aztreonam	*Gluconobacter* spp., *Chromobacterium violaceum*	1981
Glycopeptides	Vancomycin	*Amycolatopsis orientalis*	Mid-1950s
	Teicoplanin, avoparcin	*Amycolatopsis coloradensis* subsp. *labeda*	1975
Macrolides	Erythromycin	*Streptomyces erythraeus*	1952
	Spiramycin	*Streptomyces ambofaciens*	1955
Lincosamides	Lincomycin	*Streptomyces lincolnensis*	1963
Streptogramins	Streptogramins A and B	*Streptomyces diastaticus*	1953
	Virginiamycins A and B	*Streptomyces virginiae*	1955
Tetracyclines	Chlortetracycline	*Streptomyces aureofaciens*	1948
	Oxytetracycline	*Streptomyces rimosus*	
Phenicols	Chloramphenicol	*Streptomyces venezuelae*	1947
Aminoglycosides	Streptomycin	*Streptomyces griseus*	1943
	Neomycin	*Streptomyces fradiae*	1949
	Kanamycin	*Streptomyces kanamyceticus*	1957
	Gentamicin	*Micromonospora purpurea*	1963
	Tobramycin	*Streptomyces tenebrarius*	1967
Aminocyclitols	Spectinomycin	*Streptomyces spectabilis*	1961
Pleuromutilins	Tiamulin	*Pleurotus* spp.	
Polypeptide antibiotics	Polymyxin B	*Bacillus polymyxa (aerosporus)*	1947
	Polymyxin E (colistin)	*Bacillus polymyxa* subsp. *colistinus*	1949
	Bacitracin	*Bacillus licheniformis*	1943
Sulfonamides	Prontosil, sulfamethoxazole, etc.	Synthetic	1935
Trimethoprim	Trimethoprim	Synthetic	1956
Quinolones	Nalidixic acid	Synthetic	1962
Fluoroquinolones	Flumequine, enrofloxacin, etc.	Synthetic	1973
Oxazolidinones	Linezolid	Synthetic	1987, 1996

Bactericidal vs. Bacteriostatic antimicrobials

Class	Origin	Activity
Aminoglycosides	*Streptomyces*, *Micromonospora sp*	Bactericidal
Cephalosporins	*Cephalosporium* sp	Bactericidal
Macrolides	Various Actinomycetes	Bacteriostatic
Penicillins	*Penicillium* sp	Bactericidal
Phenicols	*Streptomyces venezuelae**	Bacteriostatic
Quinolones	Synthetic	Bactericidal
Rifamycins	*Amycolatopsis mediterranei*	Bactericidal
Sulfonamides	Synthetic	Bacteriostatic
Tetracyclines	*Streptomyces* sp	Bacteriostatic

Table 1. Antimicrobial agents approved for use in human and veterinary medicine.

Antimicrobial class/drug	Examples of antimicrobials used in human medicine	Examples of antimicrobials used in veterinary medicine or as growth promoters
Antimicrobial classes classified as 'critically Important' for human health by the WHO		
Aminoglycosides	Amikacin, arbekacin, gentamicin, kanamycin, netilmicin, neomycin , tobramycin , streptomycin	Amikacin, apramycin, gentamicin, neomycin, streptomycin, dihydrostreptomycin, kanamycin, framycetin, paromomycin (aminosidine)
Ansamycins	Rifabutin, rifampin, rifaximin	Rifampicin
Carbapenems and other penems	Ertapenem, faropenem, imipenem, meropenem, doripenem	None approved or known to be used
Cephalosporins, third generation	Cefixime, cefotaxime, cefpodoxime, ceftazidime, ceftizoxime, cefoperazone, cefoperazone/sulbactam, ceftriaxone	Cefpodoxime, ceftiofur, cefoperazone, cefovecin
Cephalosporins, fourth generation	Cefipime, cefpirome, cefoselis	Cefquinome
Lipopeptides	Daptomycin	None approved or known to be used
Glycopeptides	Teicoplanin, vancomycin	Avoparcin*
Macrolides, including 14-, 15-, 16-membered compounds, ketolides	Azithromycin, clarithromycin, erythromycin, midecamycin, roxithromycin, spiramycin, telithromycin	Erythromycin, pirlimycin, spiramycin, tylosin, tulathromycin, kitasamycin, oleandomycin, tilmicosin, jasamycin
Oxazolidinones	Linezolid	None approved or known to be used
Penicillins, amino	Ampicillin/amoxicillin, ampicillin/sulbactam, amoxicillin/clavulanate, piperacillin, piperacillin/tazobactam	Ampicillin/amoxicillin, ampicillin/sulbactam, amoxicillin/clavulanate
Penicillins, natural	Penicillin G, penicillin V	Penicillin G, penicillin V
Quinolones	Cinoxacin, nalidixic acid, pipedemic acid, ciprofloxacin, enoxacin, gatifloxacin, gemifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, sparfloxacin	Nalidixic acid, oxolinic acid, flumequine, pipemidic acid, danofloxacin, difloxacin, enrofloxacin, ibafloxacin, marbofloxacin, sarafloxacin, orbifloxacin, moxifloxacin
Streptogramins	Quinupristin/dalfopristin, pristinamycin	Virginiamycin‡
Drugs used solely to treat tuberculosis or other mycobacterial disease	Cycloserine, ethambutol, ethionamide, isoniazid, para-aminosalicylic acid, pyrazinamide	None approved or known to be used
Antimicrobial classes classified as 'highly important' for human health by the WHO		
Cephalosporins, first generation	Cefazolin, cephalexin, cephalothin, cephradine	Cephalothin, cephalonium, cephalexin, cefadroxil, cefazolin
Cephalosporins, second generation	Cefador, cefamandole, cefuroxime, loracarbef	Cefuroxime
Cephamycins	Cefotetan, cefoxitin	None approved or known to be used
Clofazimine	Clofazidime	None approved or known to be used
Monobactams	Aztreonam	None approved or known to be used
Penicillins, amino	Mecillinam	None approved or known to be used
Penicillins, antipseudomonal†	Azlocillin, carbenicillin, mezlocillin, ticarcillin, ticarcillin/clavulanate	None approved or known to be used
Polymyxins	Polymyxin B, colistin	Polymyxin B, colistin

*Up until 2000, avoparcin was used extensively as a growth promoter around the world (except in North America).
‡Up until 2000, virginiamycin was used extensively as a growth promoter in Europe. It is still used extensively in North America, Australia and many other parts of the world.
†Extensively used for growth promotion and/or control of coccidiosis around the world.

Targets of antimicrobial action

Cell wall synthesis
β-lactams:
Penicillin
Cephalosporins
Monobaktams
Carbapenems
Vancomycin

DNA replication
Fluoquinolones
Metronidazole

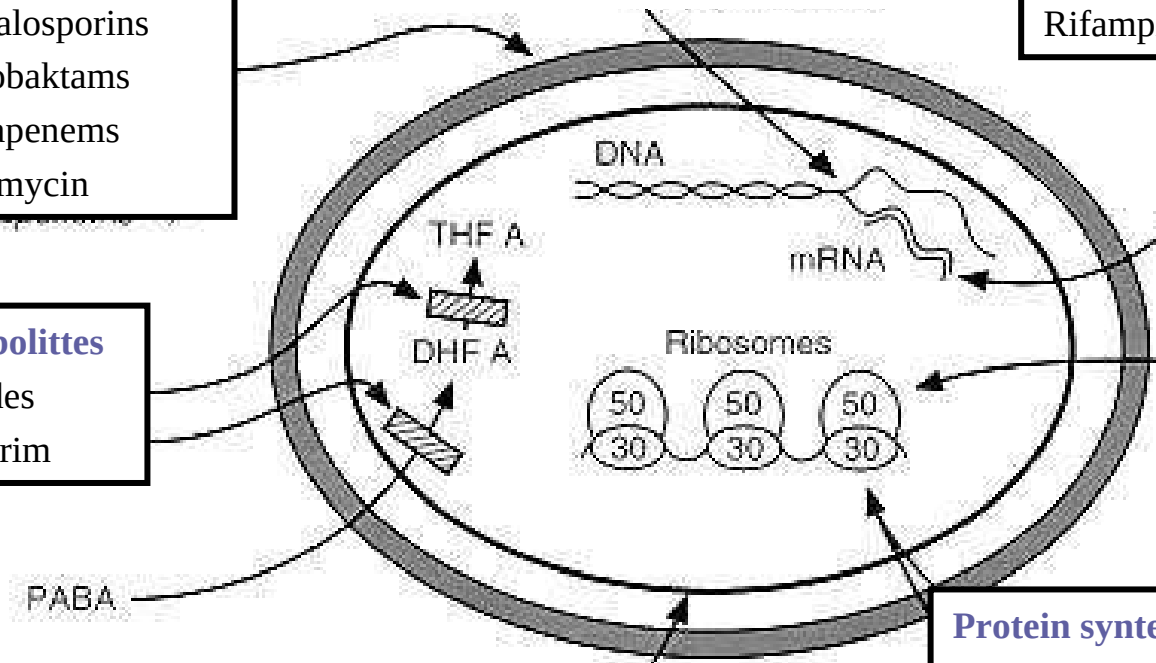
RNA-polymerase
Rifampicin

Protein syntese Inhibitors (50s)
Macrolides
chloramphenikol
Clindamycin

Anti metabolittes
Sulfonamides
Trimethroprim

Protein syntese Inhibitors (30s)
Tetracyclines
Aminoglykocides
Fucidinic acid

Cell membrane
Polymyxin
Amphotericin



Modes of antimicrobial action

- Inhibition of cell wall synthesis
- Inhibition of DNA synthesis
- Inhibition of protein synthesis
- Inhibition of RNA synthesis
- Inhibition of folic acid synthesis
- DNA breakage
- Disruption of osmotic integrity

Note - A limited number of targets

What is antibiotic resistance?

Microbiological definition:

- Resistance is the property of a bacterial strain to survive at higher antibiotic concentrations compared with most other members of the same species (Wildtypes)

Clinical definition:

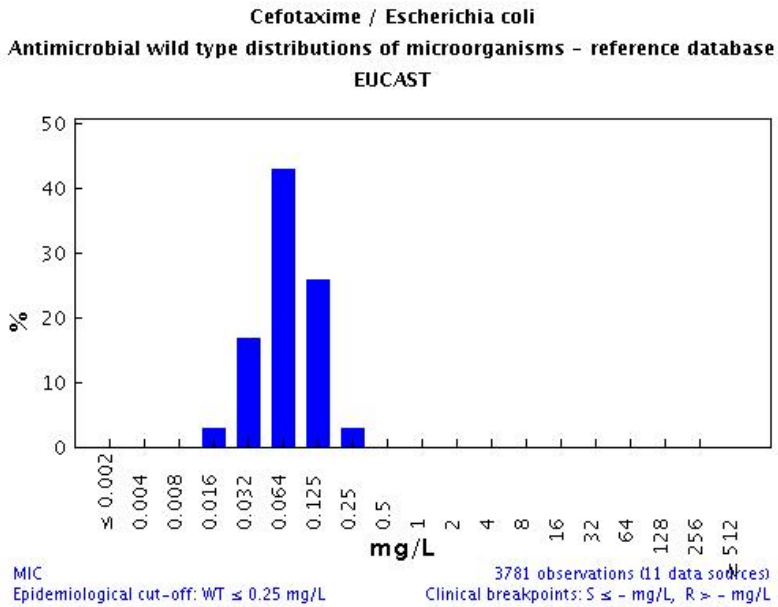
- Resistance is the ability of a bacterial strain to survive antimicrobial therapy

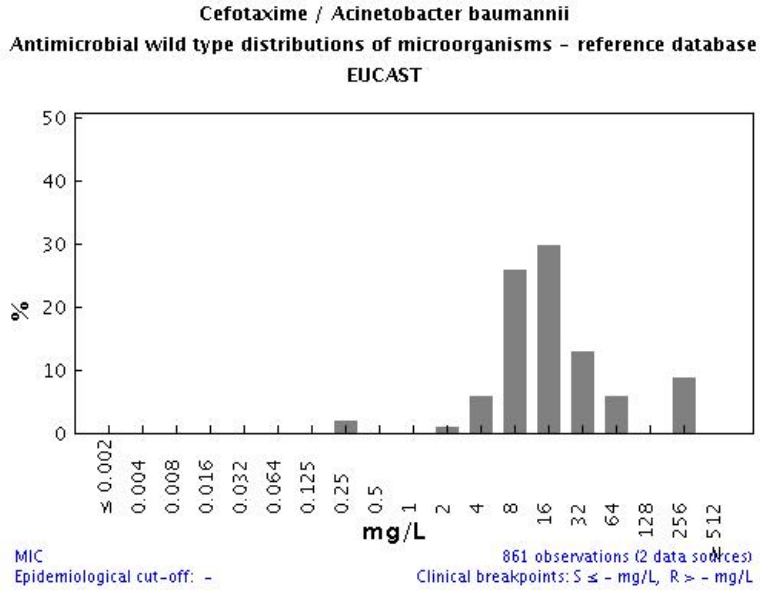
What is intrinsic resistance?

Resistance due to a structural or functional trait allowing tolerance by all members of a bacterial group (species, genus or even larger group)

- Low affinity of the target
- Impermeability
- Active exporters
- Enzymatic degradation

Example of intrinsic resistance
Cefotaxime susceptibility in
E. coli and *Acinetobacter baumannii*



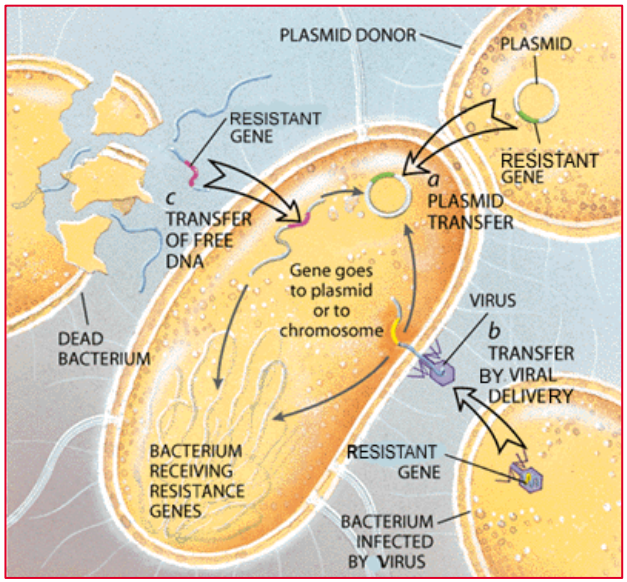


Two types of acquired resistance

Mutation (endogenous, vertical)

Gene transfer (exogenous, horizontal)

- Transformation (free DNA)
- Transduction (with phages)
- Conjugation (plasmids – active process)



What is cross-resistance

Resistance to two related (avoparcin / vancomycin) or unrelated drugs (erythromycin / lincosamides) is due to a single biological mechanism

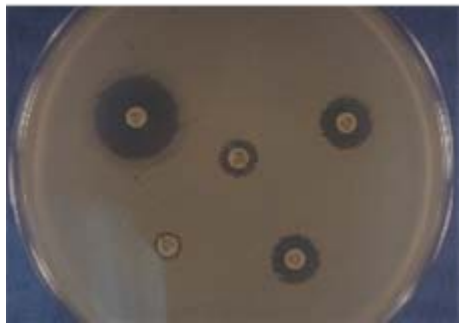
Mechanisms of resistance

- Inactivation of the drug
  - Penicillins, aminoglycosides
- Target modification
  - Tetracyclines, fluoroquinolones
- Drug trapping or titration - hyperproducers
  - Penicillins, sulphonamides
- Impermeability
  - Broad range of antibiotics (only reduced susceptibility)
- Active efflux
  - Disinfectants, metals

How do we measure antimicrobial susceptibility

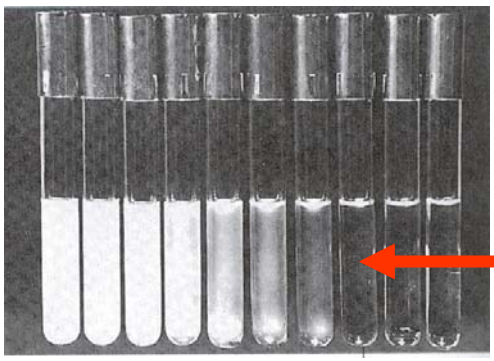
Agar diffusion method

- Disk (tablet) methods
- E-test (quantitative)



Dilution methods

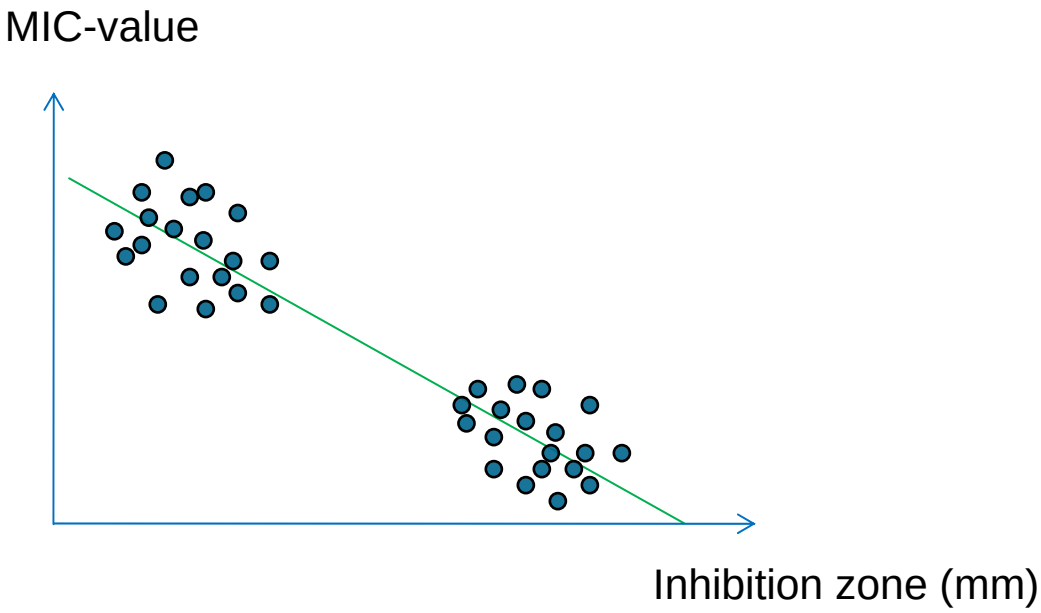
- Micro broth dilution - Liquid media (quantitative)
- Macro broth dilution - Liquid media (quantitative)
- Agar dilution - Solid media (quantitative)



MIC

Correllation between MIC and DD

MIC and inhibition zones should correllate for each antimicrobial for both slow growing and fast growing bacteria



Guidelines

International standards describe the methods (DD and MIC) in detail: media, inoculum, incubation, etc. - e.g. CLSI (Clinical and Laboratory Standards Institute)

- CLSI has defined QC reference strains and corresponding the acceptable QC results
- CLSI recommends clinical breakpoints for interpretation

Resistant: Treatment failure can be expected

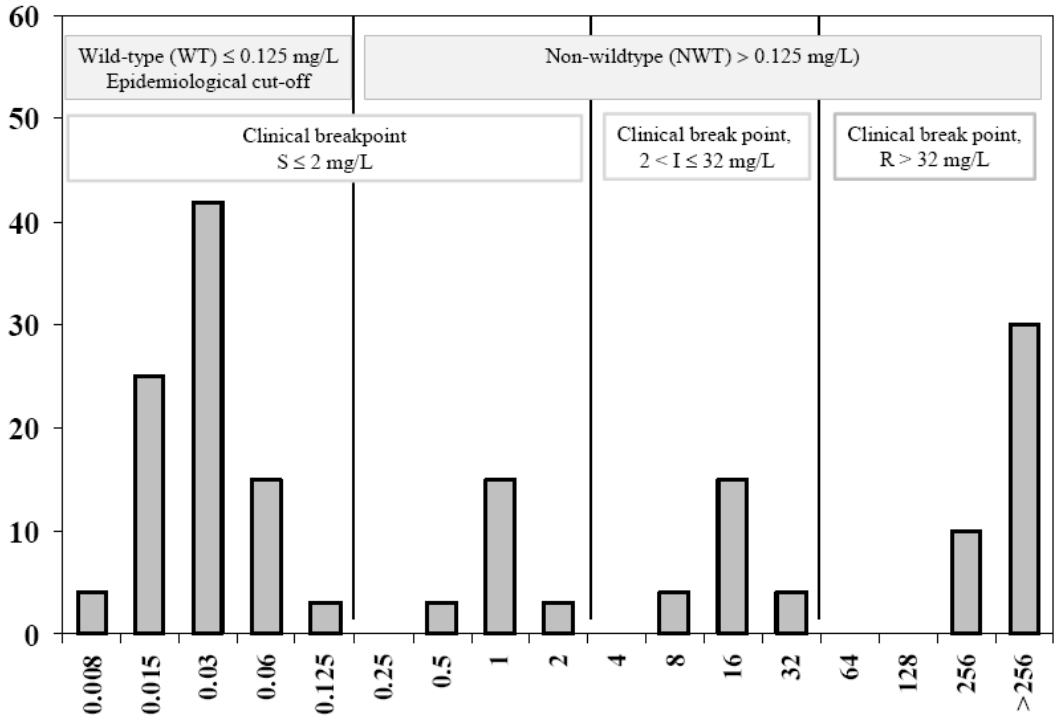
Sensitive: Succesful treatment can be expected

Intermediary: Treatment is possible if the infection is in bodysites where the antimicrobial is concentrated. Primarily a bufferzone to avoid misinterpretation

Epidemiological Cut Off Values

Defined by EUCAST (The European Committee on Antimicrobial Susceptibility Testing – www.eucast.org)

- for monitoring purpose only, no relation to clinical data
- defined by the wild type distribution of the bacteria
- all isolates with MICs above the WT distribution are called resistant



WT = Wild type organisms, i.e micro-organisms without phenotypically detectable antimicrobial resistance to the drug in question
S = Susceptible; I = Intermediate; R = Resistant

Techniques - Pros and Cons

MIC determination

- Golden standard for AST
- Data more reproducible
- Better separation of R/S
- More information
- Expensive
- Only pure cultures
- Contaminations more difficult to detect

Diffusion techniques

- Cheaper
- Primary material
- See contaminations
- Quick screening (4 hours)
- Qualitative information
- Less reproducible data
- Standardisation more difficult

Disk diffusion - Considerations

Disk diffusion methods gives reliable and reproducible data, if standardization and quality assurance is used

- Use international guidelines (e.g. CLSI)
- Standardize the method (inoculum, media, incubation, reading of results)
- Use ring tests (define criteria for acceptance, evaluate results)
- Use reference strains for method validation
- Act on deviations on the QC-strain
- Document corrective actions

Standardisation

All methods are extremely sensitive to variations in performance

Factors that influence the result:

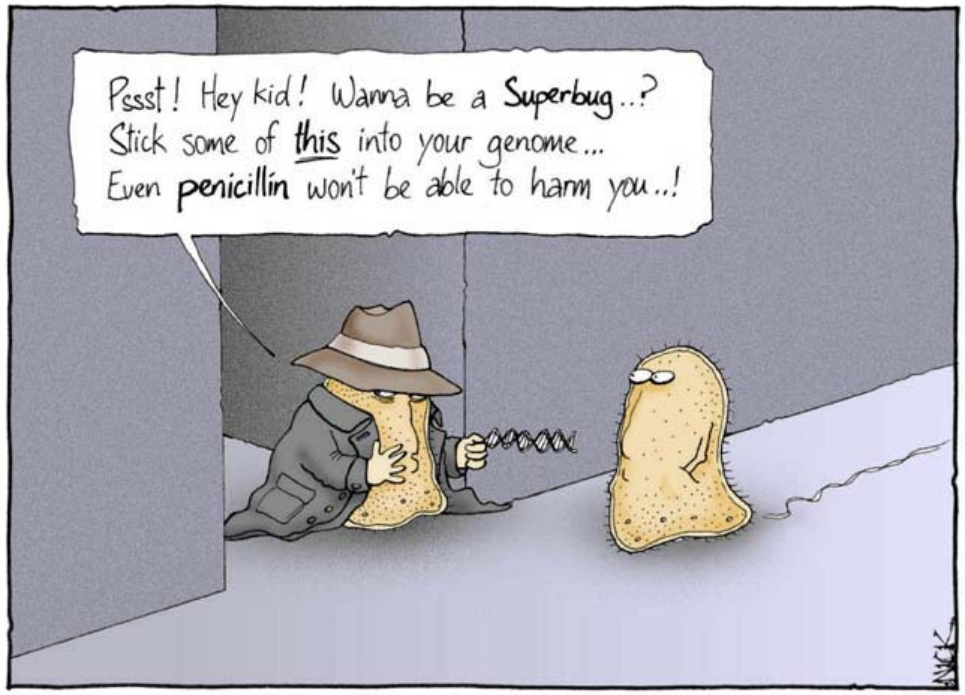
- Size of inoculum
- Contents and acidity (pH) of the broth or agar
- Incubation time and temperature
- Reading procedures

Moreover, for the *diffusion methods*:

- Diffusion rate of the antimicrobial into the agar
- Depth of the agar
- Dryness of the agar
- Growth rate of the bacteria

Community Reference Laboratory Antibiotic Resistance

WHO Global Salm-Surv
www.who.int/salmsurv



Thank you!

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