SPITALUL JUDEȚEAN DE URGENȚĂ
„SFÂNTUL IOAN CEL NOU” – SUCEAVA

UNIVERSTATEA ȘTEFAN CEL MARE SUCEAVA

REZISTENŢA LA ANTIBIOTICE PRIN PRODUCEREA DE BETA LACTAMAZĂ

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Take home messages:

- Clasificarea beta lactamazelor- evoluţie
- Emergenţa rezistenţei la bacterii Gram negativ
- Bacterii producătoare de carbapenemaze
- Beta lactamze rezistente la inhibitori
- Mecanisme de rezistenţă asociate
- Bacterii cu multiple beta lactamaze
- Rolul **laboratorului**

Clinicianul- “cere şi ţi se va da”

Tehnicile de biologie moleculară “ from bench to molecular chess”

"Lasciate ogni speranza, o voi che entrate"Divina Commedia di Dante Alighieri.

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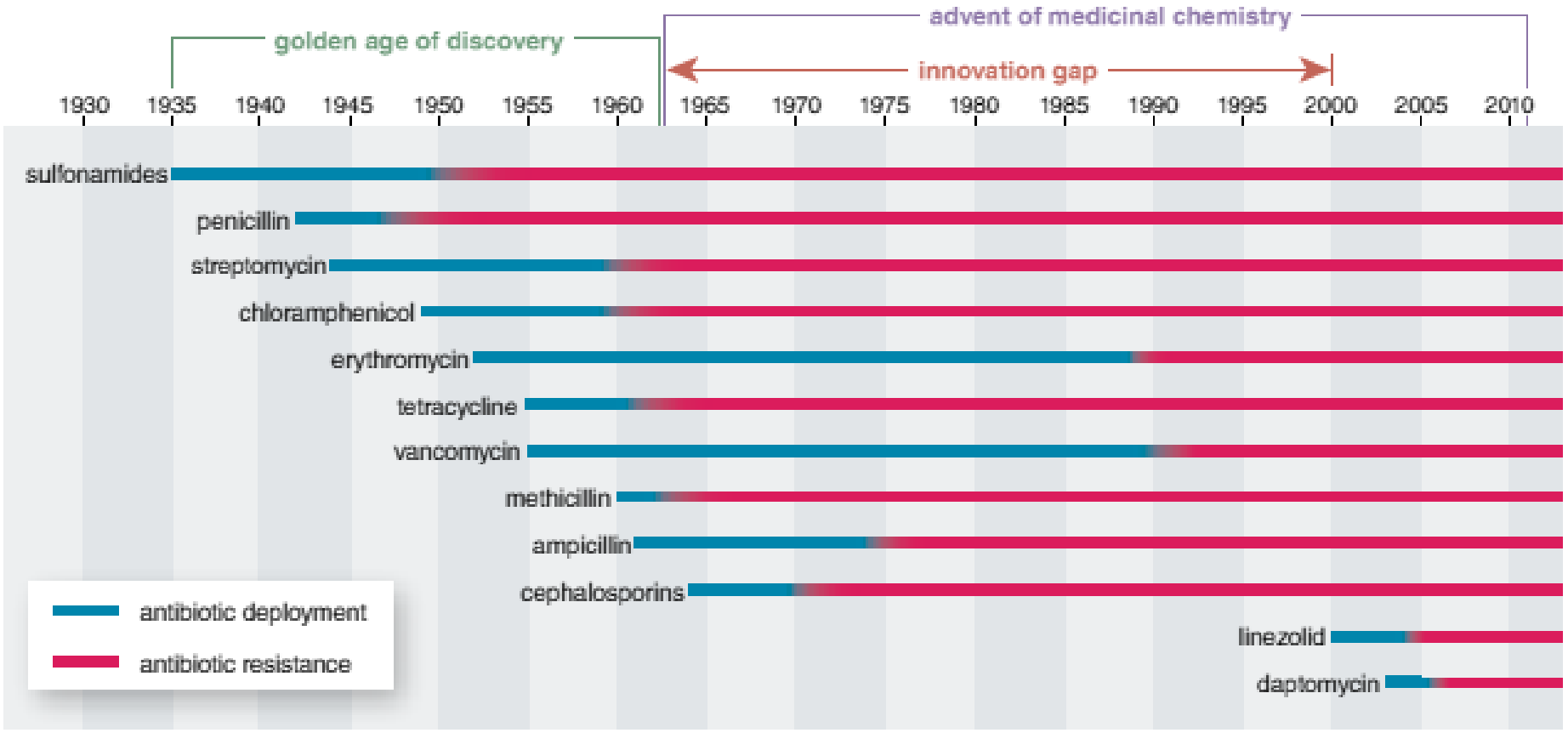
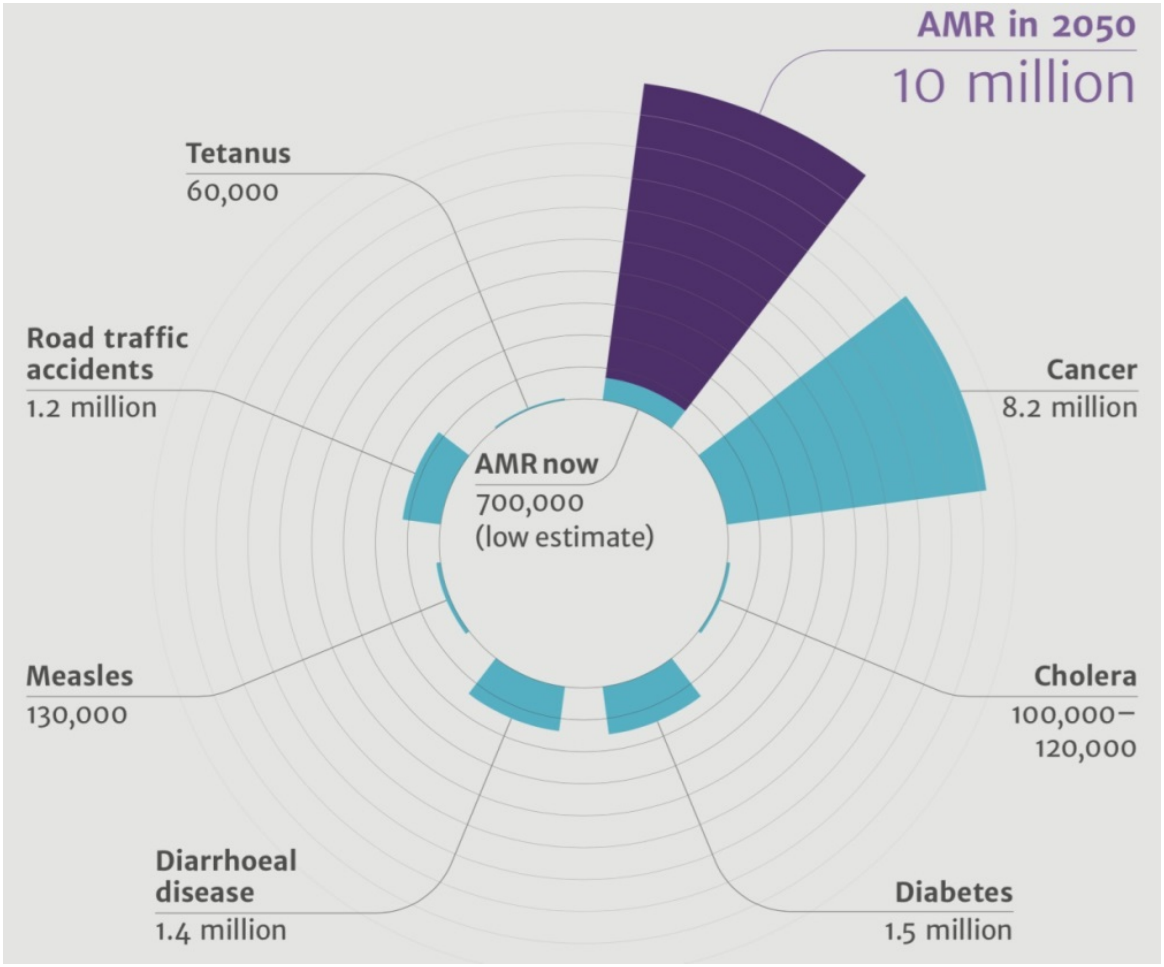
The rate at which microbes are acquiring resistance is GREATER than the rate at which antimicrobials are being discovered





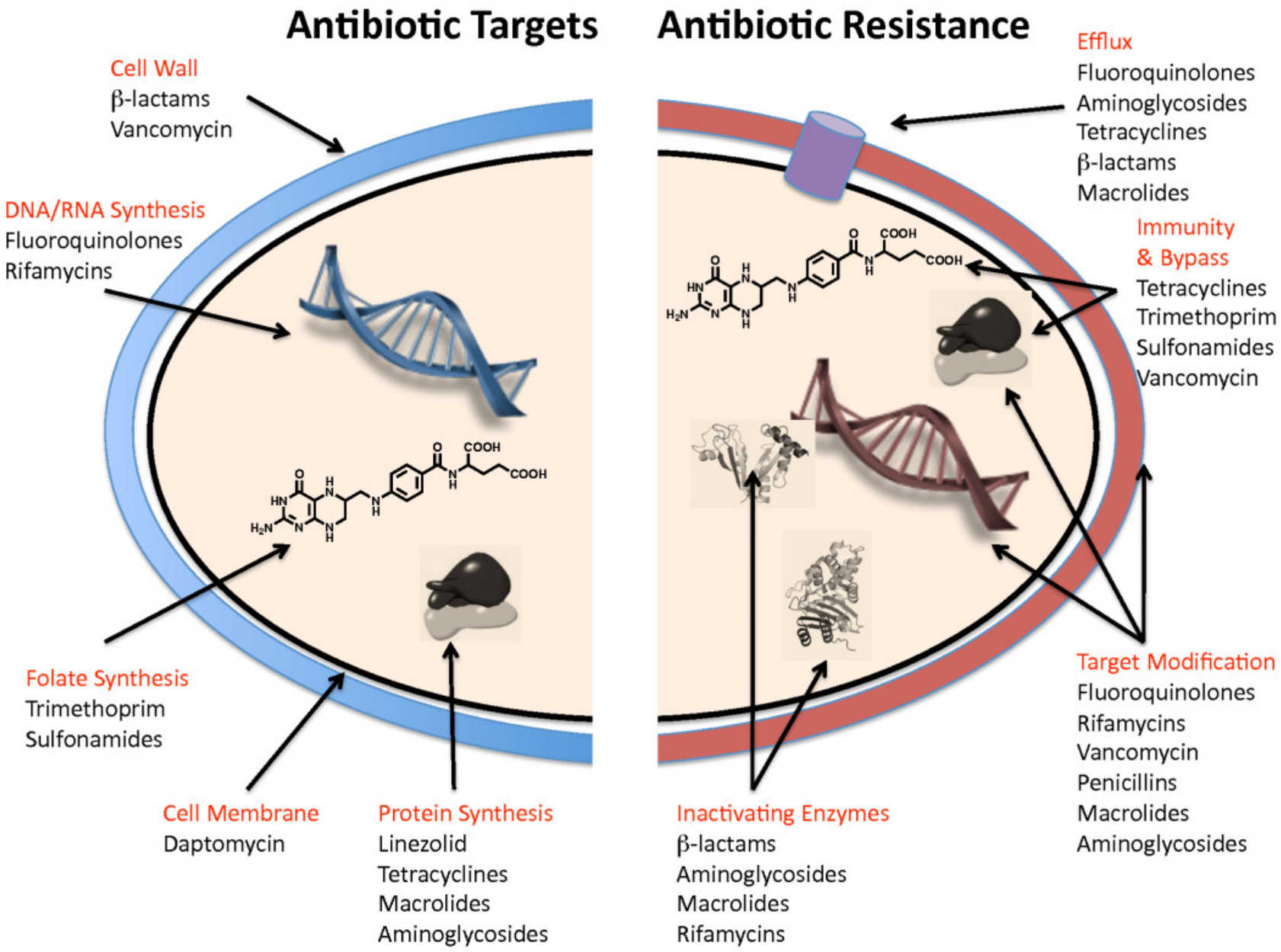
From **7%** to **50%** deaths related to infection

>**25 000 death/y** from multidrug resistant bacteria (EMA/ECDC report, Cassini et al., 2019)

Increased mortality rates

Estimated annual cost for healthcare systems and gross domestic product loss of $300 billion (Naylor et al., 2018).

Surgery, transplants, and chemotherapy - no longer viable due to infection



Gerard D Wright - http://www.biomedcentral.com/content/figures/1741-7007-8-123-1-l.jpg Antibiotic targets and mechanisms of resistance. See text for details. Wright BMC Biology 2010 8:123

How does resistance occur

Resistance = **natural** phenomenon **accelerated** by the **misuse** of antimicrobial drugs - WHO Fact Sheet No. 194 (2014)

Natural

naturally occurring resistance genes*

RNA-methylase, ABC-ATP Binding Cassette type transporters, aminoglycoside-phosphotransferases and β-lactamases

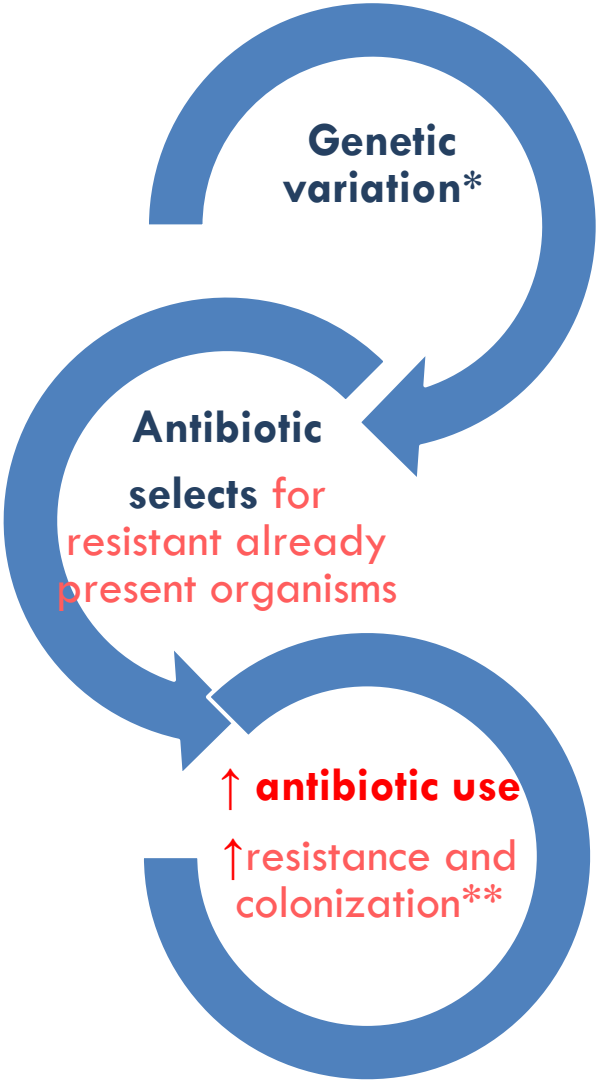
random mutations of biosynthesis genes providing a selective advantage to cells

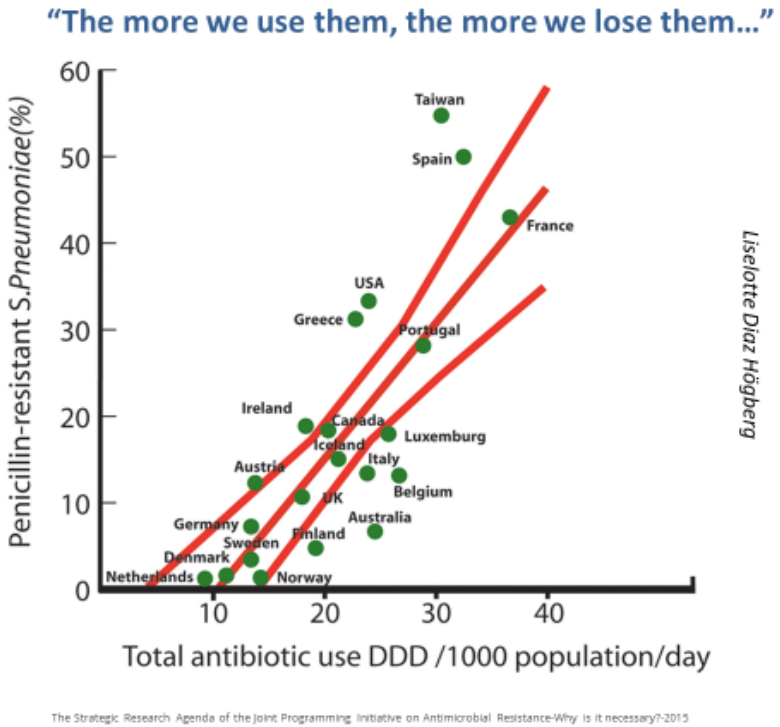
co-selection of antibiotics and antibiotic resistance genes (Fajardo and Martinez, 2008).

Acquired

Initially susceptible bacteria become resistant

How does resistance evolve?





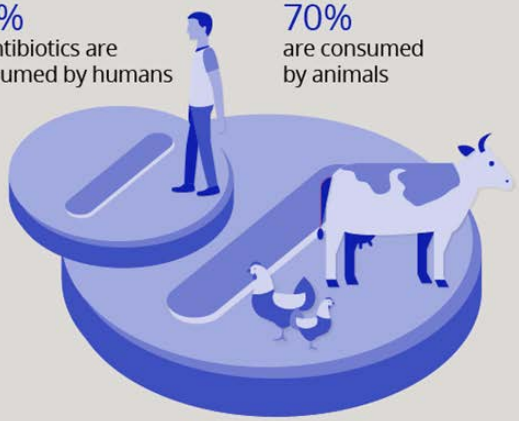
Antibiotics in humans and animals

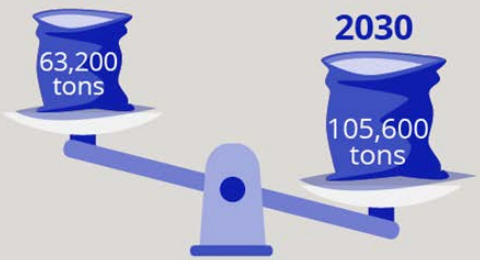
LONDON SCHOOL of HYGIENE & TROPICAL MEDICINE

2012

30%
of antibiotics are consumed by humans

70%
are consumed by animals



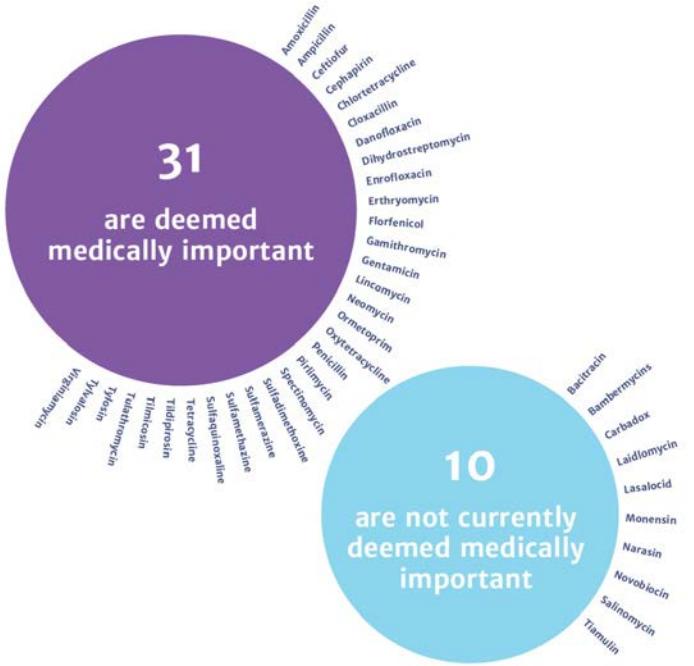


By 2030
Global consumption of antibiotics in livestock production to increase by two-thirds

Source: Review on antimicrobial resistance
Credit: Rebeccah Robinson/LSHTM

MOST ANTIBIOTICS USED IN ANIMALS ARE MEDICALLY IMPORTANT FOR HUMANS

Of the 41 antibiotics* that are approved for used in food producing animals by the FDA, 31 are categorised as being medically important for human use.

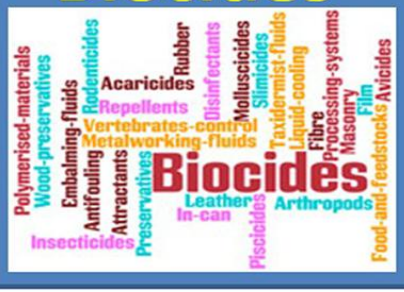


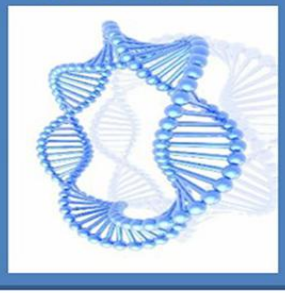
Source: FDA, 2012 Summary report on Antimicrobials sold or distributed for use in Food-producing animals.

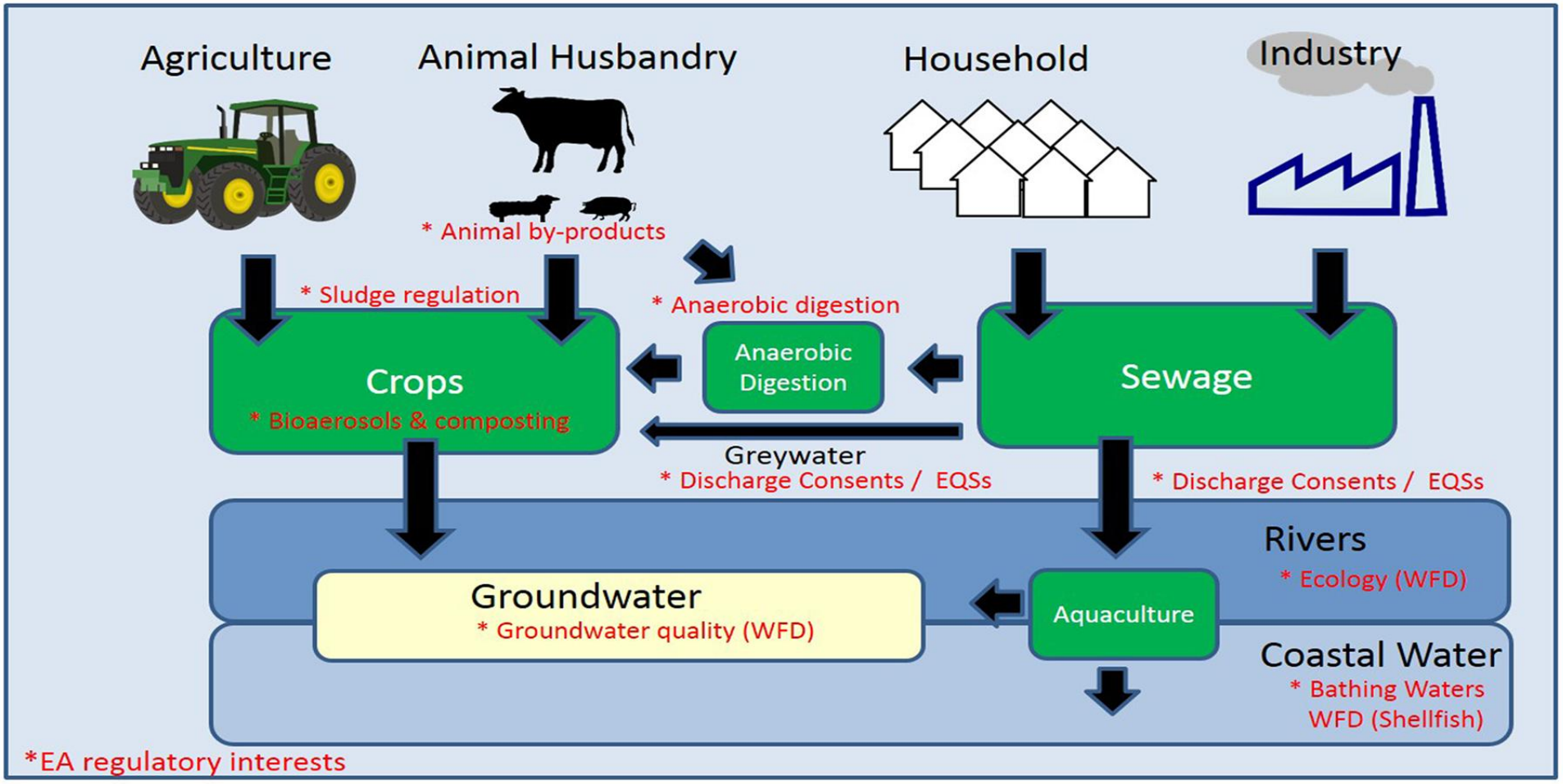
* Includes Ionophores

Review on Antimicrobial Resistance

Drivers of Antimicrobial Resistance





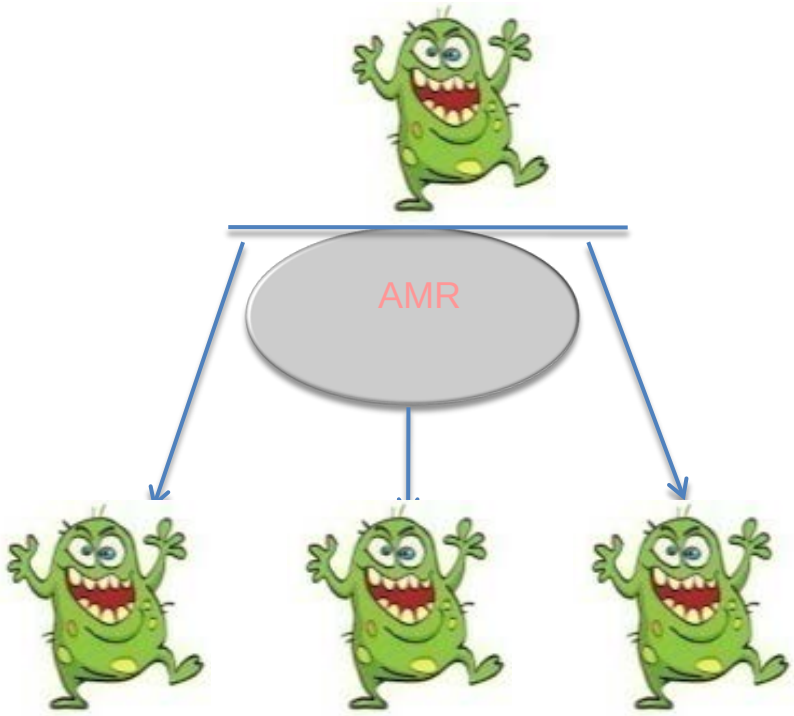


Singer et al., Front. Microbiol., 2016 | https://doi.org/10.3389/fmicb.2016.01728

GENETIC RESISTANCE

(1)VERTICAL

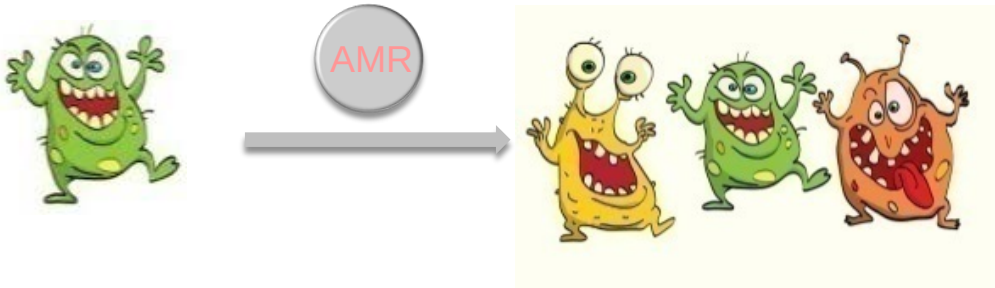
Spontaneous mutation



(2) HORIZONTAL

Horizontal transfer of AMR genes

Mobile Genetic Elements



Bacteria have generally a short generation time (less than 1h)!

Important acronymes

- MRSA- METHICILLIN-RESISTANT *S. aureus*
- PRSP- PENICILLIN RESISTANT *Str. pneumoniae*
- VISA- VANCOMYCIN (GLYCOPEPTIDE)-INTERMEDIATE *S. aureus*
- VRSA- VANCOMYCIN-RESISTANT *S. aureus*
- VRE- VANCOMYCIN R *Enterococcus faecium*
- ESBLs - Extended-spectrum β-lactamases (R Pen, Cefalosp. incl. 3rd gen, Monobactames+/-Carbapenemes)
- CRE – CARBAPENEMASE PRODUCING *ENTEROBACTERIACEAE*

Important acronymes

- *Multidrug-resistant* (MDR) – Resistant to at least three classes
- *Extensively drug-resistant* (XDR): resistant to all but one classes
- *Pan drug-resistant* (PDR): resistant to all tested antibiotics

SPICE (SPACE) Organisms - AmpC Resistance

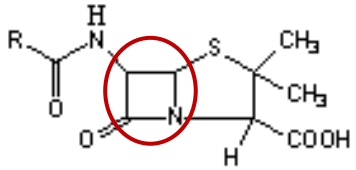
Gram-negative bacteria that have inducible, chromosomal beta-lactamase genes known as AmpC.

Resistance may not be detectable initially, but appears after a period of exposure to beta-lactam antibiotics

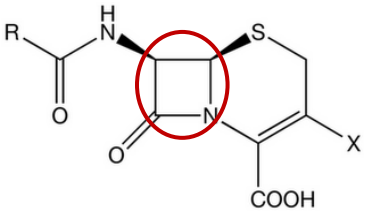
Serratia
Providencia
"**I**ndole-positive" (Proteus, Morganella, Providencia) species / **A**cinetobacter
Citrobacter
Enterobacter species

Other organisms in this class include: Acinetobacter, Cronobacter, Edwardsiella, Hafnia, Morganella, and rarely Pseudomonas

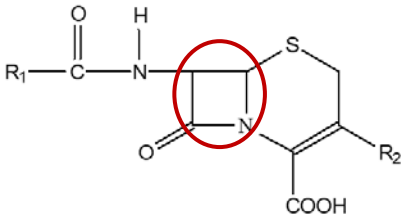
- ESKAPE(E) -*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter sp. (Escherichia coli)*
- ESCAPE -*Enterococcus faecium, Staphylococcus aureus, Clostridium difficile, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacteriaceae*



Penicillin

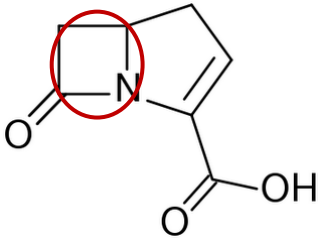


Monobactam



Cephalosporin

Beta Lactams



Carbapenem

Penicillins

PCN

Penicillin G
Benzathine PCN
VK PCN (PO)

Anti-Staph

Oxacillin (IV)
Nafcillin (IV)
Dicloxicillin (PO)

Amino-PCN

Amp +/-
Sulb (IV) #
Amox +/-
Clav (PO) #

Anti-Pseud

Pip +/- Tazo ^ #
Tic +/- Clav ^ #
(not available)

Cephalosporins

Increasing gram neg coverage

1. Cephalexin
   Cefazolin
2. Cefuroxime
3. Ceftriaxone
   Ceftazidime ^
4. Cefepime ^

---

5. Ceftaroline
   (Like CTX+MRSA)

Extended GNR

Ceftol-Tazo * ^ ~
Ceftaz-Avi * ^ ~

Monobactam

Aztreonam ^

- Aerobic Gram neg
- Pseudomonas
- Bad 4 gram pos
- Bad 4 anaerobes

KEY

* ESBL
^ Pseudomonas
~ Carbapenem-R
# Anaerobes

Carbapenems

Imipenem * ^ #

Meropenem * ^ #
+/- vaborbactam ~

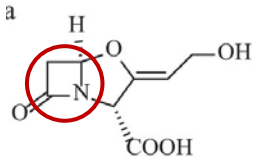
Doripenem * ^ #

[Ertapenem] * #

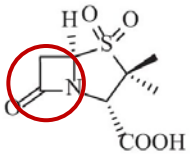
- No pseudomonas
- 1x daily dosing

D. Serota 2018

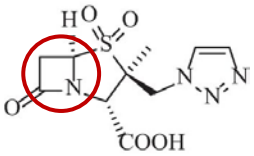
beta-lactamase target



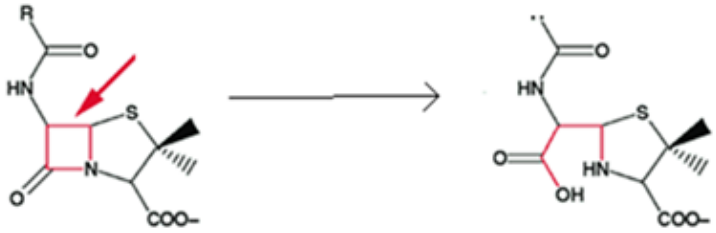
Clavulanate



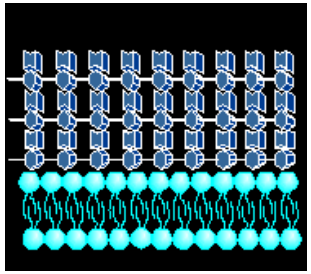
Sulbactam



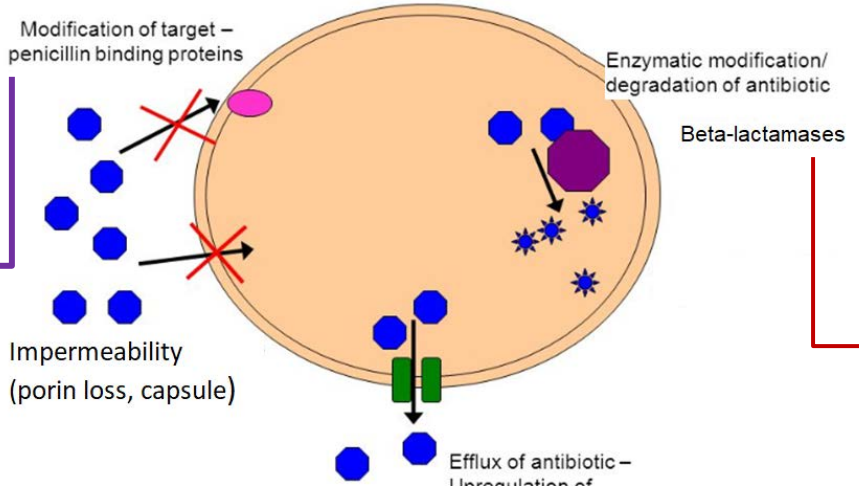
Tazobactam

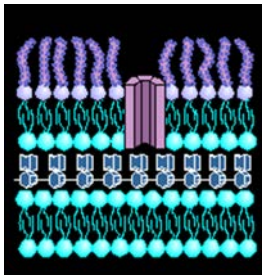


Beta-lactam resistance mechanisms

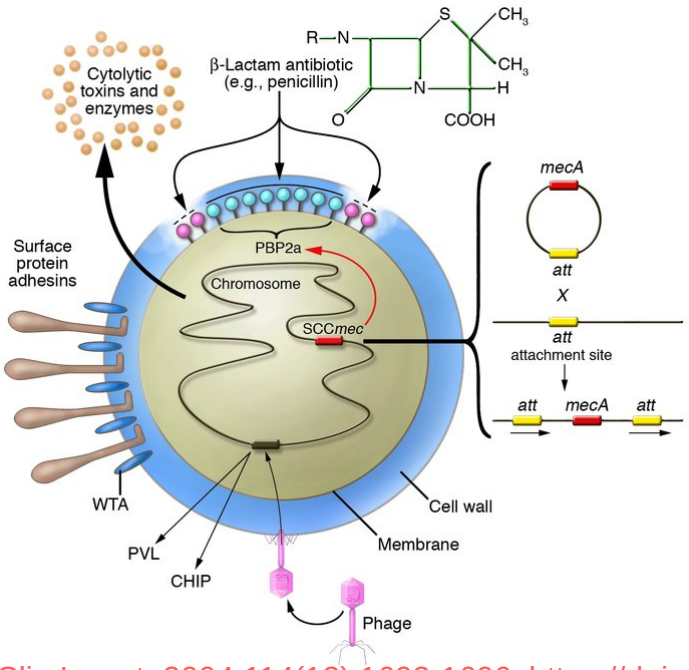


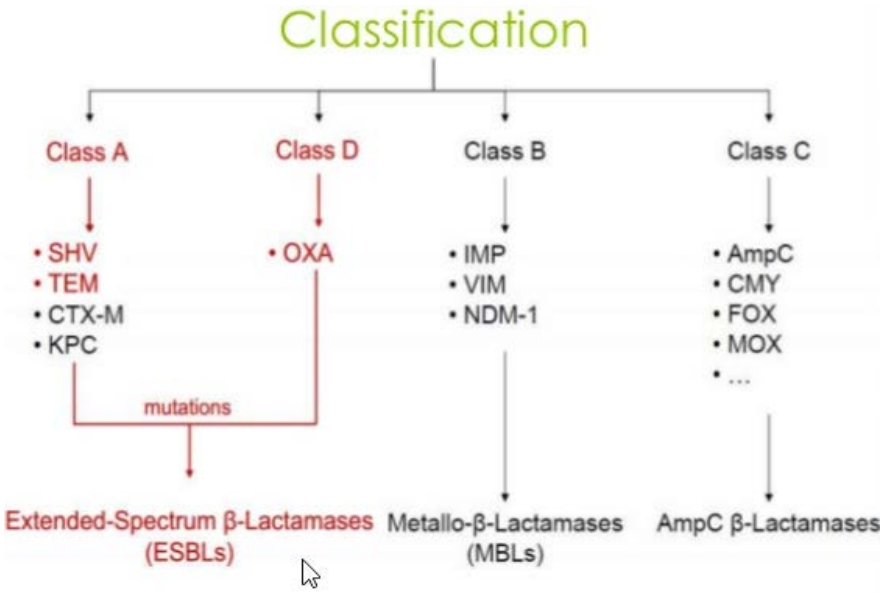
Gram-positive cellular wall





Gram-negative cellular wall



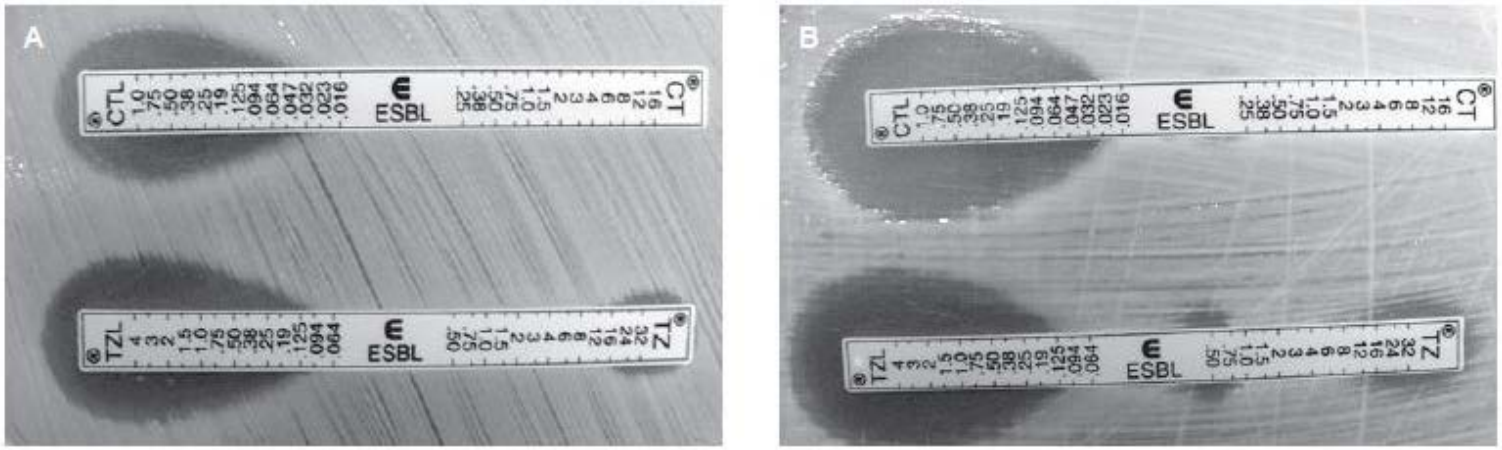


J Clin Invest. 2004;114(12):1693-1696. https://doi.org/10.1172/JCI23825.

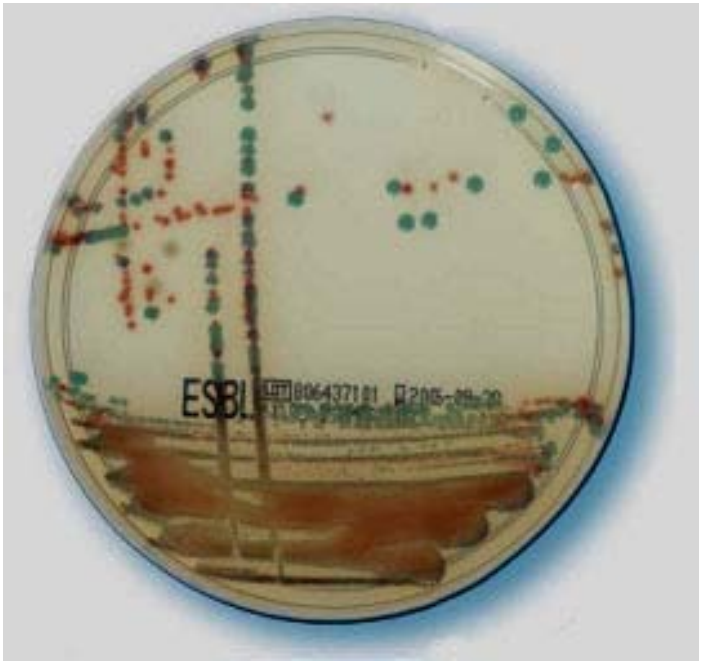
Double disk (> 5mm) between 3rd gen cephalosporin and the disk supplemented with clavulanic acid

Clavulanic acid synergism test

MIC assay (automatic, E-test etc.)



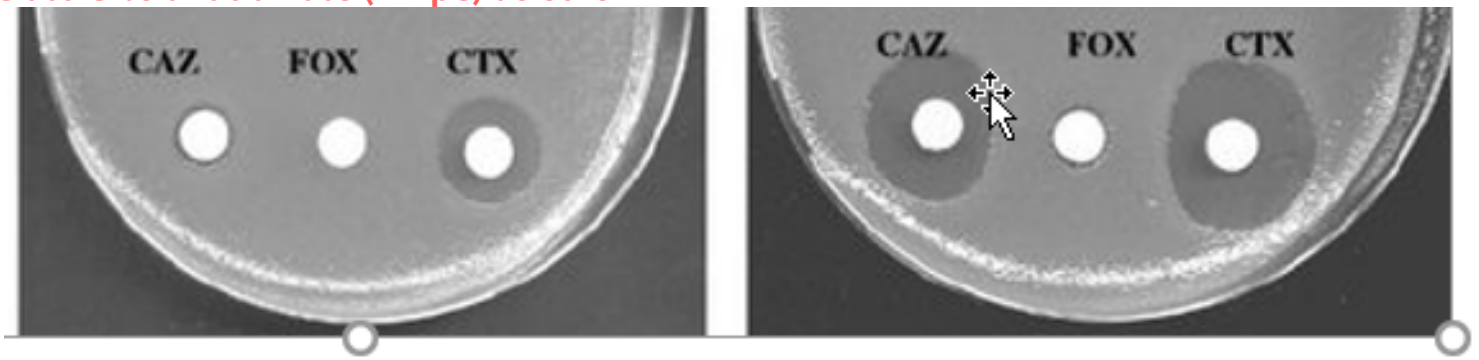
CromID ESBL



AmpC Cephalosporinase-Producing Enterobacteriaceae

- **Incidence** in children 14.2% -29%

- Transmissible AmpC, less frequent, most commonly CMY-2, but also ACT/MIR-type AmpC genes.
- **AMPCES** (*Acinetobacter, Morganella, Proteus/ Providencia/Pseudomonas, Citrobacter, Enterobacter, Serratia*)
- Inducible/derepressed
- R to Clavulanic Acid (CLA) and Cefoxitin (FOX)
- Therapeutic options:
  - 4th gen cephalosporins
  - Carbapenemes
- **Class C beta-lactamase (AmpC) detection**

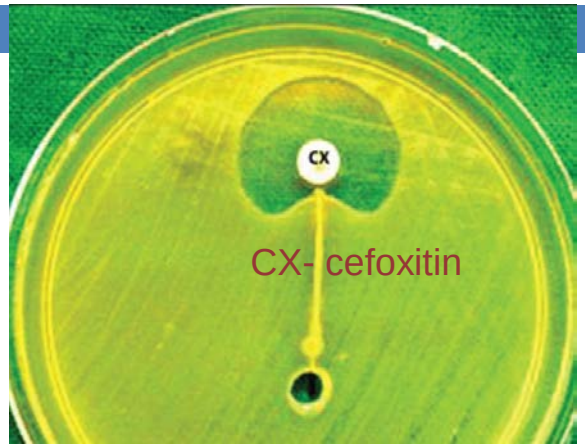


Derepressed AmpC

inducible AmpC

Class C beta-lactamase (AmpC) detection

1. Modified Hodge test

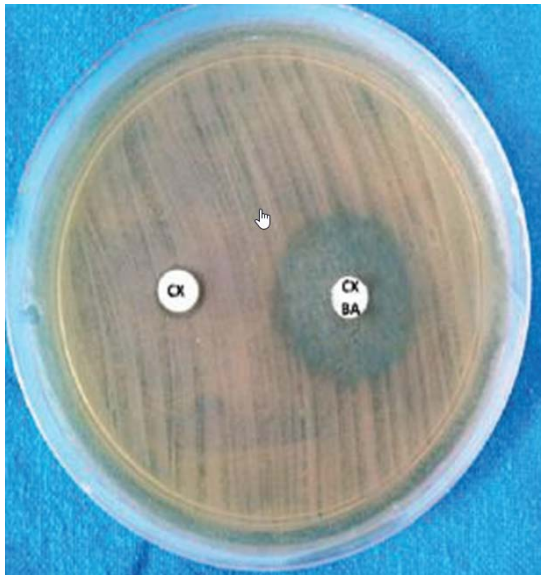


Test strain *E. cloacae* - AmpC

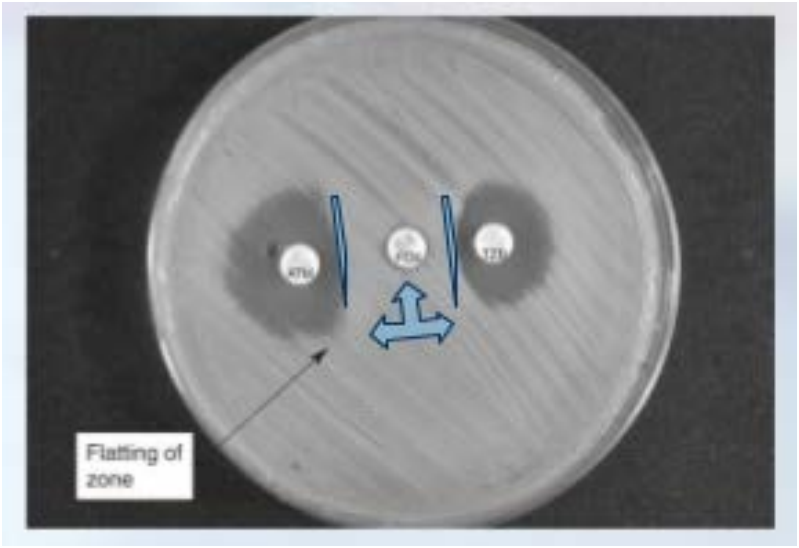
Indicator strain *E. coli* NCTC10418

Disk Cefotaxim 30 μg

2. DD test

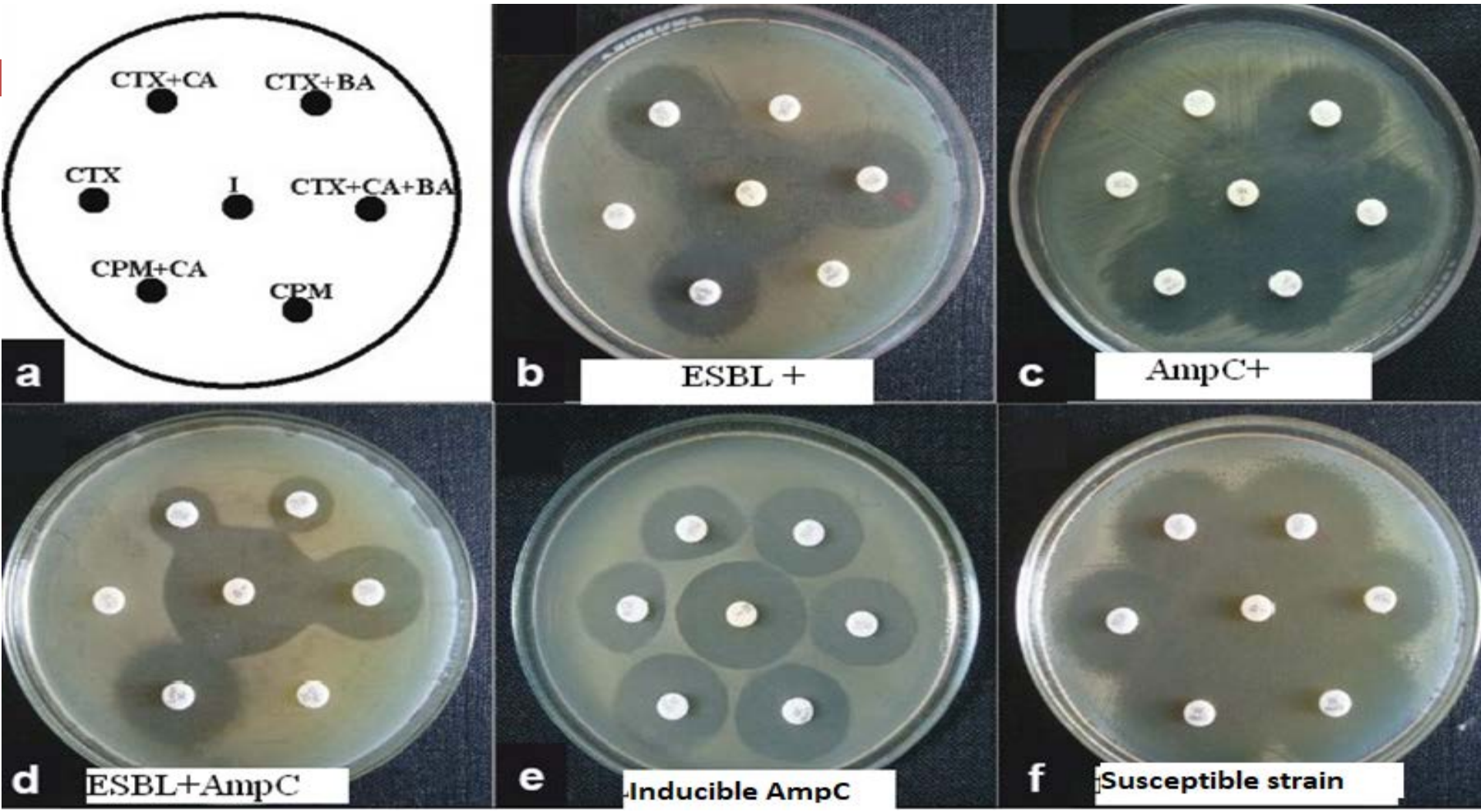


3. Antagonism



4. Commercial kits - Rosco, Mast, AB Biodisk –E-Test etc.

Differentiation of ESBL, AmpC, ESBL+AmpC phenotypes using specific inhibitors



I-imipenem;
CA/CLA- clavulanic acid;
BA – boronic acid;
CPM-cefpirome (4th gen cephalosporin);
CTX-cefotaxime (3rd gen cephalosporin).

Carbapenem Resistant Enterobacteriaceae

- Involved clones different from adults, i.e., non-ST258 KPC-Enterobacteriaceae strains
- KPC MDR, OXA-48, IMP, NDM and VIM
- Low, but increasing incidence of pediatric CRE infections over time, especially for *Enterobacter* species
- 50% mortality rate in pediatric hospitalized patients with bloodstream *CRE* infection (CDC, 2013)
- Children, especially infants-silently colonized for months to years

- **Risk factors in children**
  - medical comorbidities, prolonged hospitalizations, immunosuppression, prior antibiotic use, especially exposure to exposure to carbapenems and aminoglycosides, pulmonary and neurologic comorbidities, GI and pulmonary devices.

- Decreased sensitivity to carbapenems
- Therapeutic options for *CRE*
  - tigecycline carefully weighed for people <18 years
  - colistin and other polymyxins - optimal dosing issues for the pediatric population
  - oral fosfomycin -*CRE* bladder infection

- **Terapia necontrolată cu antibiotice determină selectarea rezistenţei atât la patogen, dar şi la flora comensală. Acest lucru afectează mediul de spital, dar şi mediul înconjurător: personalul medical, ceilalţi pacienţi, familia (Bush, Bradford, 2020);**

- **Răspândirea rezistenţei se poate realiza prin plasmide, transpozoni şi integroni-epidmeiologie genetică. TEM-1-prima beta lactamază descrisă în literatură în 1965 la un pacient din Grecia, la o tulpină de *E.coli*, s-a răspândit la > 70% dintre speciile de *Enterobacterales 30% Neisseria gonorrhoeae*.**

- **Majoritatea beta lactamazelor codificate de elementele mobile au originea în cromozomul bacterian al altor specii:**
- **Beta lactamazele SHV derivate din SHV-1 al cromozomului de *K. pneumoniae;***
- **AmpC plasmidice exprimate în *K. pneumoniae* şi *E. co*li, care sunt aproape identice cu AmpC cromozomal al complexului *Enterobacter cloacae* (ACT-1 si MIR-1), *Citrobacter freundii* (CMY), *Hafnia alv*ei (ACC-1) şi *Morganella morganii* (DHA-1);**
- **Cea mai frecvent întâlnită beta lactamază cu spectru extins, CTX-M, îşi are originea în *Kluyvera* spp (Bradford, 2001).**

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- **Beta lactamaza AmpC**

- **Aparţine clasei Ambler C şi grupul funcţional 1 clasificarea Bush Jacoby Medeiros;**
- **Conferă rezistenţă la: peniciline, oxyimino cefalosporine: Ceftazidima, Cefotaxima, Ceftriaxona; monobactame şi cefamicine-cefoxitina;**
- **Majoritatea nu sunt inhibate de clavulanata, sulbactam, tazobactam, dar sunt inhibate de: avibactam, relebactam, vaborbactam;**
- **Sunt inhibate de aztreonam (Bush et al., 1982);**
- **Frecvent sunt cromosomale la: *C. freundii, Enterobacter aerogenes, E.cloacae, Serratia marcescens* şi sunt inductibile;**
- **Genele codificatoare se regăsesc pe plasmide.**

***Acinetobacter* spp.**

- **Purtător al genei intrinseci care codifică AmpC, *bla* $_{ADC}$, gena secvenţiată prima dată în 2000;**
- **Rezistentă la cefalosporinele cu spectru extins prin supraexprimarea unui promotor localizat pe ISA *ba*1.**

***Burkholderia* spp.**

- **Majoritatea tulpinilor-beta lactamaza cromosomala clasa A;**
- **Rezistentă la peniciline şi cefalosporine de generaţia I;**
- **Pacienţi cu fibroză chistică.**

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Pseudomonas aeruginosa

- **AmpC codificată cromozomal – rezistenta la aminopeniciline şi cefalosporine;**
- **Mutaţiile – hiperproducere de AmpC-rezistentă la ticarcilină, piperacilină, aztreonam şi cefalosporine de generaţia 3 şi 4;**
- **Modificări de permeabilitate ale porinelor- mecanism asociat;**
- **Rezistenţă la cefepima –mutaţii în gena care codifică AmpC şi modificări în porine.**

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- **BLSE codificate plasmidic - Nomenclatură**

- **1989 Bush grupul 2b (Bush, 1989) capabile să hidrolizeze antibioticele cu spectru extins, dar sunt inhibate de acidul clavulanic; inițial în 2010- *K. oxytoca.***

- **Variante Inițiale TEM si SHV**
  - **SHV-2 1985 *K. ozaenae* izolată în Germania (Kliebe et al., 1985);**
  - **Aprilie 2020: 183 variante de TEM și 178 variante de SHV (https://www.ncbi.nlm.nih.gov).**

- **CTX-M**

- **Raportate în 1980;**
- **Răspândite din 2000; în prezent sunt cele mai comune BLSE (Peirano, Pitout, 2019);**
- **Hidrolizează cefotaxima şi ceftriaxona mai mult decât ceftazidime;**
- **CTX-M15 hidrolizează ceftazidima la rate mai mari decât CTX M3, ceea ce explică larga răspândire (Poirel et al., 2002);**
- **Frecvent la: *Klebsiella pneumoniae* şi *E.coli*.**

- **Carbapenemaze**
- **SME**

- *Serratia marcescens;*

- rezistentă la carbapeneme, dar sensibile la ceftazidime;

- rapoarte în Anglia, SUA, dar şi restul Europei (Bush, 2020).

- **KPC**

- 1990

- rezistentă la: cefalosporine, monobactame carbapeneme; multirezistenţă.

- **OXA48, 23, 40**

-hidrolizează penicilinele şi carbapenemele, fiind slab inhibate de inhibitori, cu excepţia avibactam.

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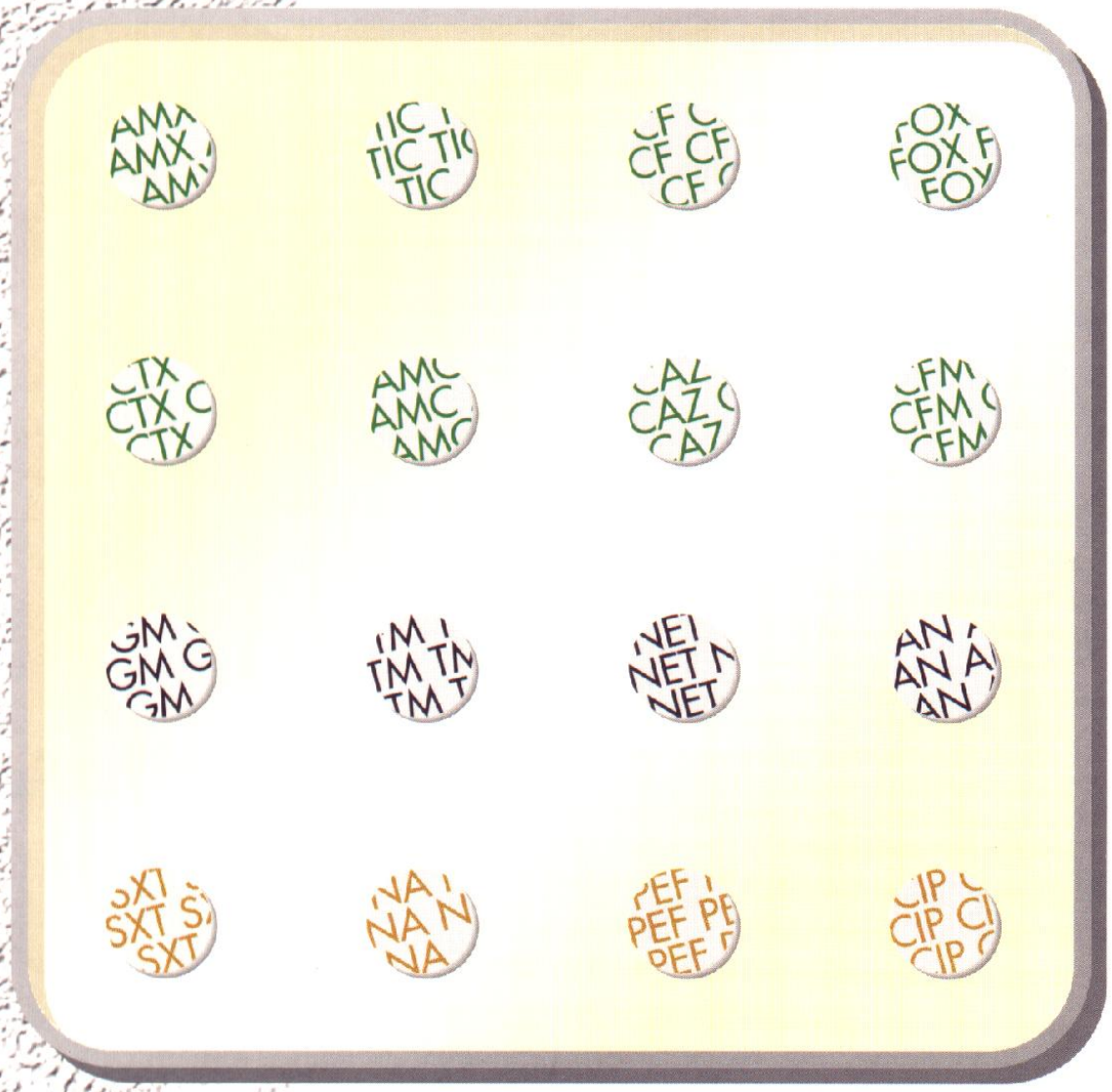
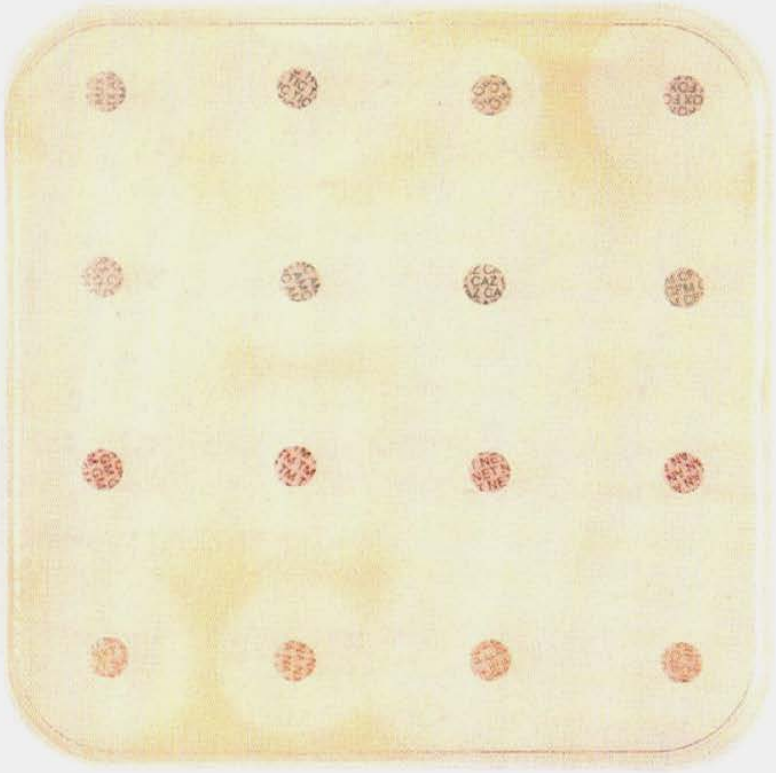
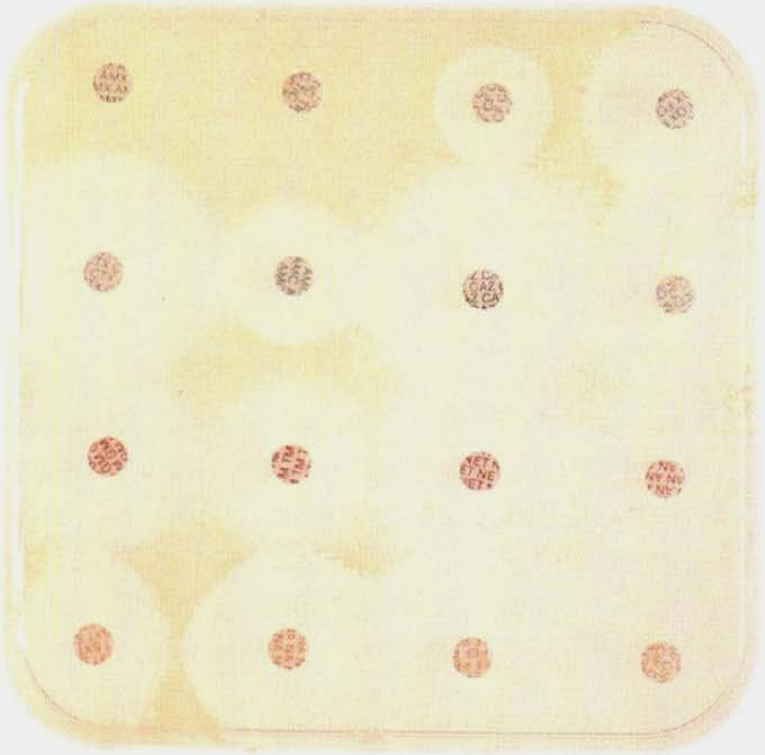


AMX	Amoxicillin	**GM**	Gentamicin
TIC	Ticarcillin	**TM**	Tobramycin
CF	Cephalotin	**NET**	Netilmicin
FOX	Cefoxitin	**AN**	Amikacin
CTX	Cefotaxime	**SXT**	Trimethoprim/ sulfamethoxazole
AMC	Amoxicillin/ clavulanic acid	**NA**	Nalidixic acid
CAZ	Ceftazidime	**PEF**	Pefloxacin
CFM	Cefixime	**CIP**	Ciprofloxacin



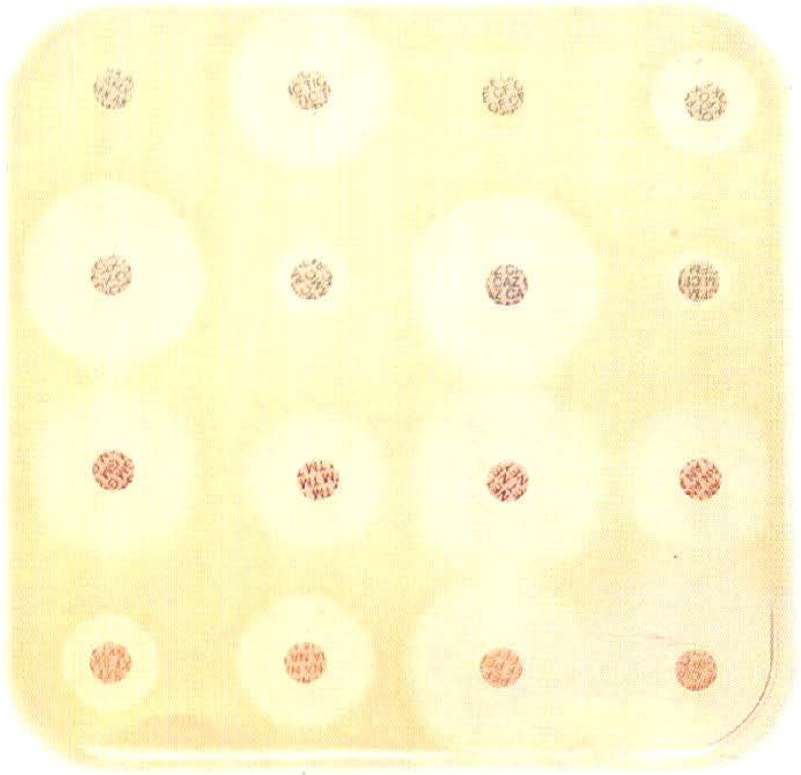
E. coli

Phenotype 1: this strain is **susceptible** to all the antimicrobials tested.



E. coli

Phenotype 2: this **penicillinase**-producing strain is resistant to amoxicillin and ticarcillin.



E. coli

Phenotype 3: this **cephalosporinase**-producing strain is resistant to amoxicillin, cephalotin, and amoxicillin/clavulanic acid.

The strain is susceptible to the other antimicrobials tested.

Rene Magritte
Belgian surrealism;
Son of man (1964)
Vizibilul invizibil

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