Antibiotic Resistance

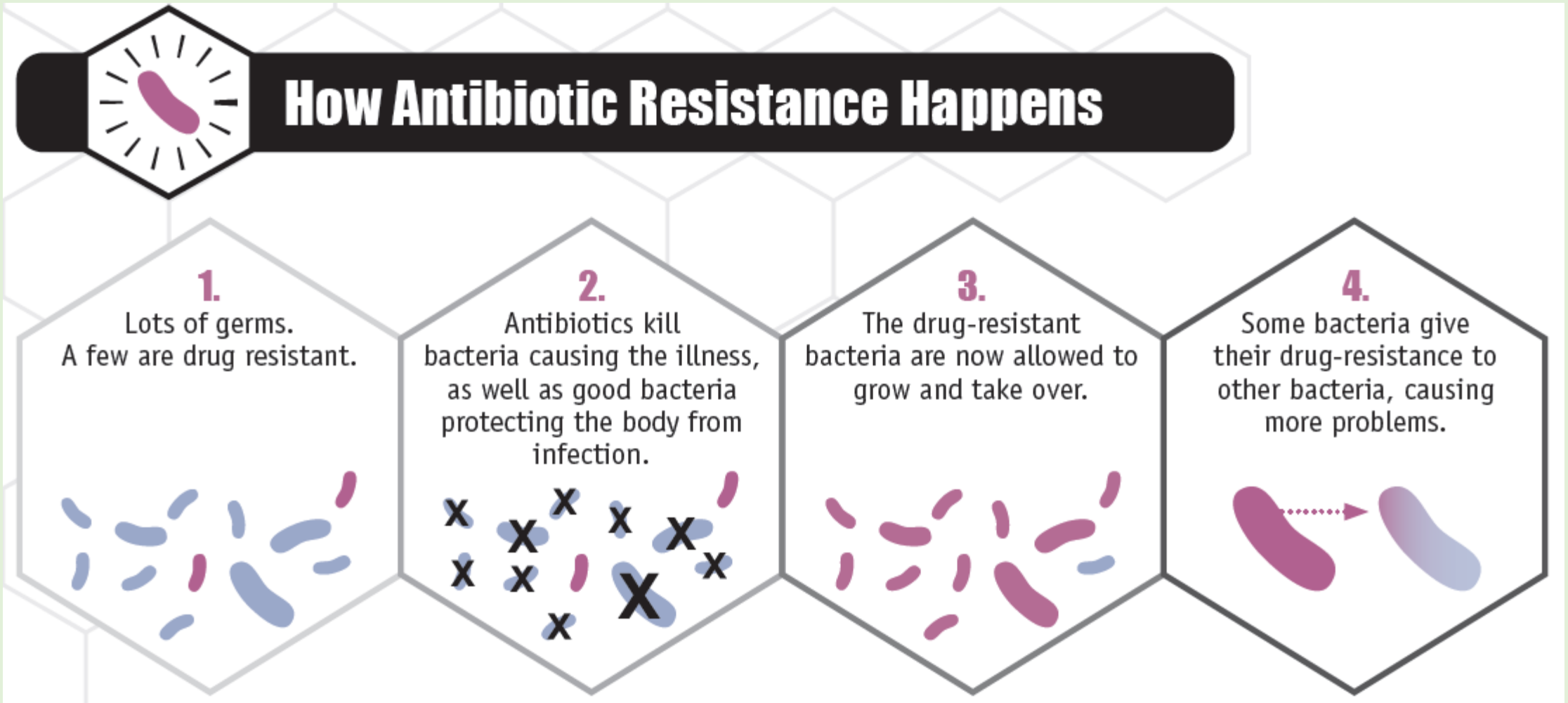
&

ABST

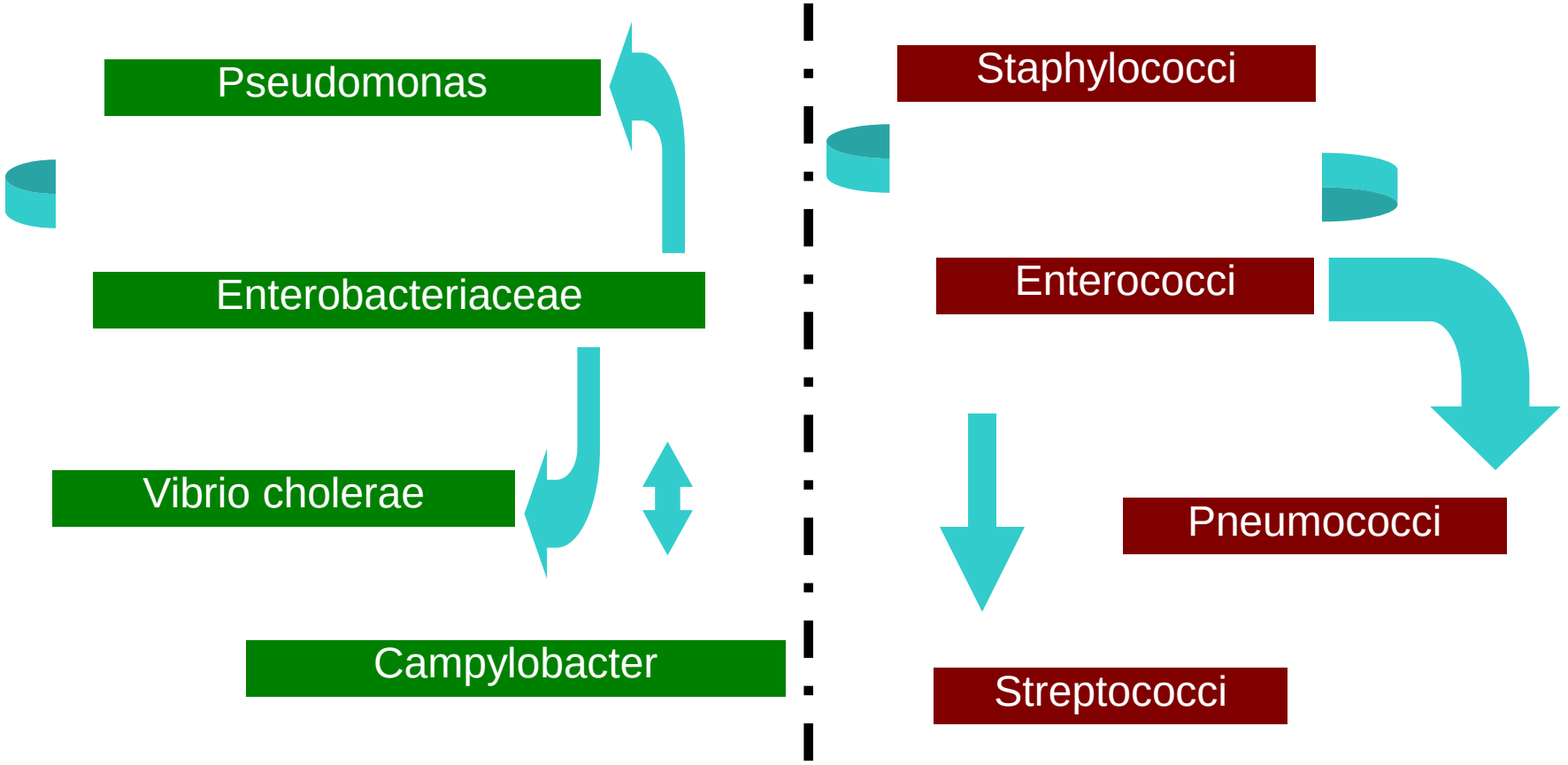
Contents

- What is Antibiotic resistance?
- What are the resistant mechanisms?
- What is an ABST (Antibiotic sensitivity testing)?
- Who are the important bugs?

  MRSA, VRSA, VISA, ESBL, VRE, CRE, MDROs

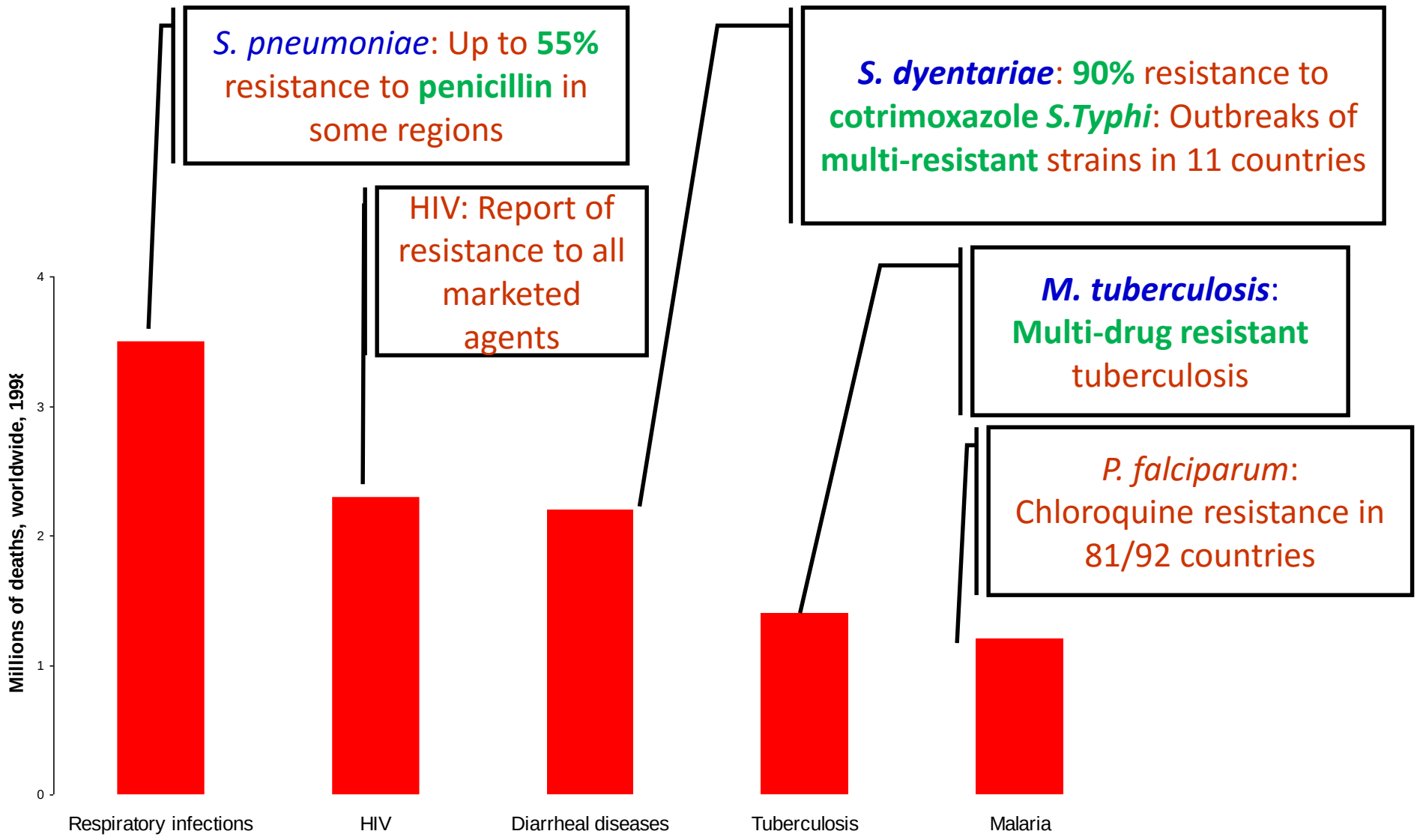

How Antibiotic Resistance Happens
1.
Lots of germs.
A few are drug resistant.
2.
Antibiotics kill
bacteria causing the illness,
as well as good bacteria
protecting the body from
infection.
3.
The drug-resistant
bacteria are now allowed to
grow and take over.
4.
Some bacteria give
their drug-resistance to
other bacteria, causing
more problems.

Genetic exchange of antimicrobial resistance genes



U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Leading global infectious diseases



Antibiotic resistant infections

Diseases	Agent	Resistances
Pneumonia	*S pneumoniae*	Penicillin
Dysentery	*S dysenteriae*	Multiple resistances
Typhoid	*S typhi*	Multiple resistances
Gonorrhea	*N gonorrhoeae*	Penicillin and tetracycline
Tuberculosis	*M tuberculosis*	Rifampicine and INH
Nosocomial infections	*S aureus*	Methicillin, vancomycin
	E species	Vancomycin
	Klebsiella, Pseudomonas	Multiple resistances

Antimicrobial resistance

- Results from **misuse**, **overuse**, **under**/ **inadequate use** of antimicrobials
- **Costs** money, undermines effectiveness of health delivery programs
- Threat to **global stability** and **national security**

Natural & acquired resistance

Natural resistance

- Chromosomic genetic support
- Affect almost all species strains
- Existed before antibiotic use (*Enterobacter sp.* - amoxicillin)

Acquired resistance (mutation)

- Chromosomic, plasmidic or transposon genetic support
- Affects a fraction of strains
- Increased with antibiotic use

  (extended spectrum beta-lactamase producing *E. coli)*

What are the antibiotic resistant mechanisms?

Mechanisms of resistance

Prevent antibiotic reaching its target

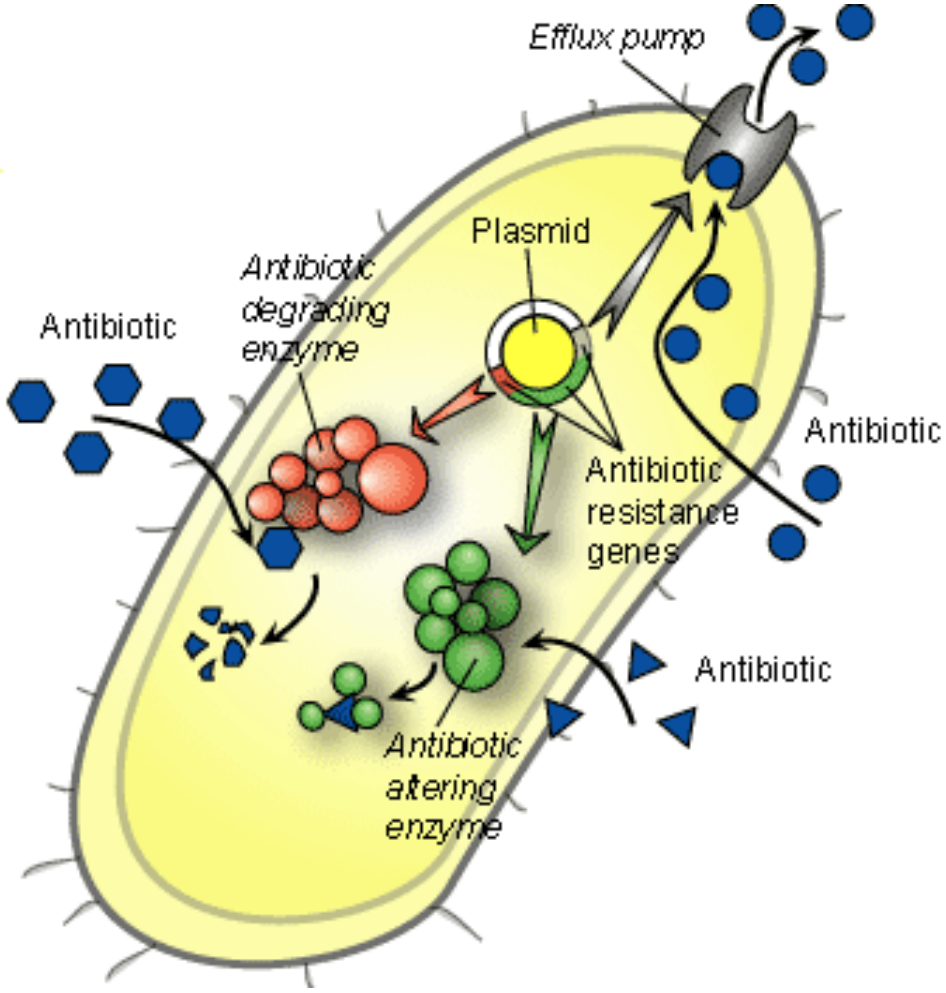
- Impaired cell membrane permeability
- Efflux phenomenon

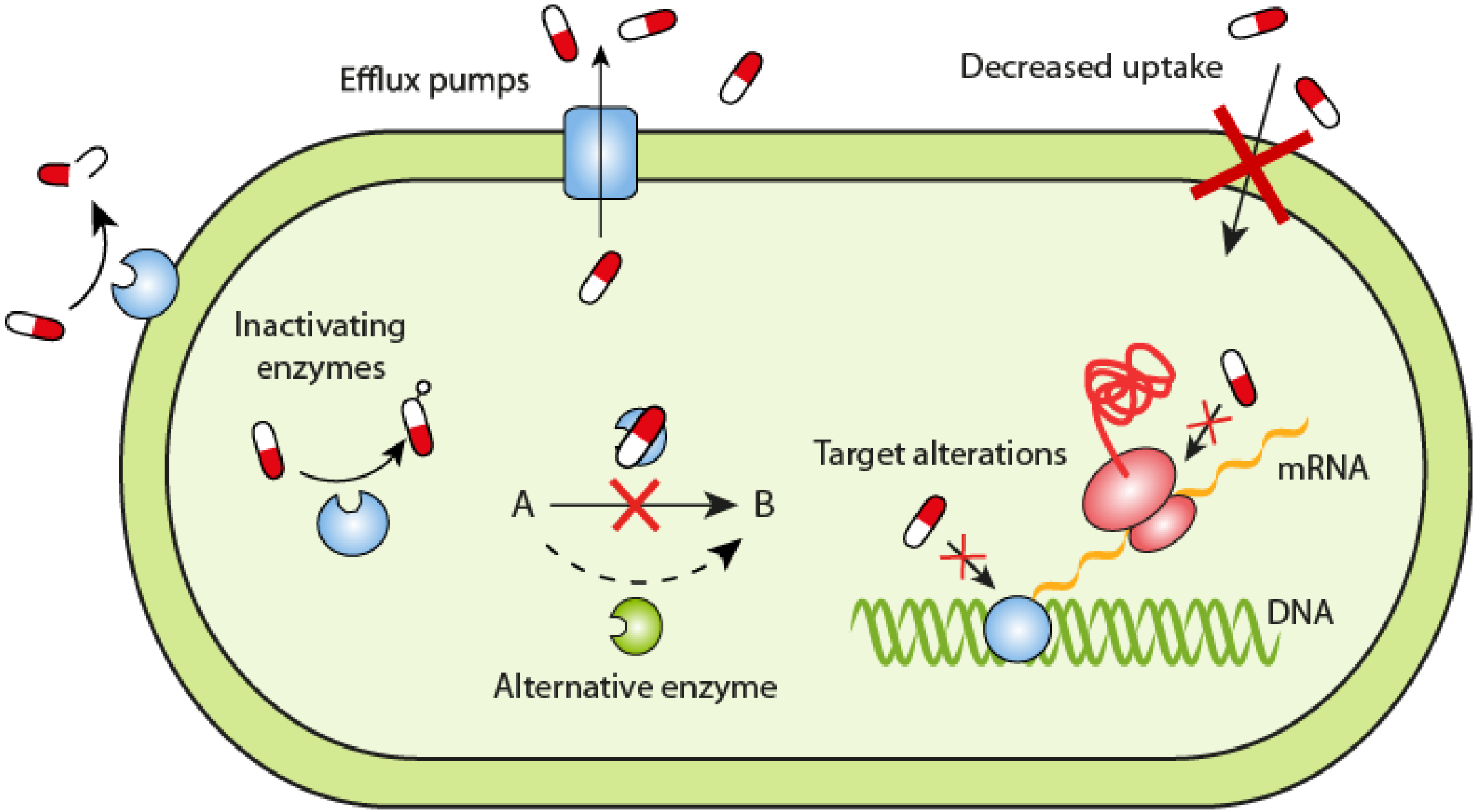
Prevent the antibiotic biding to its target

- Supplementary targets
- Decreased affinity by target modification

Inactivation before reaching the target

- Enzymatic Inhibition




Efflux pumps
Decreased uptake
Inactivating enzymes
A
B
Alternative enzyme
Target alterations
mRNA
DNA

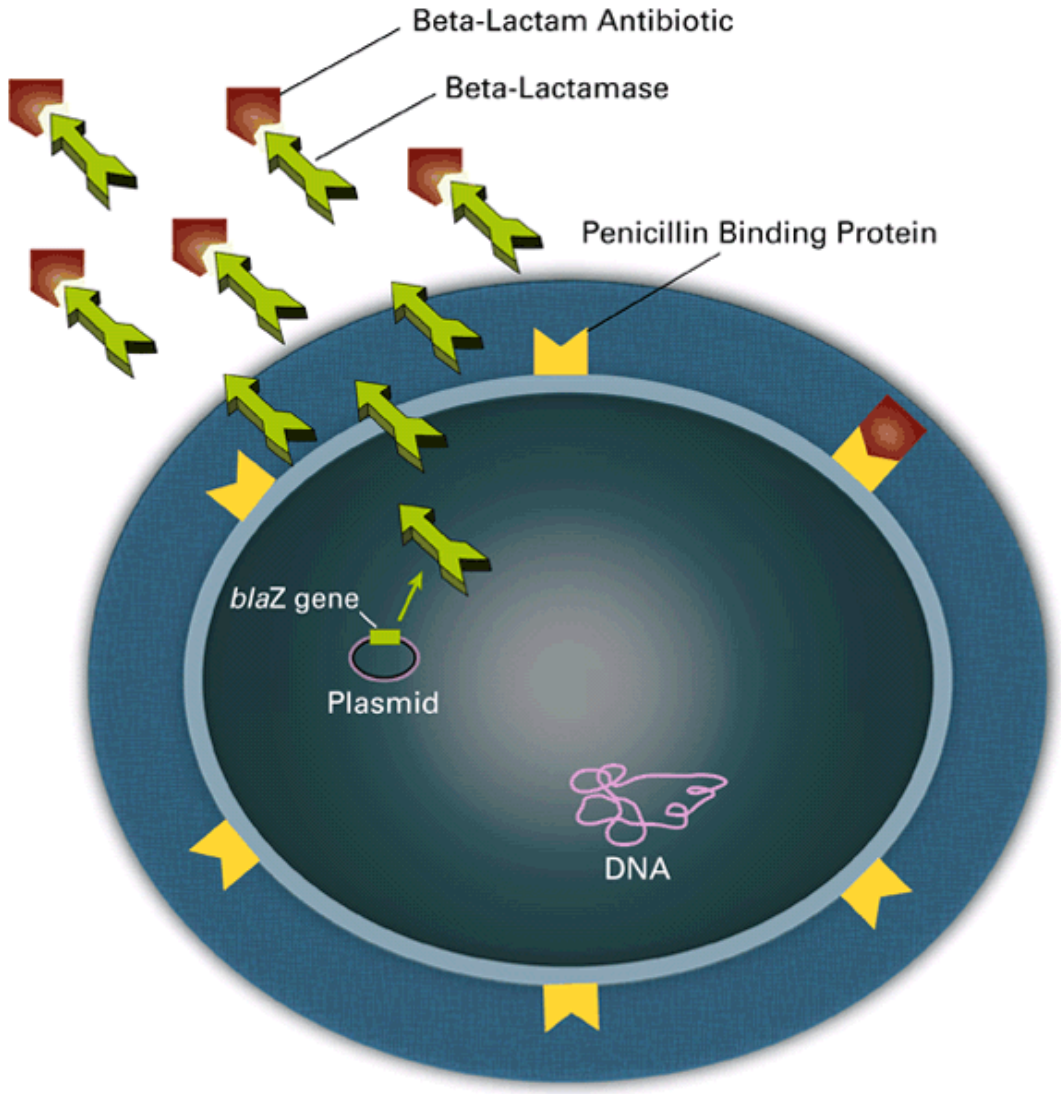
Enzymatic Inhibition

β-Lactamases

- Primarily resistance to **β-lactam antibiotics**

- Inactivate the antibiotics by **splitting beta lactam ring**

- Selective pressure by the **widespread use of antimicrobial therapy** accelerated their development and spread.



Enzymatic Inhibition

β-Lactamases

Penicillinase

- First β-lactamase was described as a "**penicillinase"**

  hydrolyzing penicillin by *Escherichia coli* in 1940

  next years, rapid spread penicillin resistance among *S. aureus* isolates

- Among gram-negative organisms - Ampicillin resistance in the 1960s

Enzymatic Inhibition

β-Lactamases

Extended-Spectrum β-Lactamases

- Capable of hydrolysing **monobactam** and **broad-spectrum Cephalosporins**
- Found primarily in

  E. coli

  K. pneumoniae

  Enterobacter aerogenes

  Morganella morganii

  Salmonella spp

Enzymatic Inhibition

β-Lactamases

Carbapenemases

- Largest antibiotic resistance spectrum
- Hydrolyze

  carbapenems (Imipenem, Meropenem, Eartapenem)
  broad-spectrum penicillins (Ampicillin)
  oxymino-cephalosporins (ceftazidime, ceftriaxone and cefotaxime)
  cephamycins (cefoxitin and cefotetan)

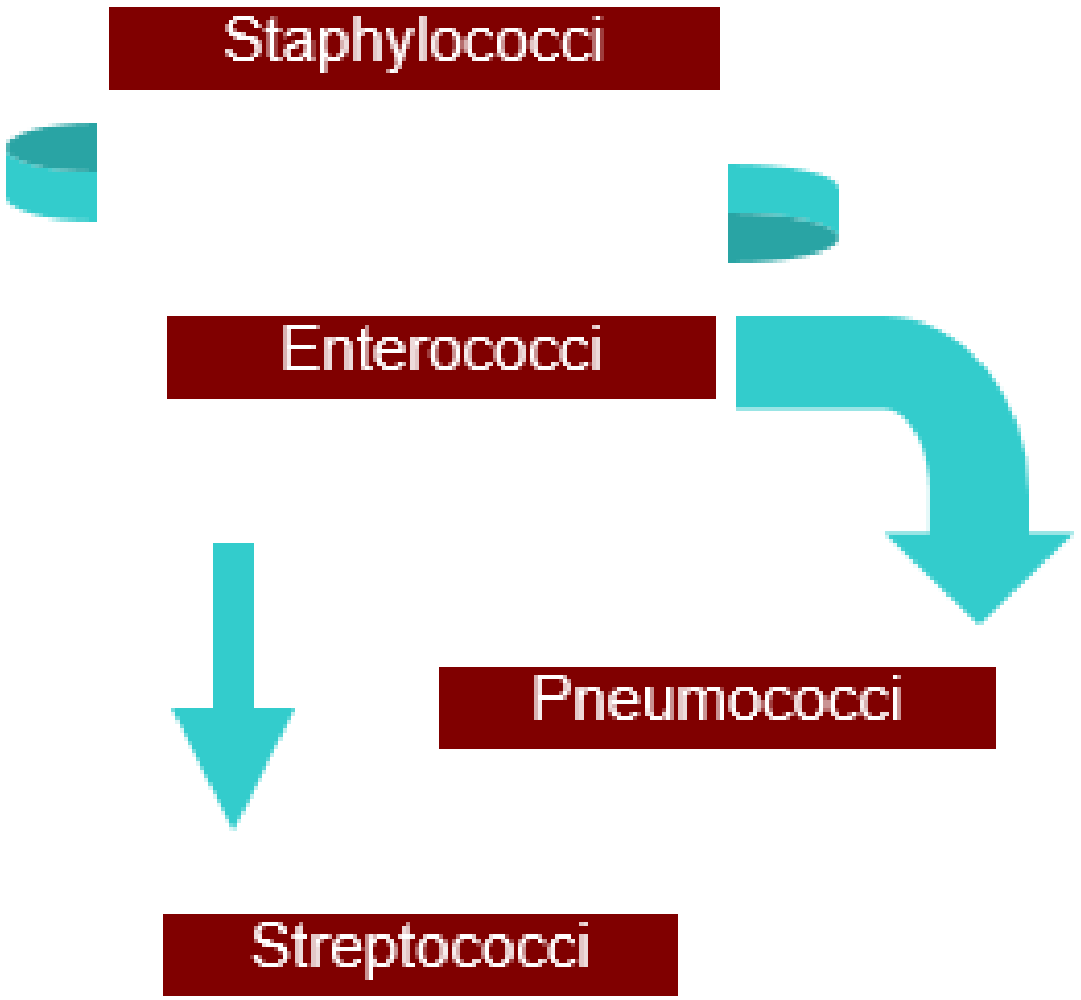
- Organisms producing **Carbapenemases**

  K. pneumoniae
  E. Coli
  Salmonella
  Serratia
  P. aeruginosa

Enzymatic Inhibition

β-lactamase in Gram-Positive Bacteria

- **Staphylococci** are the major pathogens that produce β-lactamase
- Staphylococcal β-lactamases hydrolyze penicillins
- β-lactamases of **Enterococci** seems to be of staphylococcal origin.



Enzymatic Inhibition

Aminoglycoside Modifying Enzymes

- Most common cause for aminoglycoside resistance
- Confer antibiotic resistance through three general reactions:

N-acetylation

O-nucleotidylation

O-phosphorylation

Enzymatic Inhibition

Aminoglycoside Modifying Enzymes

- Modification of the antibiotic occur in the process of transport across the cytoplasmic membrane
- Common organisms

  K. pneumoniae

  enterococci

  S. aureus

  S. epidermidis

Enzymatic Inhibition

Other enzymes

- **Chloramphenicol Acetyltransferase**

  Resistance to chloramphenicol in Gram-positive and gram-negative organisms

- **Macrolide-, Lincosamide-, Streptogramin-Inactivating enzymes**

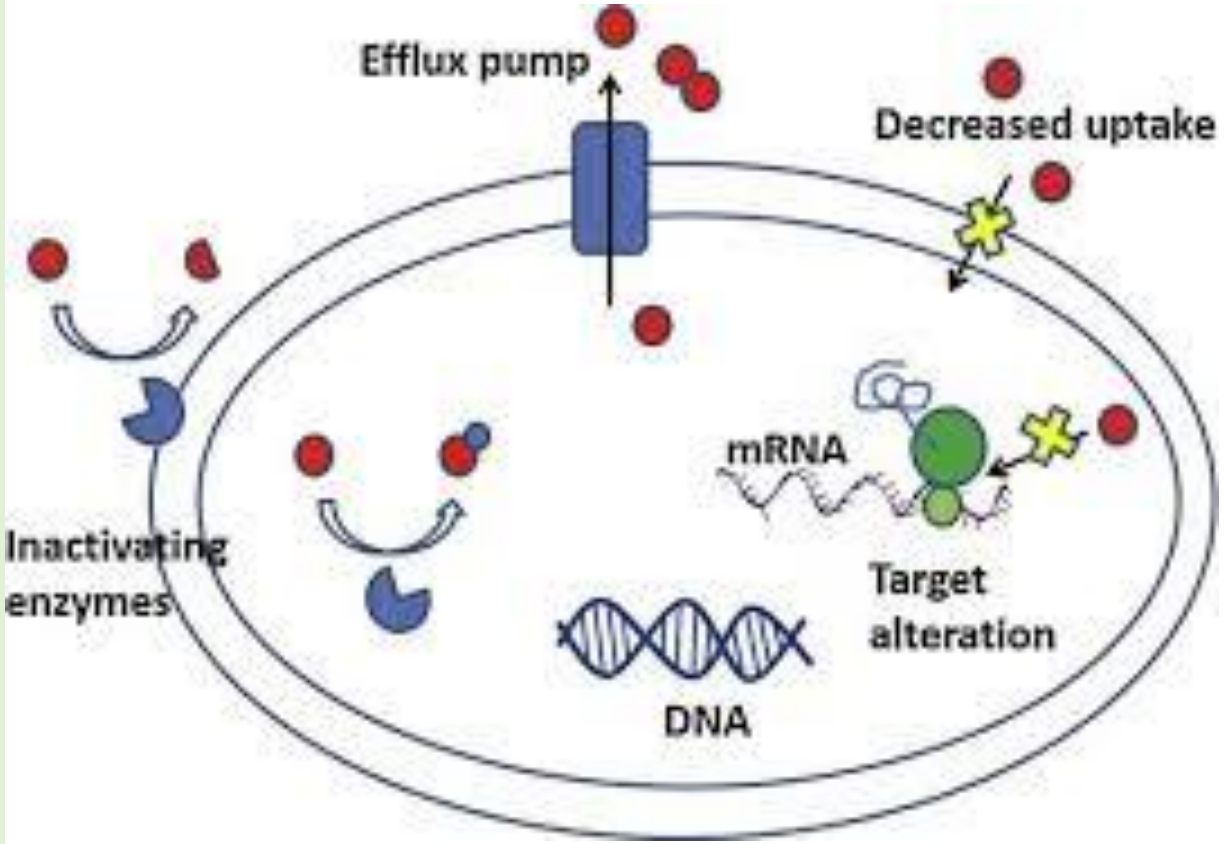
  Erythromycin esterases in *E. coli*

- **Tetracycline-inactivating enzyme (TetX)**

  Bacteroides spp

Promotion of Antibiotic Efflux

- Common mechanism of resistance in many clinically relevant pathogens.
- *E. coli, Shigella* spp., and other enteric organisms express a membrane transporter system that leads to multidrug resistance by drug efflux



Promotion of Antibiotic Efflux

Tetracyclines

- **Enteric gram-negative organisms** - decreased accumulation of **Tetracycline** through the **active efflux** of the antibiotic across the cell membrane
- **Decreased uptake** from the extracellular environment also accounts for decreased accumulation of tetracycline inside resistant cells

Promotion of Antibiotic Efflux

Macrolides and Streptogramins

- Active efflux mechanism causes resistance to macrolides, streptogramins and azalides (eg: azithromycin)

  Streptococcus pneumoniae

  Staphylococcus pyogenes

  S. aureus

  S. epidermidis

Promotion of Antibiotic Efflux

β-Lactams

Multidrug efflux pumps in the inner and outer membrane

β-lactam resistance in ***P. aeruginosa***

Periplasmic β-lactamases protect the bacterium from β-lactam agents

Promotion of Antibiotic Efflux

Fluoroquinolones

- Active efflux of fluoroquinolones

  enteric bacteria

  Staphylococci

Altered Target Sites

Alteration of Ribosomal Target Sites

Macrolides, Lincosamides, Streptogramins B (MLSB)

- Resistance to a wide variety of antimicrobial agents, including tetracyclines, macrolides, lincosamides, streptogramins, and the aminoglycosides
- **Failure** of the antibiotic **to bind** its **target site** or sites on the **ribosome** disrupts its ability to **inhibit protein synthesis** and cell growth

Altered Target Sites

Alteration of Ribosomal Target Sites

MLSB resistance – common organisms

- *S. aureus*
- *Streptococcus sanguinis*
- *B. fragilis*
- *Clostridium perfringens*
- *S. pneumoniae*

Altered Target Sites

Alteration of Ribosomal Target Sites

Aminoglycosides

- In **Enterobacteriaceae** and **nonfermenting gram-negative bacteria**
- This is now recognized as a major mechanism of resistance to all **parenteral aminoglycosides**

Altered Target Sites

Alteration of Cell Wall Precursor Targets

- Resistance of **enterococci** to **vancomycin** through target site alterations
- Resistance to vancomycin and teicoplanin in

  S. pyogenes

  S. sanguis

Alteration of Target Enzymes

Resistance to β-Lactams

- β-Lactam antibiotics inhibit bacteria by binding covalently to **PBPs** in the cytoplasmic membrane
- These target proteins catalyze the **synthesis of the peptidoglycan** that forms the **cell wall of bacteria**
- **Alterations of PBPs** can lead to **β-lactam antibiotic resistance**

Alteration of Target Enzymes

Resistance to β-Lactams

- Decrease in the affinity of the PBP
- Change in the amount of PBP produced by the bacterium

  Eg: Penicillin-resistant strains of *S. pneumoniae*

Alteration of Target Enzymes

MRSA Resistance

- In *S. aureus*, methicillin resistance is deu to **mecA gene**, which encodes **PBP2a**, a protein with **low affinity for β-lactam antibiotics**
- Confer resistance to methicillin, nafcillin, oxacillin, and cephalosporins
- The mecA gene is in the larger staphylococcal cassette chromosome mec (**SCCmec**), which appears to have been acquired by horizontal transfer from a **coagulase-negative Staphylococcus** species.

Alteration of Target Enzymes

- **Reduced penicillin-binding affinity** of PBPs of β-lactamase-negative, penicillin-resistant strains of

  N. gonorrhoeae

  Neisseria meningitides

  H. influenzae

Alteration of Target Enzymes

Quinolones

DNA gyrase (bacterial topoisomerase II)

Topoisomerase IV

- DNA gyrase is the primary site of action in Gram-negative bacteria
- **Mutations** in chromosomal loci of **DNA gyrases** cause **resistant** to **nalidixic acid** and the **newer fluoroquinolones** in members of **Enterobacteriaceae** and ***P. aeruginosa.***

Protection of Target Site

Tetracyclines

- Protect the ribosome from tetracycline action
- Eg: Gram-positive organisms, Mycoplasma, Ureaplasma, Campylobacter, Neisseria spp

Multiple resistant Mechanisms

- **Multiple mechanisms** are in operation at the same time within **individual bacterial cells.**
- Multiple antibiotic-resistance expression leads to phenotypes of multidrug resistance (**MDR**), or **panresistance**
- Common MDROs

  Gram-negative bacteria

  P. aeruginosa

Control of antibiotic resistance

- **Rational** antibiotic usage
- **Curtailment** of the unnecessary use of antibiotics in situations such as **animal husbandry**
- Development of a greater **understanding** of

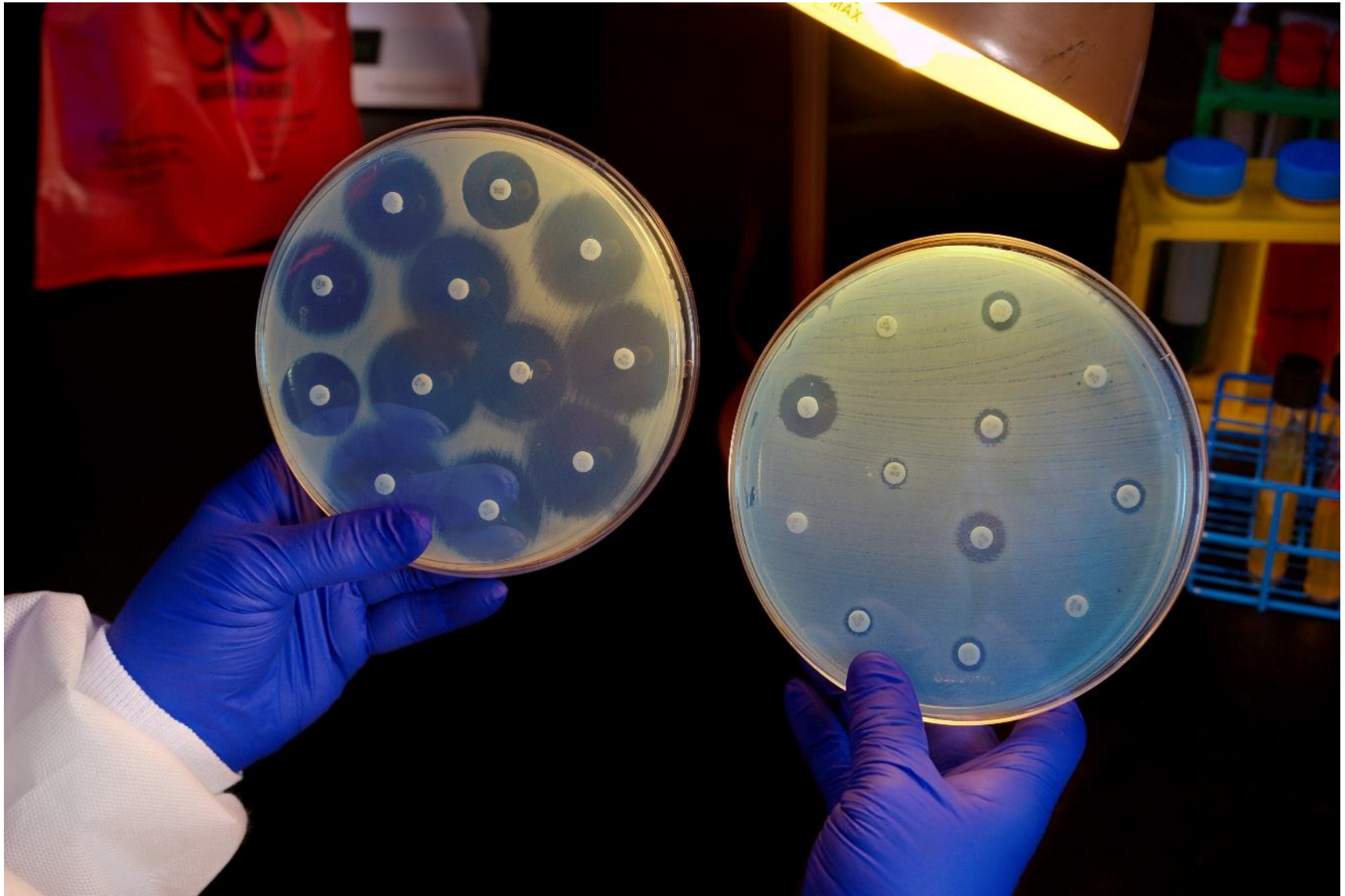
  - how antimicrobial **resistance spreads**

  - **intelligent use** and development of improved bacterial vaccines

  - **antibiotic stewardship**

  - implementation of effective **infection control** strategies

ABST

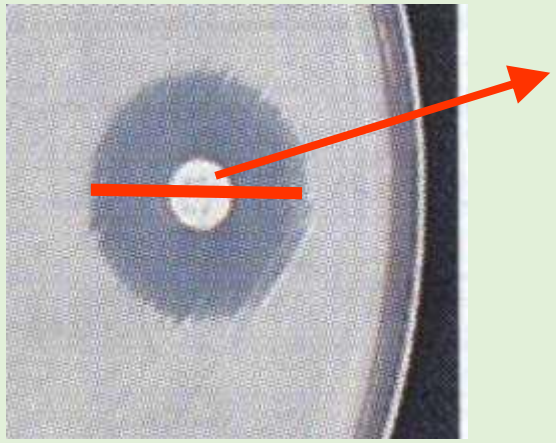


Antimicrobial susceptibility tests

- **Solid** media (diffusion)
  - Disk diffusion (Kirby-Bauer)
  - E-tests
- Minimum inhibitory concentration [**MIC**]
  - The smallest concentration of antibiotic that inhibits the growth of organism
- **Beta lactamase** production: quick screening method

Disc diffusion testing

- Antibiotic-impregnated discs placed on an agar plate
- Resulting zones of inhibition is measured
- Assess the susceptibility / resistance (standard tables)



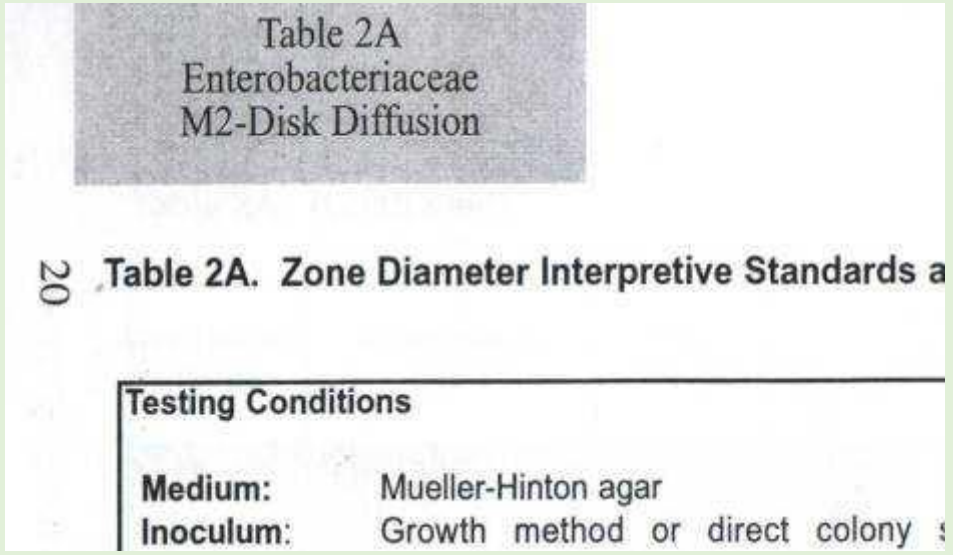


Table 2A
Enterobacteriaceae
M2-Disk Diffusion

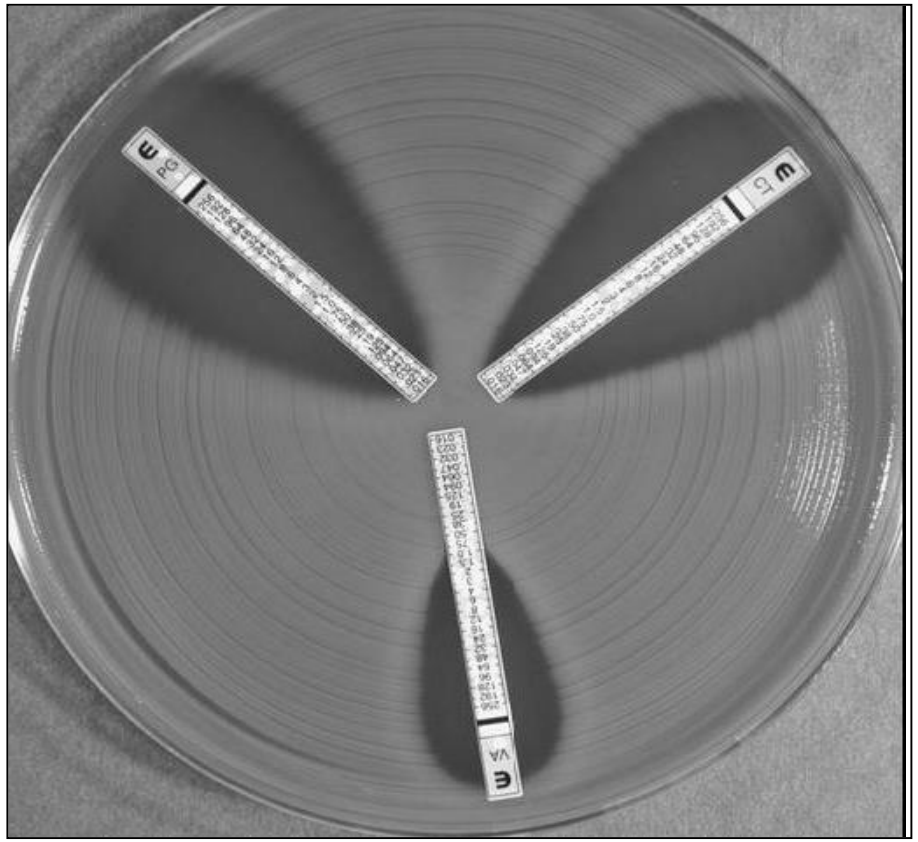
20

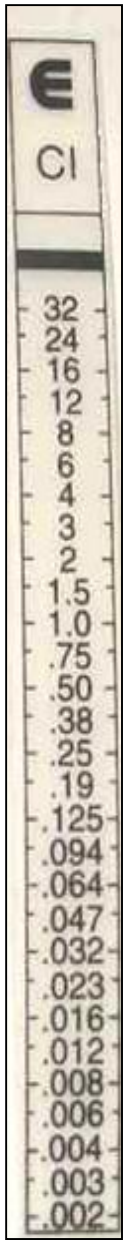
Table 2A. Zone Diameter Interpretive Standards a

Testing Conditions	
Medium:	Mueller-Hinton agar
Inoculum:	Growth method or direct colony

E-test – test MIC

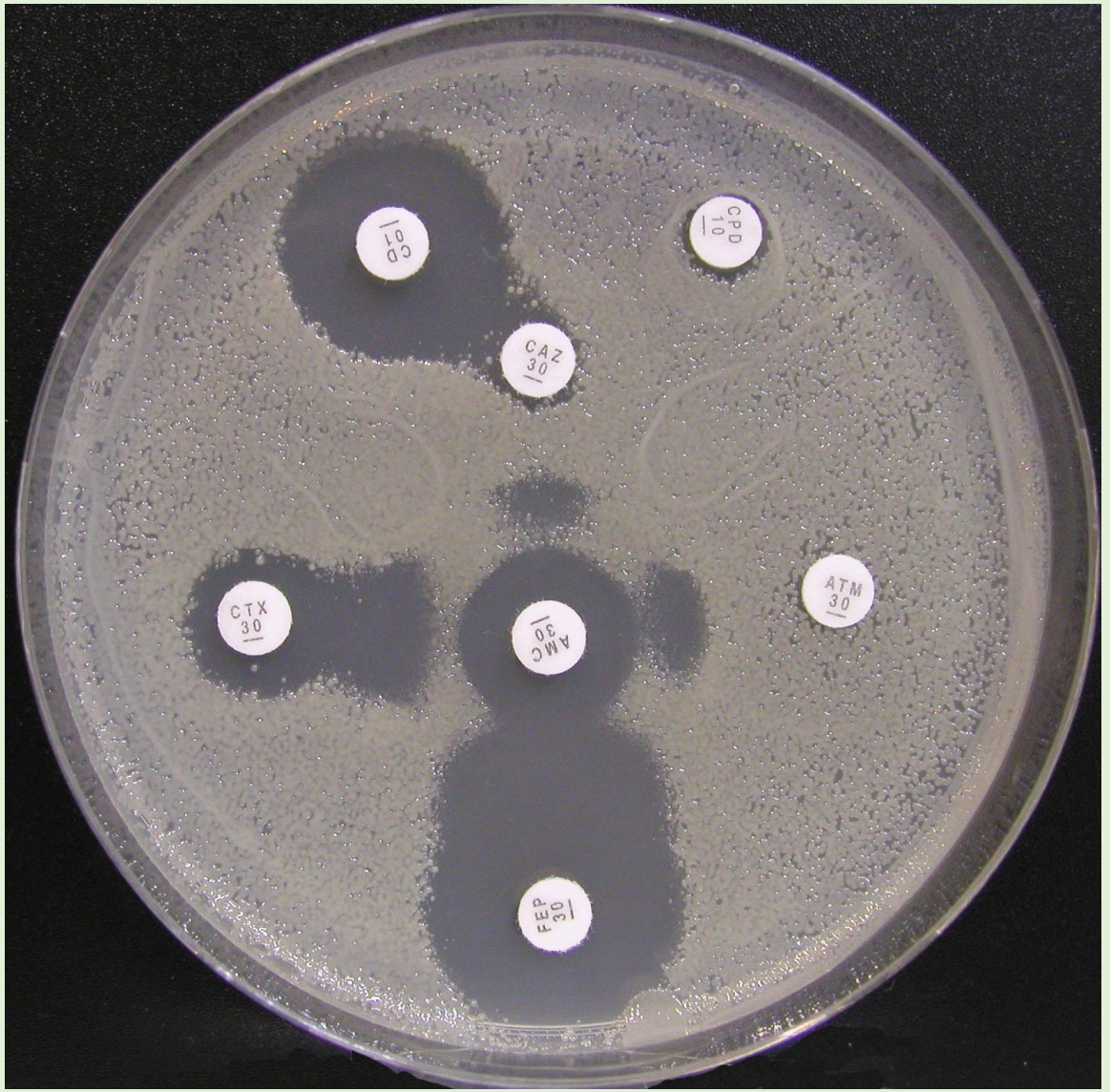
- Plastic strips with a predefined gradient of
  - One antibiotic
- One strip per antibiotic
- Wide range of antibiotics
- Easy to use
- Expensive





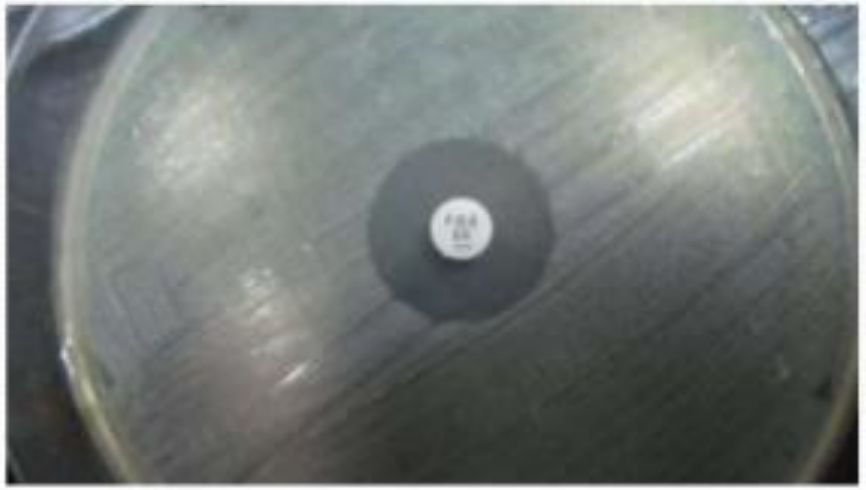
ESBL


CAZ 30
AMC 30
AM 10
CXM 30


CD 01
CrD 10
CAZ 30
CTX 30
AMC 30
ATM 30
FEP 30

MRSA

cefoxitin disk screen test



- ≥ 22 mm: sensitive.
- <22mm: resistant (MRSA).
- MIC: ≥ 4 µg/ml → MRSA

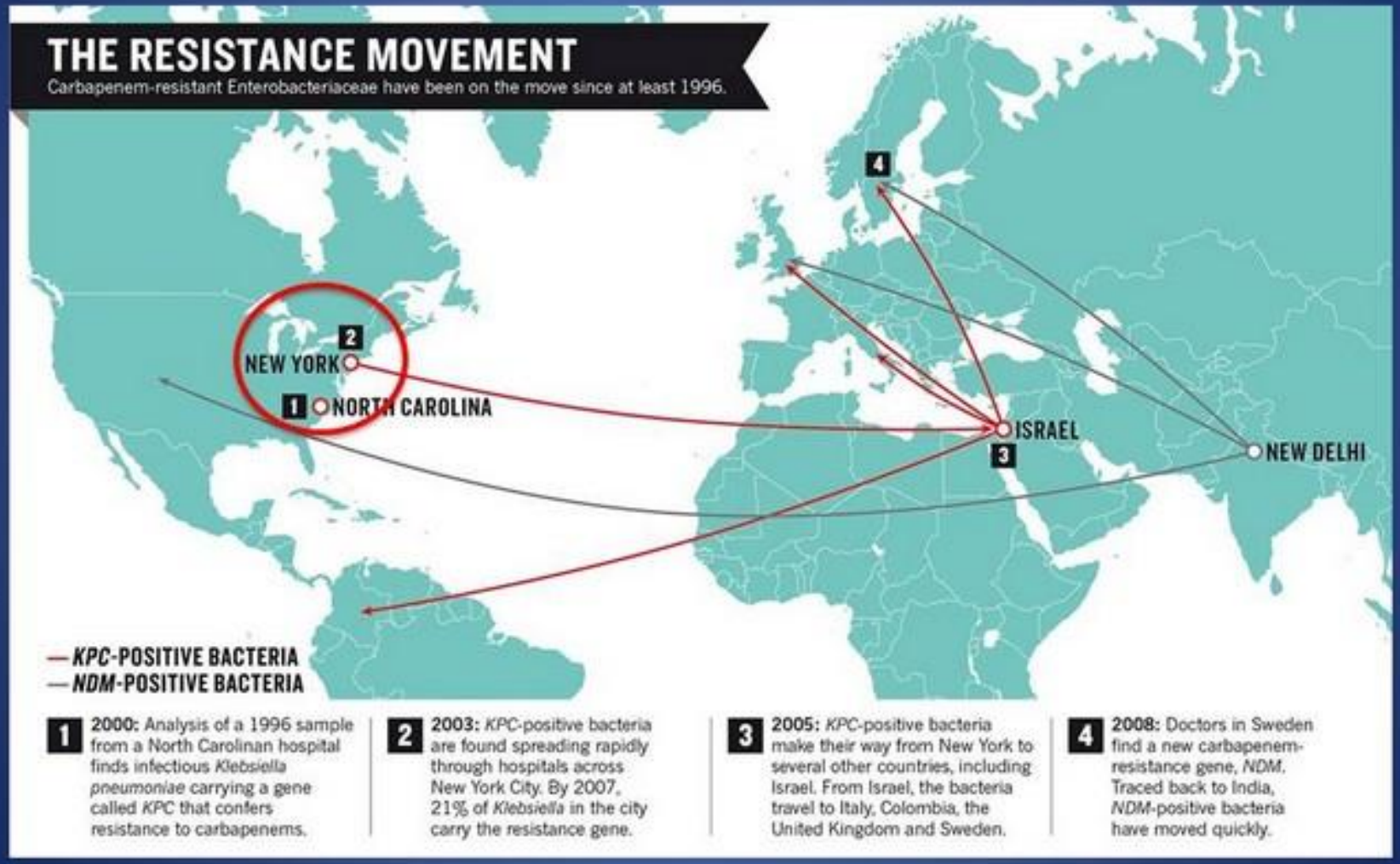
Inducible clindamycin resistance

- Though clindamycin is sensitive according to lab report, resistant in vivo
- Can be detected by D test

Figure 1: Isolate with inducible clindamycin resistance showing flattening of clindamycin zone adjacent to erythromycin disk

Carbapenem Resistant Enterobacteriaceae (CRE)

Emergence of carbapenem resistant Enterobacteriaceae



M. McKenna. Nature. Vol 499. 25 July 2013

MDROs

Eg: *Pseudomonas aeruginosa,* Acinetobacter