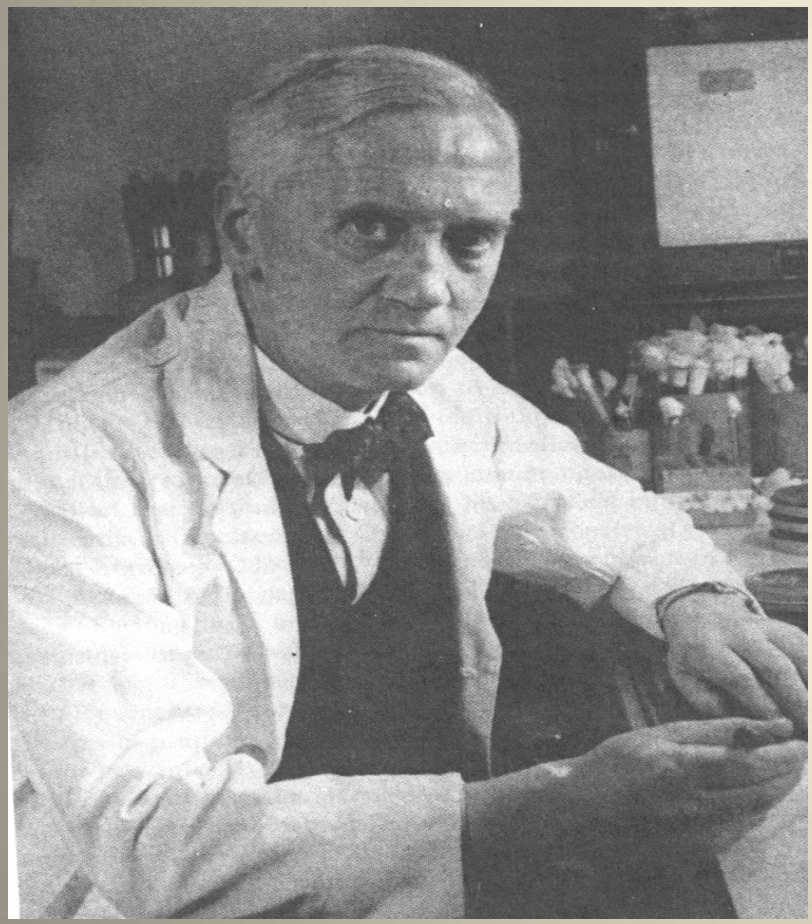
Mechanisms of Antimicrobial Resistance

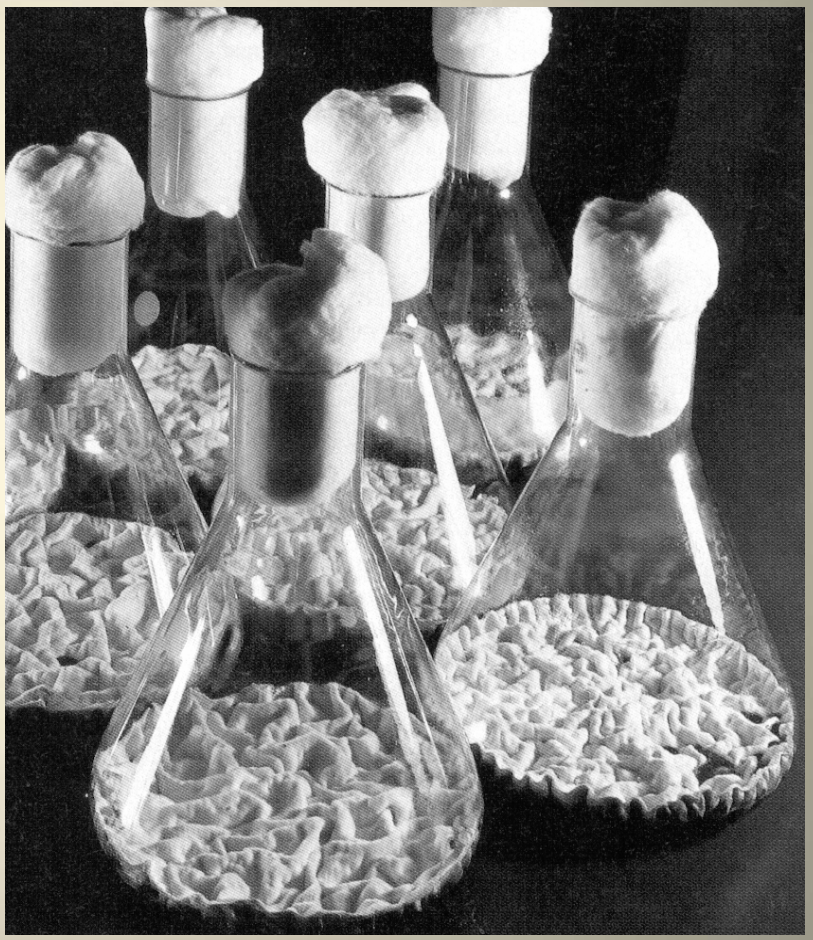
or why infection control practitioners have job security

Trevor Van Schooneveld MD

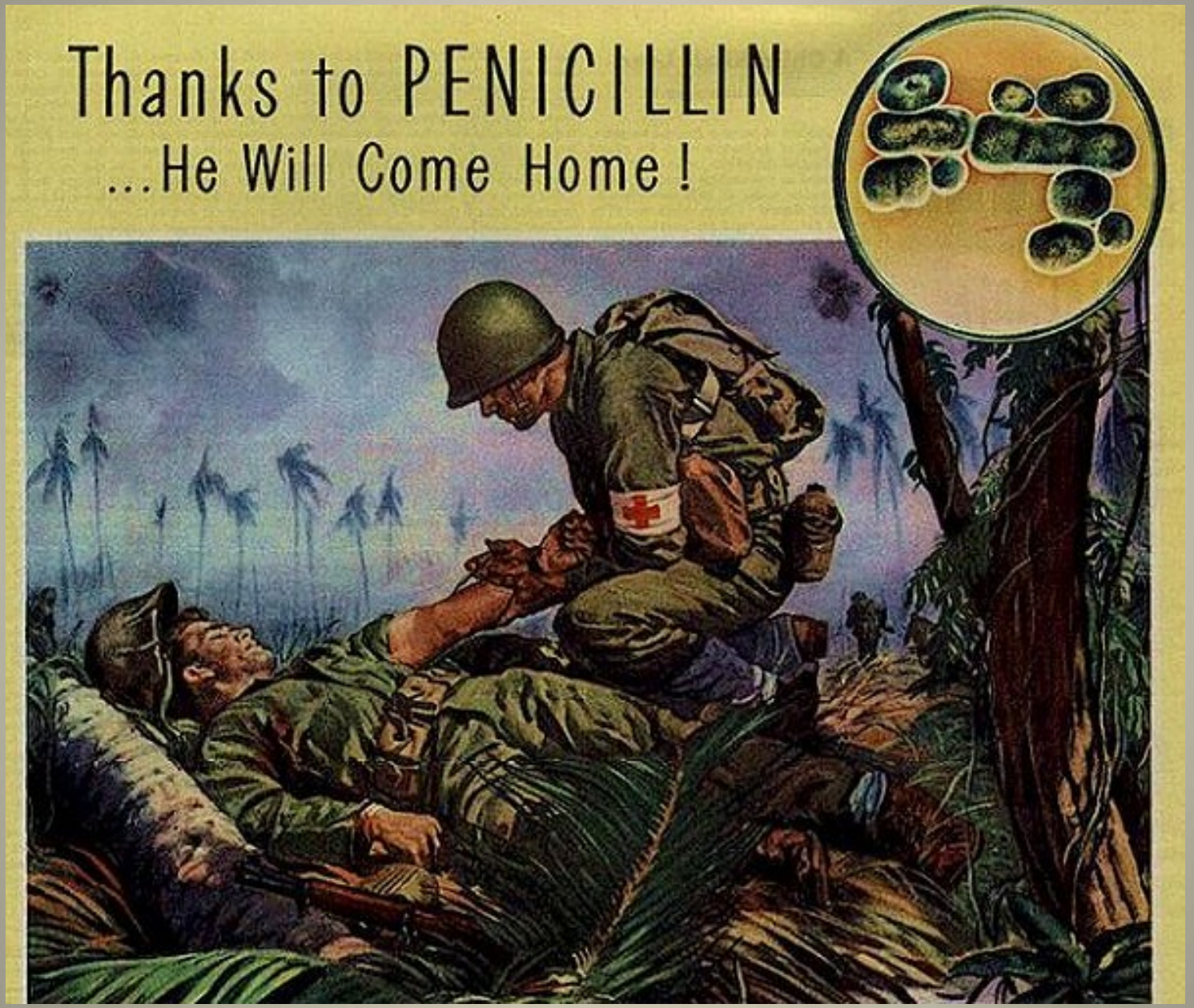
The Antibiotic Era

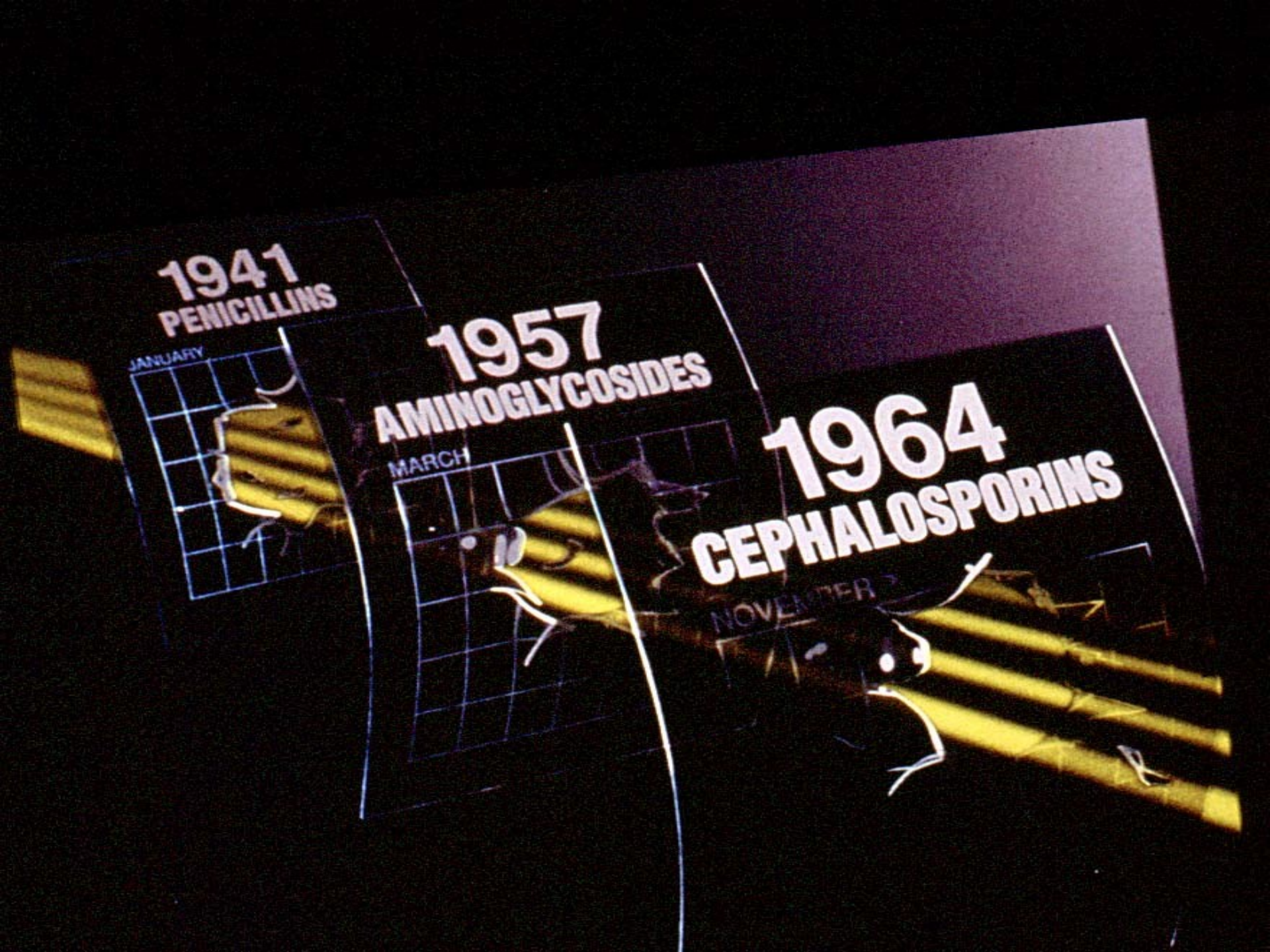


Alexander Fleming



Penicillium mold


Thanks to PENICILLIN
...He Will Come Home!

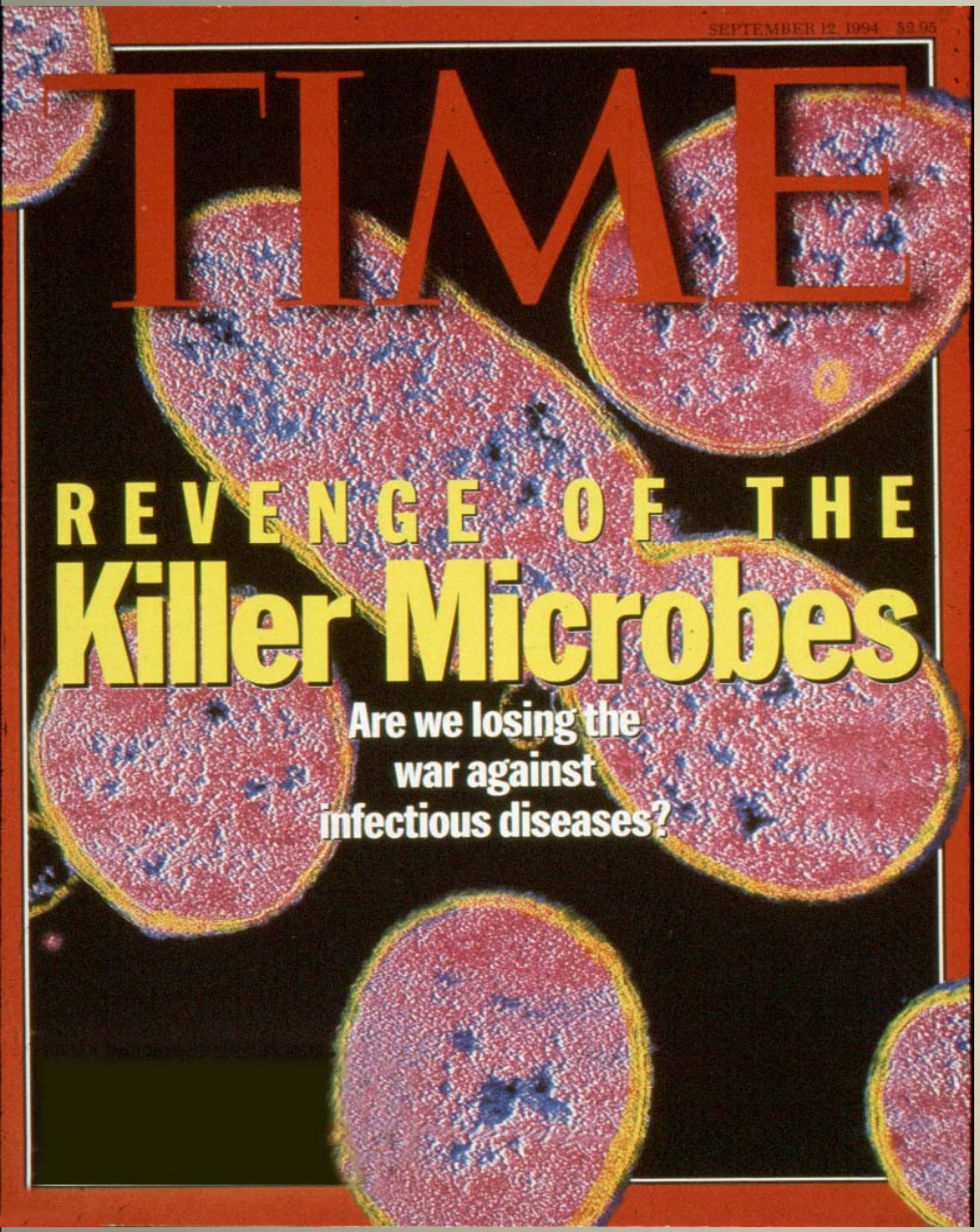

1941
PENICILLINS
JANUARY
1957
AMINOGLYCOSIDES
MARCH
1964
CEPHALOSPORINS

The Antibiotic Era

- Nearly all experts agree, by the year 2000 bacterial and viral diseases will be eliminated; in addition, atherosclerotic heart disease will have been eliminated too." TIME Magazine. Feb 25, 1966

- "It is time we close the book on infectious diseases" W.H. Stewart, US Surgeon General, 1969

- "..I cannot conceive a need for more infectious disease experts unless they spend their time culturing each other." RG Petersdorf, MD. 1978


SEPTEMBER 12, 1994 $2.95
TIME
REVENGE OF THE
Killer Microbes
Are we losing the war against infectious diseases?


Daily Mail
WEDNESDAY, JULY 25, 2007
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North West Edition 45p
THE WORLD AT WAR
FREE DVD
GET YOUR SECOND SPECIAL BONUS DISC FREE TODAY
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Hospital infection soars by 22 per cent in just three months
1,000 SUPERBUG

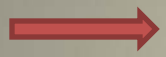

HOME
THE INDEPENDENT
Hospitals fail to halt spread of superbug that kills 4,000 a year
By Jeremy Laurance
Health Editor
Cases of C. difficile have tripled since 2001, despite attempts to enforce an NHS crackdown on cleanliness
The worst hospitals
Alarm at significant drop in number of NHS workers
By Jane Kirby
KEVIN O'SULLIVAN
Payout... Leslie Ash
£40m for NHS bug claim pot
By NICK OWENS
NHS CRISIS 'TO LAST YEARS'
17k 'killed by NHS'
DEATHS from hospital
By Jenny Hope

The Post-Antibiotic Era

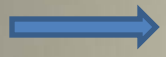
"Today we are on the verge of a medical disaster that would return physicians to the pre-penicillin days when even seemingly small infections could turn lethal for lack of effective drugs. This gruesome prediction, which would have been scoffed at a decade ago, stems from the remarkable ability of bacteria to develop resistance to almost any antibiotic medical research has thrown at them. Given time, it seems, these wily microbes will learn to chew up, spit out, or shield themselves from any drug. And when one strain learns a new resistance strategy, its not shy about sharing it with others."

Science. 1994

The March of Bacterial Resistance Over Time

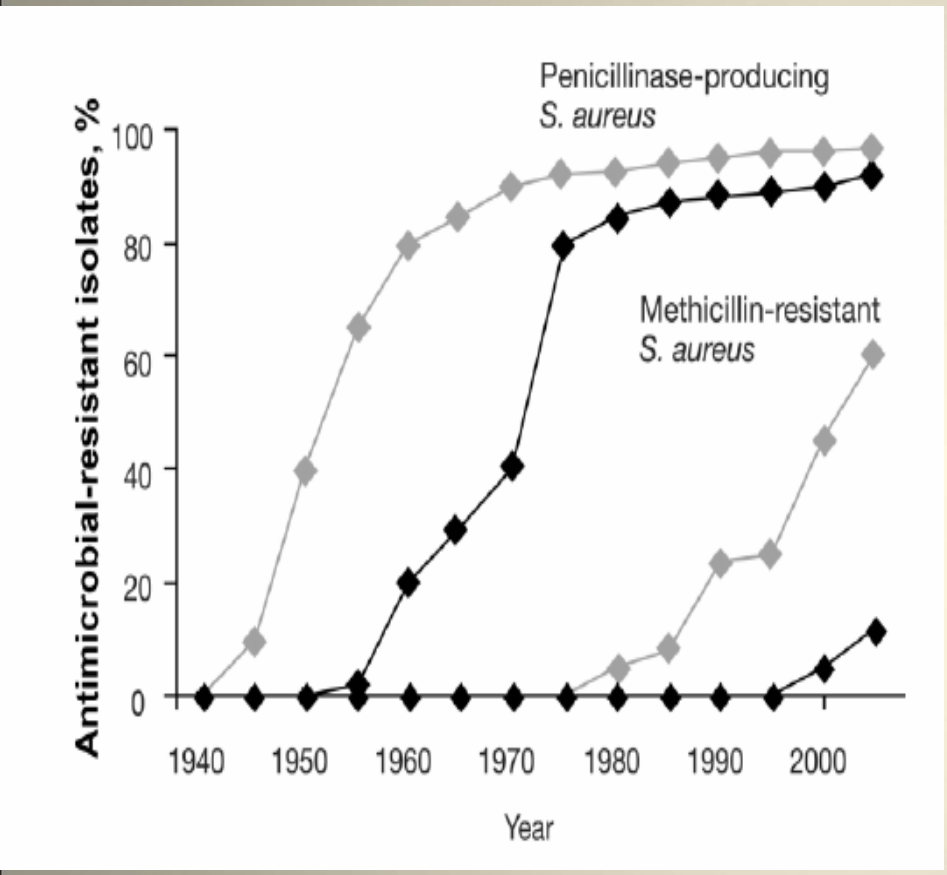


Hospital-acquired

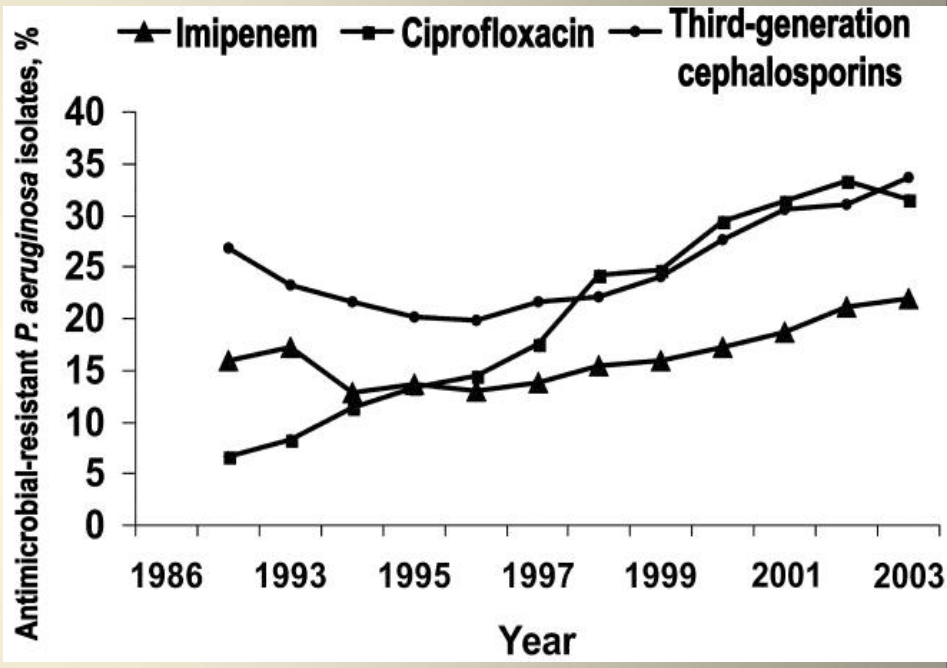


Community-acquired

Increasing Burden of Resistance



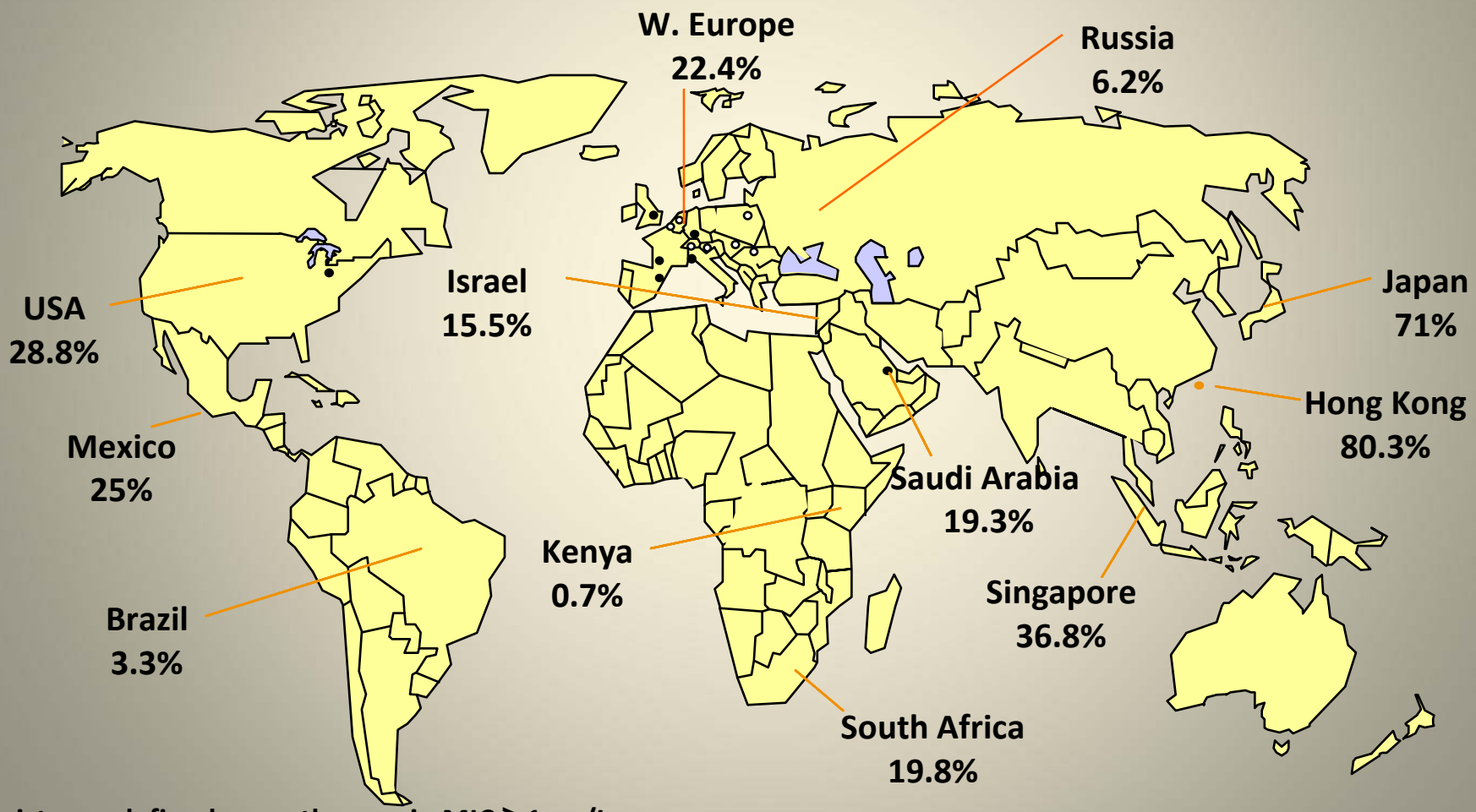
Evolution of anti-microbial resistant *S. aureus* as cause of nosocomial and community-acquired infections (*Black squares*, nosocomial infection, *Gray squares*, community-acquired infection



Resistance to antimicrobials (imipenem, ciprofloxacin, and third - generation cephalosporins) in *Pseudomonas aeruginosa* isolates recovered from intensive care unit patients

McDonald. *Clin Infect Dis*. 2006;42:S65.

The Alexander Project 1998-2000: *S. pneumoniae,* Macrolide Resistance



Resistance defined as erythromycin MIC ≥ 1mg/L.

Jacobs. *J Antimicrob Chemother*. 2003;52:229.

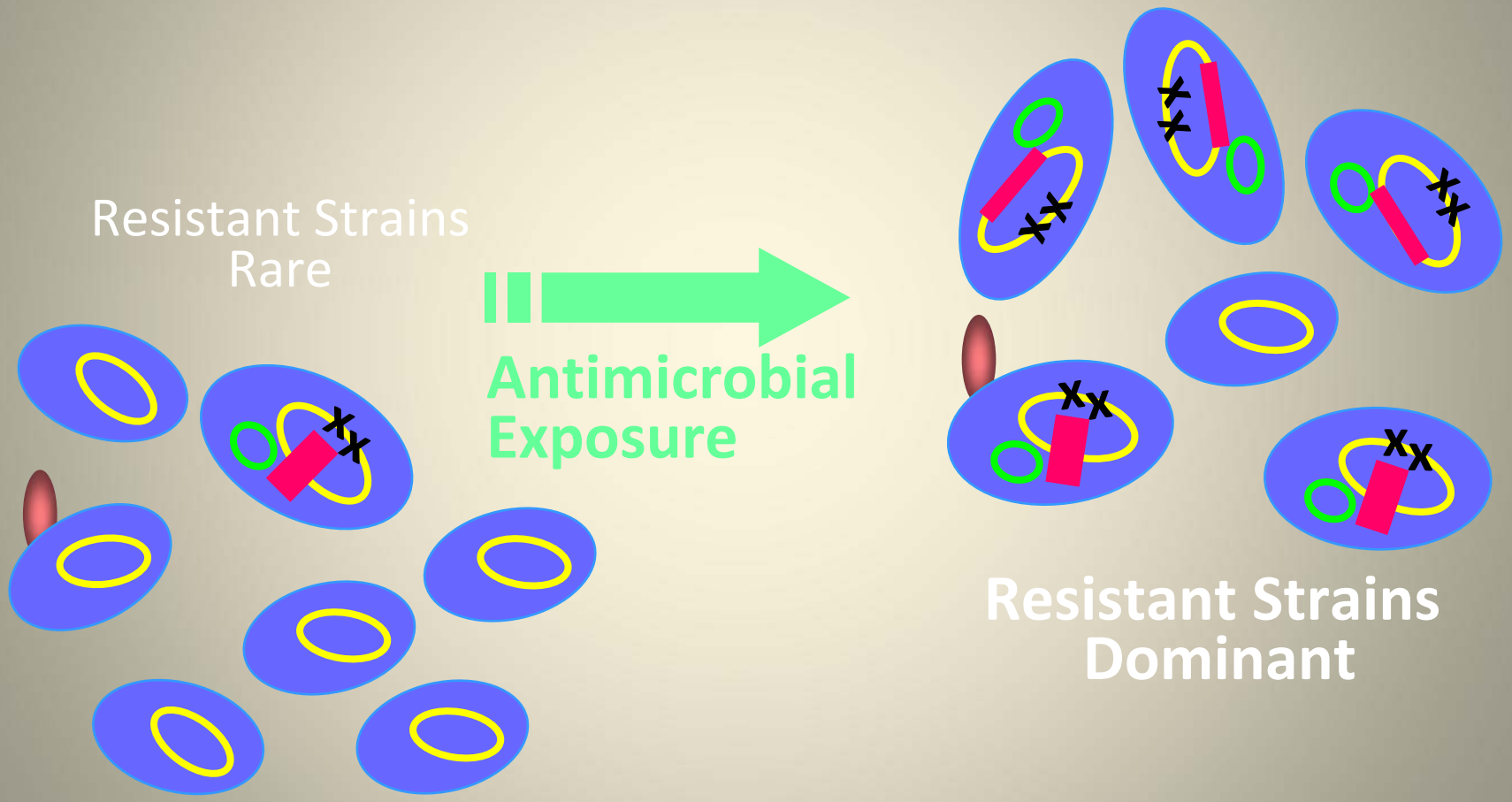
Why are resistant organisms increasing?

- Antimicrobial selection pressure
  - #1 force driving emergence of resistance
- Microbial ingenuity
  - Transmission of resistance genes
- Transmission of resistant organisms

3 Categories of Resistance

- Simple
  - Mutation produces a single resistant bacteria
  - Antibiotic pressure selects for this organism which then replaces the susceptible organisms
  - *M. tuberculosis* had predictable resistance
    - 1×10^{-6} mutants/cfu resistant to INH
    - 1×10^{-9} mutants/cfu resistant to RIF
    - Likelyhood of resistance to both = 1×10^{-15}
    - ~1×10^{12} cfu in pulmonary cavity

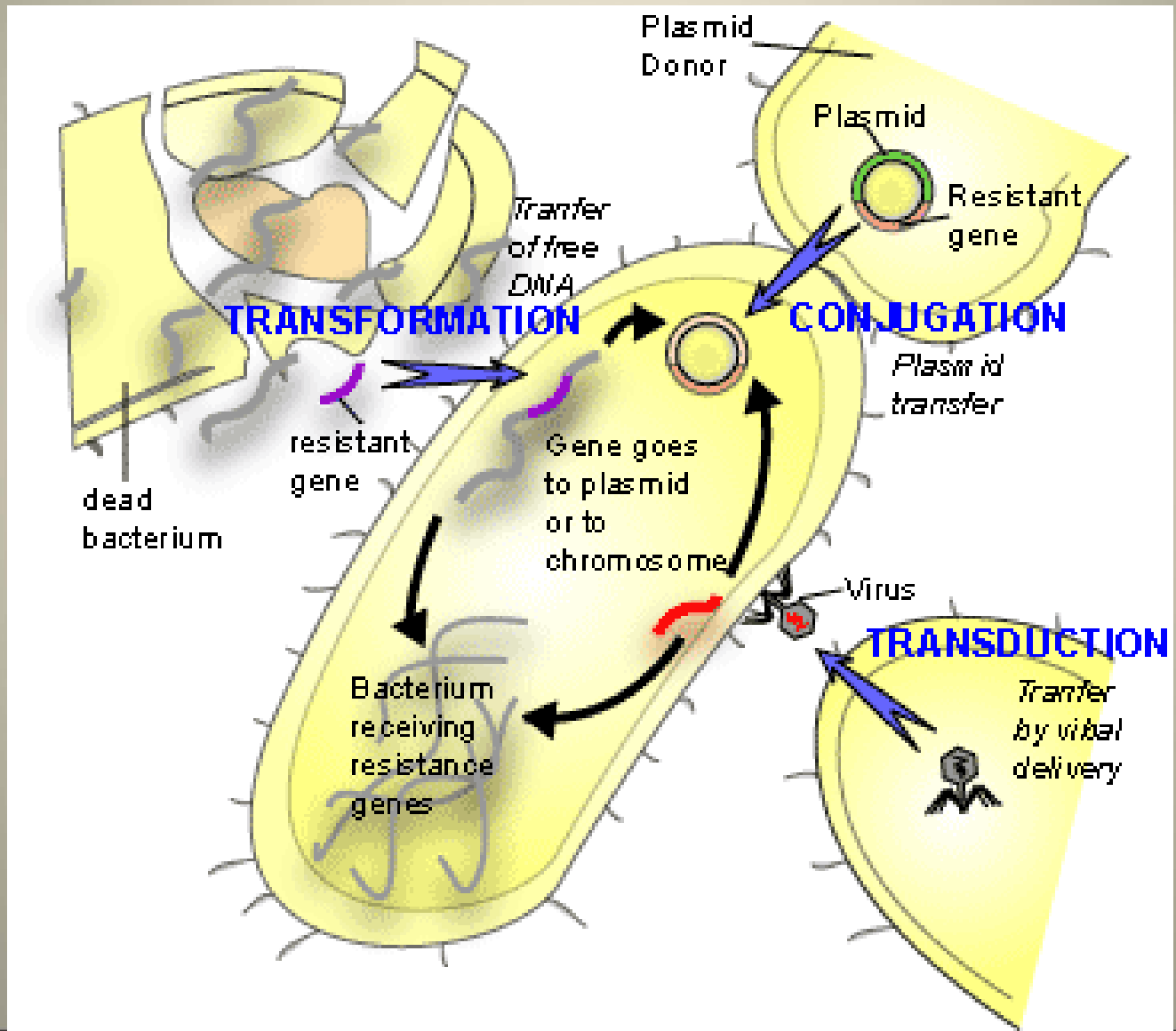
Antibiotic Selection of Resistant Strains



Moderately Complex

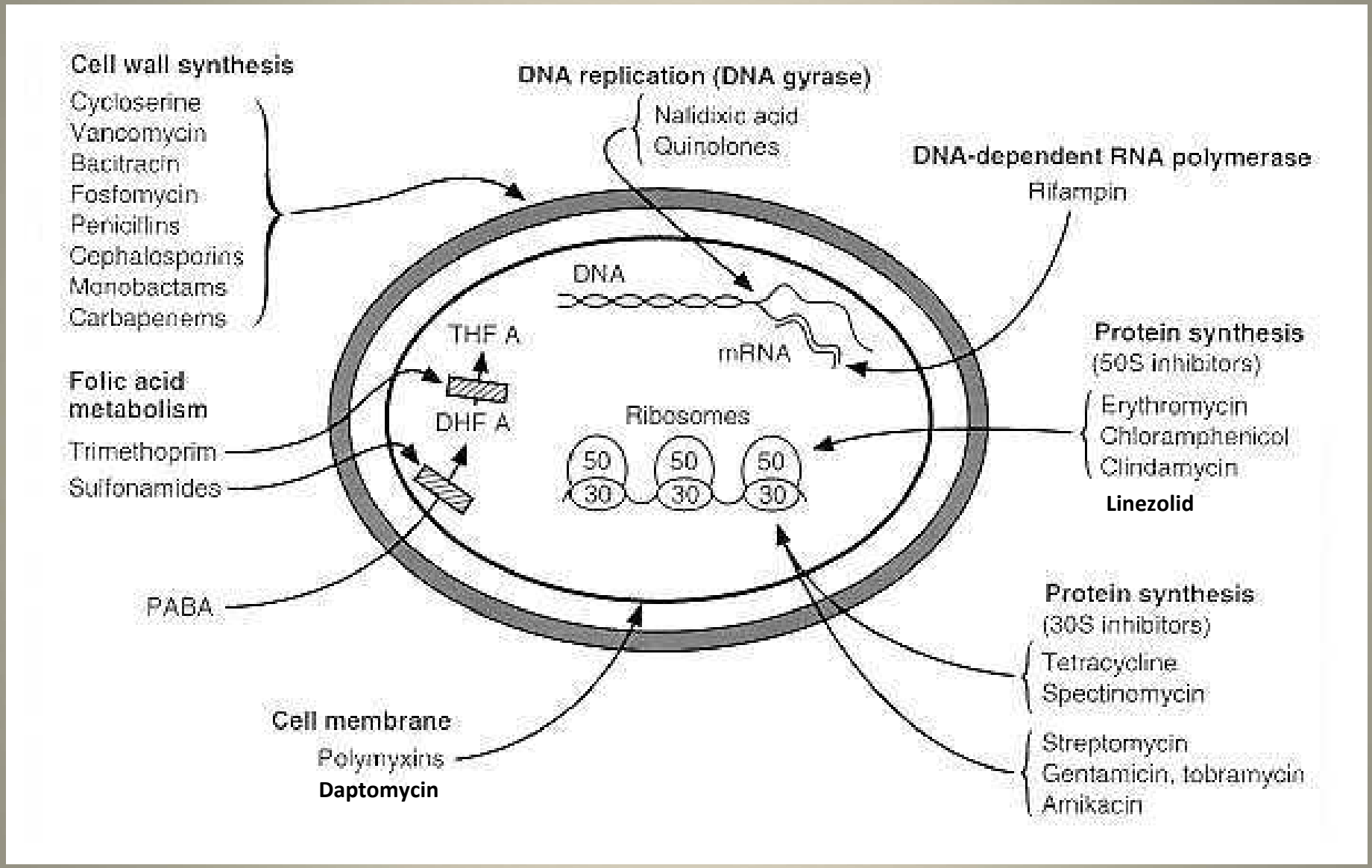
- *S. aureus* and *Enterococci* are examples
- Susceptible strains do not acquire resistance during treatment
- Do exchange genetic information with other bacteria
  - Resistant species may be present in the absence of antibiotic exposure

Mechanisms of Resistance Transfer



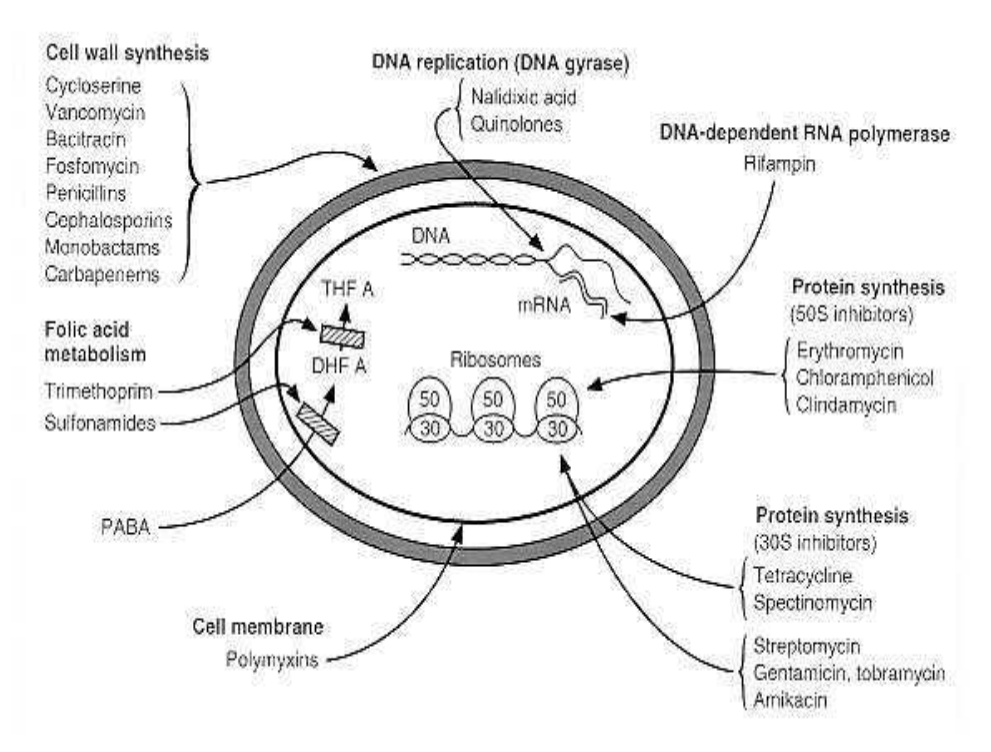
Highly Complex Resistance

- Wide array of resistance mechanisms usually present
- Can be expressed upon exposure to antibiotics
  - Allows development or resistance during treatment
  - *Pseudomonas* exposed to antibiotics
    - Decrease permeability
    - Increasing pumping out of antibiotics
    - Increase hydrolyzing enzymes
- Easily exchange DNA with other species
  - Can carry many antibiotic resistance determinants


Cell wall synthesis
Cycloserine
Vancomycin
Bacitracin
Fosfomycin
Penicillins
Cephalosporins
Monobactams
Carbapenems
DNA replication (DNA gyrase)
Nalidixic acid
Quinolones
DNA-dependent RNA polymerase
Rifampin
DNA
mRNA
THF A
DHF A
Folic acid metabolism
Trimethoprim
Sulfonamides
PABA
Ribosomes
50
30
Protein synthesis
(50S inhibitors)
Erythromycin
Chloramphenicol
Clindamycin
Linezolid
Protein synthesis
(30S inhibitors)
Tetracycline
Spectinomycin
Streptomycin
Gentamicin, tobramycin
Amikacin
Cell membrane
Polymyxins
Daptomycin

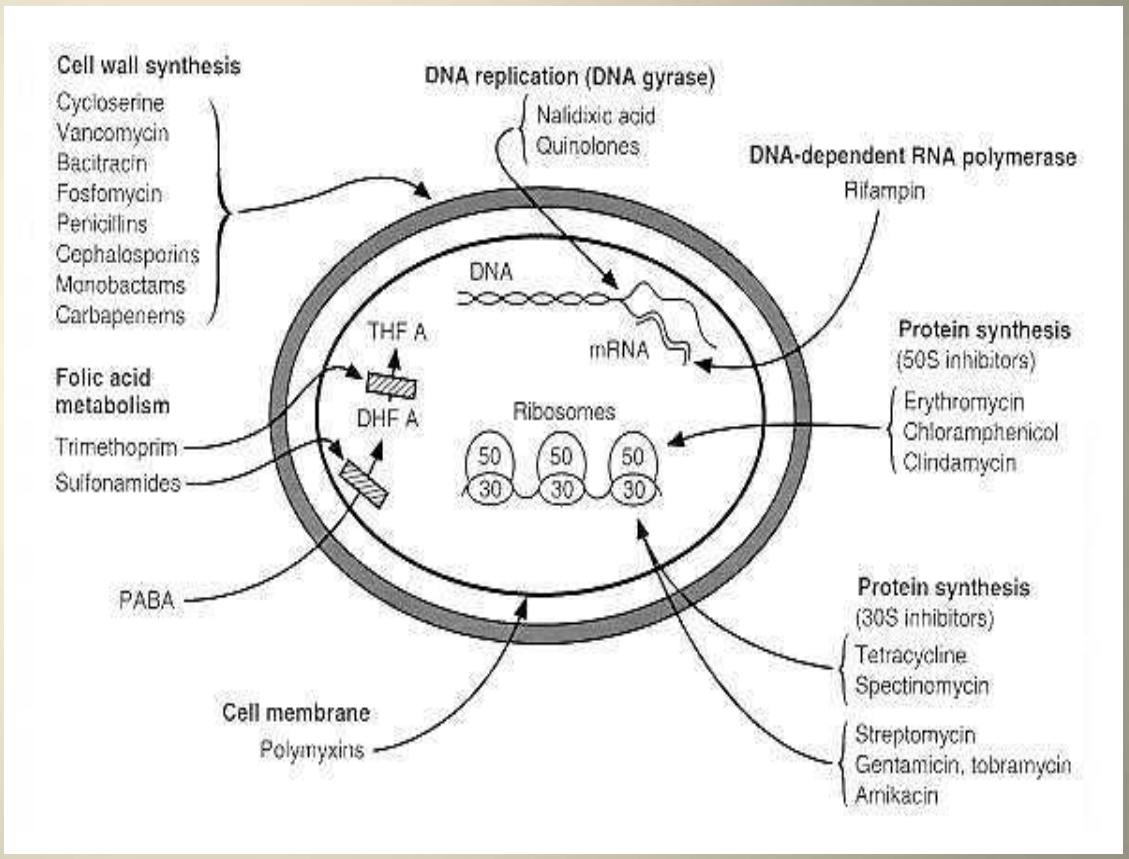
Antibiotic Mechanisms and Classes

- Cell Wall Acting Antibiotics
  - Beta-Lactams
    - Penicillins
    - Cephalosporins
    - Carbapenams
    - Aztreonam
  - Vancomycin
- Metabolic Pathway
  - Sulfa
  - Trimethoprim



Antibiotic Mechanisms and Classes

- Ribosome
  - Macrolides
  - Clindamycin
  - Aminoglycosides
  - Linezolid
  - Tetracyclines
- DNA Replication
  - Quinolones
- Cell Membrane
  - Daptomycin
  - Polymyxins

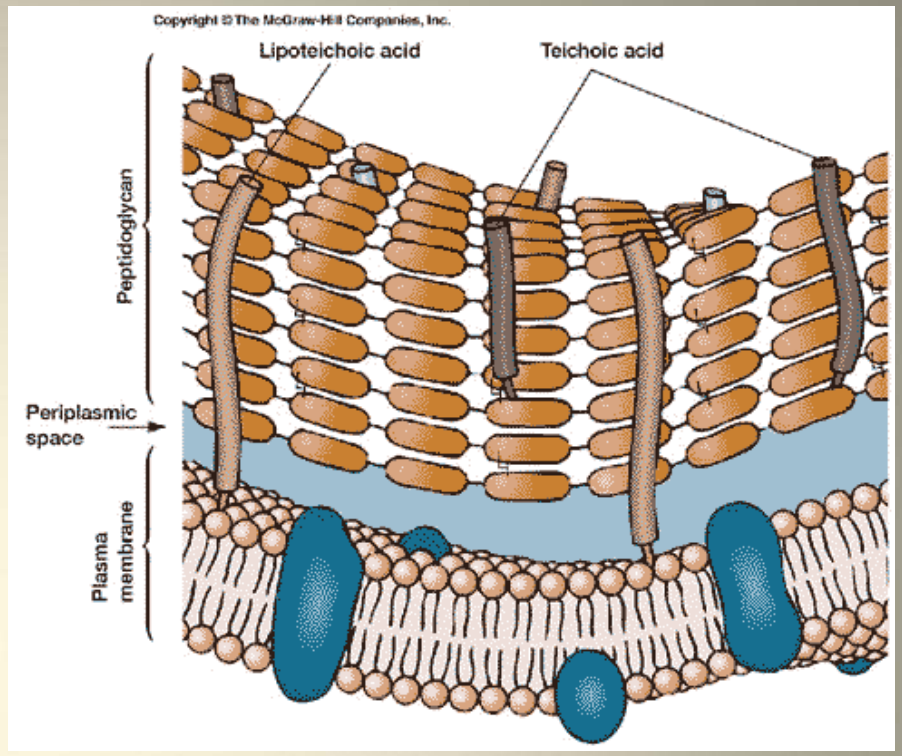


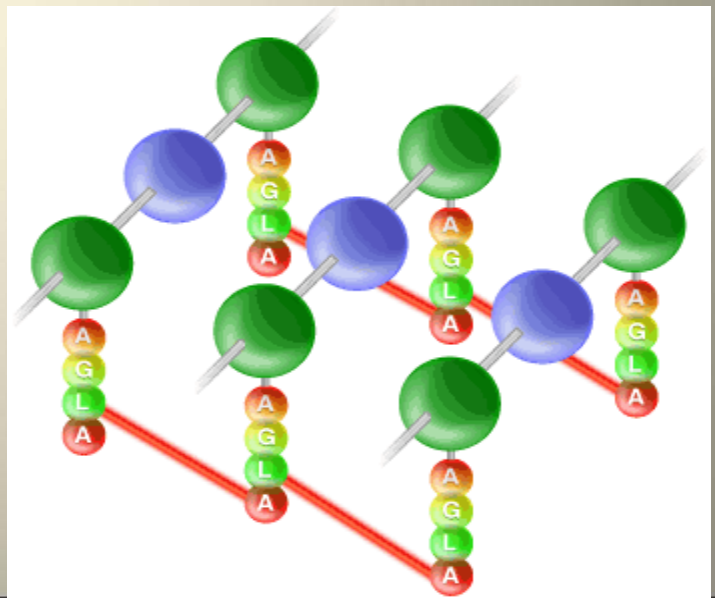
Mechanisms of Resistance

- Altered targets
- Enzymatic inactivation
- Decreased permeability of organism
- Active efflux pumps
- Bypass target process

Altered Target

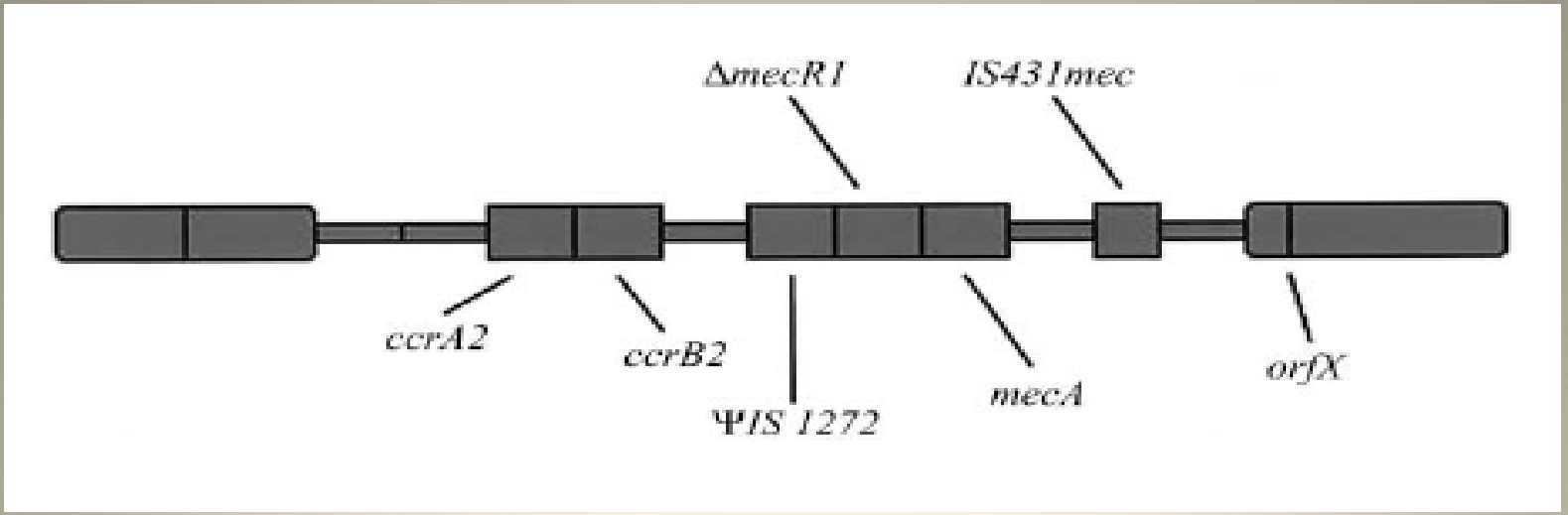
- β-lactam Mechanism
  - Binds penicillin binding proteins (PBP)
    - PBP's cross-link peptidoglycans
    - Provide stability to cell
  - β-lactam binding blocks cross-linking
    - Causes cell death





β-lactam Resistance via Altered Targets

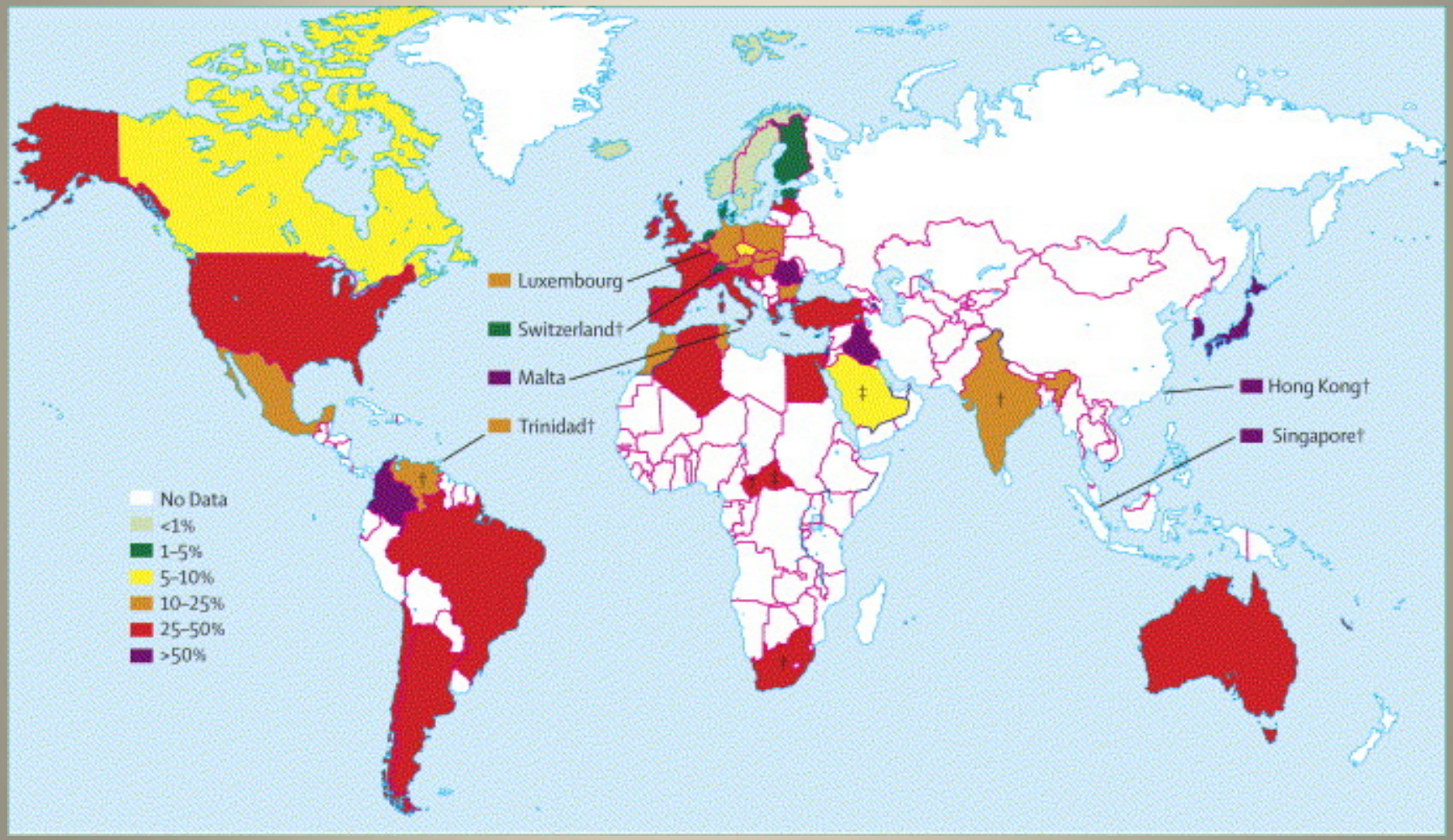
- *Staphylococcus aureus*
  - Penicillin resistance mediated by hydrolizing enzymes
  - Methicillin resistant to these
- Methicillin-resistant *S. aureus* (MRSA)
  - Altered PBP 2a
  - Deceased affinity for all β-lactams
  - Likely acquired from coag-negative Staphylococci



- PBP 2a encoded by the *mecA* gene
  - *mecA* is part of a complex of genes called the Staphylococcal cassette chromosome (SCC*mec*)
    - Mobile genetic elements which insert into the genome of *S. aureus* in very specific areas
    - Easily transferred between Staphylococci
    - These often carry other resistance determinants

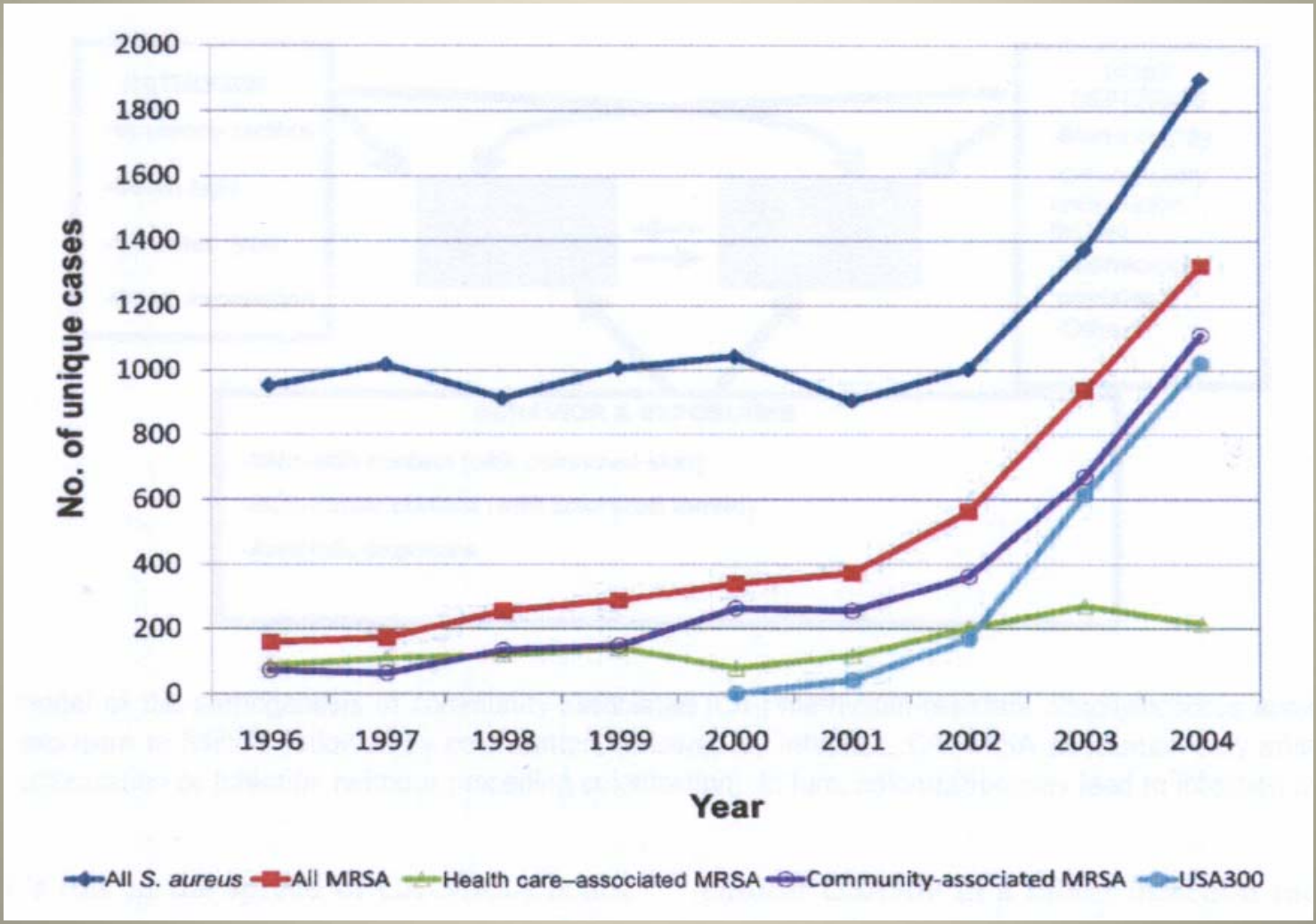
Deresinski S *Clin Infect Dis.* 2005;40:562-573

Worldwide Prevalence of MRSA displayed by country



Grundmann. *Lancet* 2006;368:874

Rapid Emergence of USA300 in Community Health Network of San Francisco



Miller. *Clin Infect Dis* 2008;46:752

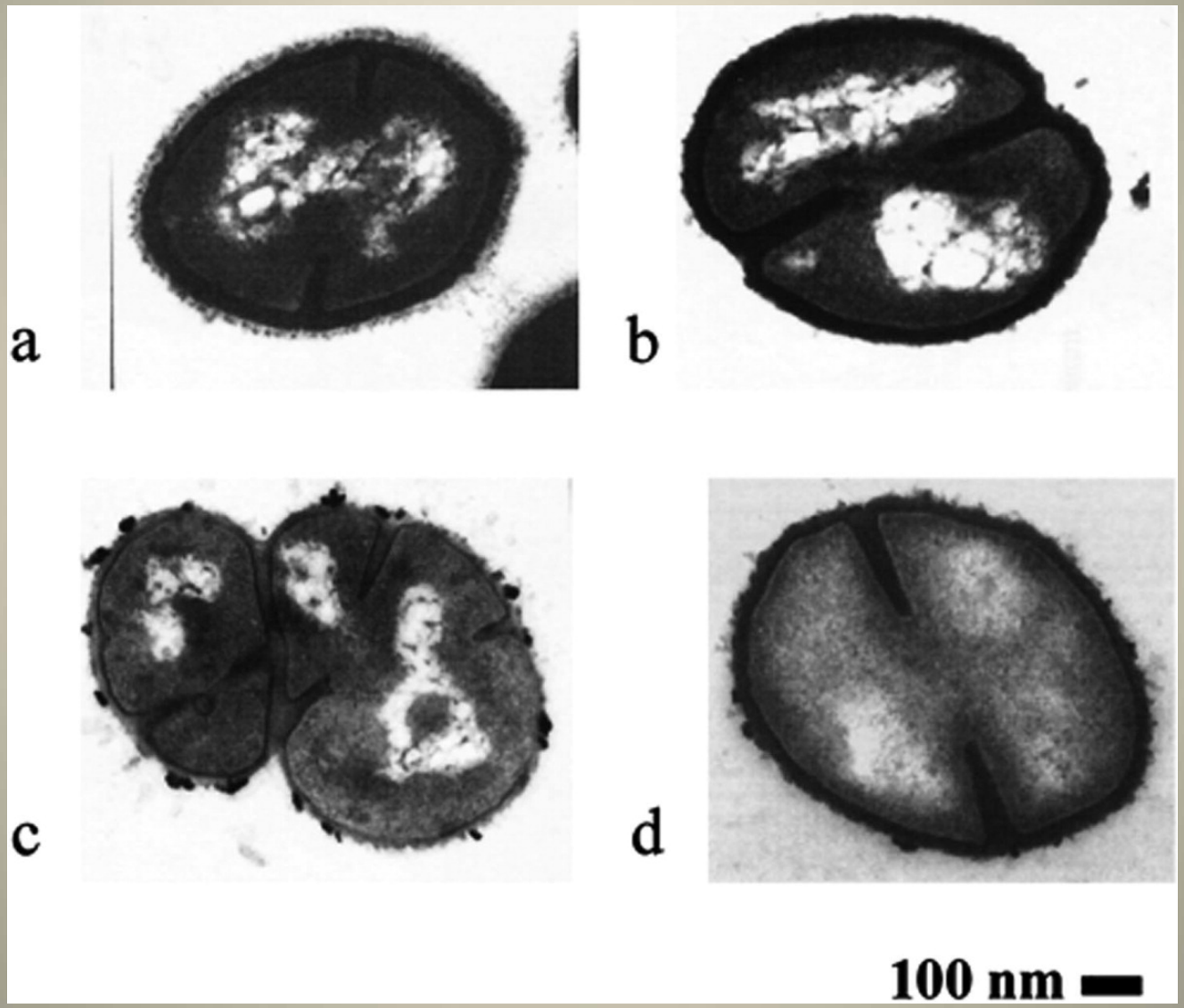
Community-Acquired MRSA

- Rates of MRSA in community rose rapidly in early 2000s
- Unique strain of MRSA – USA 300
  - *SCC mecIV* smaller and only carried methicillin resistance
  - Panton-Valentine leukocidin
  - Skin-soft tissue infections
  - Now moving into hospitals and blurring line between hospital and community MRSA

Vancomycin-intermediate *S. aureus* (VISA)

- Vancomycin was used for decades without resistance
- Intermediate resistance to vancomycin noted in late 1990's (MIC 4-8)
- Followed long courses of vancomycin
- No *vanA/B* gene detected (found in VRE)
- Significant cell wall thickening noted on EM
- Can be difficult to detect in lab

Morphological cell-wall thickening in vancomycin-intermediate *S. aureus* strains *(a* and *b),* a vancomycin-susceptible strain *(c),* and control strain *(d)*

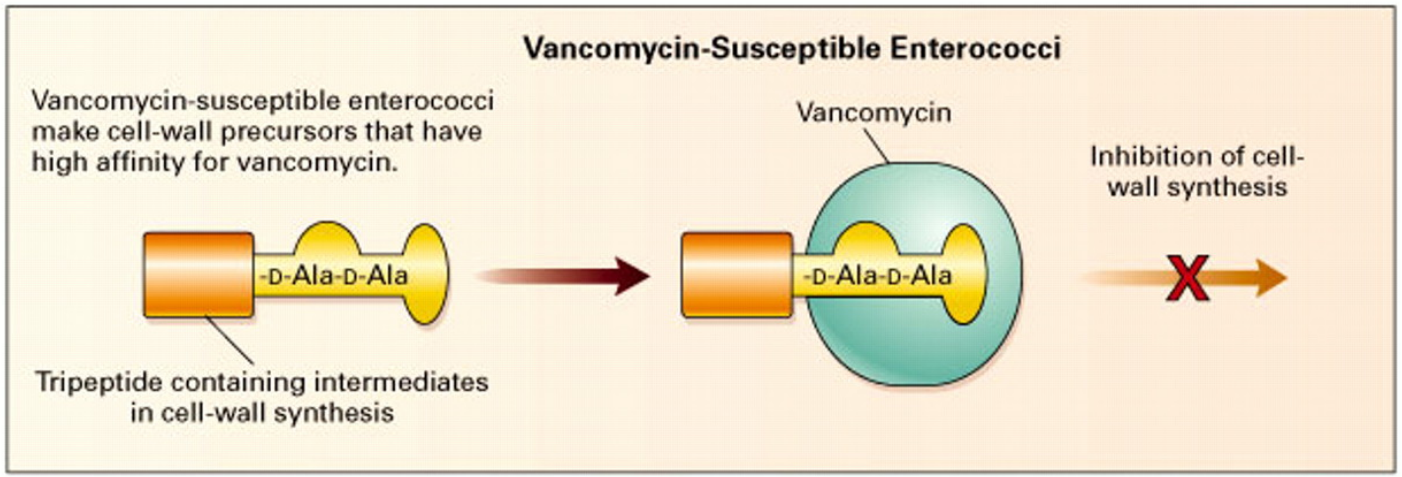


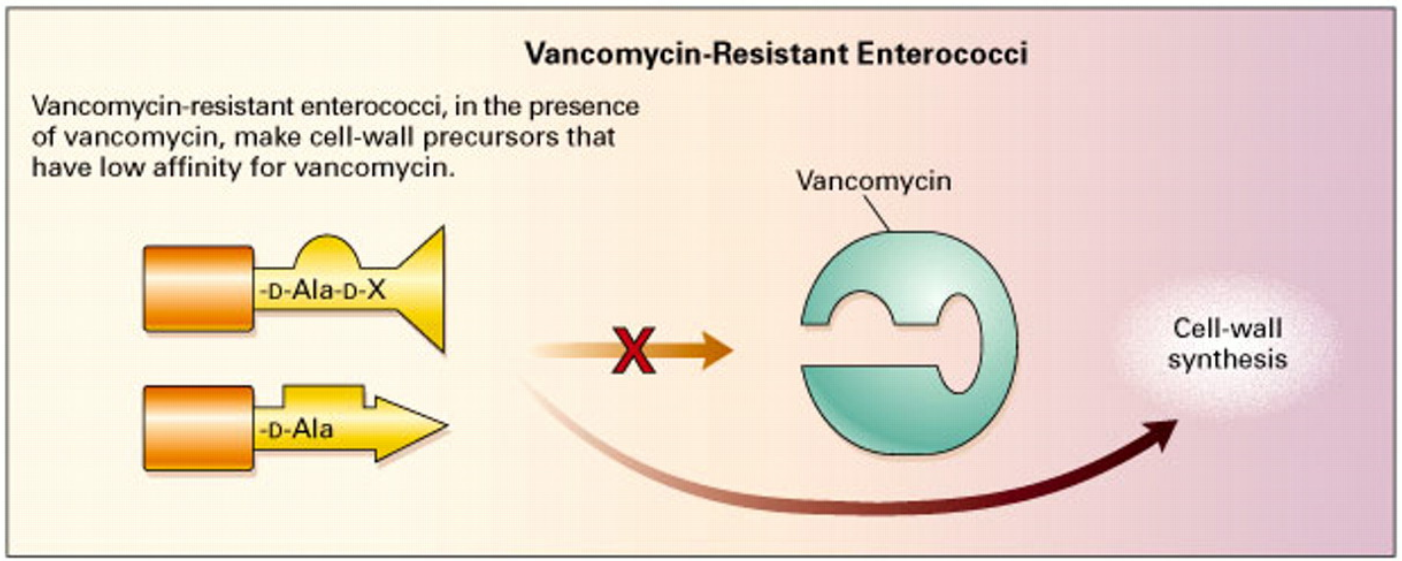
Sakoulas. *Clin Infect Dis*. 2008;46:S360.

Vancomycin Resistance

- Vancomycin binds to cell wall precursors
  - Prevents the synthesis of cell-wall preventing their incorporation into wall
- Vancomycin Resistant Enterococci (VRE)
  - Use alternative precursors for cell wall
  - *vanA* and *vanB* are most common genes
  - Acquired from and transmitted to enterococci and other strep species

Vancomycin Resistance





Murray. *NEJM*. 2000;342:710.

Vancomycin-resistant *S. aureus* VRSA

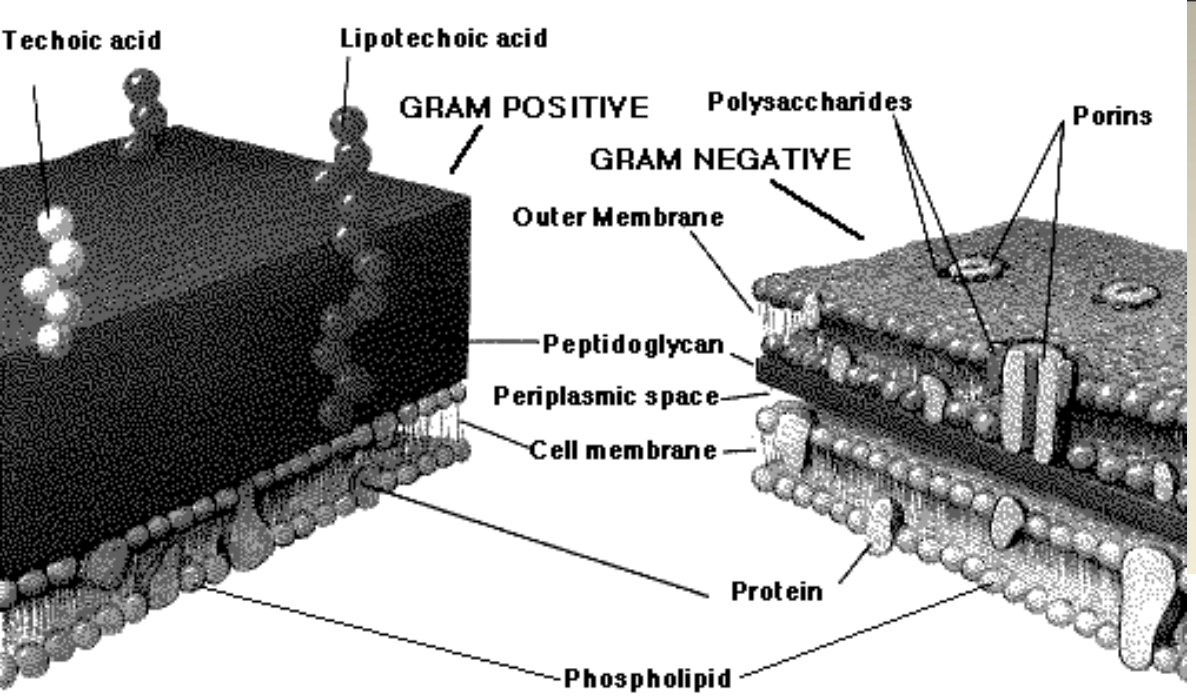
- First reported in 2002 and very few reports
- 7 VRSA isolates reported US between 2002-07
- All contained *vanA* gene
- All were
  - Colonized/infected with MRSA and enterococci
  - Had chronic health conditions
  - Prolonged exposure to antibiotics
- No person-to-person spread seen (yet)

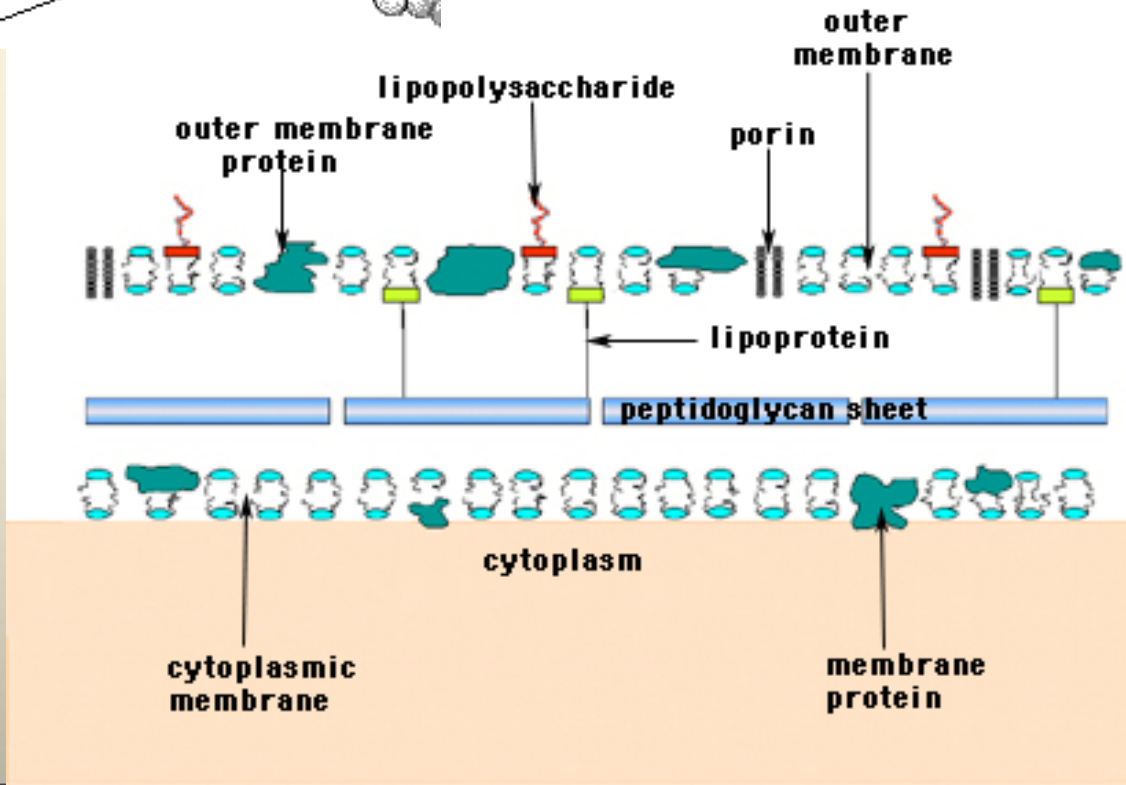
Summary

- Methicillin-resistance via altered PBP
- Vancomycin resistance via altered precursor

Enzymatic Inactivation

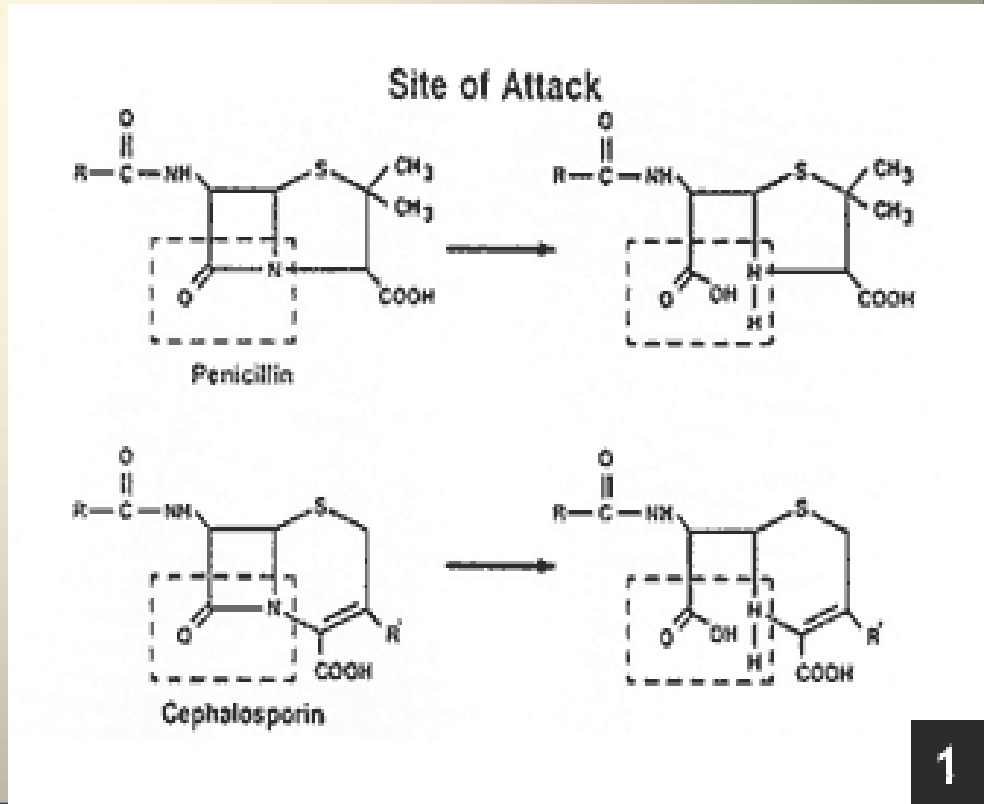
- Beta – lactamases
- Other inactivating enzymes exist (aminoglycosides, chloramphenicol)


Techoic acid
Lipotechoic acid
GRAM POSITIVE
Polysaccharides
Porins
GRAM NEGATIVE
Outer Membrane
Peptidoglycan
Periplasmic space
Cell membrane
Protein
Phospholipid


outer membrane
lipopolysaccharide
outer membrane protein
porin
lipoprotein
peptidoglycan sheet
cytoplasm
cytoplasmic membrane
membrane protein

Beta-lactamases

- Hydrolyze the beta-lactam ring
- Differing spectrums of activity
  - Penicillinase, cephalosporinase, etc.
- Location of Gene
  - Chromosomal
  - Plasmid or transposon
- Production
  - Constitutive
  - Inducible by antibiotic



Beta-lactamases

- Many naturally occurring
  - Carried on chromosome
- First plasmid mediated noted *E. coli* and *Klebsiella pneumoniae* in mid-1960s
  - TEM-1 and SHV-1
  - Inactivated all penicillins and 1st and 2nd generation cephalosporins
  - Rapidly spread other gram-negatives (*H. influenzae, N. gonorrhoeae, Psuedomonas*)
- Solution?
  - 3rd generation cephalosporin created

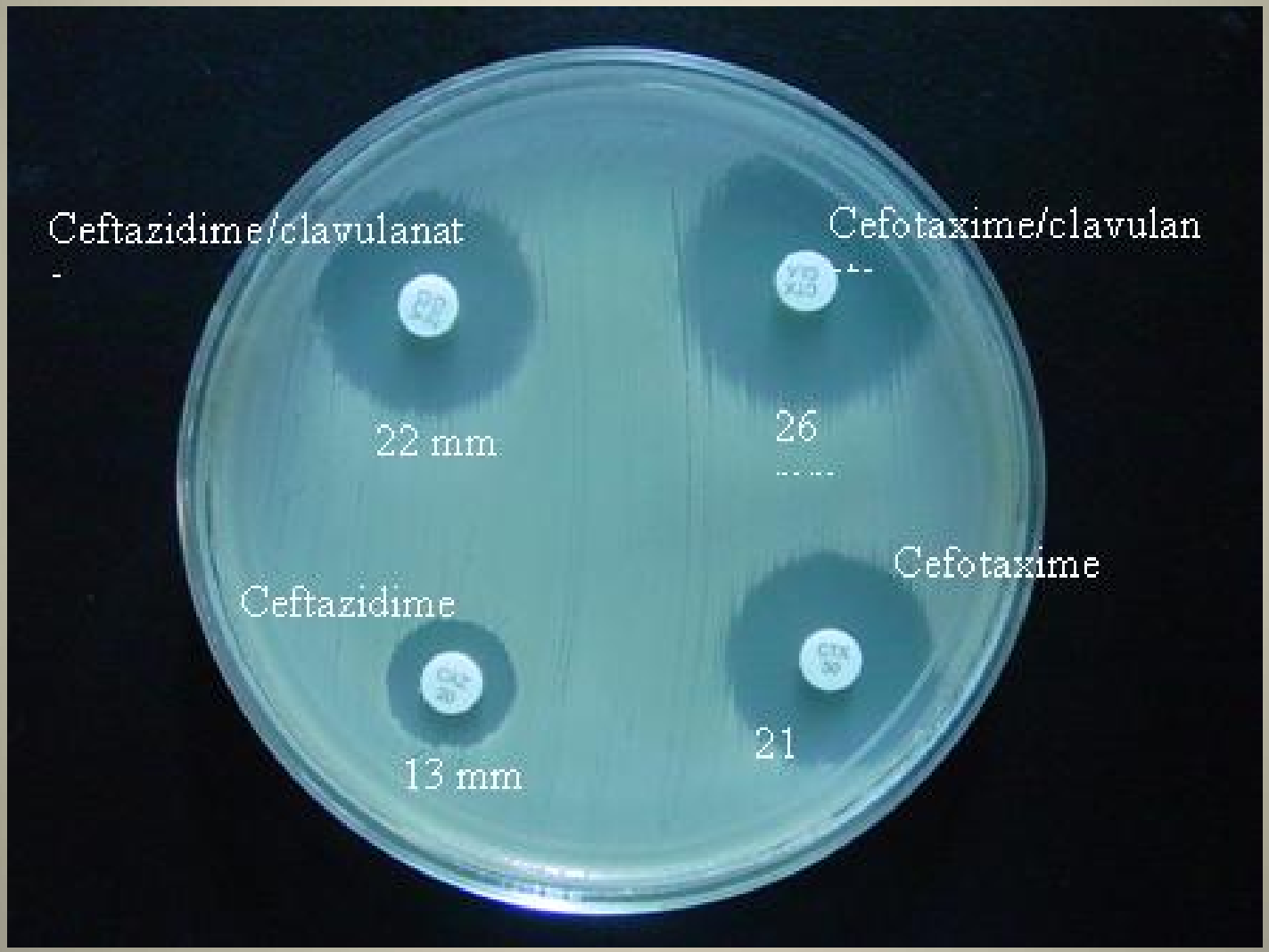
Extended Spectrum Beta-lactamases ESBL

- Mutation in beta-lactamase extended activity
  - Hydrolyzes 3rd generation cephalosporins
  - Sensitive to beta-lactamase inhibitors
- First described *Klebsiella* species 1980s
- Plasmid mediated
  - Often carry resistance to other antibiotics
- *E. coli, Klebsiella, Proteus* primarily carry
  - Incidence increasing in US even in outpatient setting

Detection and Treatment

- Can be difficult to detect
  - Activity differs for dif. 3rd generation cephalosporins
    - Some may appear susceptible and others resistant/intermediate
  - Any *E. coli, Klebsiella pneumoniae,* or *Proteus mirabilis* isolate with decreased susceptibility to 3rd-generation cephalosporin should be screened
  - If present report all 3rd-gen cephalosporin as resistant
- Treat with carbapenams

ESBL Detection



AmpC Beta-lactamase

- Extended Spectrum Beta-lactamases
  - Resistant to beta-lactamase inhibitors
- Chromosomally mediated
  - *Enterobacter, Citrobacter, Serratia*
- Usually expressed low level
  - Can be induced by antibotics
  - Can become “derepressed”
- Even more difficult to detect
  - No recommendations for screening
- Carbapenams again treatment of choice

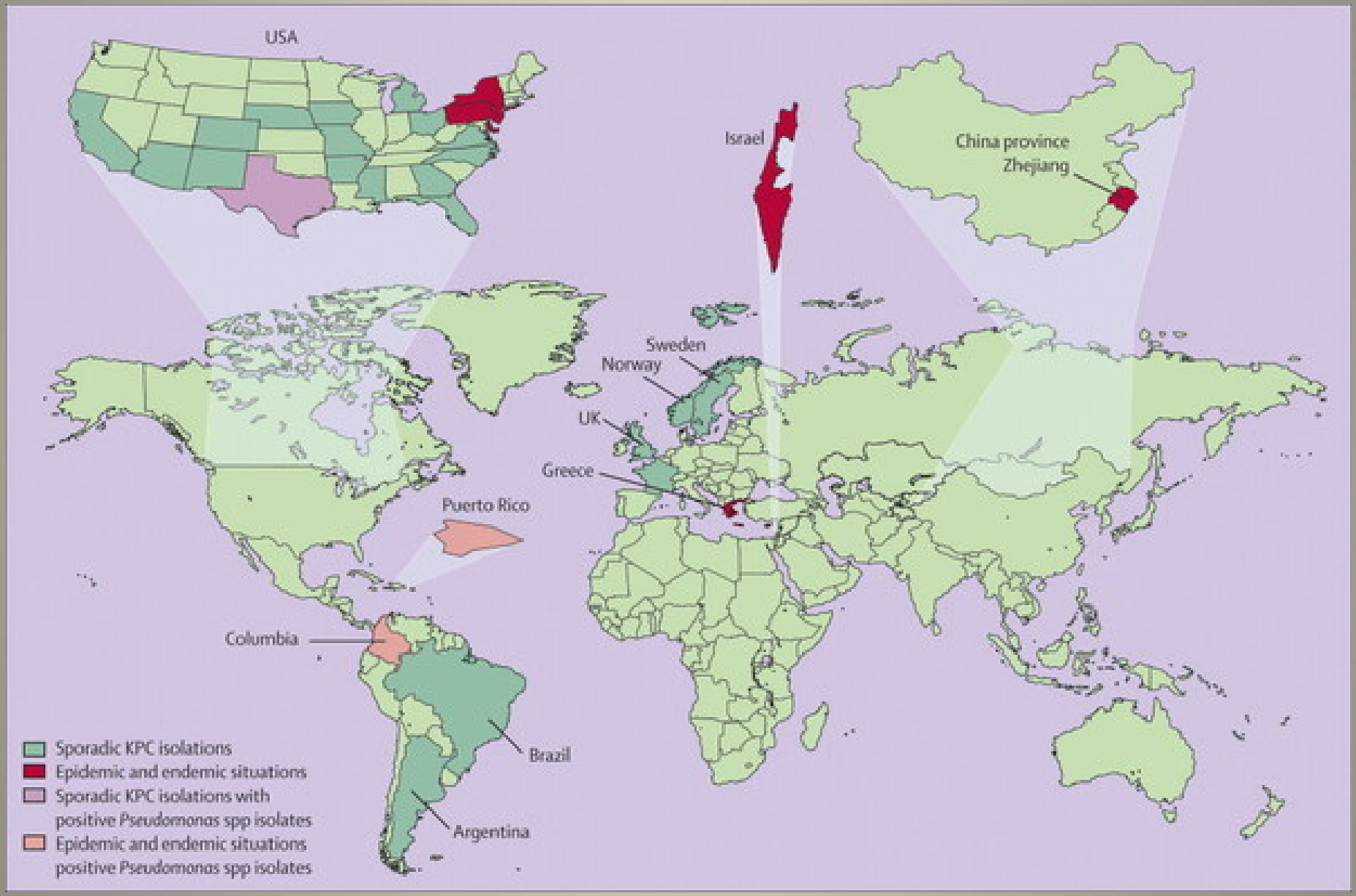
Carbapenamases

- Hydrolyze all beta-lactams
- Geographically localized in distribution
- Many different types
  - Chromosomal
    - *Psuedomonas, Acinetobacter, Stenotrophamonas, Enterobacter, Serratia*
  - Transferable
    - Transposons (*Pseudomonas, Acinetobacter*)
    - **Plasmid (*Klebsiella*)**
      - **KPC-family**

K. pneumoniae carbapenemase enzymes (KPC's)

- First noted 1996
- Plasmid mediated
- Hydrolyze all beta-lactams
- Risk Factors
  - Prolonged hospitalization/ICU stay, invasive device, immunosuppressed, multiple antibiotic exposure
- Still localized

Geographic Distribution of KPC's Worldwide

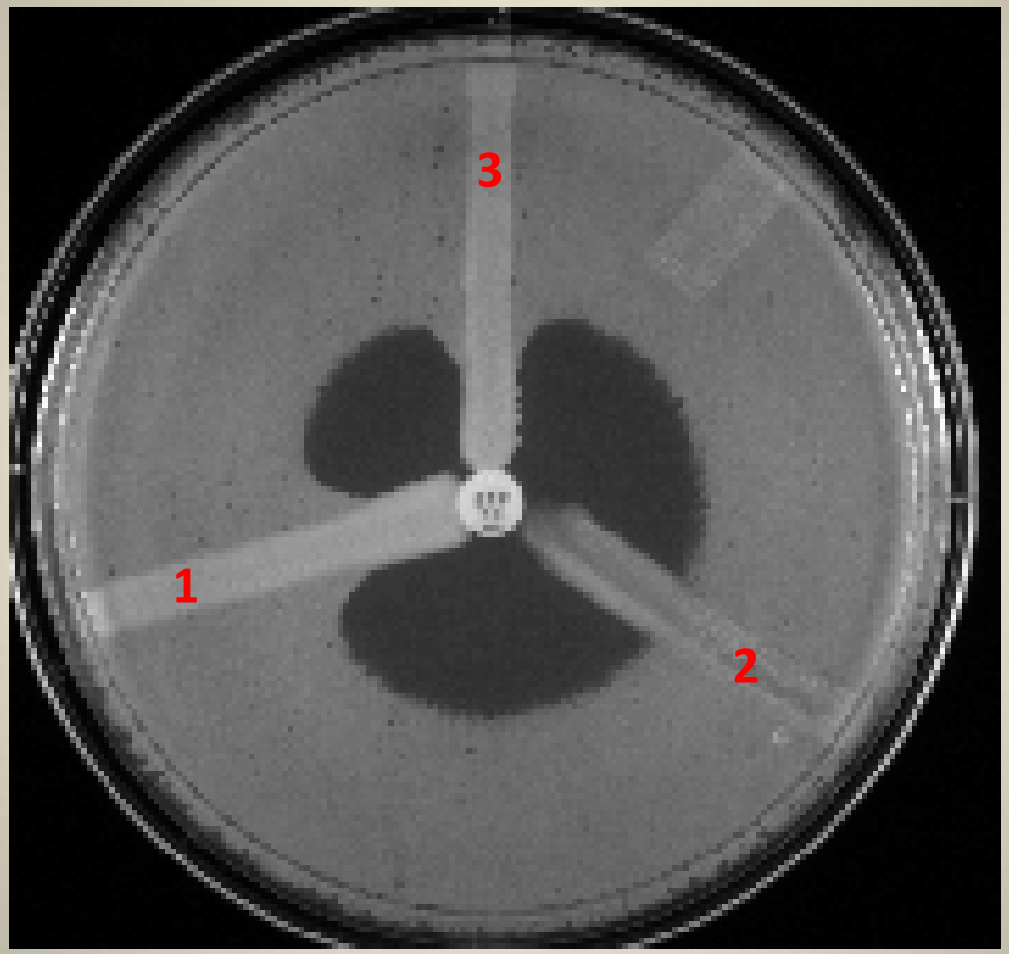


Nordmann. *Lancet Infect Dis*. 2009;9:228.

Detection

- Detection is difficult
  - May appear susceptible by usual testing
- Our lab screens any Enterobacteraciae with decreased susceptibility to any carbapenam
  - Ertapenam is best for detecting KPC's
- Modified Hodge test is screen
  - If positive confirmatory testing will be performed
    - Phenotypic and genetic tests

Modified Hodge Test



(1) *K. pneumoniaeATCC BAA 1705,* ***positive*** *result (2) K. pneumoniaeATCC BAA 1706,* ***negative*** *result; and (3) a clinical isolate,* ***positive*** *result*

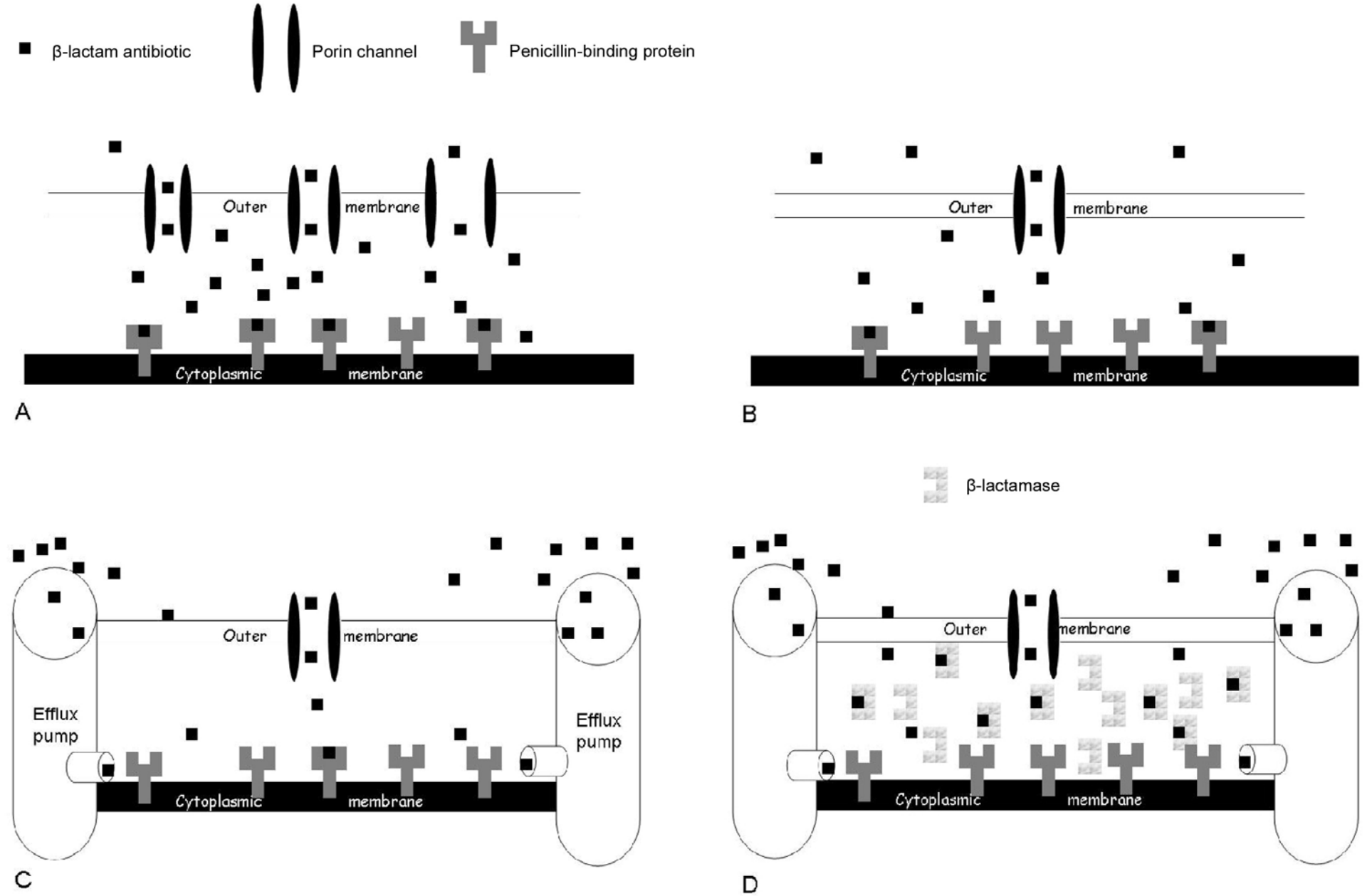
Treatment

- Treatment options limited
  - No beta-lactams
  - Frequently also resistant to FQ, AG, SMP/TMX
- Tigecycline or Colistin

Decreased Permeability

- Antibiotics must penetrate the outer membrane of gram-negative organisms to act
- Multiple mechanisms decrease permeability
  - Decreased porin expression
  - Active efflux pumps
  - Multi-drug efflux pumps
    - Frequently found *Psuedomonas*
    - Actively remove antibiotics from cell
      - Quinolones, Tetracyclines, beta-lactams, rifampin, erythromycin, chloramphenicol

Decreased Permeability



Summary

- Beta-lactamases hydrolyze drugs
  - Many types and hydrolyze different substrates
- Decreased permeability plays role in high level antibiotic resistance
  - Porin alterations
  - Active efflux pumps

Why does it all matter?

- March 2008 67 y/o male in NY hospital developed liver abscess post Whipple
  - *Klebsiella pneumoniae* and *Enterbacter cloacae* resistant to carbapenams isolated
    - Susceptible tigecycline and polymyxin B
    - Treated with tigecycline
  - Prolonged course and transferred another hospital in May 2008 with fever and hypotension
    - Undrained abscess noted
    - Drained, cultures sent, polymyxin B added

Eleman. *Clin Infect Dis*. 2009;49:271.

Why does it all matter?

- *Klebsiella pneumoniae* and *Enterbacter cloacae* again isolated
  - Producers of KPC's
  - Resistant to all antibiotics including tigecycline and polymyxin B
  - Despite continued treatment patient became bacteremic and eventually died

"It is a rarity for a physician in the developed world to have a patient die of an overwhelming infection for which there are no therapeutic options."

Bad Bugs, No Drugs

As Antibiotic Discovery Stagnates ...
A Public Health Crisis Brews

"Infectious diseases physicians are alarmed by the prospect that effective antibiotics may not be available to treat seriously ill patients in the near future. There simply aren't enough new drugs in the pharmaceutical pipeline to keep pace with drug-resistant bacterial infections, so-called 'superbugs.'"

Joseph R. Dalovisio, MD
IDSA President

How do we prevent the spread?

- Infection control measures
  - Isolation of appropriate patients
- Appropriate use of antibiotics
  - Make antibiotic choices based on local data
  - Narrow spectrum when possible
  - Use micro data to target therapy
  - Reassess need for antibiotics periodically
    - Is the patient better or is there an alternative diagnosis?