Combating antibiotic resistance

October 23, 2006

Causes of death, 2001:

Population:	6,122,210,000
Deaths:	56,554,000

USA

6.

1. **Infectious and parasitic diseases: 14.9 million**

1.

2. **Heart diseases: 11.1 million**

2.

3. **Cancers: 7.3 million**

3.

4. **Stroke: 5.5 million**

4.

5. **Respiratory diseases: 3.6 million**

5.

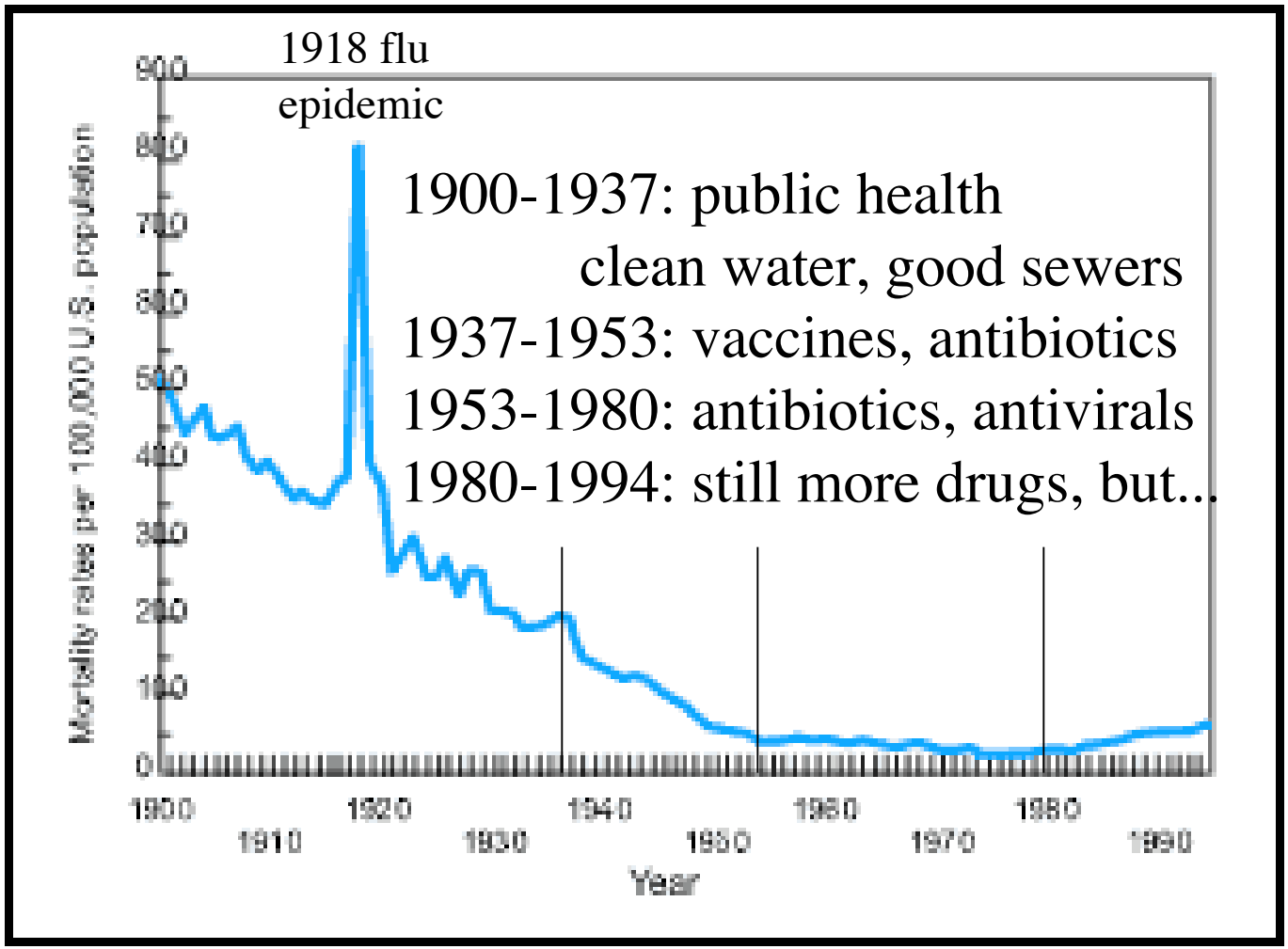
6. **Accidents, fires, drowning, etc.: 3.5 million**

7. **Maternal and perinatal: 3.0 million**

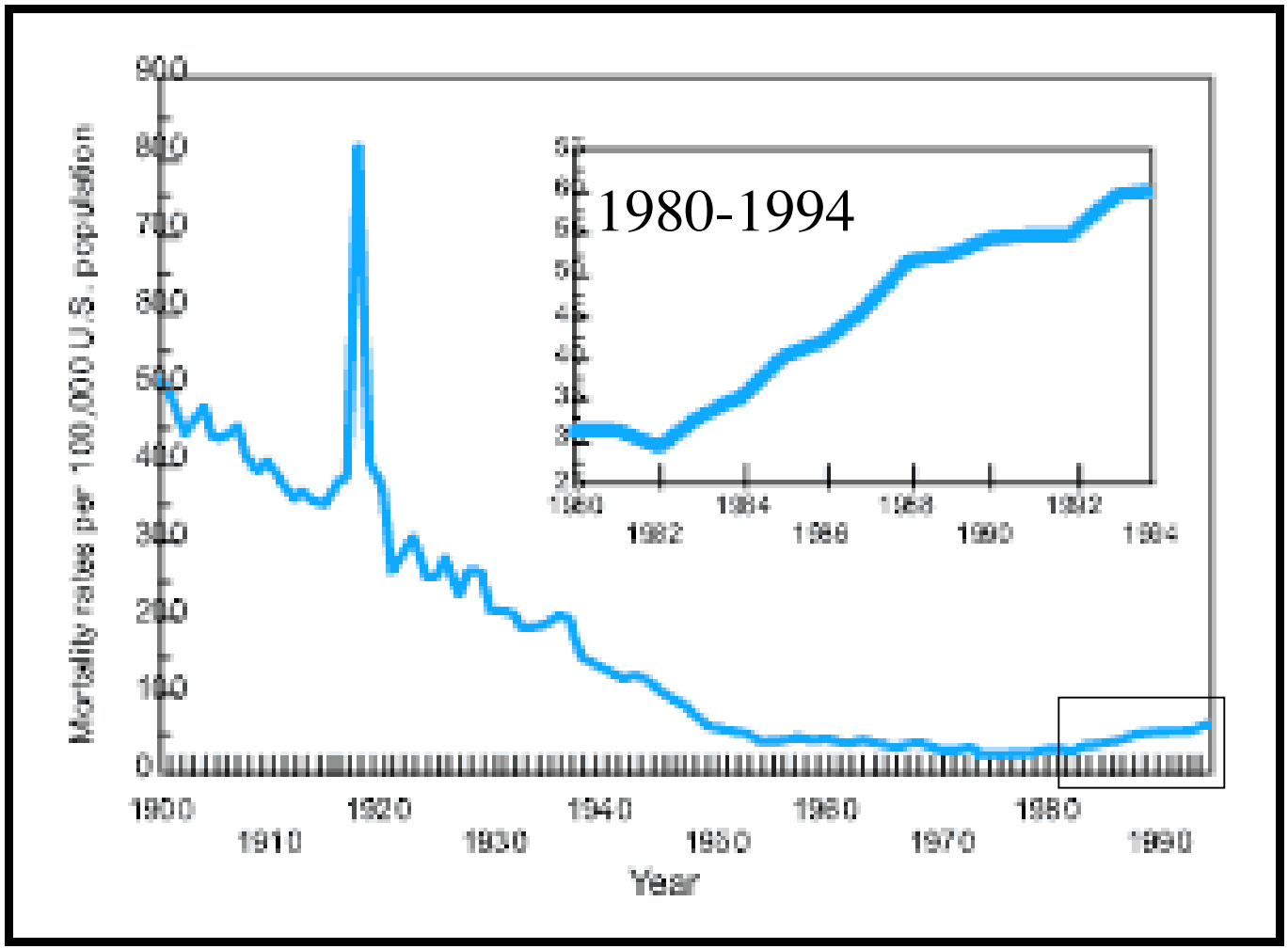
8. **Violence (war, homicide, suicide): 1.6 million**

World Health Organization
World Health Report 2002

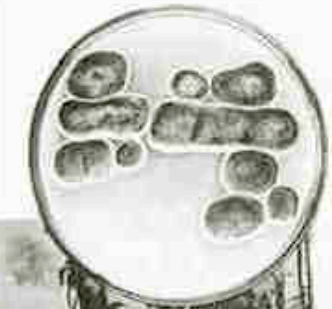
Deaths from infectious diseases in the US: 1900-1994



Deaths from infectious diseases in the US: 1900-1996



Thanks to PENICILLIN
...He Will Come Home!



FROM ORDINARY MOLD—
the Greatest Healing Agent of this War!

the tiny, green-mold-like mold above, called Penicillium notatum in the laboratory, proves the miraculous substance first discovered by Professor Alexander Fleming in 1928. Named penicillin by its discoverer, it is the most potent weapon ever developed against many of the deadliest infections. Luckily for us, the rare research mold was already a part of Schenley enterprise. Schenley Laboratories were well able to assist the problem of large-scale production of penicillin, when the great need for it arose.

When the thunderous battles of this war have subsided to pages of silent print in a history book, the greatest achievement of World War II may well be the discovery and development — not of some vicious secret weapon that destroys — but of a weapon that saves lives. That weapon, of course, is penicillin.

Every day, penicillin is performing some unbelievable act of healing on some battlefront. Thousands of men will return home who otherwise would not have had a chance. Better still, more and more of this precious drug is now available for civilian use... to save the lives of patients of every age.

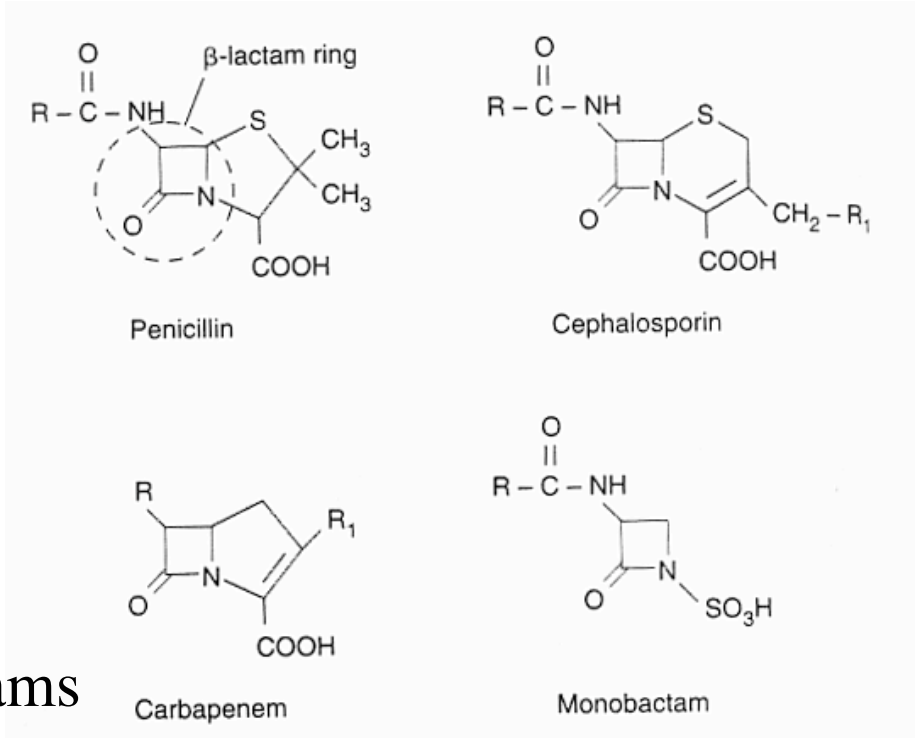
A year ago, production of penicillin was difficult, costly. Today, due to specially-devised methods of mass-production, in use by Schenley Laboratories, Inc. and the 20 other firms designated by the government to make penicillin, it is available in ever-increasing quantity, at progressively lower cost.

Listen to "THE DOCTOR HEARS" starring RAYMOND MASSEY, Tuesday evenings, C.B.S. See your papers for time and station.

Antibiotic discovery and resistance development

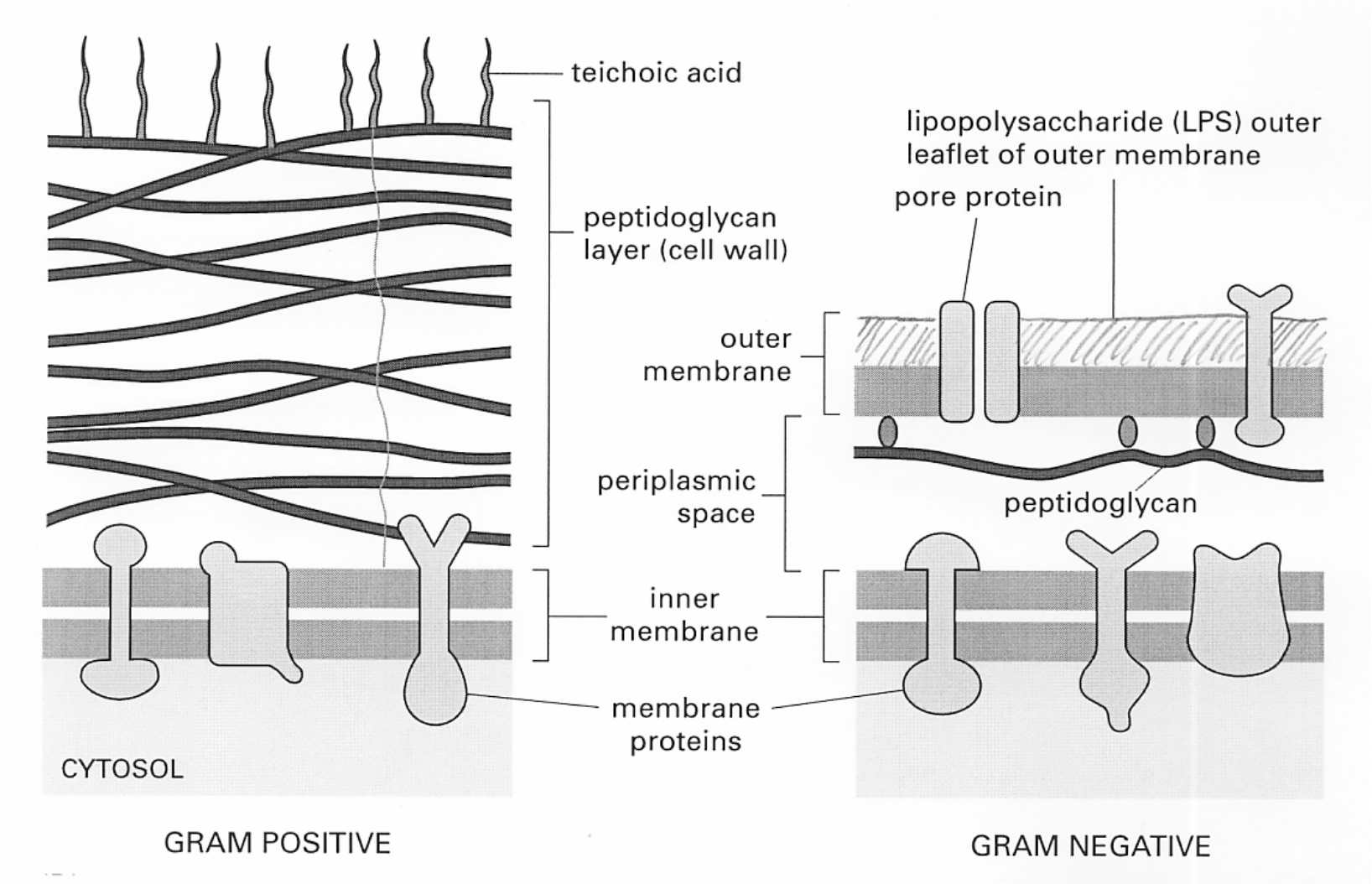
Antibiotic	*Discovered*	*First clinical use*	*Resistance*
Penicillin	1940	1943	1940
Streptomycin	1944	1947	1947
Tetracycline	1948	1952	1956
Erythromycin	1952	1955	1956
Vancomycin	1956	1972	1987
Gentamicin	1963	1967	1970

Penicillin

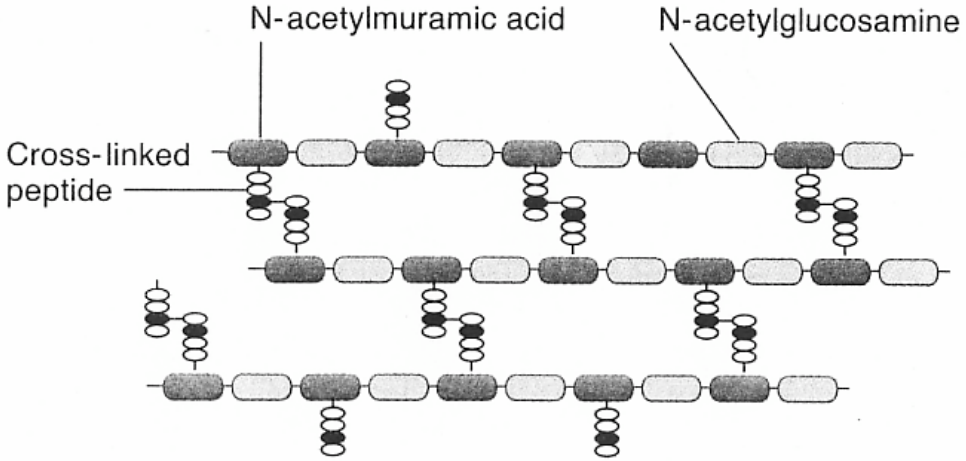


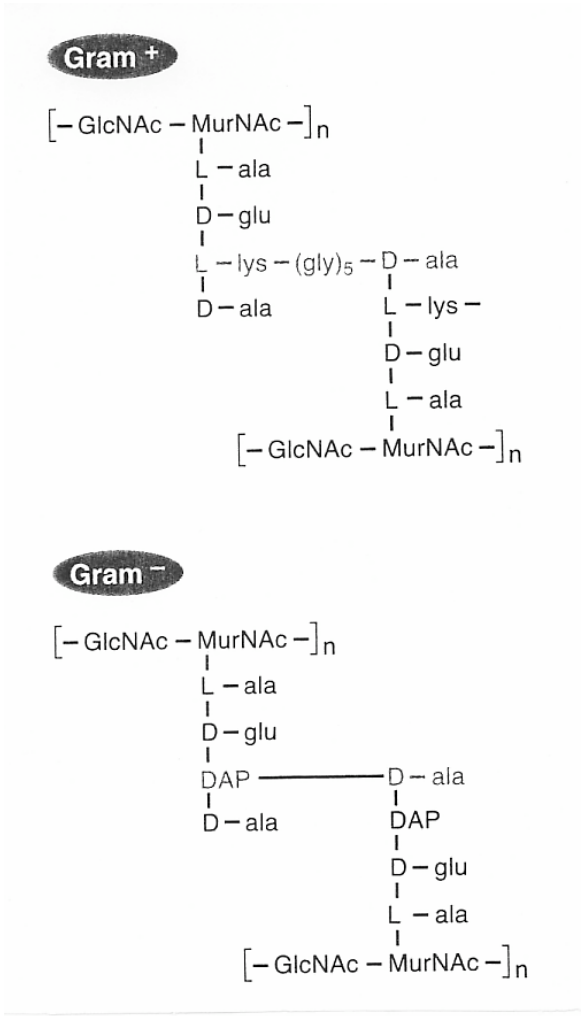
β-lactams

Bacterial cell surface structure

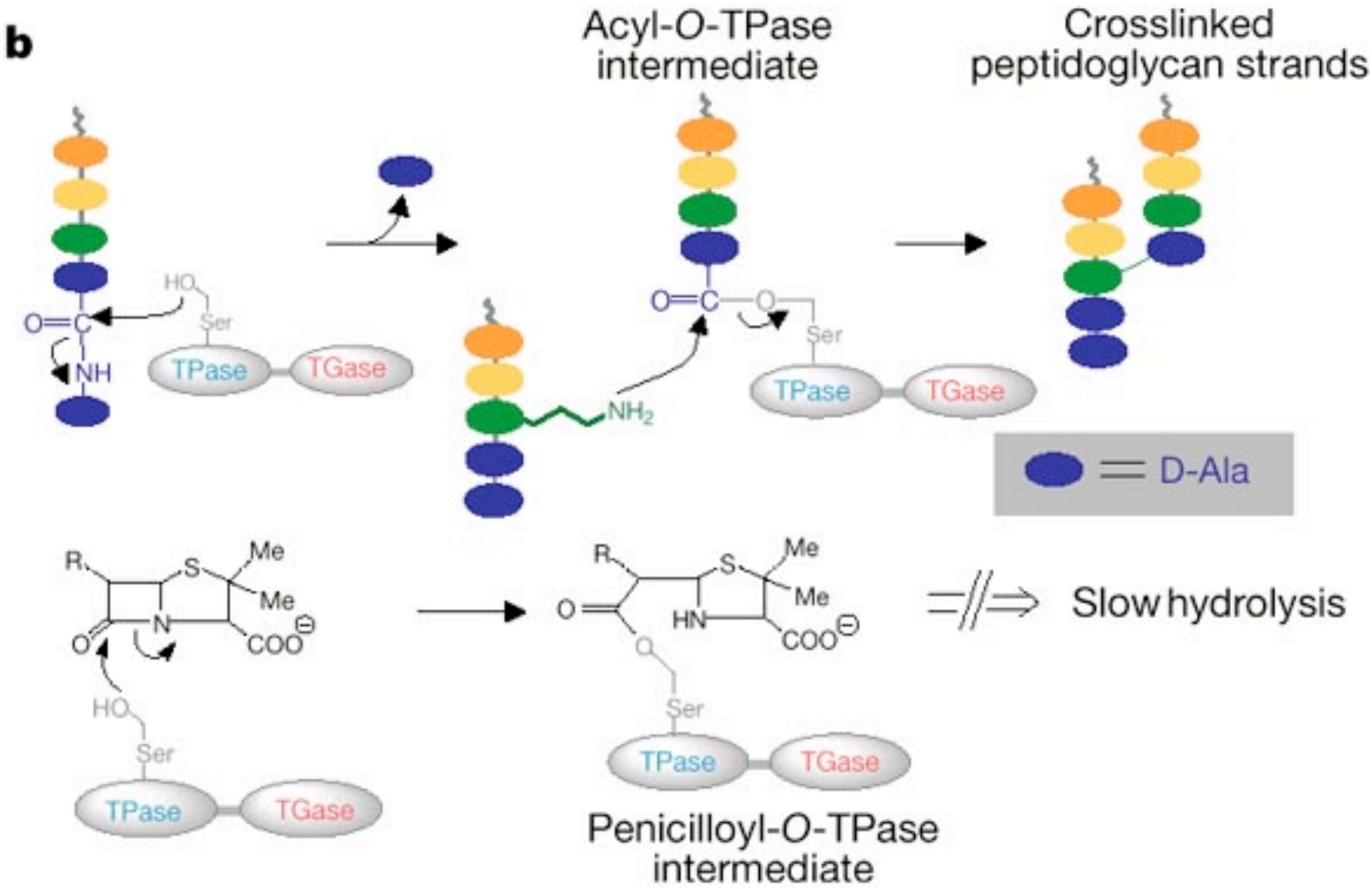


Cell wall molecular structure

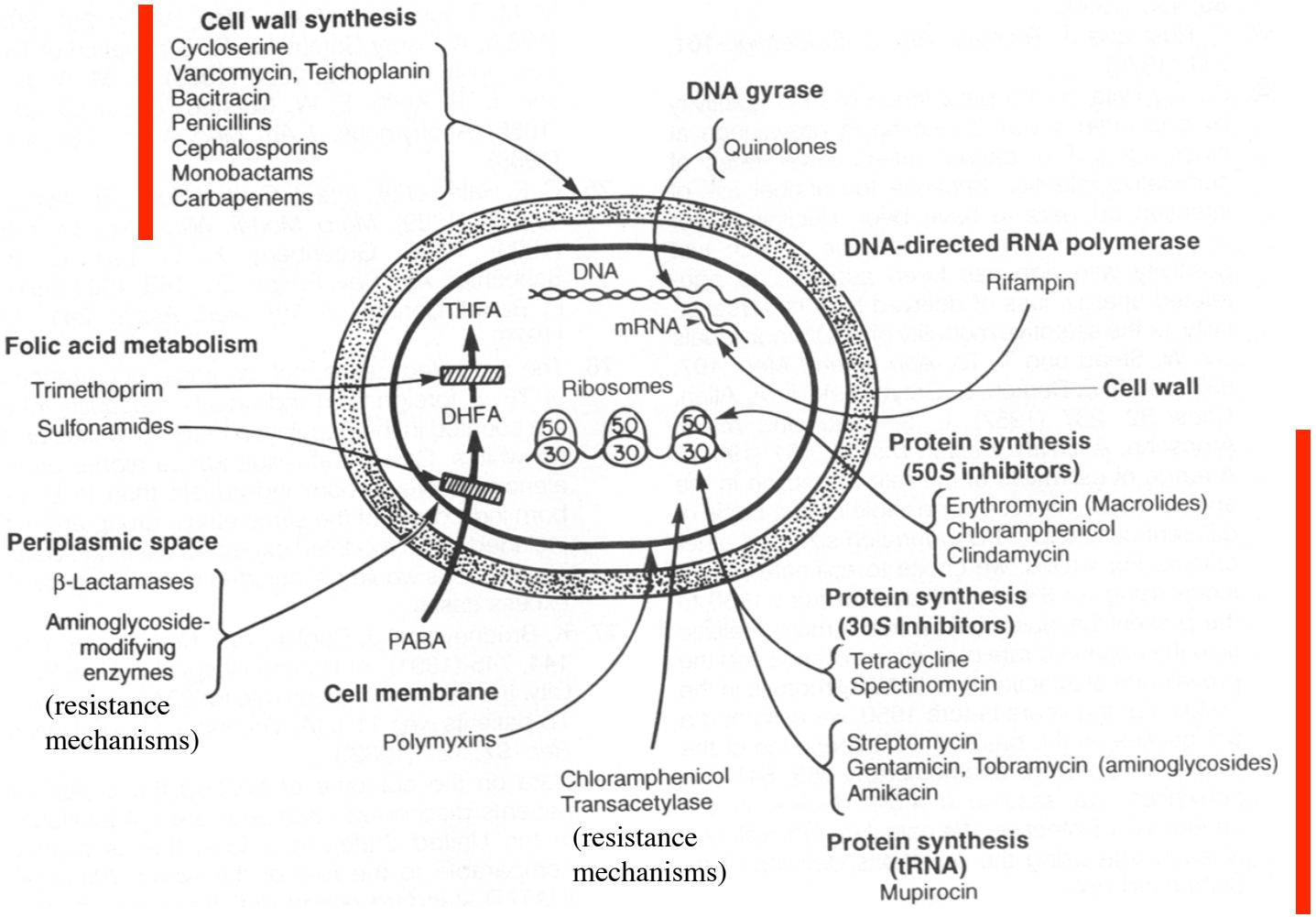




Transpeptidase reaction and penicillin inhibition



Antibiotic targets: mostly cell wall and ribosome



From: H. C. Neu, 1992, "The Crisis in Antibiotic Resistance," Science 257: 1064-1073

Modes of antibiotic resistance

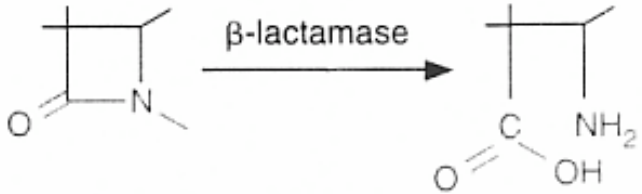
- Destroy or covalently modify the drug
- Change the target so the drug no longer binds
- Actively export the drug from the cytoplasm by a specific or non-specific efflux pump (MDR = multi-drug resistant)
- Prevent drug uptake by altering membrane permeability (rare)

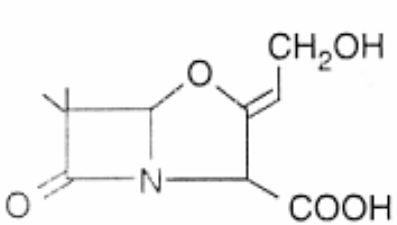
Selective pressures caused by human misuse:

- Physician overprescription
- Agricultural use as a growth enhancer
- Domestic misuse (compare the “hygiene hypothesis”)

Penicillin resistance

- Alteration in the transpeptidase (PBP)
  - Usually generates cross-resistance to all β-lactams
  - Mechanism found in MRSA (*mecA* gene acquired laterally from unknown source)
- Expression of β-lactamases
  - At least 255 different kinds
  - Derived from transpeptidases!!!
  - Rate of hydrolytic deacylation increased from 1 per hour to 1500 per second
  - Can be partially overcome by coadministration of clavulanic acid (augmentin)





Nosocomial infections

- >10 per 1000 patient-days in the hospital
- Most common in intensive care units, acute care surgical and orthopedic units
- Increasing in frequency and severity
  - Populations are more immunocompromised
  - Antibiotic resistance is becoming more prevalent
- Frequently opportunistic Gram-positives from normal flora (*Staphylococcus*, *Enterococcus*, *Streptococcus*)
- MRSA (methicillin-resistant *Staphylococcus aureus*) are often resistant to all antibiotics except vancomycin
- MRSA increasingly found in community-acquired infections as well as hospital-acquired infections

METHICILLIN-RESISTANT *S. AUREUS*

Methicillin resists most β-lactamases

1959	First clinical use of methicillin
1961	First description of MRSA
1967	First report of nosocomial infection in the US (2 cases)
1968	Increase in MRSA in the UK
1968-1979	Rise and subsequent wane of prevalence of MRSA (especially nosocomial infections) in Europe, Australia, and elsewhere (except US)
1975-1980	First reports of problems with MRSA in the US; most occurred in large tertiary care hospitals (especially burn units and ICUs)
1980-1991	MRSA increase in prevalence in US nursing homes; community-acquired MRSA infections in the US
2003-2004	Community-acquired clones of MRSA cause outbreaks of necrotizing fasciitis in Los Angeles

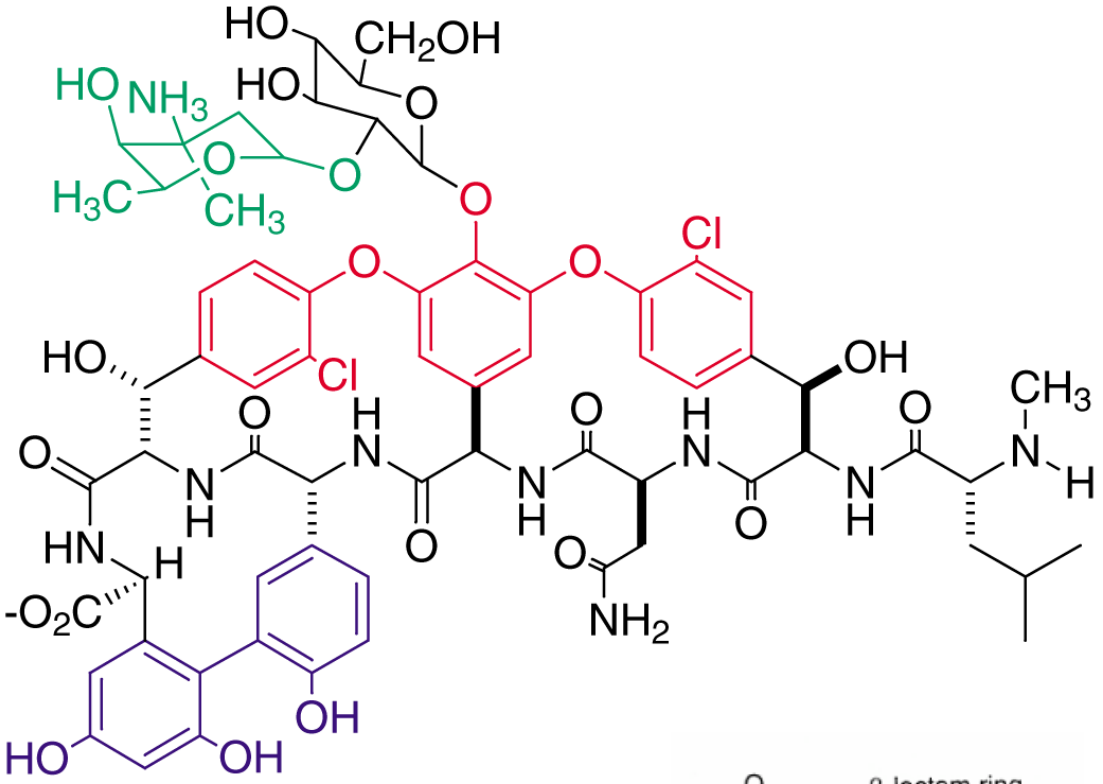
Jevons. *BMJ* 1961;1:124
Westh H et al. *Clin Infect Dis*. 1992;14:1186-1194.
Chambers HF. *Clin Microbiol Rev.* 1997;10:781-791.
Bradley SF. *Am J Med.* 1999;106(5A):2S-10S.
Miller LG et al. NEJM 2005; 352: 1445-1453.

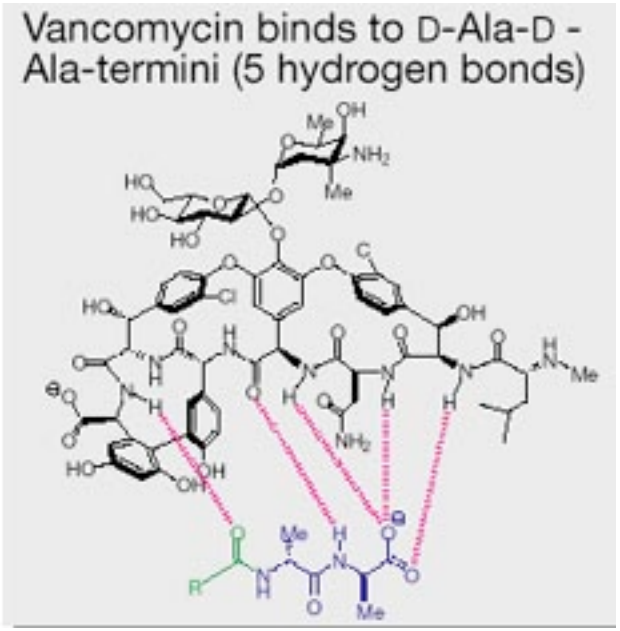
Pandemic MRSA around the world

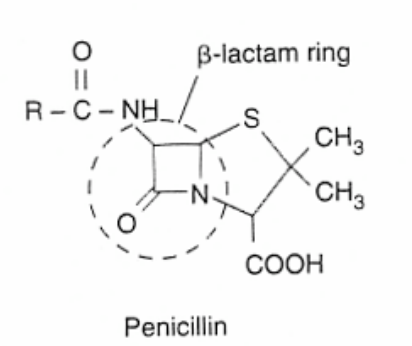
Figure 2. International spread of the pandemic MSRA clones.

Oliveira et a., 2002, Lancet Inf Dis. 2: 180

Vancomycin







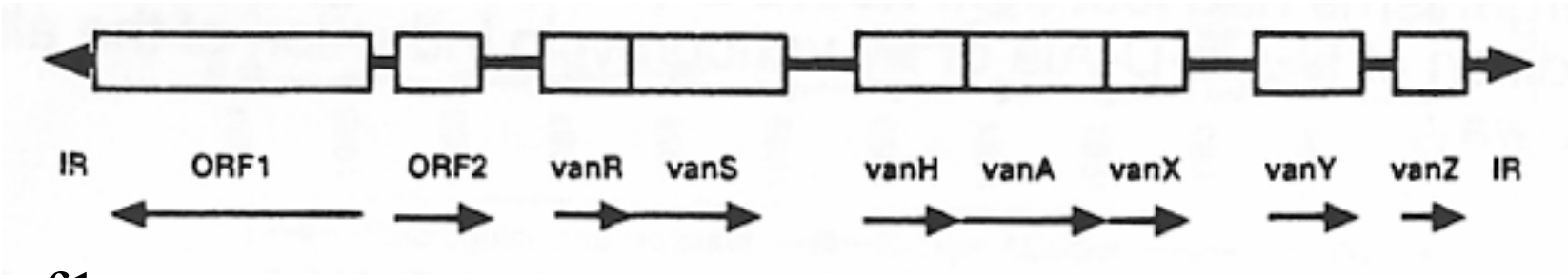
Penicillin

20-50% of a typical hospital antibiotic budget is spent on vancomycin

Vancomycin resistance in enterococci

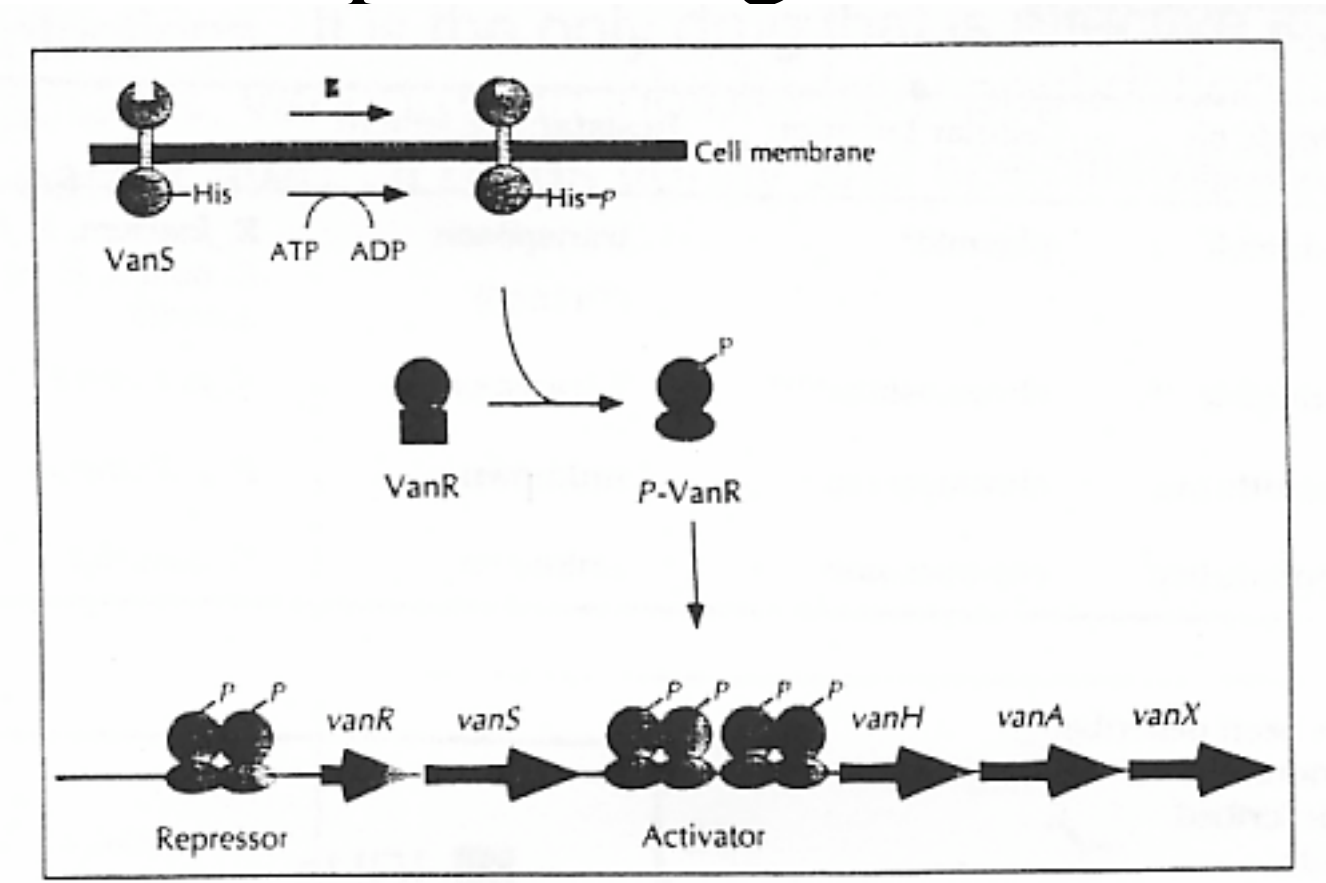
- 12 species cause bacteremia, mostly *E. faecalis* and *E. faecium*
- Vancomycin resistance described in 1986; currently 25% of clinical isolates are resistant (VRE)
- High mortality rate (10-50%)
- US: Reservoirs are hospital staff and patients (farm animals in Europe due to use of avoparcin)
- Genotypic classification of resistance:
  - vanA - inducible, cross resistance to teicoplanin, >1000 μg/ml
  - vanB - inducible, teicoplanin-sensitive, >1000 μg/ml
  - vanC, vanD - constitutive, teicoplanin-sensitive, 30-100 μg/ml

vanA: Organization of transposon Tn1546

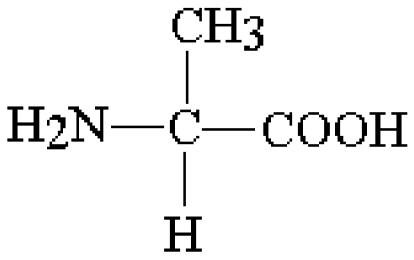


- orf1 - transposase
- orf2 - resolvase
- vanR - response regulator (transcriptional activator)
- vanS - histidine protein kinase (sensor)
- vanH - D-specific α-keto acid reductase (makes D-lactate)
- vanA - D-Ala-D-lactate peptide ligase
- vanX - D-Ala-D-Ala dipeptidase
- vanY - D-D carboxypeptidase

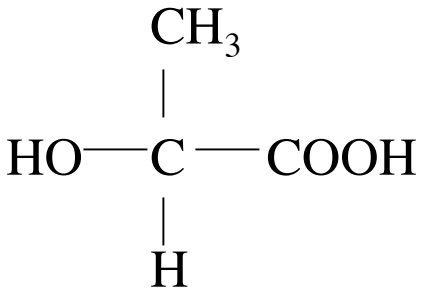
Induction of resistance genes by vancomycin via two-component response regulator



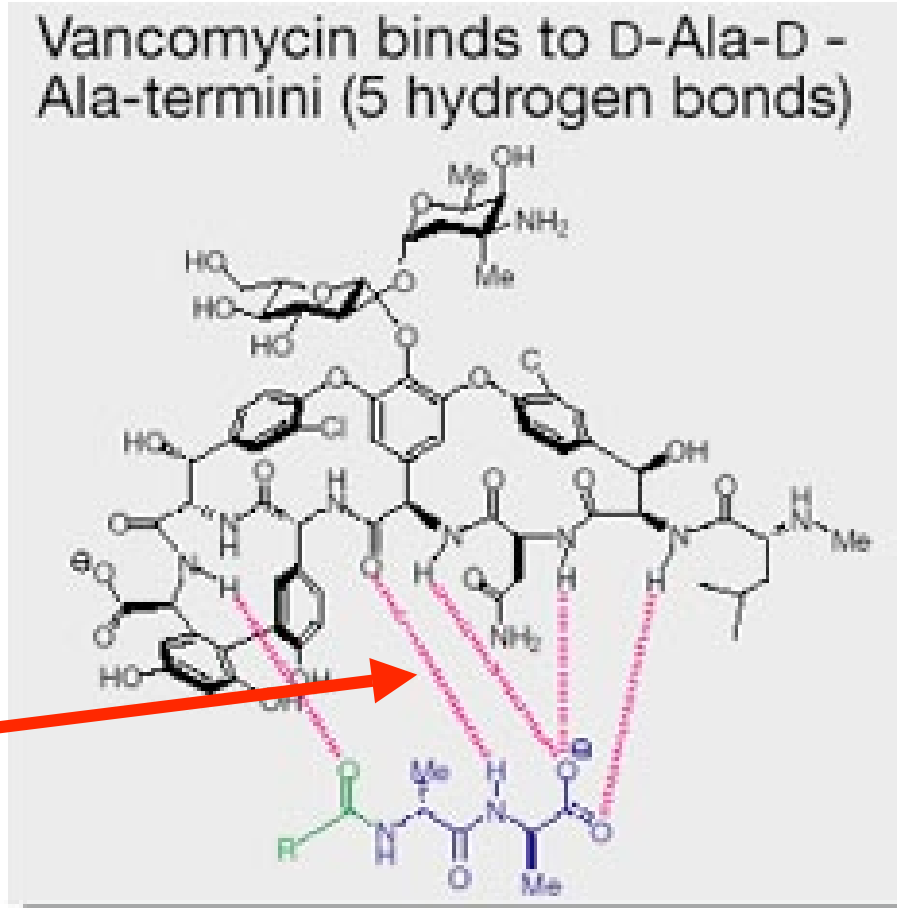
Change of cell wall peptide from D-Ala-D-Ala to D-Ala-D-lactate removes one hydrogen bond...enough!



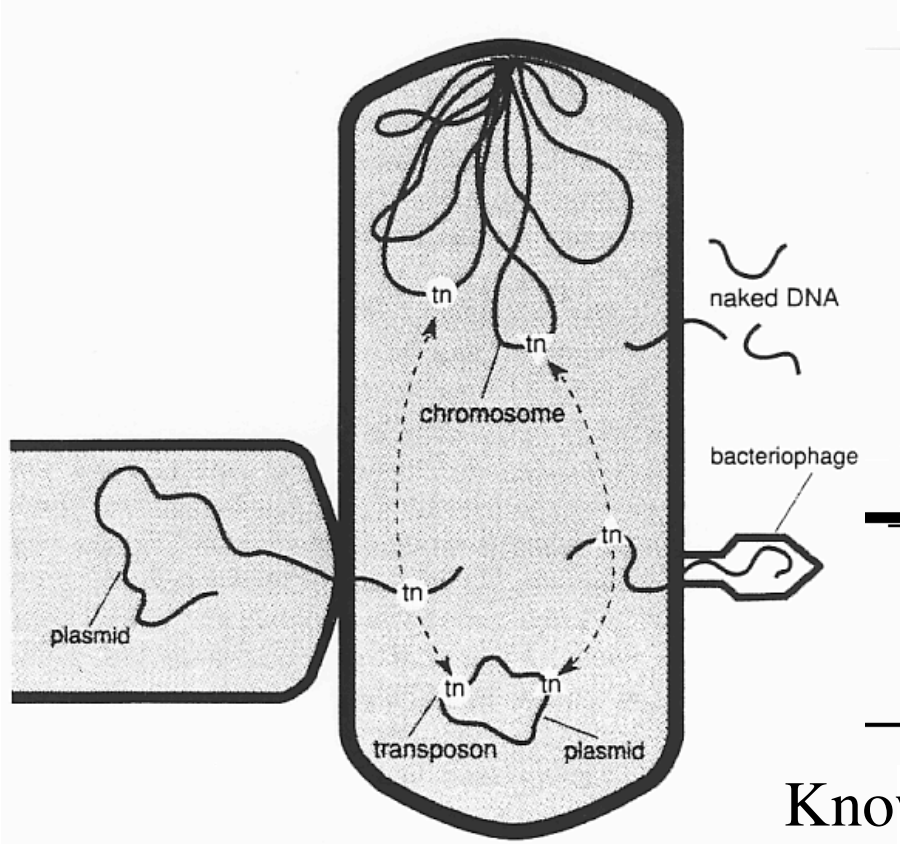
alanine

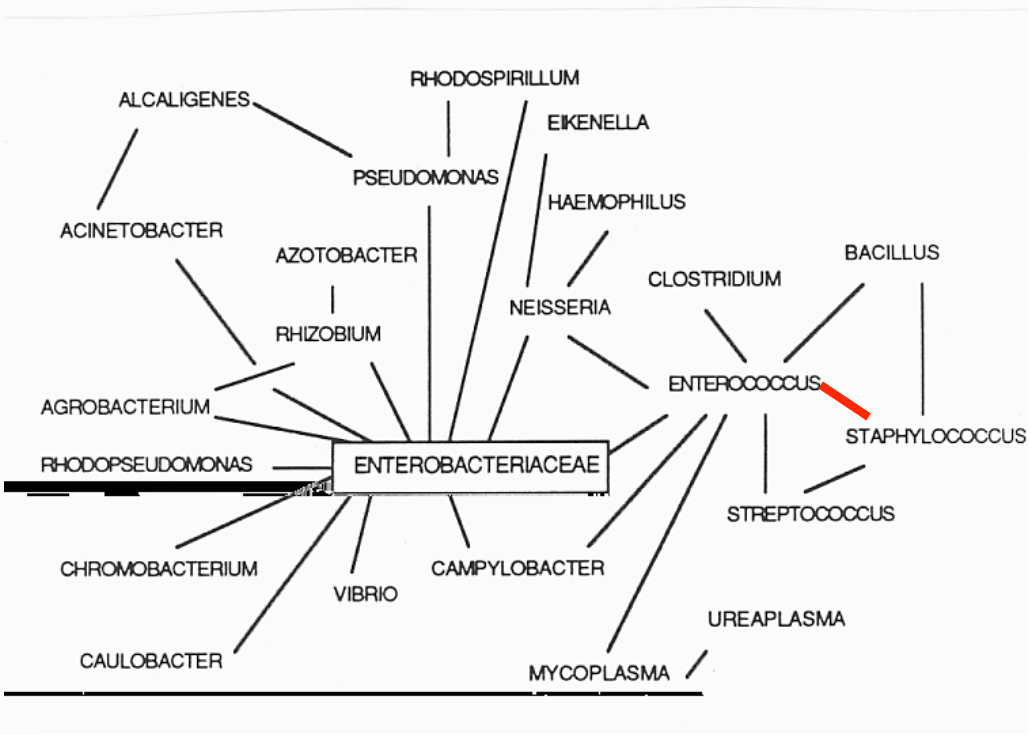


lactate



Mechanisms of genetic exchange and spread of resistance determinants





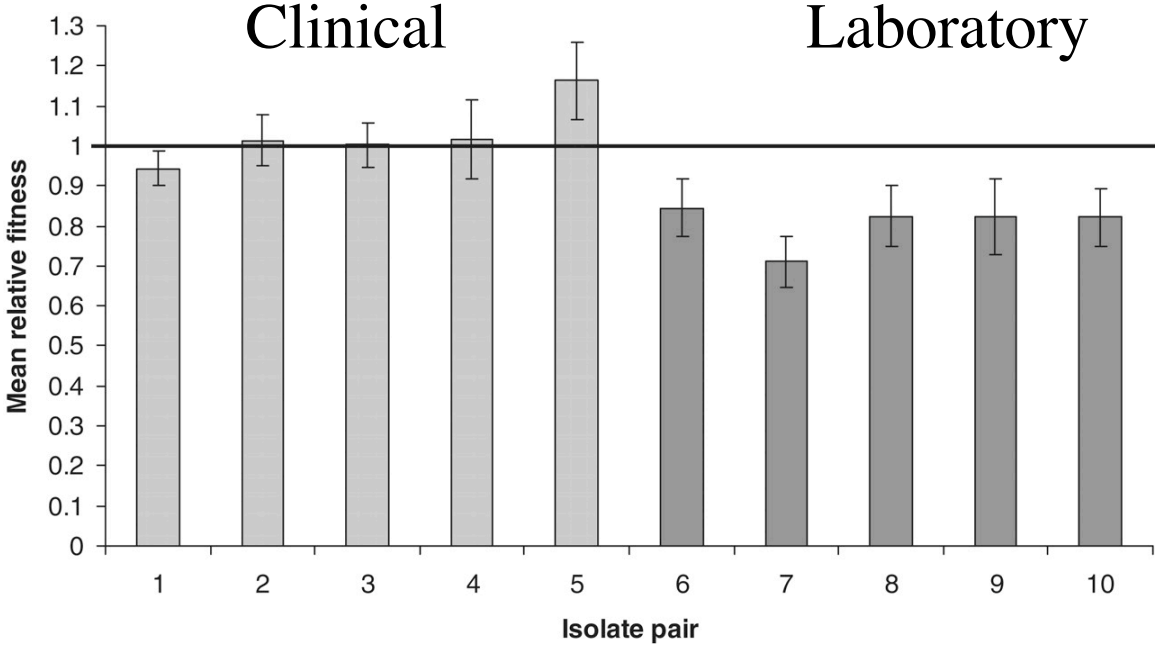
Known cross-species routes of exchange
VRE can transfer Tn1546 to MRSA in vitro
(samples immediately autoclaved)

Well, has transfer occurred?

- June 2002: 40 yo woman in Michigan
  - Hypertension, diabetes, peripheral vascular disease, chronic renal failure
  - Recurrent foot ulcers due to diabetic neuropathy; right foot amputated
  - Treated with vancomycin, gentamicin, ampicillin-sulbactam, piperacillin-tazobactam, levofloxacin, clindamycin, cefazolin, trimethoprim-sulfamethoxyzole, tobramycin and metronicazole prior to amputation
  - Cultured MRSA in April 2002, VRE in June 2002
  - VRSA appeared in June 2002: Tn1546 transferred from VRE on a conjugative plasmid (pLW1043)

Chang et 1l., 2003, NEJM 348: 1342
Weigel et al., 2003, Science 302: 1569

Isn't there a fitness cost?



Clinical isolates of rifampicin-resistant *Mycobacterium tuberculosis* have little or no fitness defect; laboratory isolates always do

Gagneux et al., 2006, *Science* 312: 1944

Also: bacteria under antibiotic stress

1) increase genetic transformability
2) increase error-prone replication mechanisms

Pseudomembranous colitis:
a disease caused by antibiotics

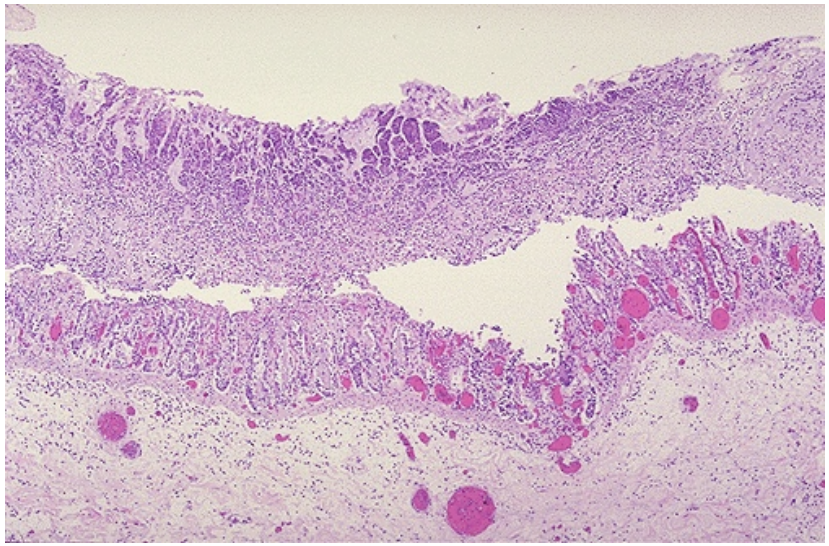
3% of healthy adults and 20-40% of hospitalized patients are colonized with spores of *Clostridium difficile*

Killing normal flora with antibiotics allows *C. difficile* outgrowth (clindamycin, cephalosporins, fluoroquinolines all implicated)

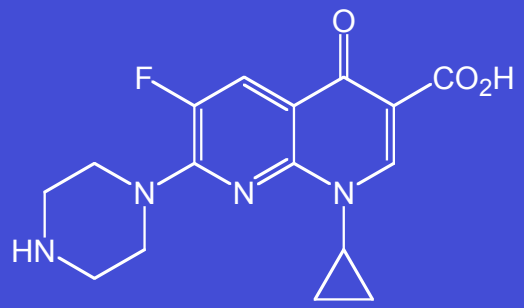
Two toxins cause cell death and inflammation

"Pseudomembrane" is made of fibrin, inflammatory cells, necrotic tissue, overlying the mucosa

PAPER 1: McDonald et al. New, more virulent strains are spreading

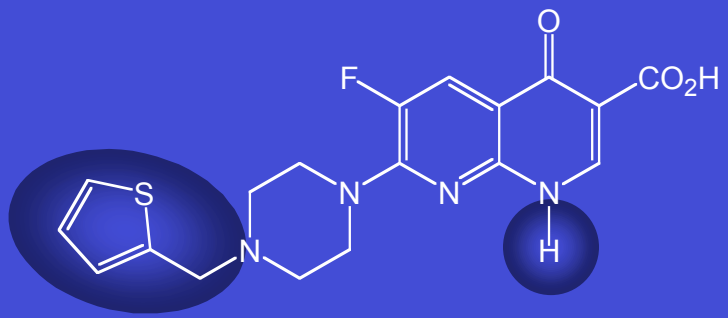


What can we do? New targets, new drugs…



Ciprofloxacin

Inhibits DNA Topoisomerase
Point mutations in GyrA
give resistance

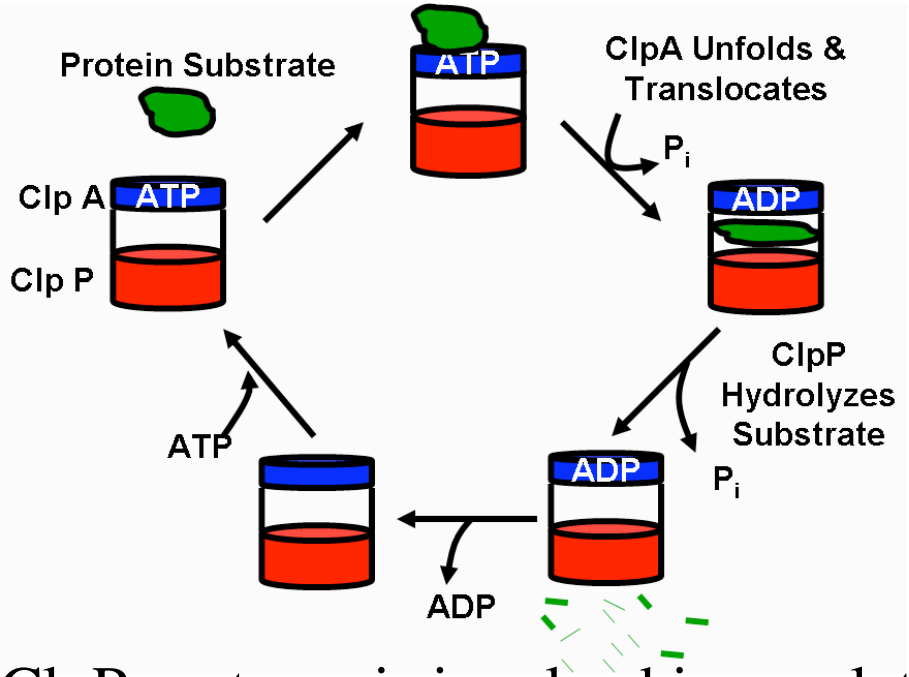


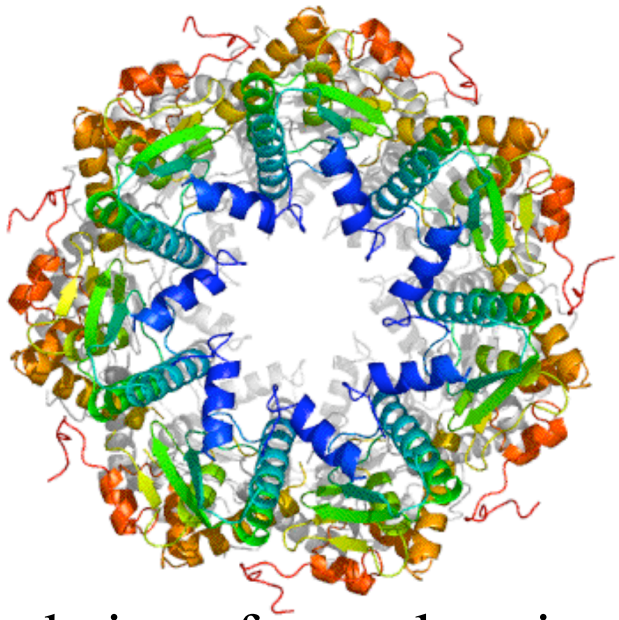
A-692345

Inhibits protein synthesis
(S. pneumoniae, H. influenzae)

Dandliker, *et. al. AAC* (**2003**), *47*, 3831.

ClpP protease a *nonessential* antibiotic target





ClpP protease is involved in regulated degradation of cytoplasmic proteins (compare the eukaryotic proteasome)

ΔclpP mutants are viable

ClpP normally requires an ATPase partner (e.g. ClpA) to activate proteolysis

PAPER 2: Brotz-Oesterhelt et al. What happens if it is misregulated?