What is the Biggest *inadvertent* Biology *Experiment ever Performed: That with the Grandest Scale?*

Inadvertent

The Biggest Experiment

Human vs. Microbe

PERIOD 1950 to date

OBJECTIVE Control of Microbes by Antibiotics

SCALE The Biosphere

PROTOCOL addition of >1 million metric tons of assorted Antibiotics into the environm

- *Rise and persistance of antibiotic resistant strains of human pathogens is a public health nightmare*
- *In this respect hospitals are on of the scariest places to be*

Oct, 22, 2007

HEALTH

Hospital screens for super bug

St. Joseph taking steps to isolate MRSA infections

MARY LANE GALLAGHER

BELLINGHAM — St. Joseph Hospital has just begun screening all intensive-care patients for the drug-resistant staph infection M in an effort to stop the spread of the dangerous bacteria.

The Centers for Disease Control released a report last week that estimates MRSA is responsible for about 94,000 serious infecti each year and 19,000 deaths. Methicillin-resistant Staphylococcus aureus is a type of souped-up staph bacteria that resists the antibiotics traditionally used to fight staph infections.

MRSA (pronounced "mer-sa") infections commonly show up as small pimples or boils on the skin. But invasive MRSA that gets ir the body through a surgical wound, for example, can be deadly.

It's difficult to know how widespread the bug is in Washington state because MRSA infections don't have to be reported to state h authorities. But there have been reports of MRSA in Whatcom County since at least 2003, said Greg Stern, Whatcom County hea officer.

Nearly half, 47 percent, of the staph infections confirmed in the laboratory at St. Joseph Hospital were MRSA in 2006, hospital off say.

But that may not tell the whole story, either, because not all patients are tested for the presence of MRSA, which can live in some nose or folds of skin without causing an infection.

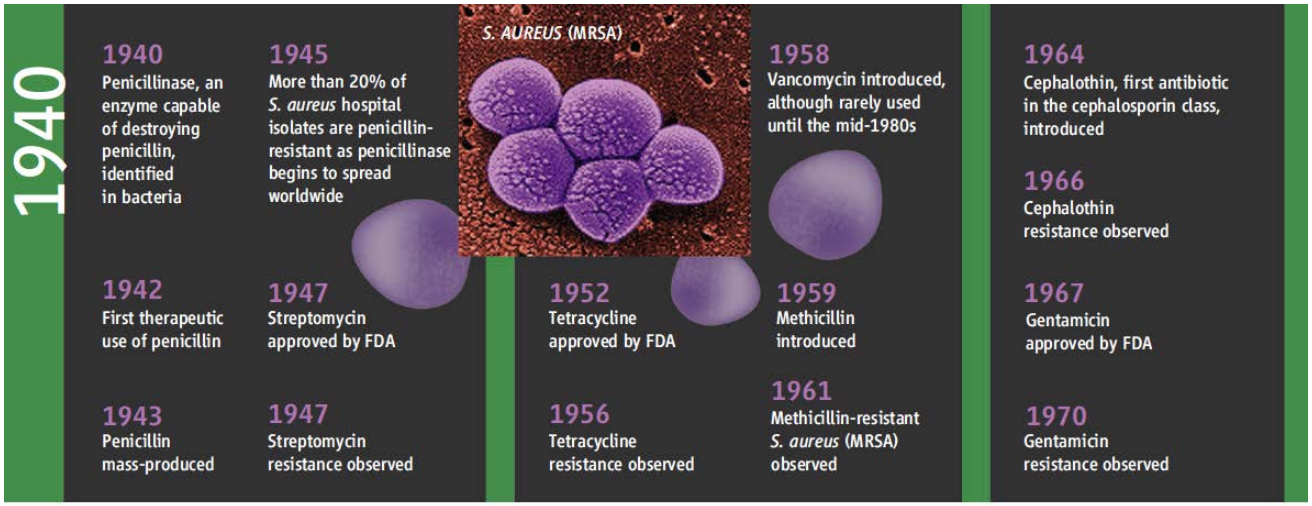
"It's very difficult to determine how big of a problem it is," said Joni Och, director of the hospital's center for health-care improveme "There may be colonized patients that show no signs or symptoms. It's kind of difficult to put your hands on."

The hospital routinely tests heart surgery patients whose lives could be jeopardized by a MRSA infection around the heart. Other surgeons can order a MRSA test on a patient, but not all surgery patients are screened.

The hospital just started screening all patients coming into the intensive-care unit on the recommendation of a national quality eff the Institute for Healthcare Improvement.

A patient who is found to have the bacteria in the body is put in a single-patient room with a sign outside telling all those who ente wear a gown and gloves to help stop the spread of the infection.

Some health-care advocates say hospitals should fight the infection by testing all patients, as have European countries who have nearly eradicated MRSA.


1940
1940
Penicillinase, an enzyme capable of destroying penicillin, identified in bacteria
1942
First therapeutic use of penicillin
1943
Penicillin mass-produced
1945
More than 20% of S. aureus hospital isolates are penicillin-resistant as penicillinase begins to spread worldwide
1947
Streptomycin approved by FDA
1947
Streptomycin resistance observed
S. AUREUS (MRSA)
1952
Tetracycline approved by FDA
1956
Tetracycline resistance observed
1958
Vancomycin introduced, although rarely used until the mid-1980s
1959
Methicillin introduced
1961
Methicillin-resistant S. aureus (MRSA) observed
1964
Cephalothin, first antibiotic in the cephalosporin class, introduced
1966
Cephalothin resistance observed
1967
Gentamicin approved by FDA
1970
Gentamicin resistance observed

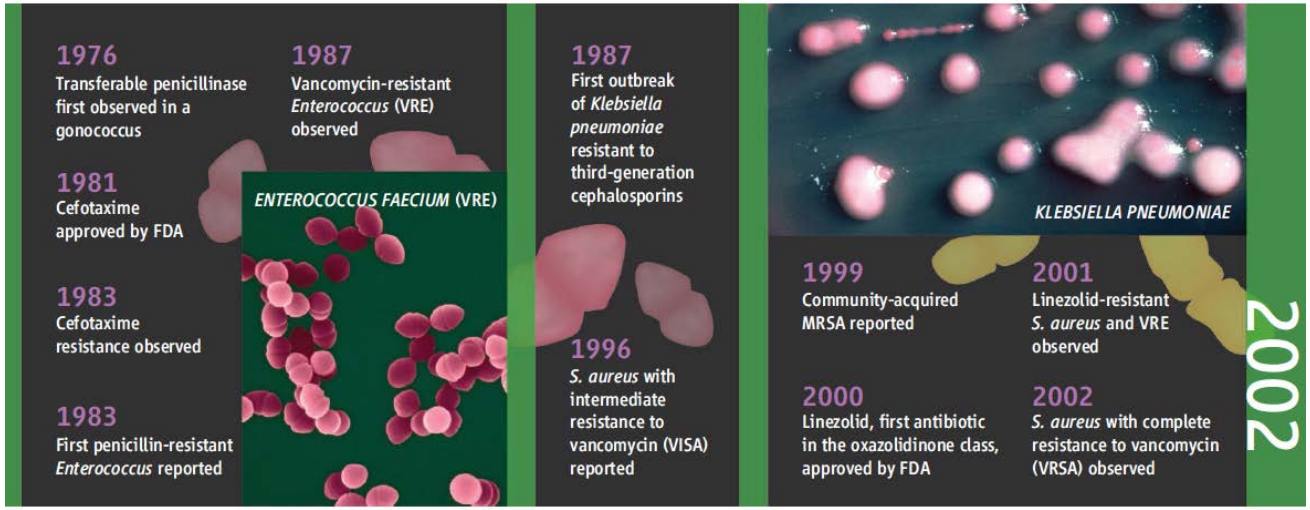

1976
Transferable penicillinase first observed in a gonococcus
1981
Cefotaxime approved by FDA
1983
Cefotaxime resistance observed
1983
First penicillin-resistant Enterococcus reported
1987
Vancomycin-resistant Enterococcus (VRE) observed
ENTEROCOCCUS FAECIUM (VRE)
1987
First outbreak of Klebsiella pneumoniae resistant to third-generation cephalosporins
1996
S. aureus with intermediate resistance to vancomycin (VISA) reported
KLEBSIELLA PNEUMONIAE
1999
Community-acquired MRSA reported
2000
Linezolid, first antibiotic in the oxazolidinone class, approved by FDA
2001
Linezolid-resistant S. aureus and VRE observed
2002
S. aureus with complete resistance to vancomycin (VRSA) observed
2002

Table 1. Dates of deployment of representative antibiotics and herbicides, and the evolution of resistance. [Source (*75*)].

EVOLUTION OF RESISTANCE TO ANTIBIOTICS AND HERBICIDES

Antibiotic or herbicide	Year deployed	Resistance observed
	Antibiotics	
Sulfonamides	1930s	1940s
Penicillin	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Cephalosporins	1960s	late 1960s

Science 293: 1786 Sept. 7, 2001

How do antibiotic resistance genes/alleles confer resistance?

How do antibiotic resistance genes/alleles confer resistance?

BMJ VOLUME 317 5 SEPTEMBER 1998 www.bmj.com

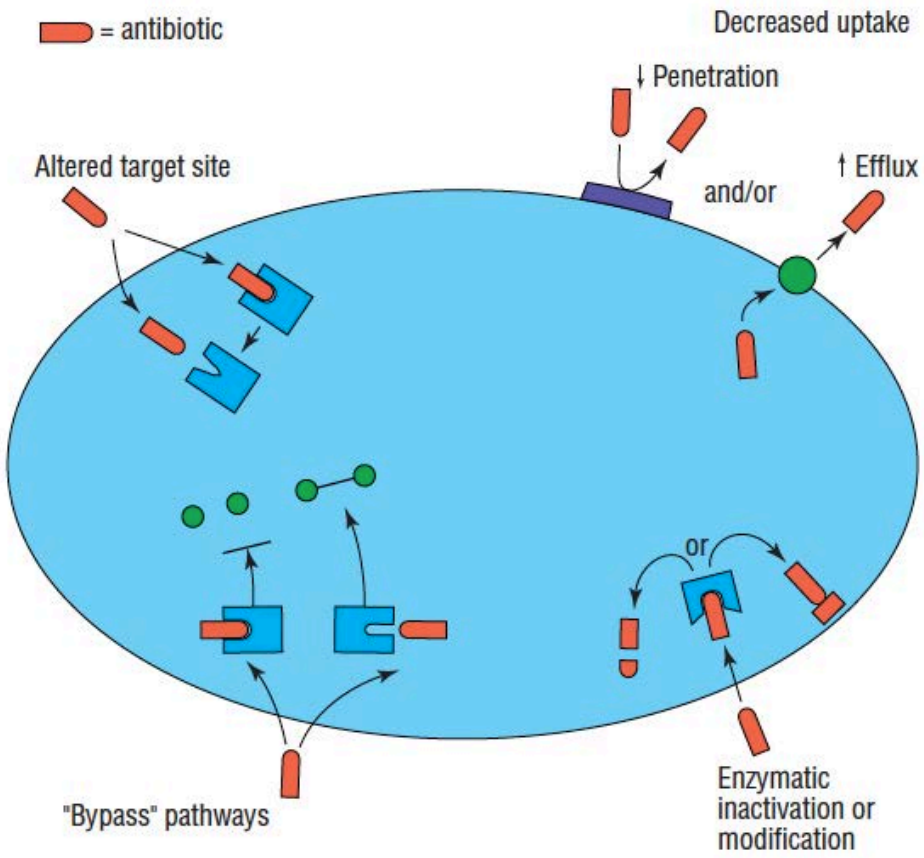


Fig 1 Four major biochemical mechanisms of antibiotic resistance

BMJ VOLUME 317 5 SEPTEMBER 1998 www.bmj.com

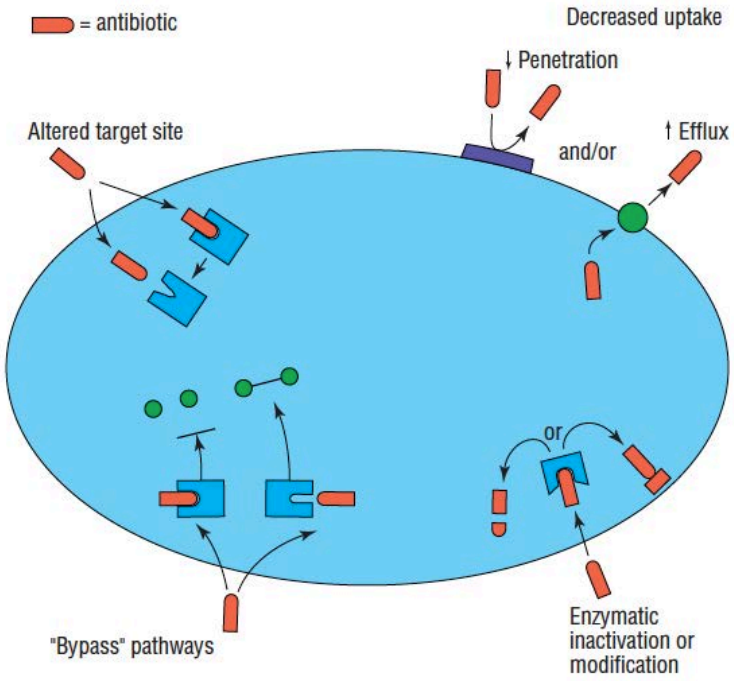


Fig 1 Four major biochemical mechanisms of antibiotic resistance

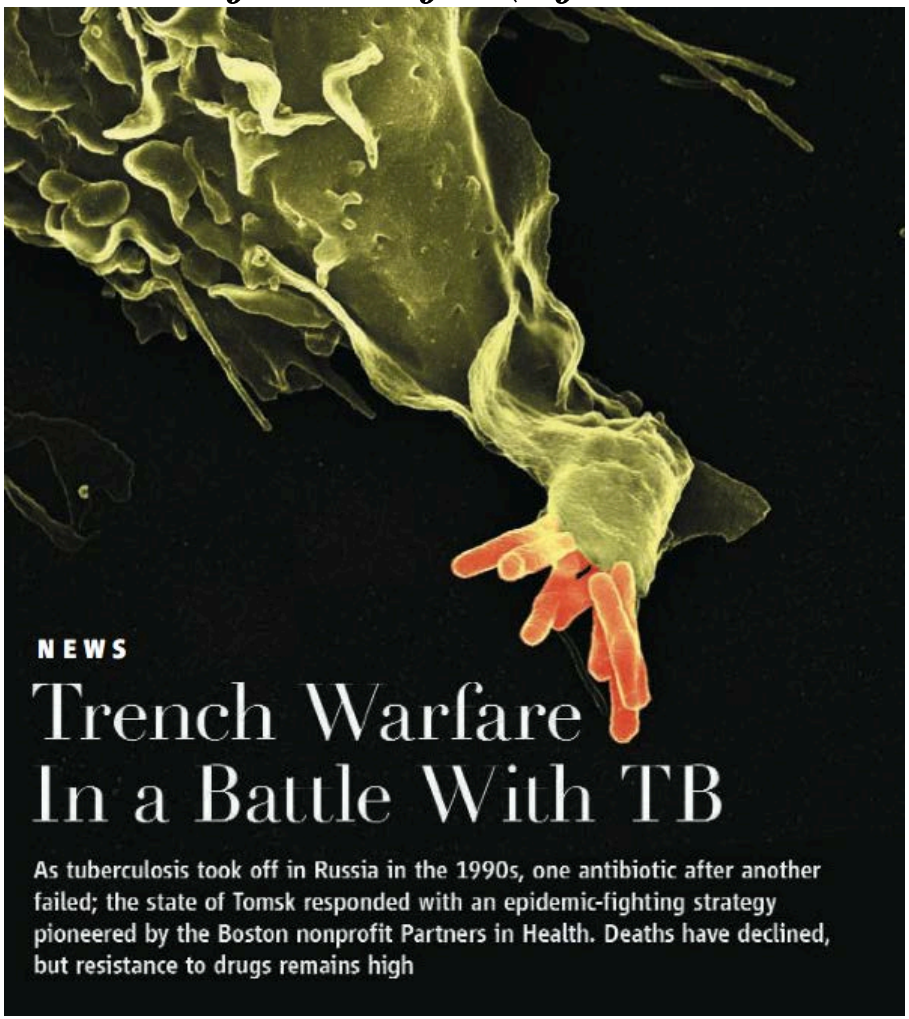
Where do these antibiotic-resistance alleles/genes come from?

Antibiotic resistance acquired by two fundamental mechanisms:

- ***Sustain a mutation that confers resistance in a gene already present in the bacteria's genome: this mechanism explored in the mutagenesis lab***
- *Acquire a new gene from somebody else via horizontal gene transfer: this strategy mechanism explored the next bacterial genetics lab*

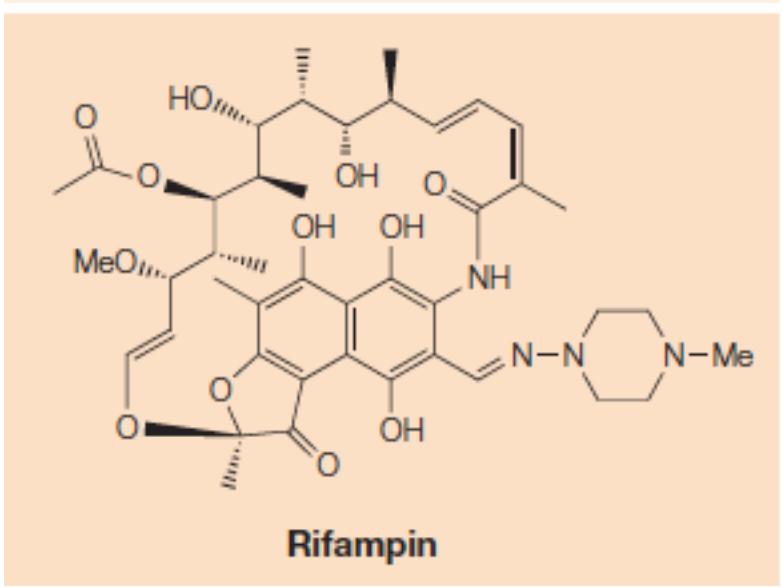
Target site alterations

rif^s → rif^r (rif = the antibiotic rifamycin = rifampin)



Rifampin (rifamycin) is a major drug used in the treatment of tuberculosis infections, and increasing rifampin resistance represents a worldwide clinical problem***. Resistance to rifampin is caused by mutations in the rpoB gene, encoding the beta-subunit of RNA polymerase.***

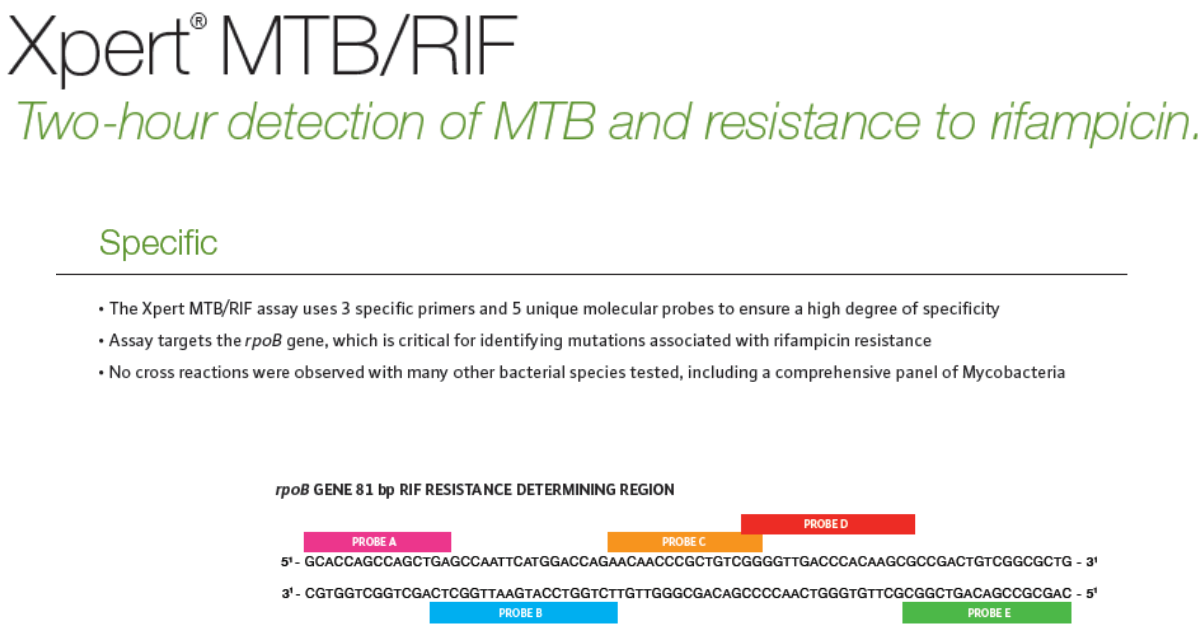
Tough bug. Mycobacterium tuberculosis (red) likes nothing better than to be ingested by a macro- phage, its usual home.

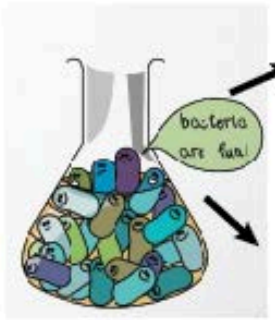


Rifampin

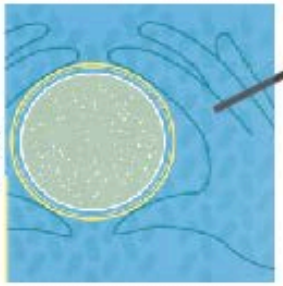
- The rifamycin antibacterial agents–rifampin (also known as rifampicin) function by binding to and inhibiting bacterial RNA polymerase
- The wildtype *Mycobacterium tuberculosis* RNA polymerase is 1000X more sensitive to this antibiotic than the wildtype *E. coli* RNA polymerase.
- Rifamycins are first-line antituberculosis agents, and are among the few antituberculosis agents that can kill nonreplicating tuberculosis bacteria.
- For all major bacterial pathogens, including the tuberculosis pathogen, strains resistant to rifamycins have arisen
- Resistance to rifamycins involves substitution of residues within the rifamycin-binding site on bacterial RNAP, i.e., substitutions that directly decrease rifamycin binding

“ What we really need,” Rich (a Russian health professional) adds, “is a good, cheap, point-of-service test.” Wealthier countries have access to polymerase chain reaction tests that monitor variable TB organism genes, signaling within 24 hours whether the strain is resistant to the first-line drugs isoniazid and rifampin (rifamycin). “





Select for rifamycin resistant mutations (mutant colonies). Measure frequency of rifS --> rifR mutations



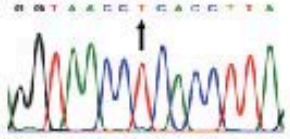
Pick individual colonies streak on rifamycin plates toconfirm phenotype andisolate pure colonies

set up liquid cultures of rif-R clones

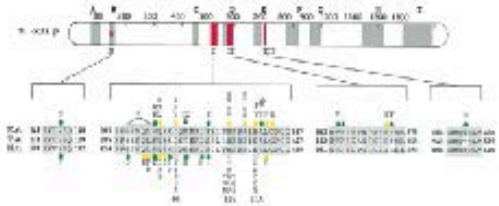
Prepare genomic DNA

Use PCR to amplify a portion of the gene coding for RNA polymerase (target of rifamycin)

Send PCR products out for sequencing



Determine site of resistance mutation

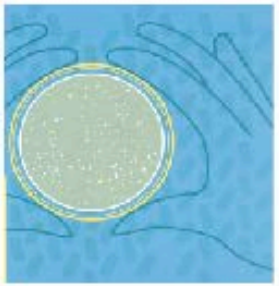


Screen for lac- mutations (mutant colonies) on MacConkey plates Measure frequency of lac+ --> lac- mutations

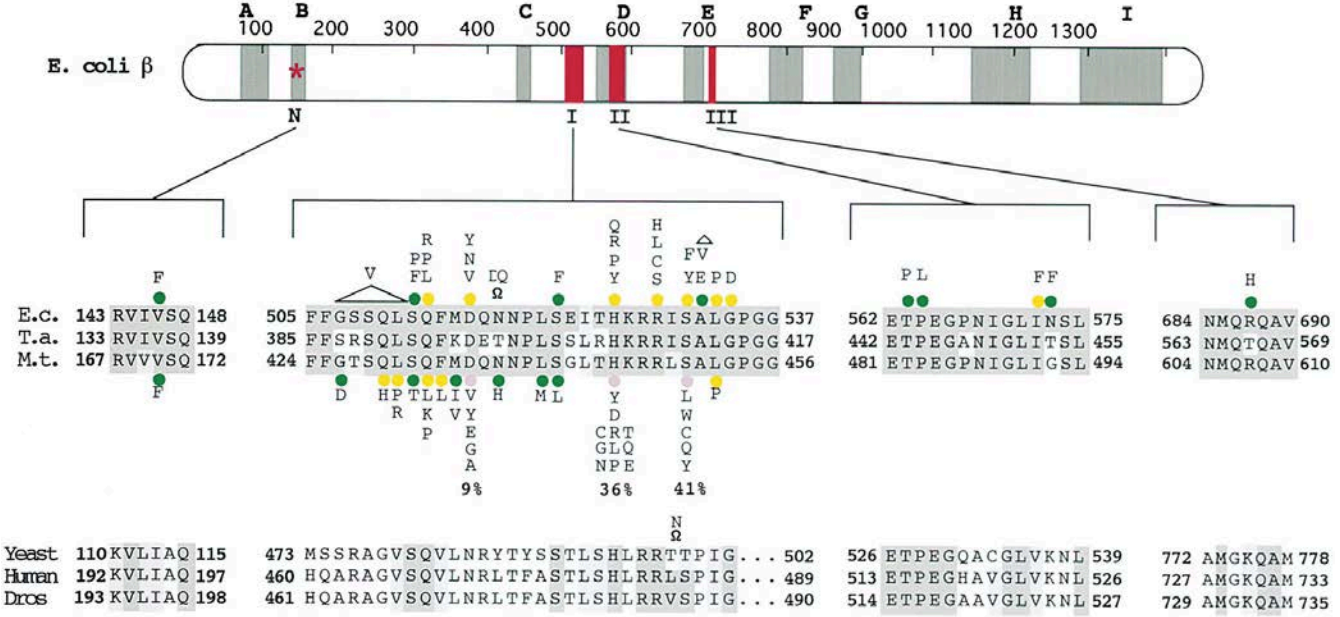
Pick individual lac- candidates and streak on MacConkey plates to confirm phenotype and isolate pure colonies

Set up liquid cultures of lac- clones

Meaure frequency of lac- –> lac+ reverse mutations by plating on selective media



Follow-up on rifR mutants *We will follow up on our rifR mutations by determining the location and nature of mutatgenic changes that can confer resistance to the antibiotic without severely compromising the ability of RNA polymerase to perform its cellular function*



Part 3: orient our PCR primers and sequence output with respect to this cartoon of the rpoB protein

Figure 1. The Rif-Resistant Regions of the RNAP b Subunit
The bar on top schematically represents the *E. coli* b subunit primary sequence with amino acid numbering shown directly above. Gray boxes indicate evolutionarily conserved regions among all prokaryotic, chloroplast, archaebacterial, and eukaryotic sequences. indicate the four clusters where RifR mutations have been identified in E. coli. Mutations that confer RifR in E. coli and M. tuberculosis are indicated directly above (for E. coli) or below (for M. tuberculosis) as follows: D for deletions, V for insertions, and colored dots for amino acid substitutions (substitutions at each position are indicated in single amino acid code in columns above or below the positions).
Color coding for the amino acid substitutions is as follows:
yellow, residues that interact directly with the bound Rif
green, residues that are too far away from the Rif for direct interaction
purple, three positions that are substituted with high frequency (noted as a % immediately below the substitutions) in clinical isolates of RifR M. tuberculosis

BMJ VOLUME 317 5 SEPTEMBER 1998 www.bmj.com

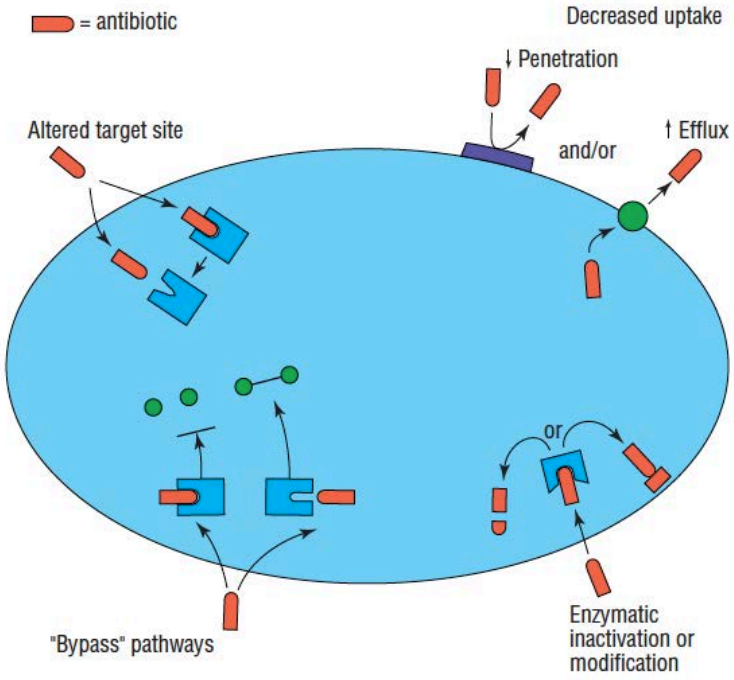


Fig 1 Four major biochemical mechanisms of antibiotic resistance

Where do these antibiotic-resistance alleles/genes come from?

Weapons of Microbial Drug Resistance Abound in Soil Flora

http://fire.biol.wwu.edu/trent/trent/microbialresistance.pdf

Multidrug Resistance in *S. aureus*

Antibiotic	MSSA (1930)	MRSA (1994)	Resistance mechanism
Penicillin	S	R	+ (1945)
Streptomycin	S	R	+ (1948)
Tetracycline	S	R	+ (1950)
Methicillin	S	R	+ (1961) *mecA*
Oxacillin	S	R	+
Cephalothin	S	R	+
Cefotaxime	S	R	+
Imipenem	S	R	+
Chloramphenicol	S	R	+
Ciprofloxacin	S	R	A
Clindamycin	S	R	+
Erythromycin	S	R	+
Gentamycin	S	R	+
Rifampin	S	R	A
Vancomycin	S	S	*A (1997)* VISA
Vancomycin	S	S	*+ (2002) vanA*
Teichoplanin	S	S	+
Trimeth/Sulfa	S	R	A

A = adaptive

implying a darwinian process

+ = acquired from an extra species source

Emergence of multidrug resistance in *Staphylococcus aureus*. The Brazilian clone of methicillin-resistant *S. aureus* (MRSA), isolated in 1994 (*2*), was resistant (R) to nearly all the antibiotics listed. Most of the resistance mechanisms were not adaptive (A), but acquired (+) from an extraspecies source. In contrast, an invasive strain of *S. aureus* (MSSA), recovered in 1930, was susceptible (S) to all the agents.

A recent article on antibiotic resistance is titled

The bacteria fight back

This is misleading: as we have already seen in the mutagenesis lab, "adaptive" resistance happens because mutation happens and the antibiotic is simply selecting for preexisting mutations in the vast bacterial populations

Antibiotic resistance acquired by two fundamental mechanisms:

- *Sustain a new mutation that confers resistance: this mechanism explored in the mutagenesis lab*
- ***Acquire a new gene from somebody else via horizontal gene transfer: this strategy mechanism explored the next bacterial genetics lab***

Multidrug Resistance in *S. aureus*			
Antibiotic	MSSA (1930)	MRSA (1994)	Resistance mechanism
Penicillin	S	R	+ (1945)
Streptomycin	S	R	+ (1948)
Tetracycline	S	R	+ (1950)
Methicillin	S	R	+ (1961) *mecA*
Oxacillin	S	R	+
Cephalothin	S	R	+
Cefotaxime	S	R	+
Imipenem	S	R	+
Chloramphenicol	S	R	+
Ciprofloxacin	S	R	A
Clindamycin	S	R	+
Erythromycin	S	R	+
Gentamycin	S	R	+
Rifampin	S	R	A
Vancomycin	S	S	*A (1997)* VISA
Vancomycin	S	S	*+ (2002) vanA*
Teichoplanin	S	S	+
Trimeth/Sulfa	S	R	A

Emergence of multidrug resistance in *Staphylococcus aureus*. The Brazilian clone of methicillin-resistant *S. aureus* (MRSA), isolated in 1994 (*2*), was resistant (R) to nearly all the antibiotics listed. Most of the resistance mechanisms were not adaptive (A), but acquired (+) from an extraspecies source. In contrast, an invasive strain of *S. aureus* (MSSA), recovered in 1930, was susceptible (S) to all the agents.

(+) = acquired from an extraspecies source
What do we know about the transfer mechanisms?
What types of genetic elements are involved?

Similar Themes in Eukaryotic and Prokaryotic Genetics:

- Use model organisms to explore basic questions about heredity
- Use phenotypic variation to explore hereditary processes and to define the existence of specific genes
- Cross males and females with different phenotypes!

In other ways, doing genetics with bacteria is fundamentally different

- number of organisms handled is off the scale used by eukaryotic geneticists
- ability to detect very rare events is one of the most powerful aspects of bacterial genetics

---

Prokaryotes the unseen majority

From PNAS 95: 6578 June 1998

Most of the earth's prokaryotes occur in

- open ocean: 1.2×10^{29} cells
- soil: 2.6×10^{29} cells
- oceanic subsurfaces (marine sediments below 10cm): 3.5×10^{30} cells
- terrestrial subsurfaces (below 8 meters): $0.25 - 2.5 \times 10^{30}$ cells

Other habitats

- animals -- many vertebrate and invertebrate animals contain dense populations of prokaryotes that play important roles in nutrition and disease
- leaves
- air

Microbes maketh man

People are not just people. They are an awful lot of microbes, too

Aug 18th 2012 | The Economist

POLITICAL revolutionaries turn the world upside down. Scientific ones more often turn it inside out. And that, almost literally, is happening to the idea of what, biologically speaking, a human being is.

The traditional view is that a human body is a collection of ***10 trillion cells which are themselves the products of 23,000 genes.*** If the revolutionaries are correct, these numbers radically underestimate the truth. For in the nooks and crannies of every human being, and especially in his or her guts, dwells the microbiome: ***100 trillion bacteria of several hundred species bearing 3million non-human genes……***humans are not single organisms, but superorganisms made up of lots of smaller organisms working together.

We and other complex organisms are composed of an interconnected ecosystem of eukaryotic and prokaryotic cells:

Density of prokaryotes on human skin:
10^3-10^4 per cm^2
[except in groin and axilla where it is 10^6 per cm^2]

Total estimated number of prokaryotes on the skin of an individual is 3 X 10^8 cells

In the human colon: 3.2 X 10^{11} cells/g
(colon is about 220 g)

Compare to # of people on earth-- which is?

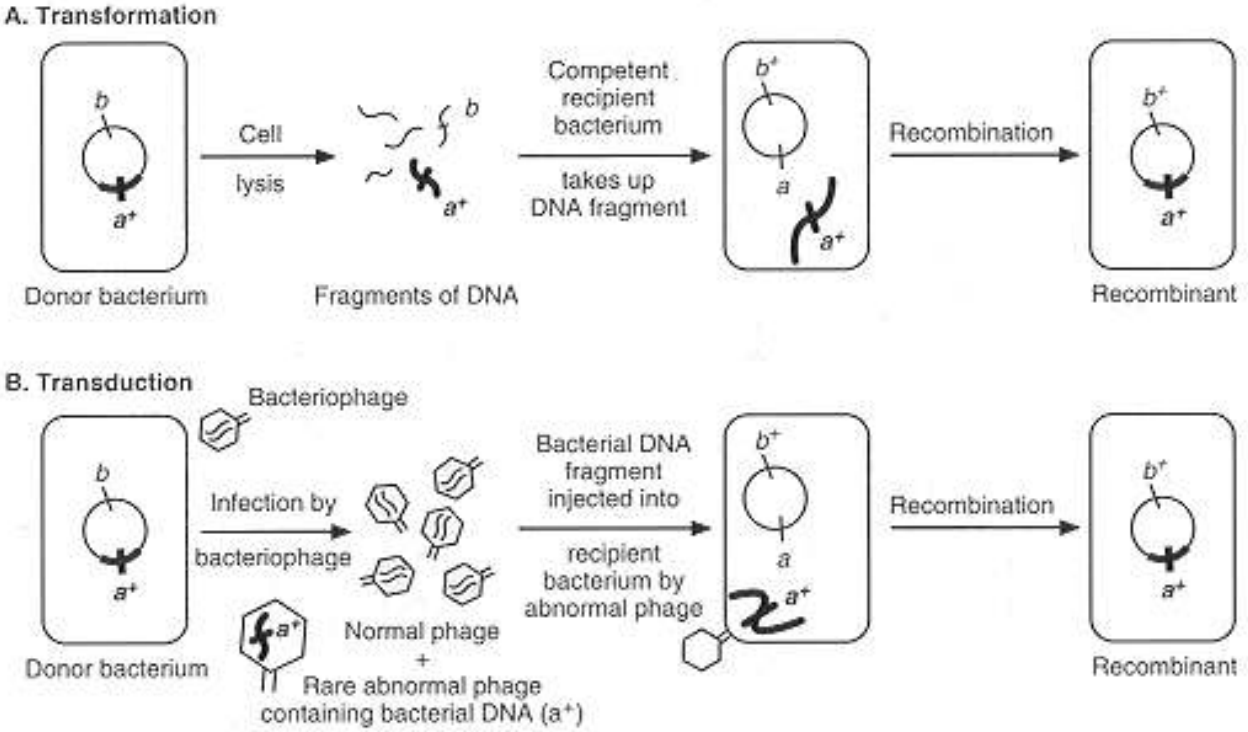
http://www.census.gov/main/www/popclock.html

Antibiotic resistance acquired by two fundamental mechanisms:

- *Sustain a new mutation that confers resistance: this mechanism explored in the mutagenesis lab*
- ***Acquire a new gene from somebody else via horizontal gene transfer: this strategy mechanism explored the next bacterial genetics lab***

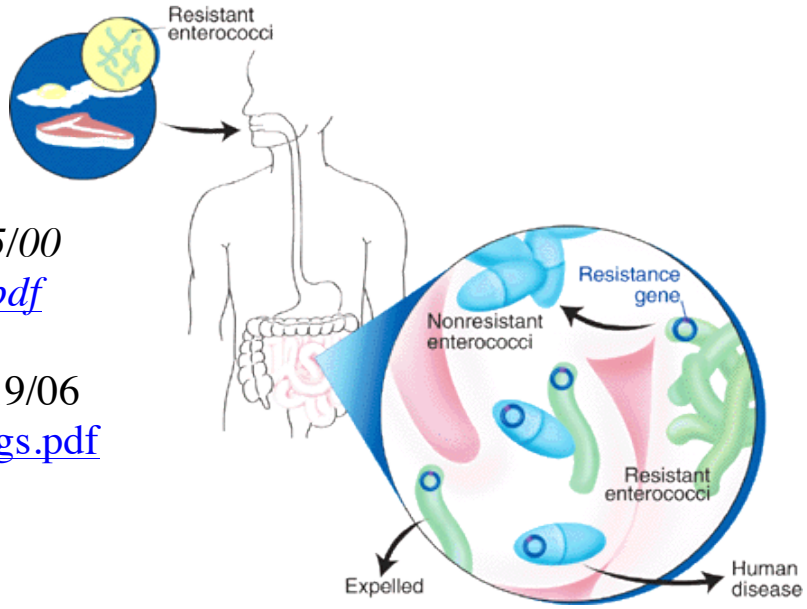
How do bacterial acquire genes from each other?

What are the mechanisms of horizontal gene transmission?


A. Transformation
b
a+
Donor bacterium
Cell
lysis
b
a+
Fragments of DNA
Competent
recipient
bacterium
takes up
DNA fragment
b+
a
a+
Recombination
b+
a+
Recombinant
B. Transduction
Bacteriophage
b
a+
Donor bacterium
Infection by
bacteriophage
Normal phage
+
a+
Rare abnormal phage
containing bacterial DNA (a+)
Bacterial DNA
fragment
injected into
recipient
bacterium by
abnormal phage
b+
a
a+
Recombination
b+
a+
Recombinant
C. Conjugation
b
a+
Donor
bacterium
+
b+
a
Recipient
bacterium
Direct transfer
of a+ DNA
from donor
to recipient
b
a+
b+
a
Separation
of mating
bacteria
b
b+
a+
a
Recom-
bination
b+
a+
Recombinant

We will examine plasmid transfer between E. coli cells in the lab

It is well documented that many cases of AR acquisition involve transfer of plasmid encoded genes between cells that are not necessarily closely related



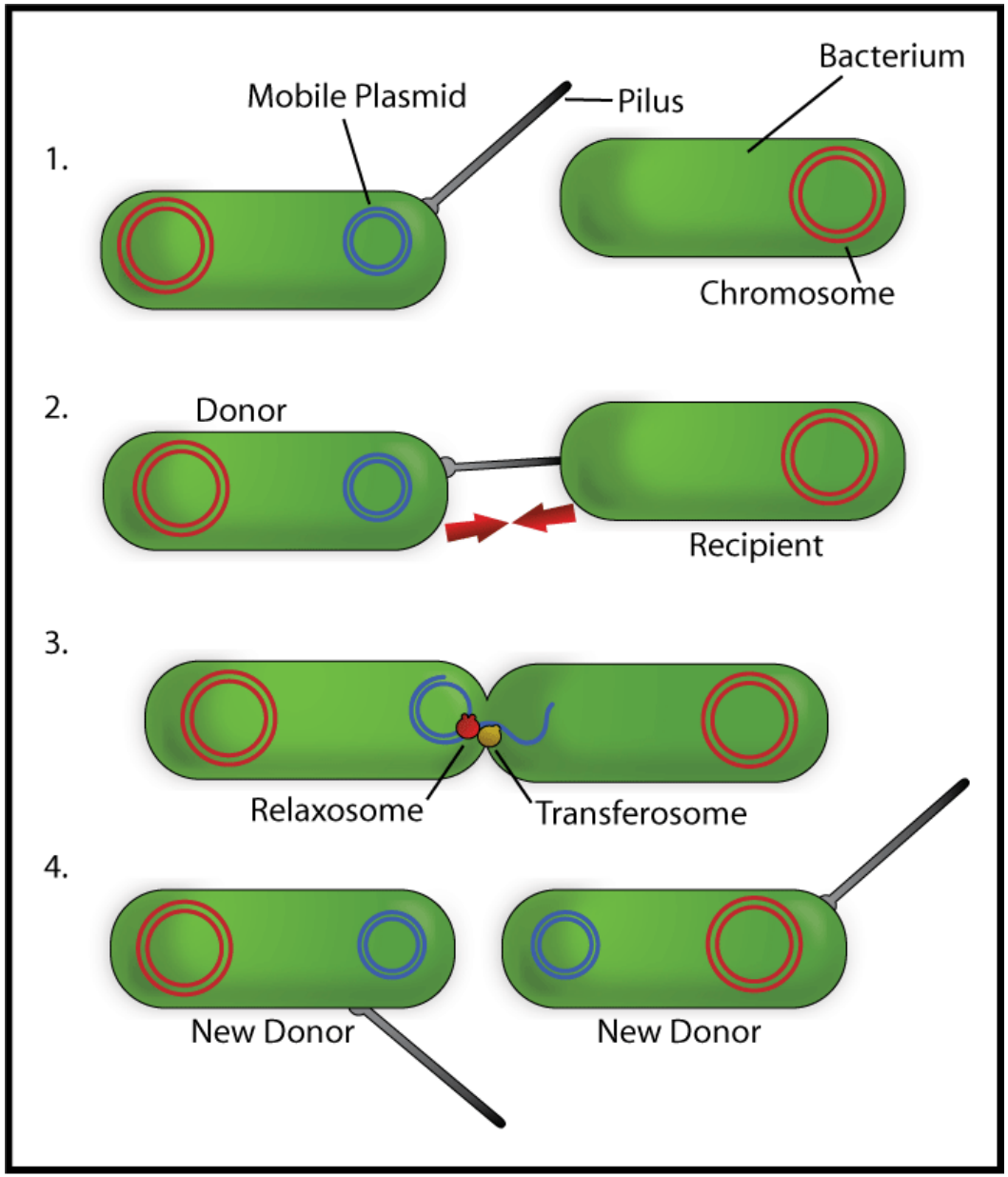
Reading Assignments

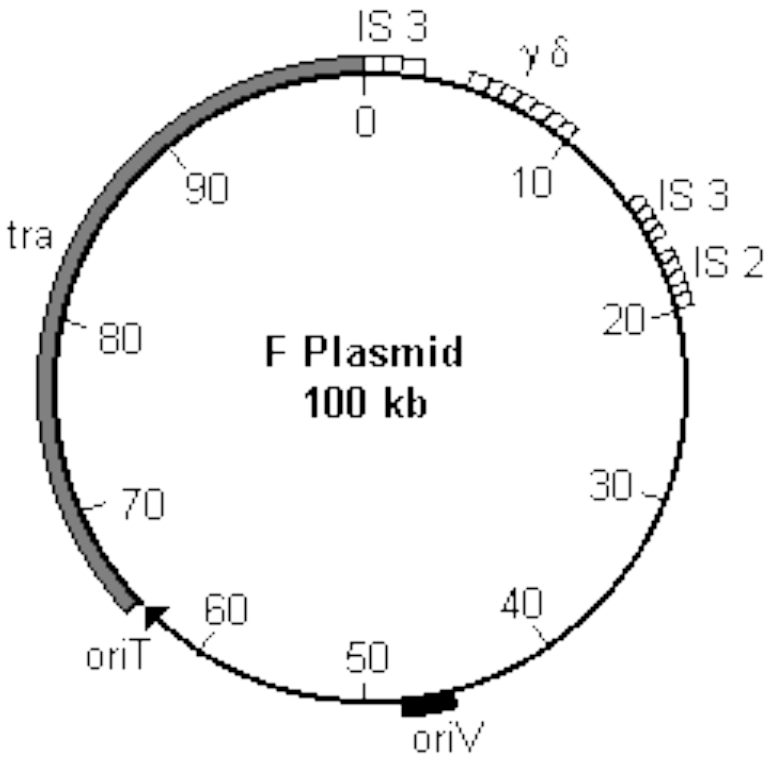
Super bugs on the Hoof? Science 288: 792 5/5/00
http://fire.biol.wwu.edu/trent/trent/superbugs.pdf

SuperBugs Abound in Soil Nature News 1/19/06
http://fire.biol.wwu.edu/trent/trent/soilsuperbugs.pdf

Decoding bacterial genotypes:
We know that sex in humans is determined by the presence or absence of the Y chromosome and by the number of X chromosomes on fruitflies and worms,

But how is sex determined in bacteria?


1.
Mobile Plasmid
Pilus
Bacterium
Chromosome
2.
Donor
Recipient
3.
Relaxosome
Transferosome
4.
New Donor
New Donor



IS 3 & IS 2 = insertion sequences
γδ = transposon Tn1000
oriV = origin of replication
oriT = origin of conjugal transfer
tra = tra functions

The tra (transfer) gene specify structural proteins that are required for pili formation and various enzymes required for "mobilization" of the DNA

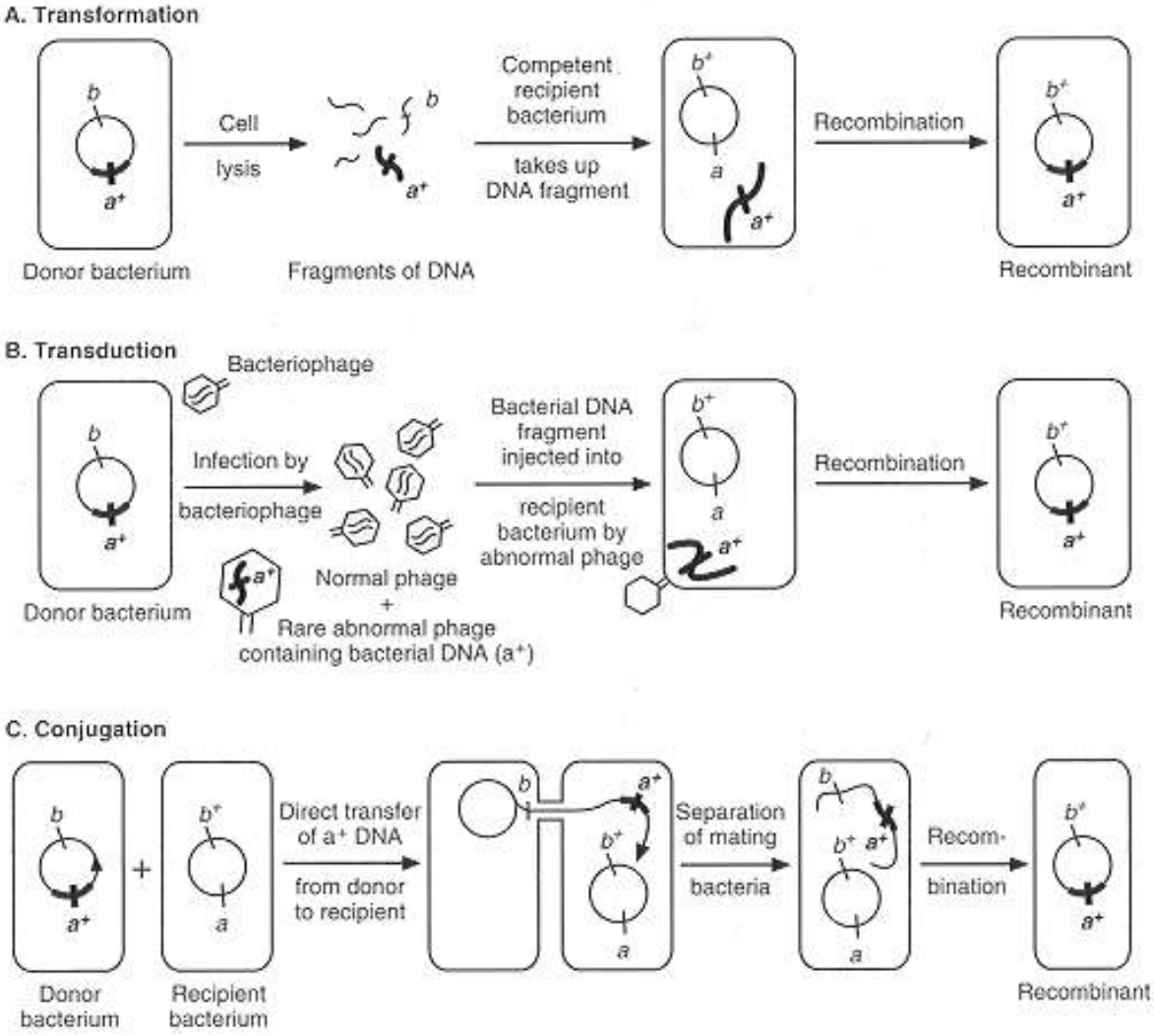
Transfer of the plasmid DNA starts at ori-T and proceeds counterclockwise on the drawing shown above

Horizontal gene transfer during conjugation

F’ X F- or F^+ X F-

Animation of gene transfer
http://www.blackwellpublishing.com/trun/artwork/Animations/conjugation/conjugation.html
Some thoughts for the curious: Why aren't all bacterial cells male?
http://fire.biol.wwu.edu/trent/trent/Ffactorpopulationgenetics.pdf

We will examine plasmid transfer between E. coli cells in the lab


A. Transformation
b
a+
Cell
lysis
b
a+
Competent
recipient
bacterium
takes up
DNA fragment
b+
a
a+
Recombination
b+
a+
Donor bacterium
Fragments of DNA
Recombinant
B. Transduction
Bacteriophage
b
a+
Infection by
bacteriophage
Normal phage
+
Rare abnormal phage
containing bacterial DNA (a+)
Donor bacterium
Bacterial DNA
fragment
injected into
recipient
bacterium by
abnormal phage
b+
a
a+
Recombination
b+
a+
Recombinant
C. Conjugation
b
a+
b+
a
Donor
bacterium
Recipient
bacterium
Direct transfer
of a+ DNA
from donor
to recipient
b
a+
b+
a
Separation
of mating
bacteria
b
b+
a+
a
Recom-
bination
b+
a+
Recombinant

Whole plasmid transfer does not require sequence homology between incoming DNA and resident genome to generate a stable recombinant

Why is the homology issue so important in the movement of antibiotic resistance genes?

Important implications of whole plasmid transfer

- ***This is a great way for a bacterial cell to acquire novel genes:*** since no requirement for cross over, resident genome does not have to have homology to incoming genes
- Also plasmid packaged AR genes come with their own transfer genes

---

Pathogenecity genes are often coded on plasmid or phage genes

see Nature News 6/14/11: Phage on the Rampage
http://fire.biol.wwu.edu/trent/trent/PhagerampageNatureNews.pdf

Page encoded genes code for Shiga toxin in pathogenic *E. coli*