The Antibiotic Resistome

Adam Ratner, M.D., M.P.H.
Departments of Pediatrics and Microbiology
Columbia University

Antibiotic Resistance is a Clinical Problem

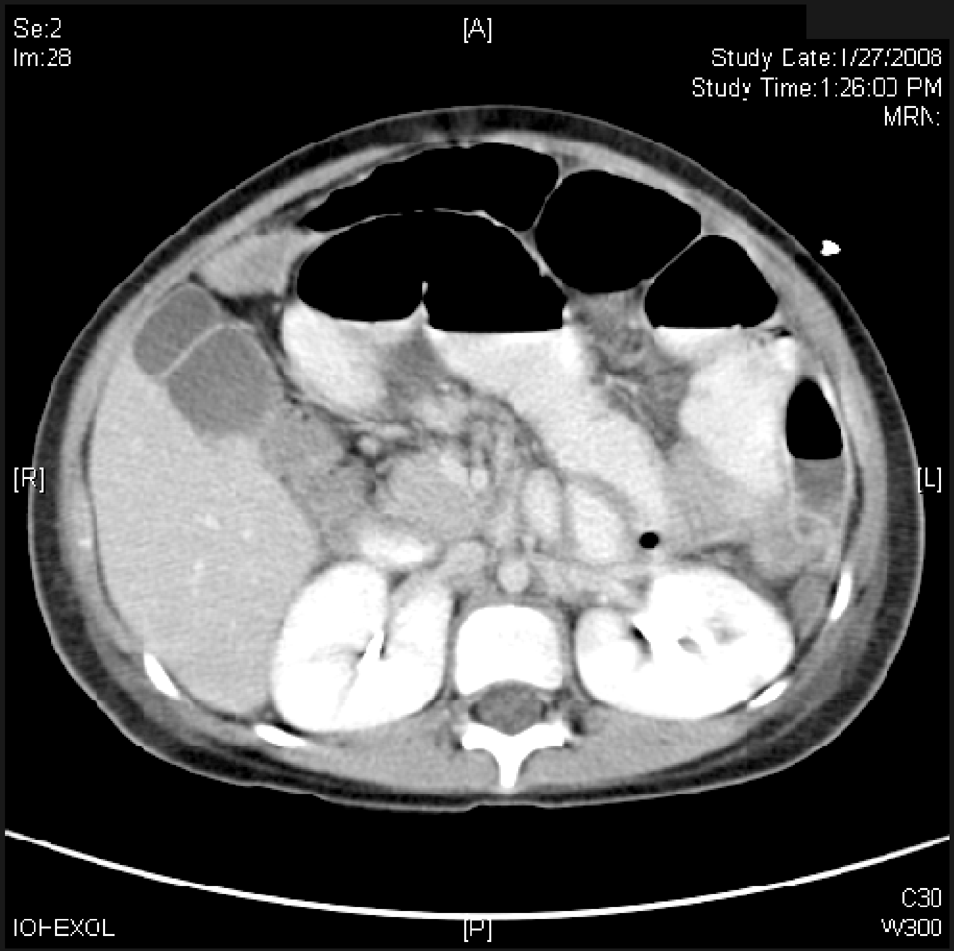
Newborn infant girl with fussiness, 1 day of low-grade fever.

CSF CULTURE & SMEAR 2008-01-25 14:00	
SPECIMEN DESCRIPTION:	CEREBROSPINAL FLUID
GRAM SMEAR:	FEW WBCS SEEN
GRAM SMEAR:	FEW GRAM POSITIVE COCCI IN CHAINS IN PAIRS
GRAM SMEAR:	CALLED WITH READ BACK TO [redacted] @ 15:00 ON 01/25/08
CULTURE:	LIGHT GROWTH OF STREPTOCOCCUS GROUP B (*sens*)
CULTURE:	CALLED WITH READ BACK TO [redacted]N 01/26/08 @ 0815.
Collection time: 2008-01-25 14:00 Received time: 2008-01-25 14:00	
Status: Final, Accno: [redacted]	
[redacted]	

LIGHT GROWTH OF STREPTOCOCCUS GROUP B				
METHOD:E-TEST MIC				
PEN	VAN	CTX	ERY	LVX
.19 S	.50 S	.125 S	.25 S	.50 S

Antibiotic Resistance is a Clinical Problem

5 year old girl presents with perforated appendicitis, intraabdominal abscesses.



CULTURE & SMEAR SITE 2008-01-28 16:00	
SPECIMEN DESCRIPTION:	ABSCESS ABDOMEN
GRAM SMEAR:	MANY POLYS
GRAM SMEAR:	MANY GRAM POSITIVE COCCI IN CHAINS IN PAIRS
GRAM SMEAR:	MANY GRAM NEGATIVE RODS
CULTURE:	HEAVY ESCHERICHIA COLI (*sens*)
CULTURE:	MODERATE STREPTOCOCCUS VIRIDANS GROUP (*sens*)
Collection time: 2008-01-28 16:00 Received time: 2008-01-28 16:00	
Status: Final, Accno: [redacted]	
[redacted]	

HEAVY ESCHERICHIA COLI

METHOD:VITEK MIC

AMP	A/S	P/T	CFZ	CPM	CFT	CEZ	CTX	IMP	AZM	AMI	GEN	TOB	CIP	LVX	T/S	CEFUROXIME SODIUM	MER
>=32 R	>=32 R	32 I	32 R	<=1 S	S	<=1 S	<=1 S	<=1 S	<=1 S	<=2 S	<=1 S	<=1 S	<=0.25S	<=0.25S	>=320R	4 S	<=0.25S

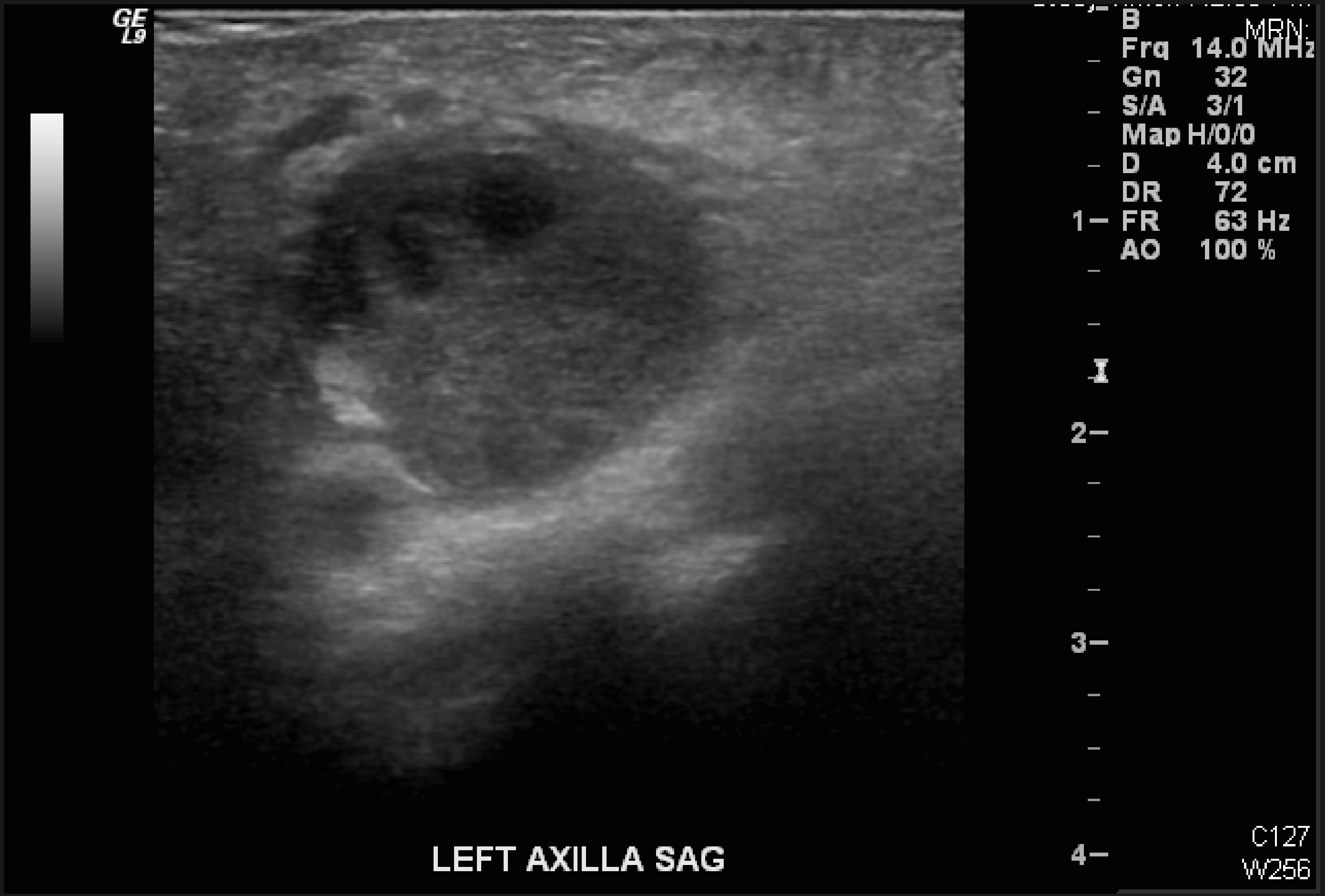
MODERATE STREPTOCOCCUS VIRIDANS GROUP

METHOD:E-TEST MIC

PEN	VAN	CTX	ERY	LVX	CIP
.047 S	1.0 S	.38 S	.032 S	.38 S	

Antibiotic Resistance is a Clinical Problem

18 month old girl with fevers and swelling under her left arm.



Antibiotic Resistance is a Clinical Problem

CULTURE & SMEAR SITE 2007-02-24 16:30	
SPECIMEN DESCRIPTION:	WOUND NO.2
GRAM SMEAR:	NO POLYS SEEN
GRAM SMEAR:	FEW GRAM POSITIVE COCCI IN CLUSTERS IN PAIRS
CULTURE:	HEAVY STAPHYLOCOCCUS AUREUS, METHICILLIN RESISTANT (sens)
Collection time: 2007-02-24 16:30 Received time: 2007-02-24 17:17	
Status: Final, Accno: [redacted]	

HEAVY STAPHYLOCOCCUS AUREUS, METHICILLIN RESISTANT

METHOD:MICROSCAN MIC

T/S	RIF	TET	OXA	PEN	VAN	ERY	CLN	GEN	CIP	LVX
<=2/38S	<=1 S	<=4 S	>2 R	>8 R	<=2 S	>4 R	<=0.25S	2 S	<=1 S	<=2 S

Antibiotic Resistance is a Clinical Problem

Teenage boy with cystic fibrosis, long history of lung infections has worsening function, awaits transplant.

Antibiotic Resistance is a Clinical Problem

CULTURE & SMEAR 2008-02-10 02:24	
SPECIMEN DESCRIPTION:	SPUTUM
GRAM SMEAR:	<25 SQUAMOUS EPITHELIAL CELLS/LPF
GRAM SMEAR:	NO POLYS SEEN
GRAM SMEAR:	MODERATE GRAM NEGATIVE RODS
CULTURE:	HEAVY MUCOID PSEUDOMONAS AERUGINOSA (*sens*)
CULTURE:	MIXED COMMENSAL FLORA
Collection time: 2008-02-10 02:24 Received time: 2008-02-10 12:07	
Status: Final, Accno [redacted]	

HEAVY MUCOID PSEUDOMONAS AERUGINOSA
METHOD:E-TEST MIC
POLYMIXIN B
.38 S

HEAVY MUCOID PSEUDOMONAS AERUGINOSA										
METHOD:KIRBY BAUER										
AMI	AZM	CPM	CEZ	GEN	IMP	LVX	MER	P/T	TOB	T/S
S	R	R	R	R	R	R	R	R	R	R

Antibiotic Resistance is a Clinical Problem

Table 1. Selected Current Problems with Antimicrobial-Drug Resistance, According to Drug Class.

Antibiotic Class	Mechanism of Resistance
Cephalosporins	Extended-spectrum β-lactamases, chromosomal cephalosporinases
β-Lactamase inhibitors	Hyperproducers of β-lactamases, new β-lactamases resistant to inhibitors, chromosomal cephalosporinases
Carbapenems	Zinc metalloenzymes and other β-lactamases
Vancomycin, teicoplanin	Modified cell-wall precursors with decreased affinity for vancomycin
Quinolones	Alterations in DNA topoisomerase, efflux mechanisms, permeability changes
Trimethoprim–sulfamethoxazole	Resistant enzymes in folate-synthesis pathway
Erythromycin, new macrolides	Methylation of the bacterial ribosome producing resistance to macrolides, clindamycin, and streptogramin B antibiotics
Aminoglycosides	Aminoglycoside-modifying enzymes

Invasive Methicillin-Resistant *Staphylococcus aureus* Infections in the United States

R. Monina Klevens, DDS, MPH
Melissa A. Morrison, MPH
Joelle Nadle, MPH
Susan Petit, MPH
Ken Gershman, MD, MPH
Susan Ray, MD
Lee H. Harrison, MD
Ruth Lynfield, MD
Ghinwa Dumyati, MD
John M. Townes, MD
Allen S. Craig, MD
Elizabeth R. Zell, MSTAT
Gregory E. Fosheim, MPH
Linda K. McDougal, MS
Roberta B. Carey, PhD
Scott K. Fridkin, MD
for the Active Bacterial Core surveillance (ABCs) MRSA Investigators

Context As the epidemiology of infections with methicillin-resistant *Staphylococcus aureus* (MRSA) changes, accurate information on the scope and magnitude of MRSA infections in the US population is needed.

Objectives To describe the incidence and distribution of invasive MRSA disease in 9 US communities and to estimate the burden of invasive MRSA infections in the United States in 2005.

Design and Setting Active, population-based surveillance for invasive MRSA in 9 sites participating in the Active Bacterial Core surveillance (ABCs)/Emerging Infections Program Network from July 2004 through December 2005. Reports of MRSA were investigated and classified as either health care–associated (either hospital-onset or community-onset) or community-associated (patients without established health care risk factors for MRSA).

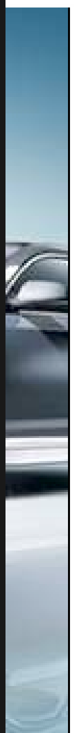
Main Outcome Measures Incidence rates and estimated number of invasive MRSA infections and in-hospital deaths among patients with MRSA in the United States in 2005; interval estimates of incidence excluding 1 site that appeared to be an outlier with the highest incidence; molecular characterization of infecting strains.

Results There were 8987 observed cases of invasive MRSA reported during the surveillance period. Most MRSA infections were health care–associated: 5250 (58.4%) were community-onset infections, 2389 (26.6%) were hospital-onset infections; 1234 (13.7%) were community-associated infections, and 114 (1.3%) could not be classified. In 2005, the standardized incidence rate of invasive MRSA was 31.8 per 100 000 (interval estimate, 24.4-35.2). Incidence rates were highest among persons 65 years and older (127.7 per 100 000; interval estimate, 92.6-156.9), blacks (66.5 per 100 000; interval estimate, 43.5-63.1), and males (37.5 per 100 000; interval estimate, 26.8-39.5). There were 1598 in-hospital deaths among patients with MRSA infection during the surveillance period. In 2005, the standardized mortality rate was 6.3 per 100 000 (interval estimate, 3.3-7.5). Molecular testing identified strains historically associated with community-associated disease outbreaks recovered from cultures in both hospital-onset and community-onset health care–associated infections in all surveillance areas.

Conclusions Invasive MRSA infection affects certain populations disproportionately. It is a major public health problem primarily related to health care but no longer confined to intensive care units, acute care hospitals, or any health care institution.

JAMA. 2007;298(15):1763-1771 www.jama.com

NEW YORK POST
24 HOURS A DAY
Monday, February 25, 2008
Last Update: 08:50 AM EST



SHOCKING SUPERBUG NY TALLY

By SUSAN EDELMAN

November 25, 2007 -- Outbreaks of the superbug MRSA infected at least 242 patients - and killed seven - in New York hospitals over the last three years, officials said.

The state Department of Health released data showing nearly 50 reported outbreaks of the drug-resistant bacteria in hospitals statewide since 2003.

Some hospital data was not available, state officials said.

Health Department spokeswoman Claire Pospisil said hospitals that report outbreaks should take extra steps to enforce infection controls such as hand-washing and other hygiene to prevent the spread of germs.

MRSA, or methicillin-resistant Staphylococcus aureus, has caused

Where does antibiotic resistance come from?

Inappropriate use?

Bad doctors?


I will wash my hands between patients...
I will wash my hands between patients...
I will wash my hands between patients...
I will wash my hands between patients...
I will wash my hands between patients...
I will wash my hands between patients...
I will wash my hands between patients...
I will wash my hands between patients...
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Where does antibiotic resistance come from?

Was there antibiotic resistance before antibiotics were used widely (ca. 1940s)?

Nothing in biology makes sense except in the light of evolution.

-Theodosius Dobzhansky (1900-1975)

"There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring 'fastness' [resistance]."

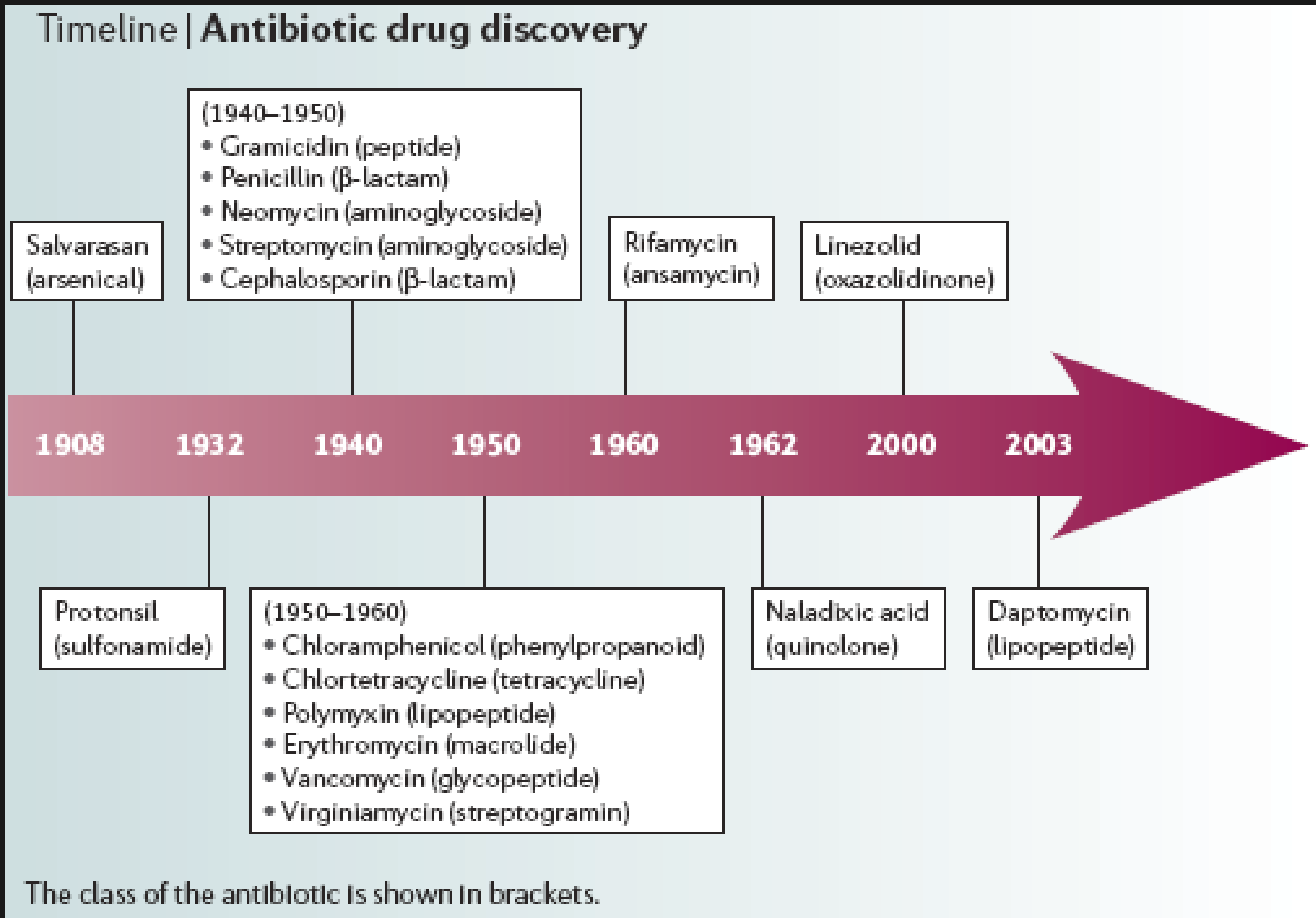
—Alexander Fleming, 1946

Antibiotic Resistance

- Mechanisms
- Transfer of resistance mechanisms

Evolution and Ecology of Antibiotic Resistance

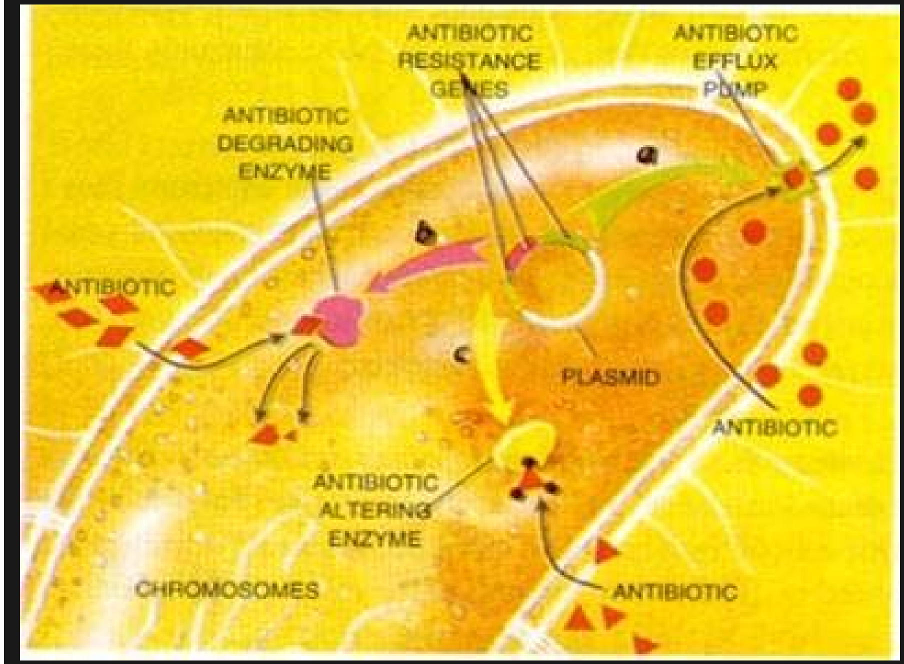
- Selective pressure vs. fitness cost
- Concept of the pan-microbial resistome
- Predicting the evolution of resistance
- Strategies to combat the evolution of resistance

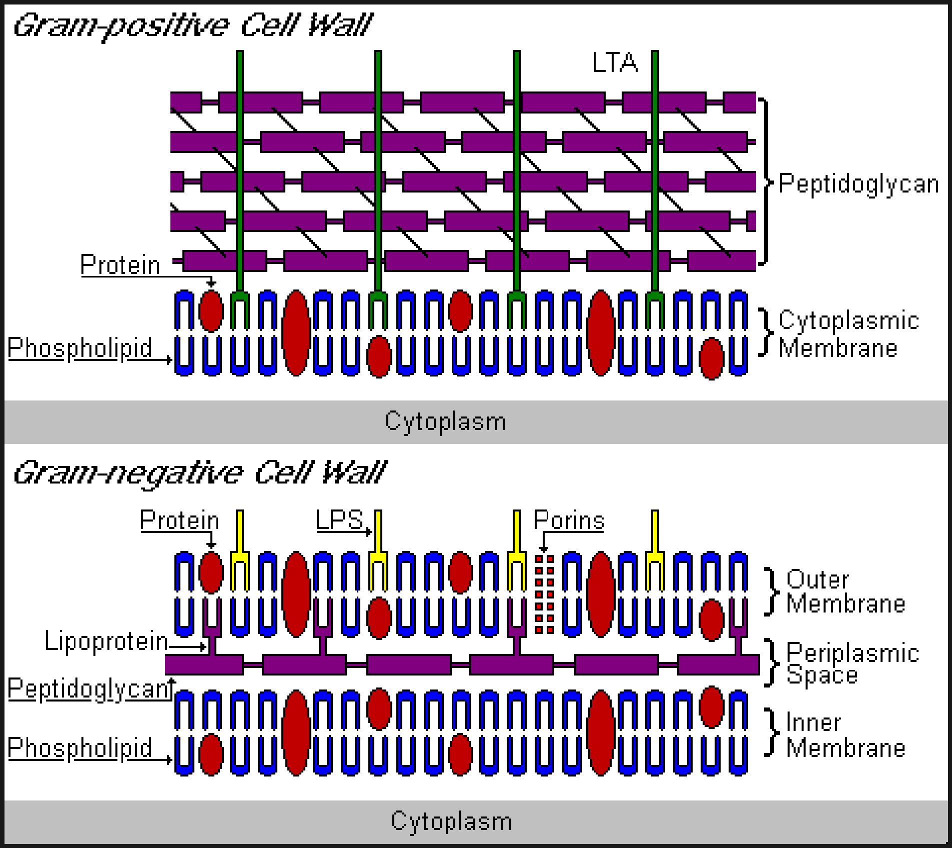


Wright GD. *Nat Rev Microbiol* 2007.

Antibiotic Resistance Mechanisms

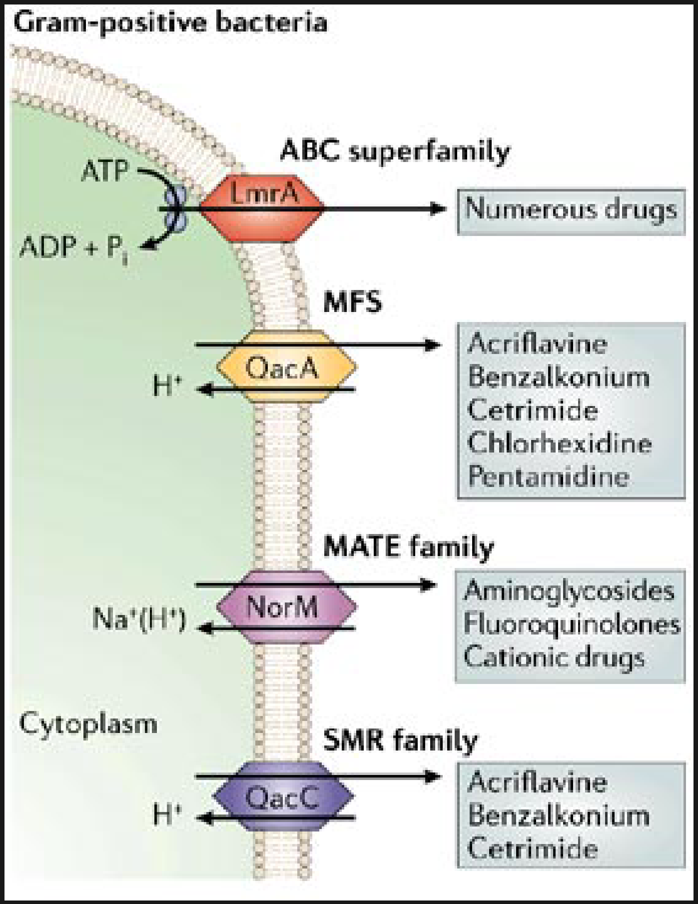
Physical barriers

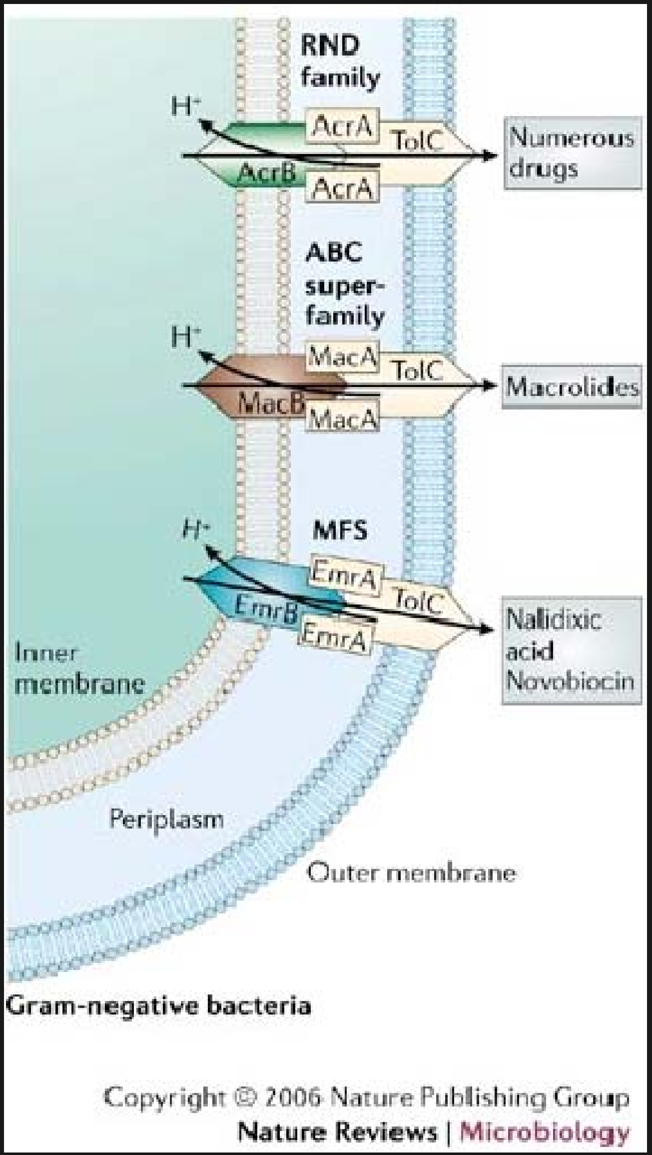




Antibiotic Resistance Mechanisms

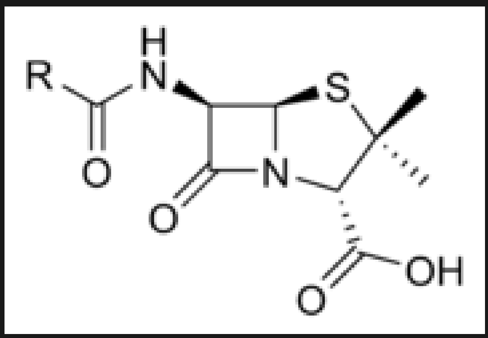
Efflux pumps





Antibiotic Resistance Mechanisms

- Target modification
  - PBP, ribosome, DNA gyrase, cell wall
- Enzymatic inactivation
  - β-lactamase



An Enzyme from Bacteria able to Destroy Penicillin

E. P. Abraham.
E. Chain.
Sir William Dunn School of Pathology,
Oxford.

Dec. 5.

No. 3713, Dec. 28, 1940 NATURE

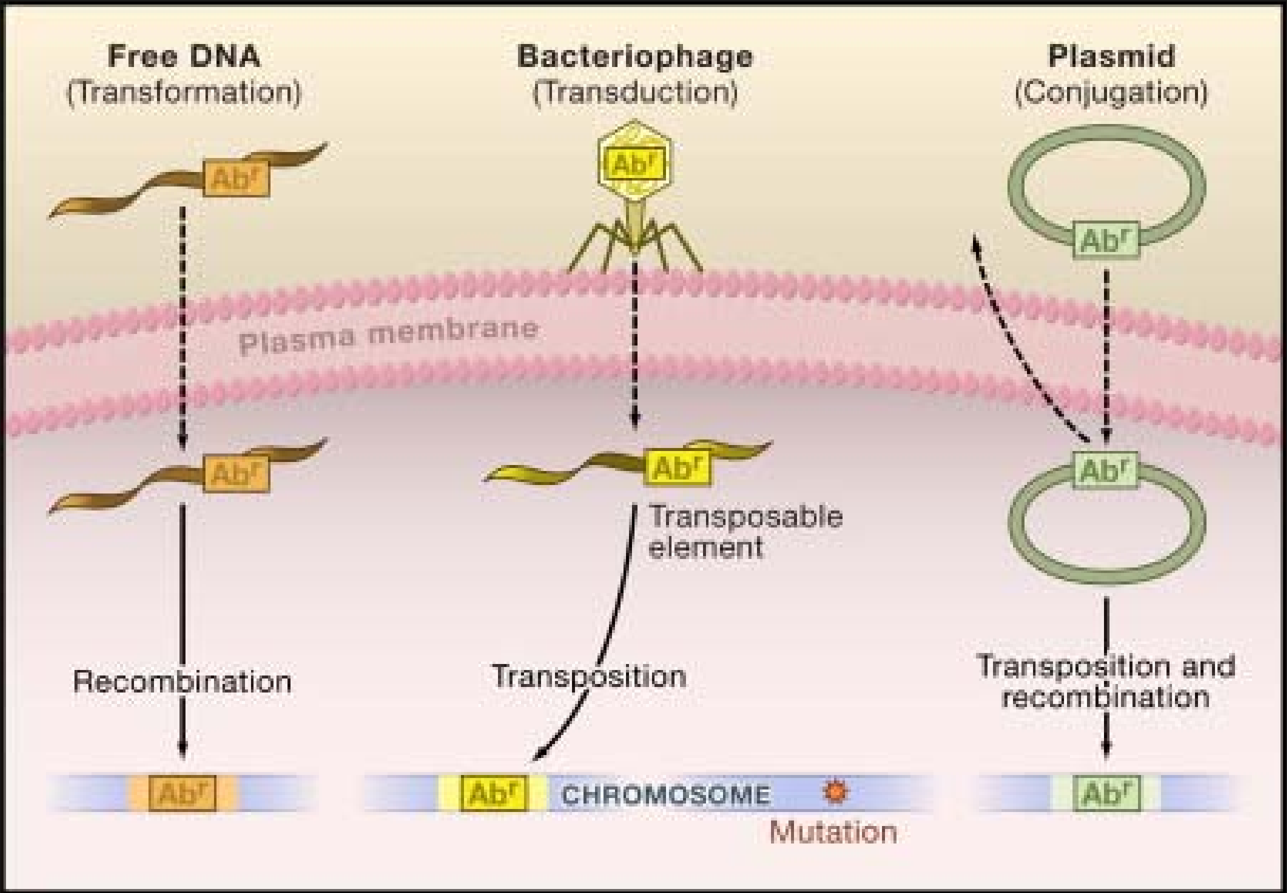
Transfer of Resistance Mechanisms

Mutation (no transfer required)

Transformation

Transduction

Conjugation



Why understanding resistance mechanisms matters:

1. Predicting the future
2. Slowing the progression
3. Intervening intelligently

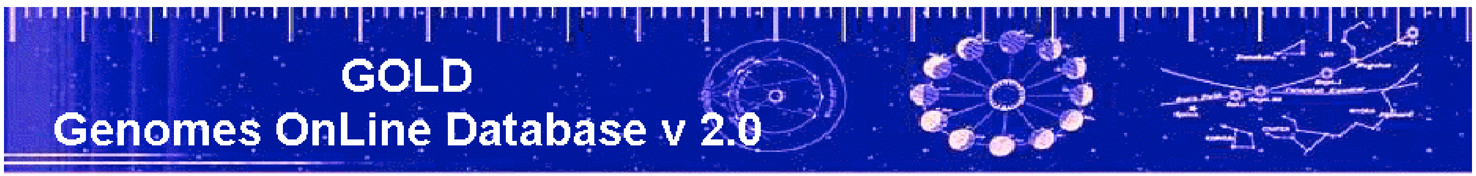
Multidrug Resistance in *S. aureus*

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

Bacterial Genomes and Infectious Diseases

Whole-Genome Random Sequencing and Assembly of *Haemophilus influenzae* Rd

Robert D. Fleischmann, Mark D. Adams, Owen White, Rebecca A. Clayton, Ewen F. Kirkness, Anthony R. Kerlavage, Carol J. Bult, Jean-Francois Tomb, Brian A. Dougherty, Joseph M. Merrick, Keith McKenney, Granger Sutton, Will FitzHugh, Chris Fields,* Jeannine D. Gocayne, John Scott, Robert Shirley, Li-Ing Liu, Anna Glodek, Jenny M. Kelley, Janice F. Weidman, Cheryl A. Phillips, Tracy Spriggs, Eva Hedblom, Matthew D. Cotton, Teresa R. Utterback, Michael C. Hanna, David T. Nguyen, Deborah M. Saudek, Rhonda C. Brandon, Leah D. Fine, Janice L. Fritchman, Joyce L. Fuhrmann, N. S. M. Geoghagen, Cheryl L. Gnehm, Lisa A. McDonald, Keith V. Small, Claire M. Fraser, Hamilton O. Smith, J. Craig Venter†



Contact: Genomesonline	Last Update: February 3, 2008	Location www.genomesonline.org
722 Published Complete Genomes	Search GOLD: 3595 genome projects	115 Metagenomes
92 Archaeal Ongoing Genomes	1757 Bacterial Ongoing Genomes	905 Eukaryotic Ongoing Genomes

-omes

Genome
Transcriptome
Proteome
Metabolome

Microbiome
(Resistome)

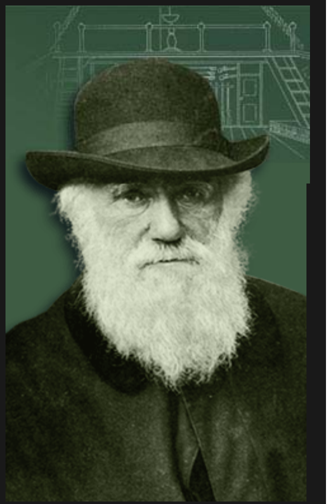
Concept of a species or microbial “pan-genome”

Evolution and Ecology of Antibiotic Resistance

- Selective pressure vs. fitness cost
- Concept of the pan-microbial resistome
- Predicting the evolution of resistance
- Strategies to combat the evolution of resistance

How does natural selection work?

- Variation
- Inheritance
- Selection
- Time
- Adaptation



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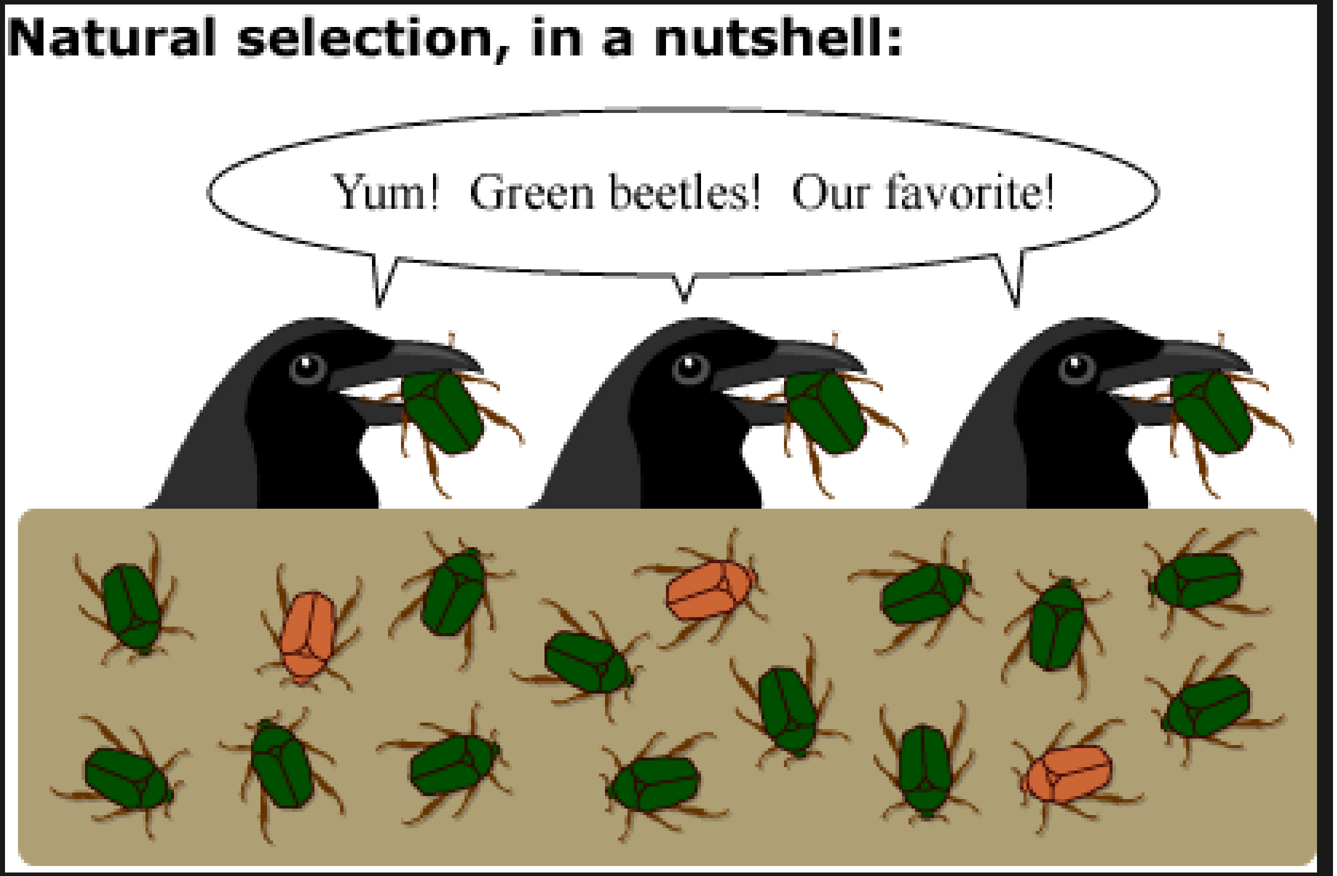
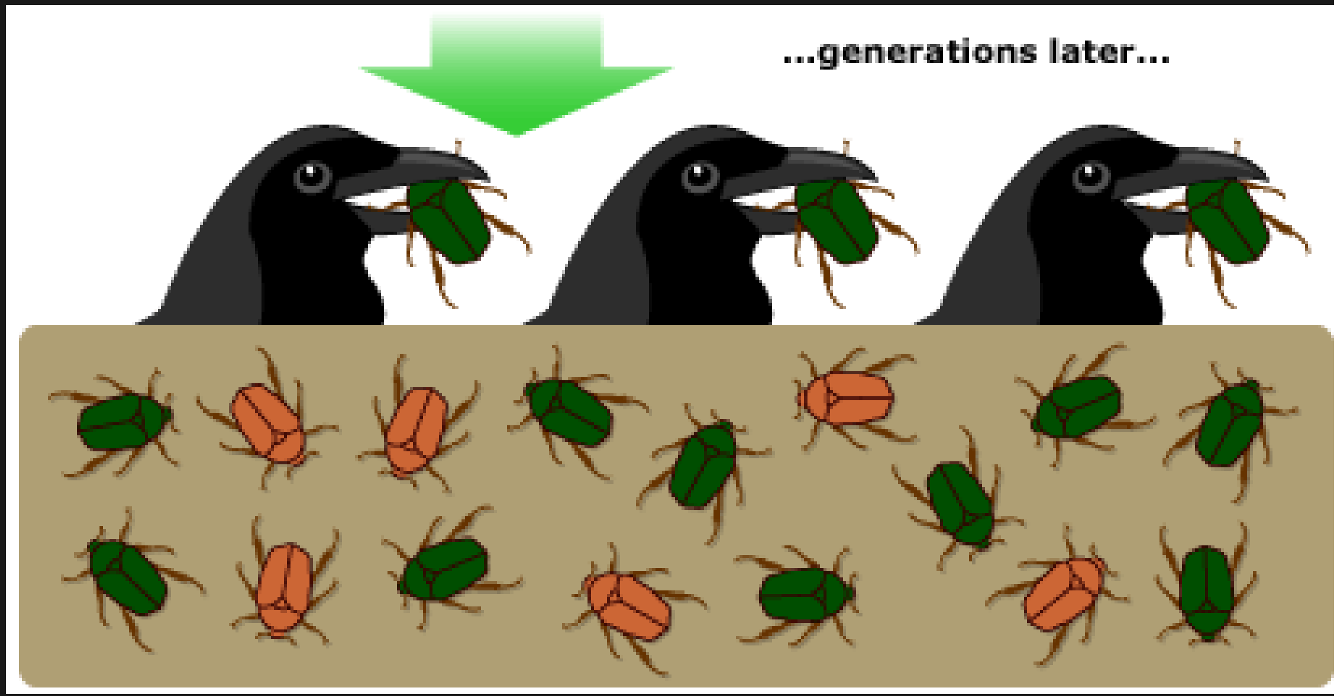


From “Battling bacterial evolution: The work of Carl Bergstrom”
Understanding Evolution, University of California.

How does natural selection work?

Variation
Inheritance
Selection
Time
Adaptation



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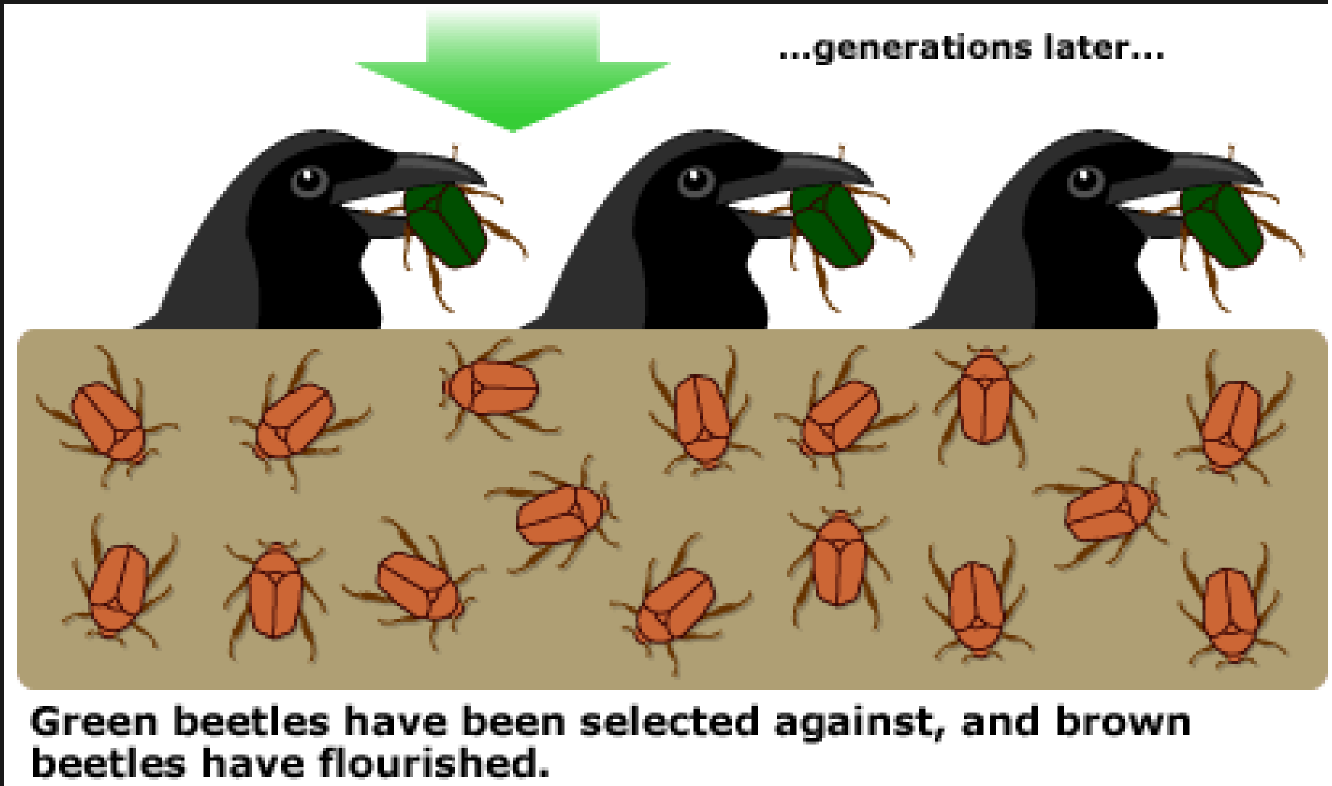
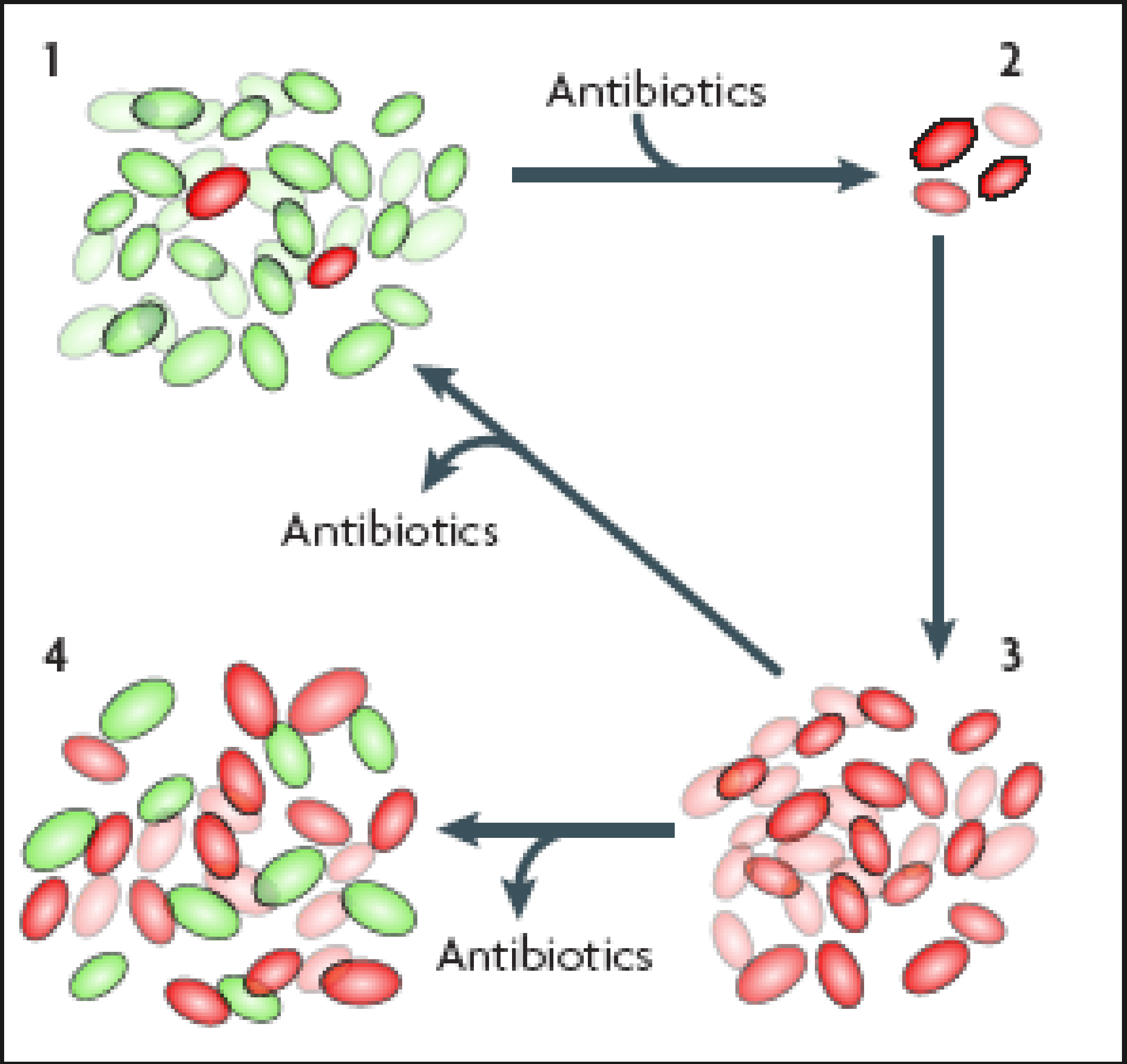


Selective pressure vs. fitness cost in resistance



How is resistance maintained?

Continued selective pressure?
- direct pressure (antibiotics)
- co-selection (clusters of genes)

Lack of fitness cost?
- compensatory mutations

Effects of Environment on Compensatory Mutations to Ameliorate Costs of Antibiotic Resistance

J. Björkman,[1,2]* I. Nagaev,[2]* O. G. Berg,[3] D. Hughes,[2] D. I. Andersson[1]†

Rationale for examining the soil resistome

1. Antibiotics are ancient.

2. Antibiotics are made by environmental microbes.

3. Resistance is essential for those microbes and their neighbors.

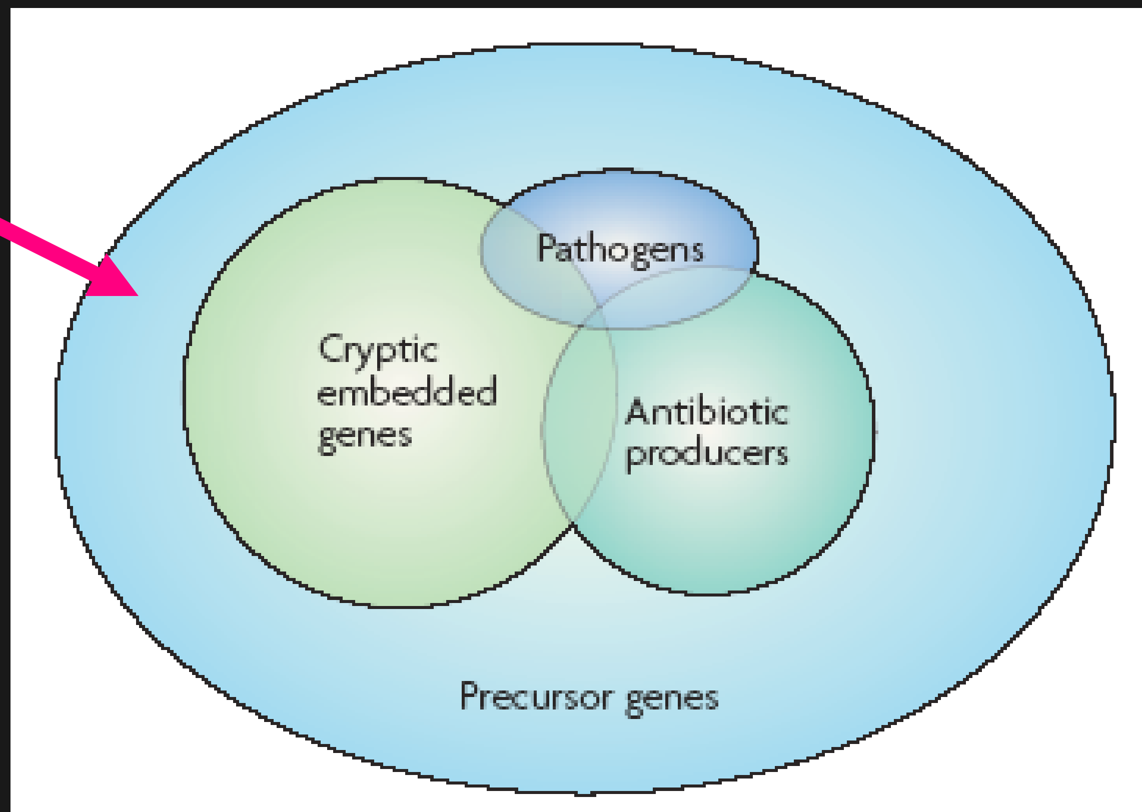
4. Resistance can be transmitted from environmental microbes to potential pathogens.

Sampling the Antibiotic Resistome

Vanessa M. D'Costa,[1] Katherine M. McGrann,[1] Donald W. Hughes,[2] Gerard D. Wright[1]*

20 JANUARY 2006 VOL 311 SCIENCE

precursor genes may have important functions in environmental survival (efflux pumps, ability to use different substrates for cell wall)



Sampling the Antibiotic Resistome

Vanessa M. D'Costa,[1] Katherine M. McGrann,[1] Donald W. Hughes,[2] Gerard D. Wright[1]*

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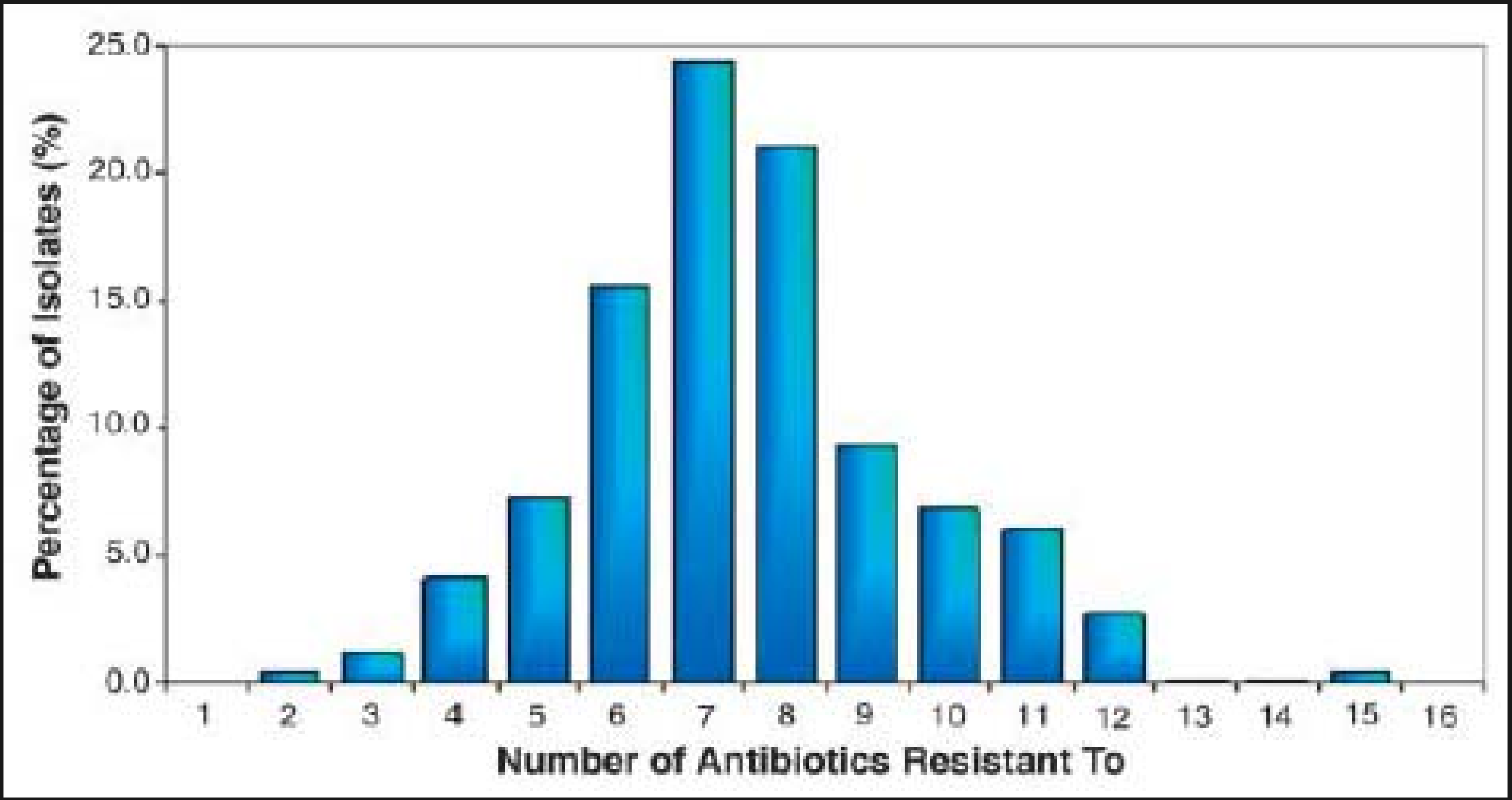

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Antibiotic	Number of strains		Complete inactivation: %	
	Resistant	Screened for inactivation	Of isolates screened	Of library
Cephalexin	442	16	18.8	N/A*
Ciprofloxacin	52	52	0.0	0.0
Clindamycin	107	46	0.0	N/A
Daptomycin	480	80	80.0	N/A
Erythromycin	128	128	7.0	1.9
Novobiocin	12	12	0.0	0.0
Rifampicin	49	49	40.8	4.2
Synercid	294	71	18.3	N/A
Telithromycin	83	83	4.8	0.8
Trimethoprim	478	80	0.0	N/A
Vancomycin	5	5	0.0	0.0

*Not applicable. Statistic cannot be determined, because all resistant isolates were not assayed.

Sampling the Antibiotic Resistome

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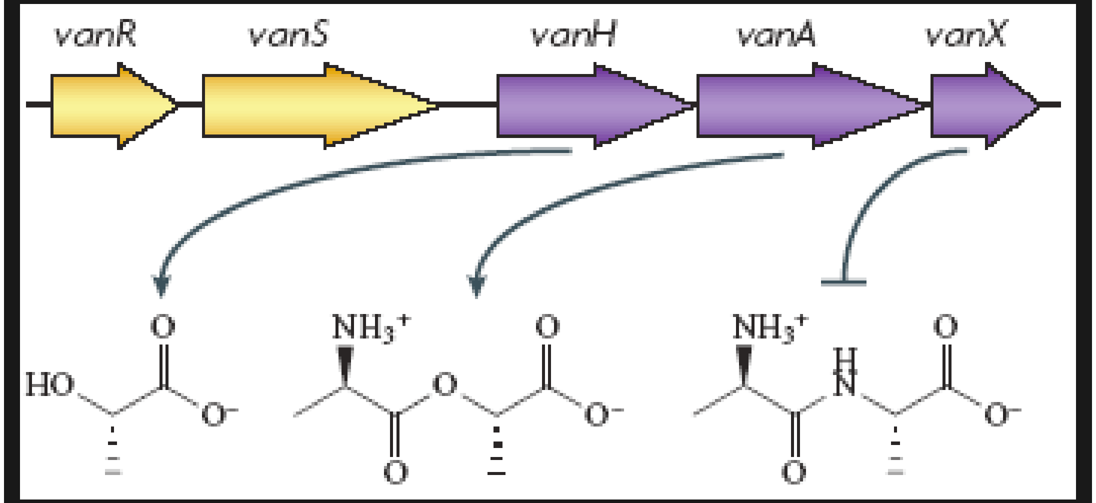
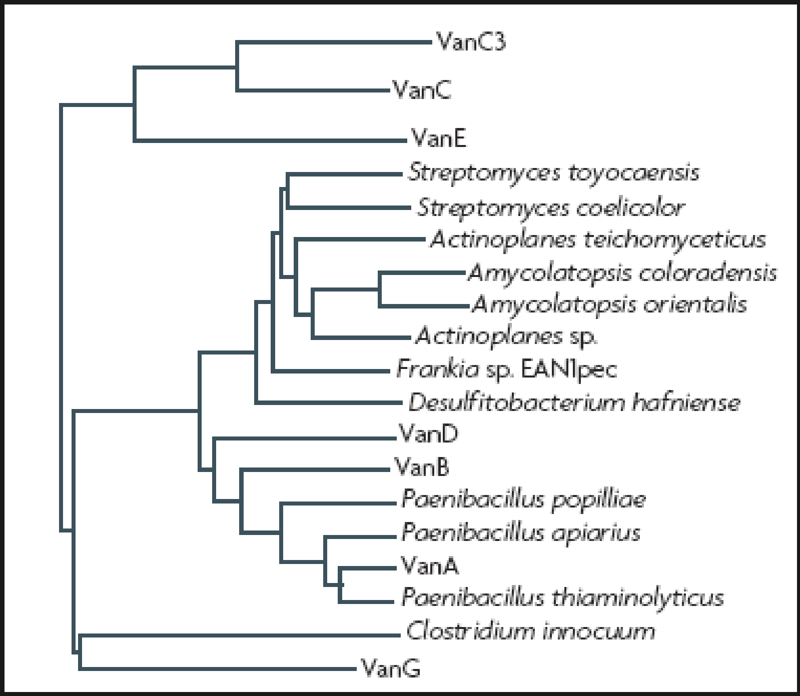
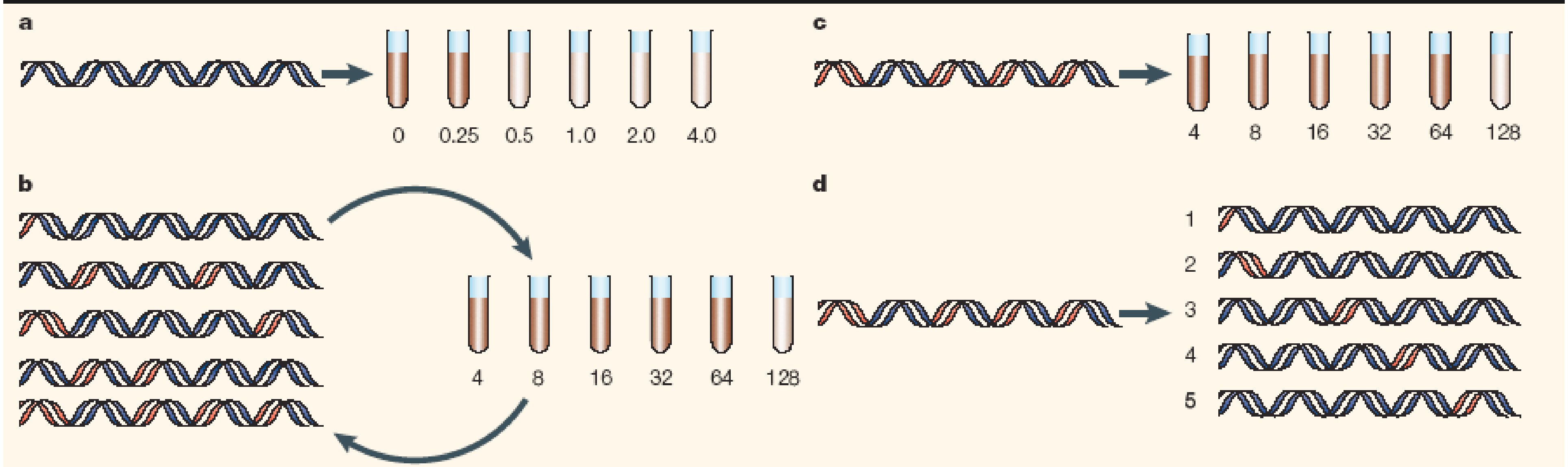




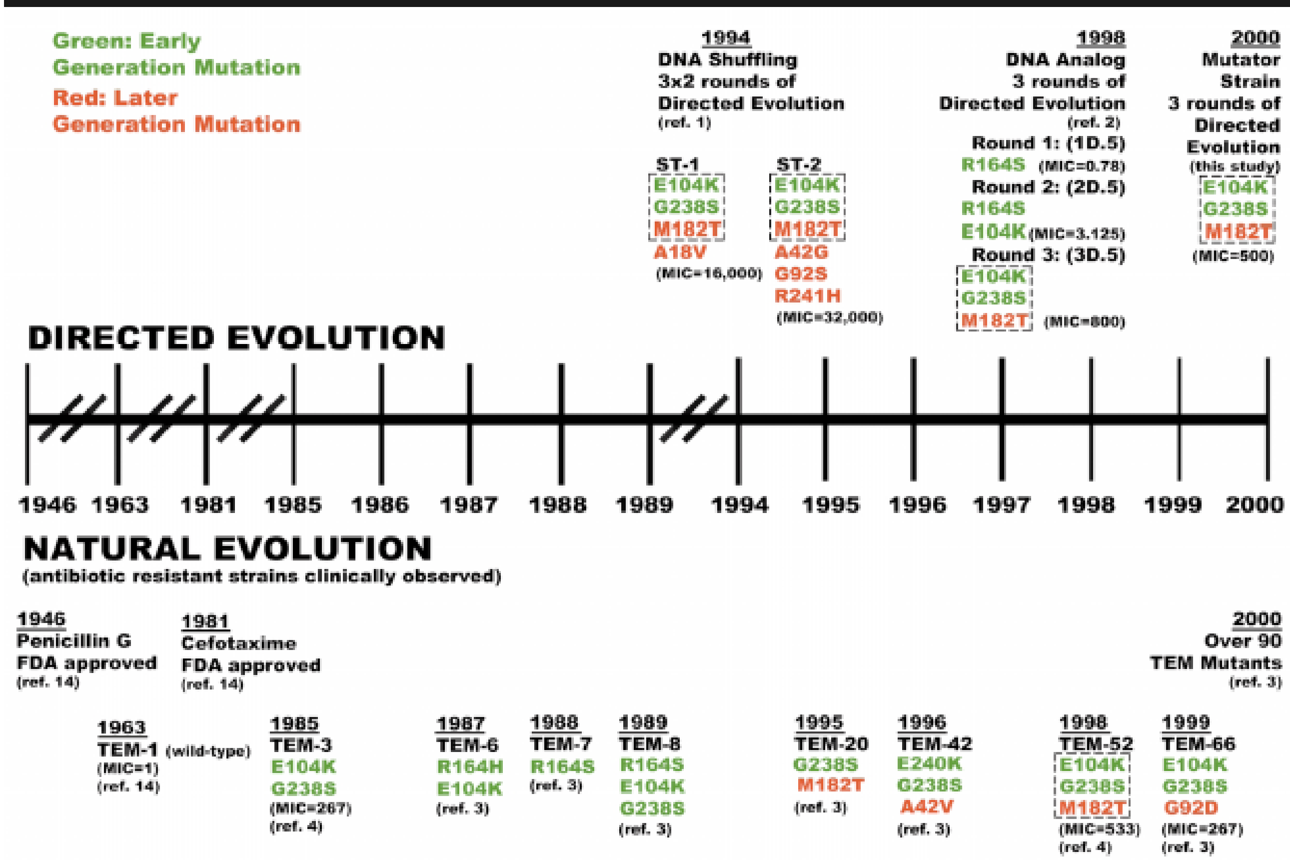
Predicting the evolution of resistance



Predicting the evolution of antibiotic resistance genes

Barry G. Hall

Predicting the evolution of resistance



Predicting the emergence of antibiotic resistance by directed evolution and structural analysis

M. Cecilia Orencia[1], Jun S. Yoon[1], Jon E. Ness[2], Willem P. C. Stemmer[2] and Raymond C. Stevens[1]

[1]Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037, USA. [2]Maxygen, Redwood City, California 94063, USA.

Strategies to combat the evolution of resistance

1. Minimize environmental selective pressure
   - hospital, outpatient, agriculture
   - vaccination

2. Understand the antibiotic resistome to predict future resistance

3. New strategies for antibiotics
   - specific targeting of virulence factors
   - targeting of resistance mechanisms
   - inhibition of mutation and/or stress responses

Inhibition of Mutation and Combating the Evolution of Antibiotic Resistance

Ryan T. Cirz[1], Jodie K. Chin[1], David R. Andes[2], Valérie de Crécy-Lagard[3], William A. Craig[2], Floyd E. Romesberg[1*]

Acknowledgements

CIRAR

Departments of Pediatrics and Microbiology

NIH
FAMRI