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Concomitant Antibiotic and Mercury Resistance among Gastrointestinal Microflora of Feral Brook Trout, *Salvelinus fontinalis*

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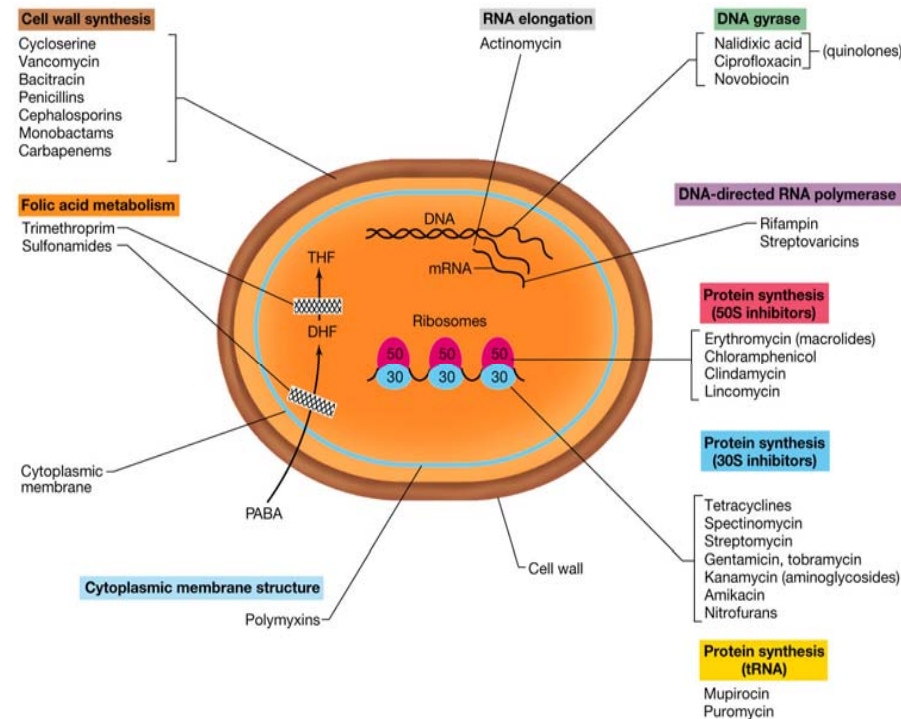
Concomitant Antibiotic and Mercury Resistance among Gastrointestinal Microflora of Feral Brook Trout, *Salvelinus fontinalis*



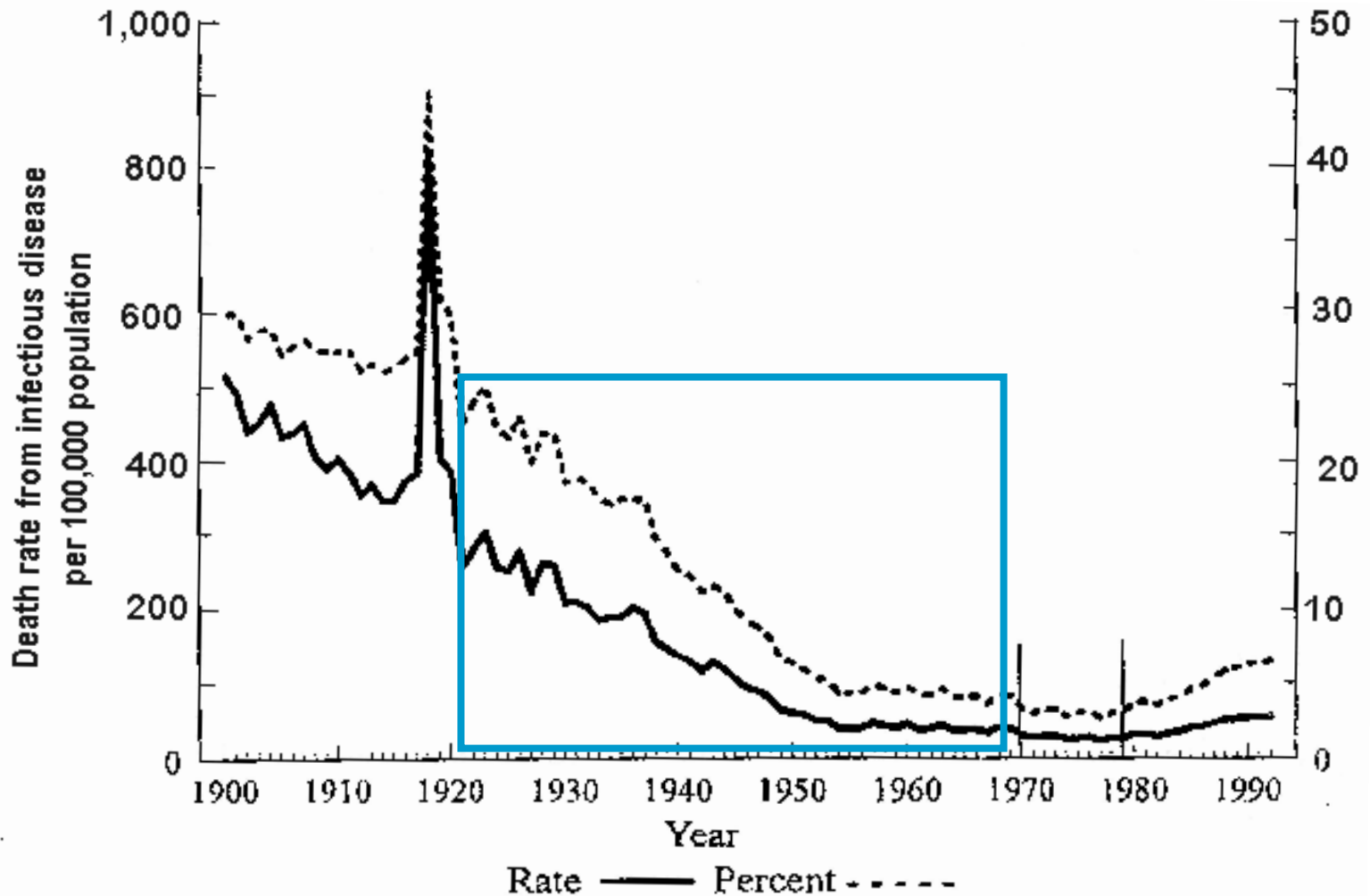
Matt Meredith
Colby College
Undergraduate Research Symposium
May 5th, 2006

Antibiotics (Ab)

- “Low molecular weight compounds produced by microorganisms to inhibit the growth of other microorganisms”
- Selective toxicity
- Confer competitive advantage
- Antimicrobial = synthetic Ab

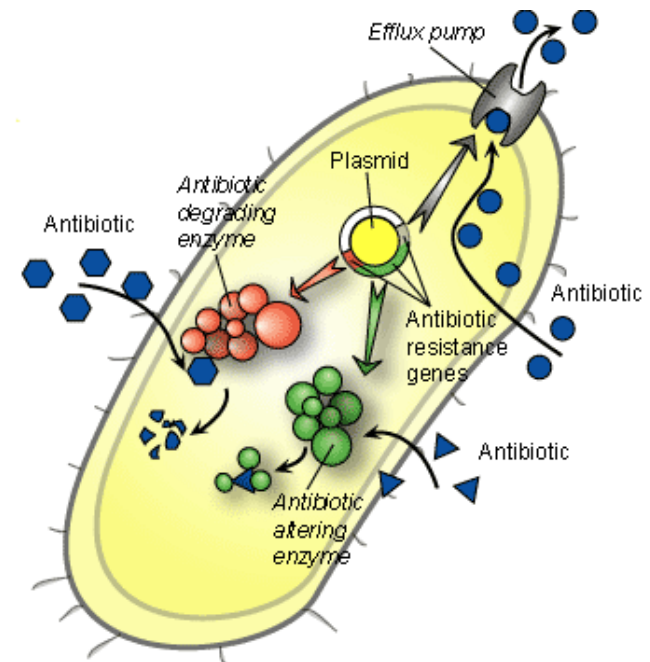


US Infectious Disease Mortality in the 20th Century

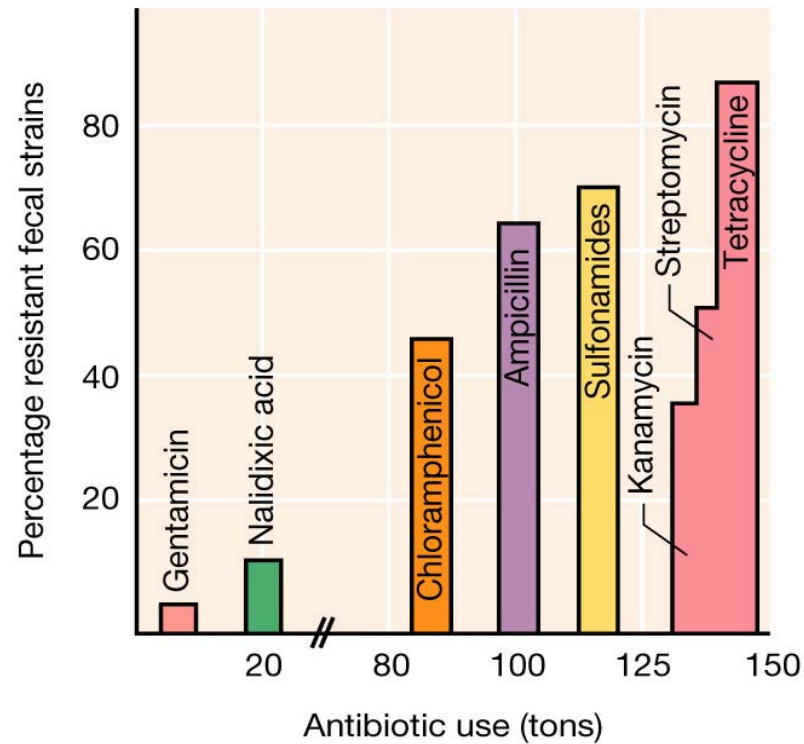


Antibiotic Resistance (Ab^r)

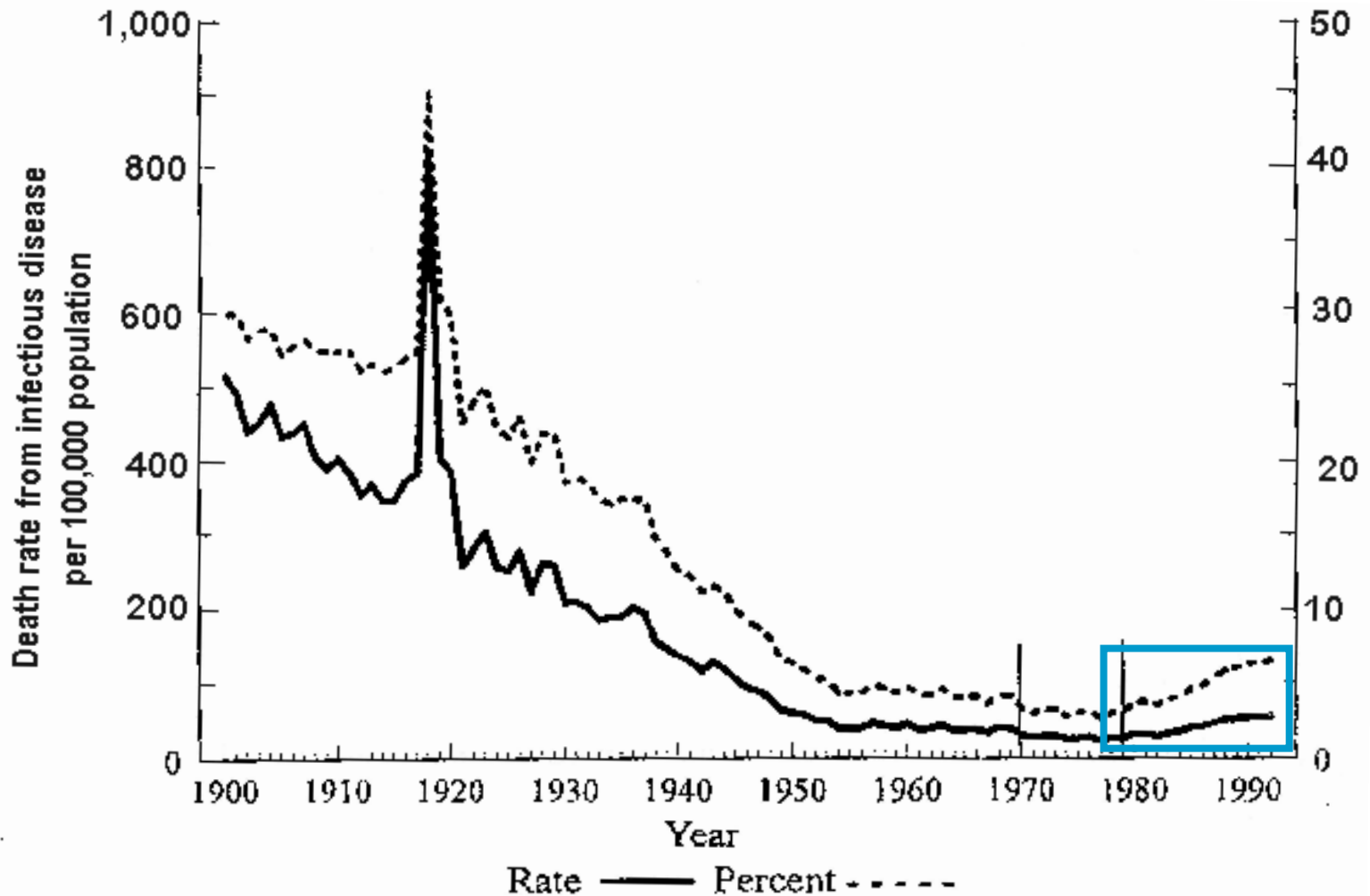
- Ab^r gene origin in Ab -producing organisms to prevent self-destruction
- Selected by excessive and imprudent use of Ab



Ab Selection for Ab^r

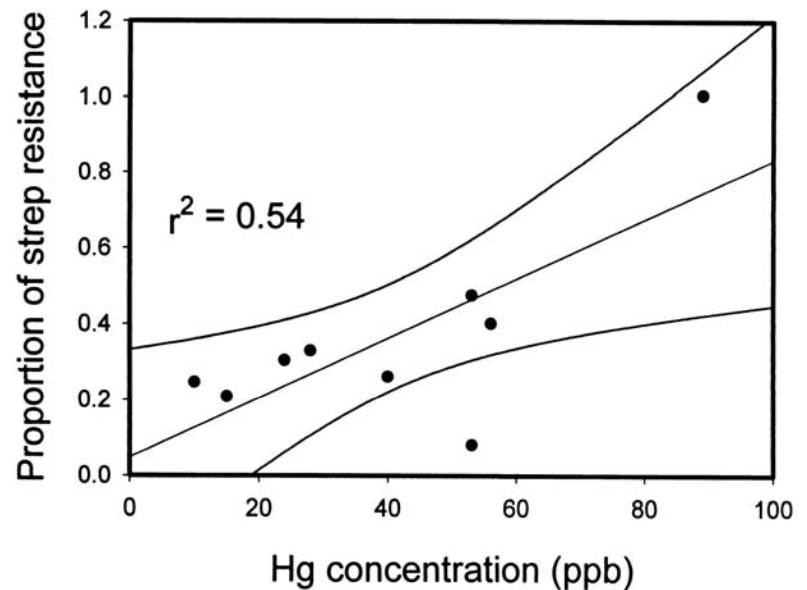


US Infectious Disease Mortality in the 20th Century



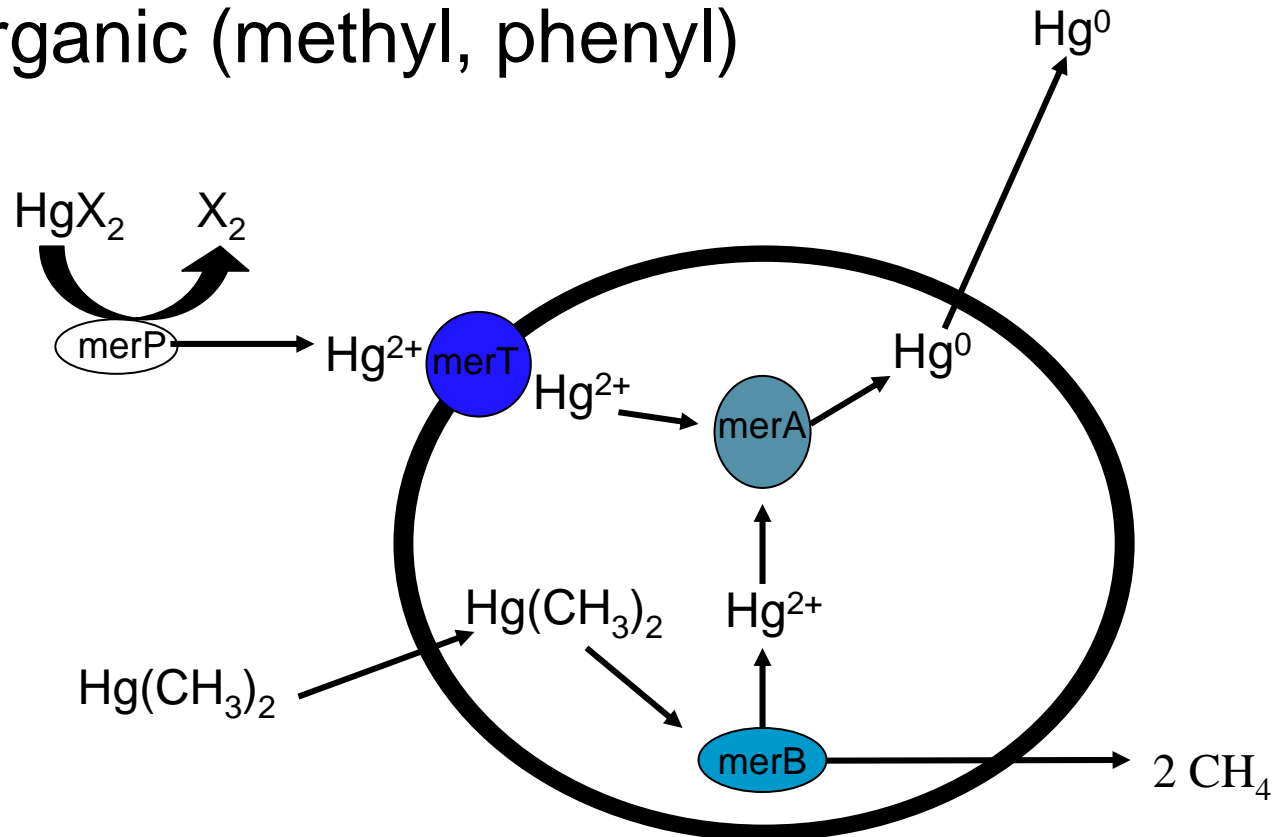
Ab^r and Mercury Resistance (Hg^r)

- Hg as indirect selective agent for Ab^r
- Ab^r and Hg^r proximate on mobile genetic element?

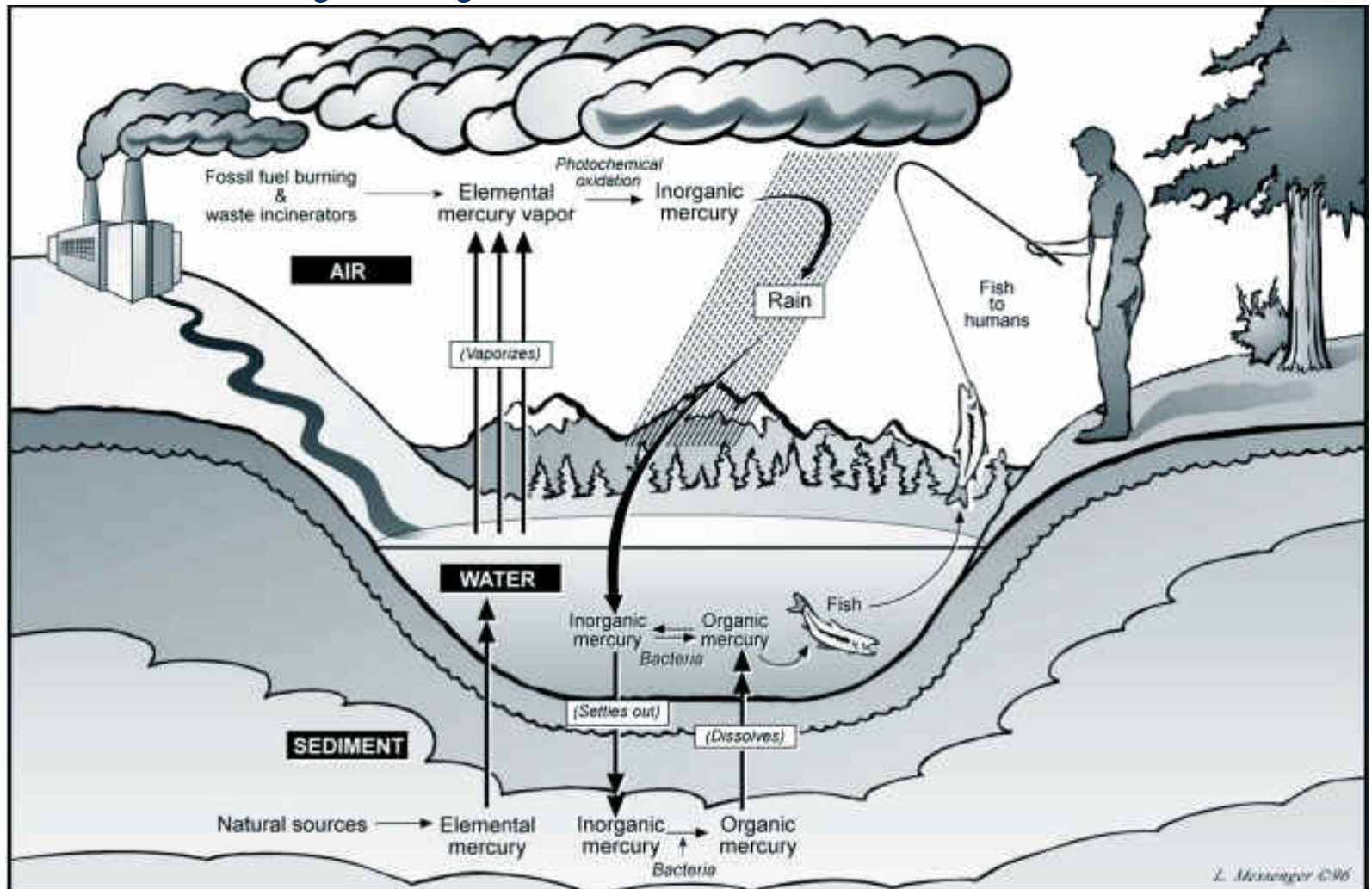


Forms of Hg and Hg^r

- Elemental (Hg⁰)
- Ionic (Hg²⁺)
- Organic (methyl, phenyl)



Mercury Cycle



Mercury in Maine



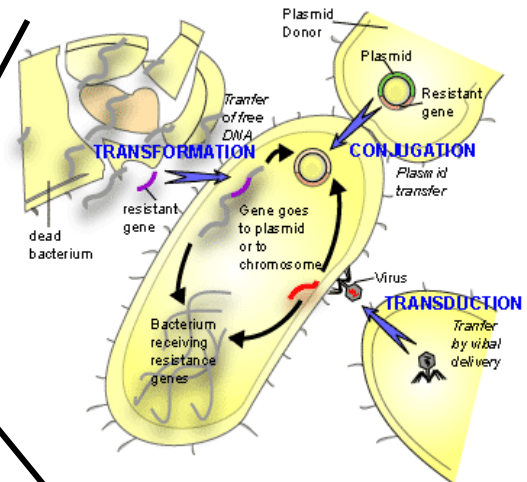
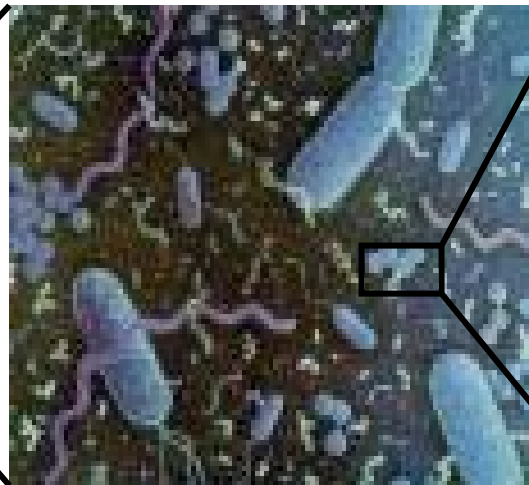
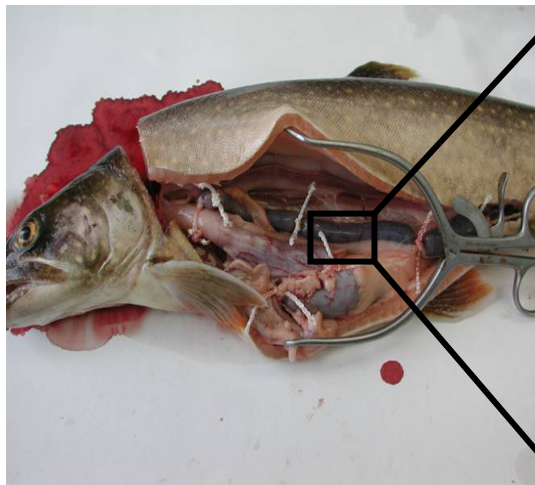
Lake Kennebago, Maine

- 1.5 ng l⁻¹ total Hg
- Pristine
- Oligotrophic
- No Ab-usage



Fish GI tract system

- Commensal populations stable
- Commensal gene reservoir stable
- GI bacterial commensals exposed to Hg
- Simultaneous selection for Hg^r and Ab^r ?
 - Feral brook trout: *Salvelinus fontinalis*





Isolate Isolation

- FBT dissection, GI tract extraction
- GI tract/ingesta dilution in PO_4 buffer
- Serial dilution on TSA plating media containing 0 and 25 μM HgCl_2
- Incubation at 22°C

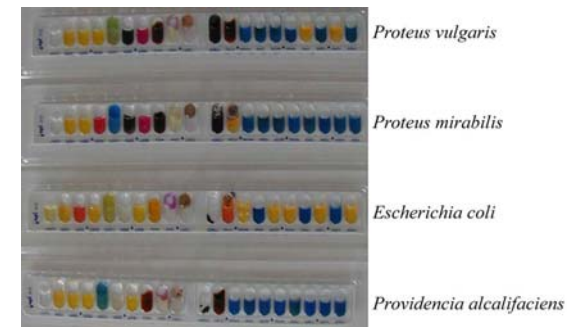
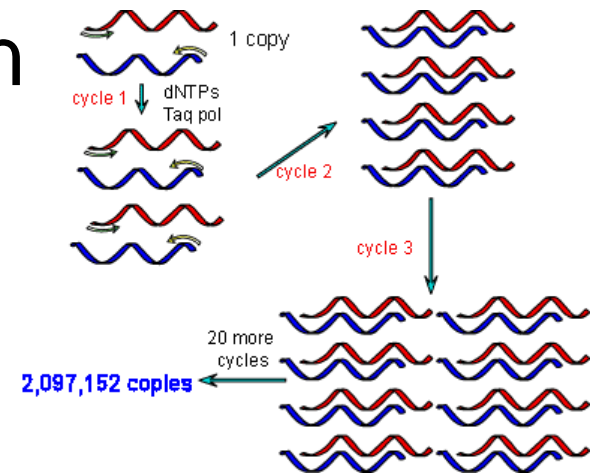


29 Bacterial Isolates

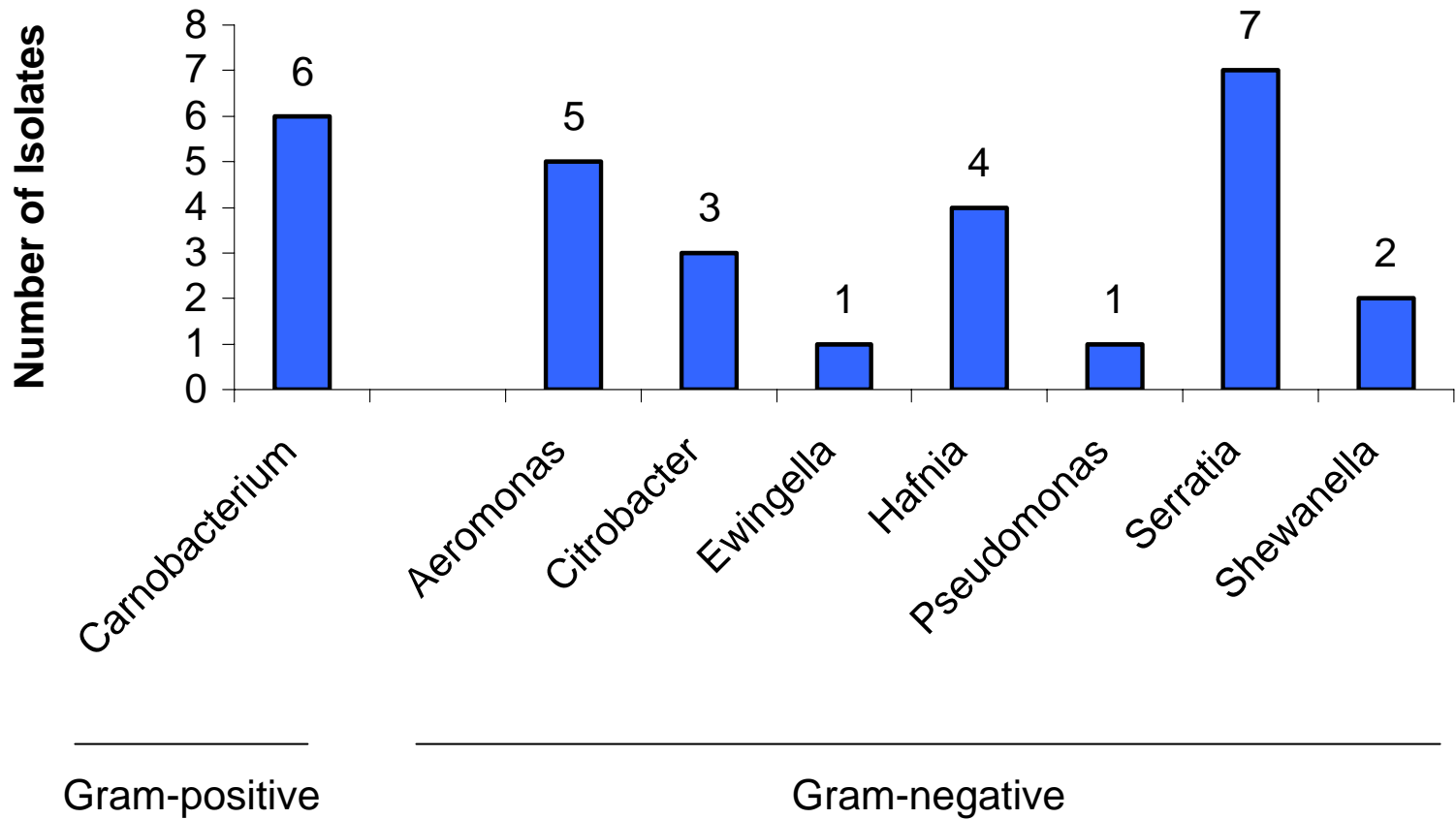
- August 2004: 3 fish – 6 FBT1
- September 2005: 4 fish – 9 FBT3
- October 2005: 2 fish – 14 FBT4

Isolate Identification

- Genomic DNA extraction
- 16S rDNA PCR
- 16S rDNA sequencing
- Blastn database search
- API 20E strip

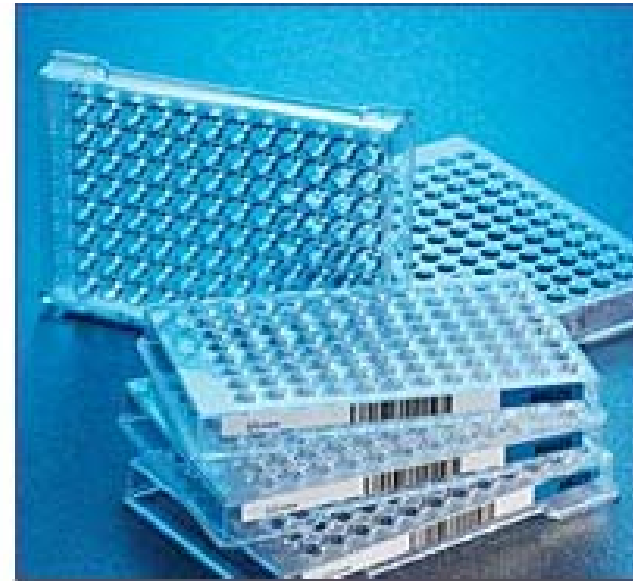


8 Genera



Phenotypic Characterization of Ab^r

- Ab MIC panel:
GPN2F (Gram-positive)
MJ (Gram-negative)



Antibiogram (GPN2F)

Isolate	Genus	AMP 0.12- 16	PEN 0.06- 8	OXA+ 0.25- 8	FAZ 2- 16	AXO 8- 64	GEN 2- 16	CIP 0.5- 2	LEVO 0.25- 8	GAT 1- 8	VAN 1- 32	ERY 0.25- 8	CLI 0.5- 4	SYN 0.12- 4	LZD 0.5- 8
FBT1-19	<i>Carnobacterium</i>	>16	>8	>8	16	>64	>16	2	2		2	0.5	>4	2	2
FBT1-22	<i>Carnobacterium</i>	8	4	>8	8	>64	>16		1			0.5	>4	2	1
FBT3-9	<i>Carnobacterium</i>	0.5	1	>8	16	>64	>16	2	2	2	2		>4	1	
FBT3-14	<i>Carnobacterium</i>	1	2	>8		>64	>16		0.5		2	0.5	>4	1	
FBT4-1	<i>Carnobacterium</i>	>16	>8	>8	4	>64	>16	>2	2			0.5	>4	2	2
FBT4-18	<i>Carnobacterium</i>	4	8	>8	4	>64	>16		2			0.5	>4	2	1

AMP, ampicillin; AXO, ceftriaxone; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FAZ, cefazolin; GAT, gatifloxacin; GEN, gentamicin; LEVO, levofloxacin; LZD, linezolid; OXA+, oxacillin+2%NaCl; PEN, penicillin; SYN, quinupristin/dalfopristin; VAN, vancomycin.

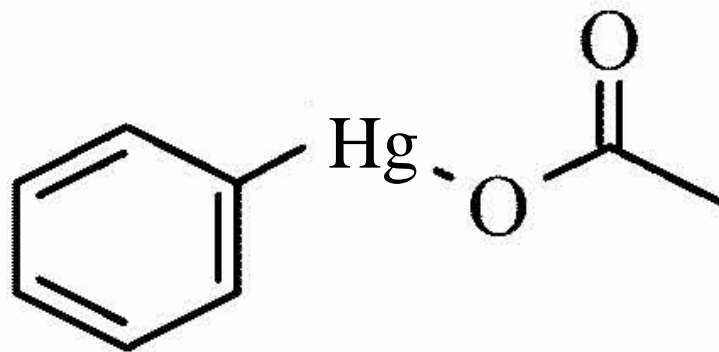
Antibiogram (MJ)

Isolate	Genus	AMP 0.5- 16	A/S 8/4- 16/8	MEZ 4- 64	TIM 4/2- 64/2	AUG 0.5/0.25- 16/8	PIP 4- 64	FAZ 1- 16	CEP 8- 16	FOX 2- 16	TAZ 1- 16	AXO 4- 32	FUR 2- 16	AMI 4- 32	GEN 0.25- 8	TET 0.25- 8	CIP 0.06- 2	OFL 0.25- 4	LOM 0.5- 4	COT 0.5/9.5- 2/38	NIT 32- 64	FIS 0- 256
FBT1-8	<i>Serratia</i>	>16	16/8	16	8/2	16/8	8	>16	>16	>16			>16		1	>8	0.12	0.5	1		>64	
FBT1-15	<i>Ewingella</i>	>16		8		>16/8		>16	>16	8			>16		0.5	2					64	
FBT1-21	<i>Serratia</i>	>16	>16/8	32	8/2	>16/8	16	>16	>16	16		>32	>16		0.5	4					64	
FBT1-23	<i>Serratia</i>	>16	>16/8	16	16/2	>16/8	16	>16	>16	16		>32	>16		0.5	8					64	>256
FBT3-1	<i>Serratia</i>	>16	>16/8	>64	8/2	16/8	32	>16	>16	16		>32	>16		0.5	8					64	
FBT3-2	<i>Aeromonas</i>	>16	>16/8		16/2	16/8		8							1							
FBT3-5	<i>Aeromonas</i>	>16	>16/8	16	>64/2	16/8	8	>16	>16	16				8	2							>256
FBT3-6	<i>Shewanella</i>					2/1		16							1	2						>256
FBT3-8	<i>Serratia</i>	>16	>16/8	>64	8/2	16/8	>64	>16	>16	16		>32	>16		0.5	8					64	
FBT3-16	<i>Serratia</i>	>16	>16/8	64	16/2	>16/8	>64	>16	>16	16		>32	>16		0.5	8					64	
FBT3-17	<i>Hafnia</i>	>16	>16/8	8	16/2	>16/8	8	>16	>16	4	8		16		0.5	>8						>256
FBT4-2	<i>Hafnia</i>	4		32	8/2	4/2	16	16	>16				4		1	>8				>2/38	64	>256
FBT4-3	<i>Aeromonas</i>	>16	>16/8	16	64/2	8/4		>16	>16						2	0.5						>256
FBT4-5	<i>Aeromonas</i>	>16	>16/8		8/2	8/4		8						16	4	0.5						
FBT4-6	<i>Citrobacter</i>	>16	16/8		8/2	>16/8	8	>16	>16	>16			8		1	2		0.5				
FBT4-9	<i>Shewanella</i>	8			8/2	16/8		>16	>16						1	1	0.12					>256
FBT4-10	<i>Citrobacter</i>	4				4/2		2	16	4						2						>256
FBT4-11	<i>Hafnia</i>	>16	>16/8	8	32/2	>16/8	8	>16	>16	8	2		>16		0.5	>8				1/19		>256
FBT4-12	<i>Citrobacter</i>	8				8/4		8	>16	4			4		0.5	4	0.12					>256
FBT4-19	<i>Serratia</i>	>16	>16/8	64	16/2	>16/8	64	>16	>16	16		>32	>16		0.5	4					64	>256
FBT4-21	<i>Aeromonas</i>	>16	>16/8	32	64/2	>16/8	16	>16	>16	8				8	2							>256
FBT4-22	<i>Hafnia</i>	>16	>16/8	8	32/2	>16/8	8	>16	>16	4	8		16		0.5	>8						>256
FBT4-23	<i>Pseudomonas</i>	>16	>16/8	>64	>64/2	>16/8	32	>16	>16	>16	>16	>32	>16		1	8	0.5	2	4	>2/38	>64	>256

AMI, amikacin; AMP, ampicillin; A/S, ampicillin/sulbactam; AUG, amoxicillin/clavulanic acid; AXO, ceftriaxone; CEP, cephalothin; CIP, ciprofloxacin; COT, trimethoprim/sulfamethoxazole; FAZ, cefazolin; FIS, sulfisoxazole; FOX, ceftazidime; FUR, cefuroxime(parenteral); GEN, gentamicin; LOM, lomefloxacin; MEZ, mezlocillin; NIT, nitrofurantoin; OFL, ofloxacin; PIP, piperacillin; TAZ, ceftazidime; TET, tetracycline; TIM, ticarcillin/ clavulanic acid.

Phenotypic Characterization of Hg^r

- TSA plating media
- HgCl₂ (0-1000 μM) and phenylmercuric acetate (PMA) (0-16 μM)
- Inoculated with droplet of bacterial suspension
- Incubation at 22°C





Hg MICs

- HgCl_2 : 100 μM (70%)
250 μM (30%)
- PMA : 8 μM (10%)
16 μM (20%)
>16 μM (70%)



Isolate Exposure to Hg

- Lake Kennebago water : 1.5 ng l⁻¹ total Hg
- *S. fontinalis* muscle tissue : 395 ng g⁻¹
- *S. fontinalis* diet (e.g. crawfish)



Hg-susceptible isolates?

- Population counts on 0 and 25 μM HgCl_2
- TSA plating media inoculated with GI/ingesta- PO_4 buffer suspension
- Triplicate
- Incubation at 22°C

- No significant difference between culturable bacterial growth on 0 and 25 μM HgCl_2 by paired Student's t test ($P=0.088$, $\text{df}=5$)



Genotypic Characterization of Hg^r

- *merA* PCR amplification
- Two primer sets: long and short
- Long set: Amplification in 12 isolates
- Short set: Amplification and
sequencing in 5 isolates

merA Amplification

Gram Negative Isolates

Isolate	Genus
FBT1-8	<i>Serratia</i>
FBT1-15	<i>Ewingella</i>
FBT1-21	<i>Serratia</i>
FBT1-23	<i>Serratia</i>
FBT3-1	<i>Serratia</i>
FBT3-2	<i>Aeromonas</i>
FBT3-5	<i>Aeromonas</i>
FBT3-6	<i>Shewanella</i>
FBT3-8	<i>Serratia</i>
FBT3-16	<i>Serratia</i>
FBT3-17	<i>Hafnia</i>
FBT4-2	<i>Hafnia</i>
FBT4-3	<i>Aeromonas</i>
FBT4-5	<i>Aeromonas</i>
FBT4-6	<i>Citrobacter</i>
FBT4-9	<i>Shewanella</i>
FBT4-10	<i>Citrobacter</i>
FBT4-11	<i>Hafnia</i>
FBT4-12	<i>Citrobacter</i>
FBT4-19	<i>Serratia</i>
FBT4-21	<i>Aeromonas</i>
FBT4-22	<i>Hafnia</i>
FBT4-23	<i>Pseudomonas</i>

Gram Positive Isolates

Isolate	Genus
FBT1-19	<i>Carnobacterium</i>
FBT1-22	<i>Carnobacterium</i>
FBT3-9	<i>Carnobacterium</i>
FBT3-14	<i>Carnobacterium</i>
FBT4-1	<i>Carnobacterium</i>
FBT4-18	<i>Carnobacterium</i>

Sequence Similarity

FBT1-21	86%
FBT1-23	85%
FBT3-1	84%
FBT3-8	91%
FBT3-16	89%



Conclusions

- Presence of Ab^r in absence of Ab-use
- Presence of Hg^r due to Hg exposure
- Established relationship between Hg^r and Ab^r
- Hg likely indirect selective agent for Ab^r determinants



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- Frank Fekete (Colby College)
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- Tamar Barkay (Rutgers University)



Questions?



Thank you.

Ab^r Prevalence

- Methicillin-resistant *Staphylococcus aureus* strains endemic in hospitals and are increasing in other settings such as locker rooms.
- Vancomycin-resistance *S. aureus* and enterococci emerged in 2002
- Incidence of sepsis has nearly tripled between 1979 to 2000.

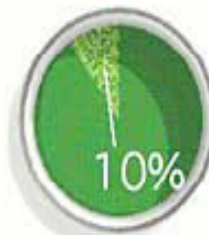
STAPHYLOCOCCUS AUREUS
VS. PENICILLIN



ENTEROCOCCUS FAECIUM
VS. CIPROFLOXACIN (CIPRO)



STREPTOCOCCUS PNEUMONIAE
VS. TETRACYCLINE



STAPHYLOCOCCUS AUREUS
VS. METHICILLIN



ENTEROCOCCUS FAECIUM
VS. AMPICILLIN



STREPTOCOCCUS PNEUMONIAE
VS. PENICILLIN

