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Multi-Drug Resistant *Salmonella*

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Salmonella and Public Health Concerns

Leading cause of death from food-borne illness

In 2002 *Salmonella* Newport and *Salmonella* Typhimurium were 2 of the top 3 Human Isolates

- Both Associated with MDR
- 27% of Typhimurium were resistant to >5 antimicrobial drugs
- 22% of Newport were resistant to >8 antimicrobial drugs

Antimicrobial Drug Resistance

Emerged as the *highest priority* issue for public health officials in developed countries

Antimicrobial drug resistance increases:

- Cost of health care
- Morbidity (severity and length), mortality

Resistance is not new, but have seen rapid emergence of organisms of consequence in health care

- Resistant variants of *Salmonella*, *Campylobacter have been primary food-borne pathogens of concern*

Multi-drug resistant *Salmonella*

Salmonella Typhimurium DT104

- Resistance genes on the chromosome
- ACSSuT resistance pattern

Salmonella Newport (esp. MDR-AmpC)

- Resistance plasmid/chromosomally mediated
- ACSSuTAxKGCfCro R-Type not uncommon
- Outbreaks assoc. with beef
- *Salmonella* Newport increasingly commonly recovered from human salmonellosis

Fluoroquinolone-resist *Salmonella*

- *Fluoroquniolones primary for salmonellosis in adults*

Ceftriaxone-resist *Salmonella*

- *Ceftriaxone primary for salmonellosis in children*

Antibiotic Resistance Mechanisms

- Alteration of the drug target
- By-pass the drug target
- Inactivation of the drug
- Reduced permeability
- Increased efflux
- Increased stress regulon response

Emergence, Dissemination, Maintenance of Antimicrobial Drug Resistance

Common-place view is that:

- Genetic mutation
- Antibiotic use

While this does happen to some degree, probably represents minority of cases

Emergence, Dissemination, Maintenance of Antimicrobial Drug Resistance

In majority of instances, resistant bacteria present at very low levels in humans, animals and the environment

- Some factor allows expansion of the resistant population
- Antimicrobial use is just one factor

In general, resistance can be described as:

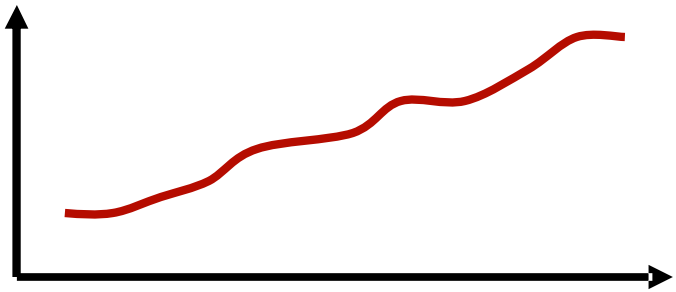
- Intrinsic (natural attribute)
- Acquired mutation or acquisition of DNA.

Acquired resistance

Genetic mutation

Probably a minor contributor to overall resistance

- E.g., fluoroquinolone resistance following point mutation(s) to the *gyr*A
  - *Transfer between bacteria rare but has been demonstrated* qnr *and proposed to become more common*
- Stepwise, gradual decrease in susceptibility



Acquired resistance

Acquisition of exogenous genetic material

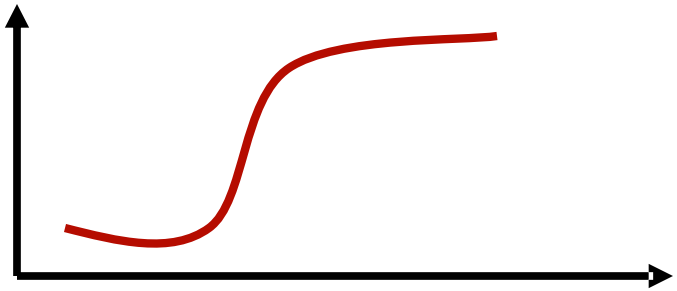
- DNA exchange

Likely much more common than mutations

Can occur within species & between species

Mobile genetic elements

Most important mechanism for resistance dissemination



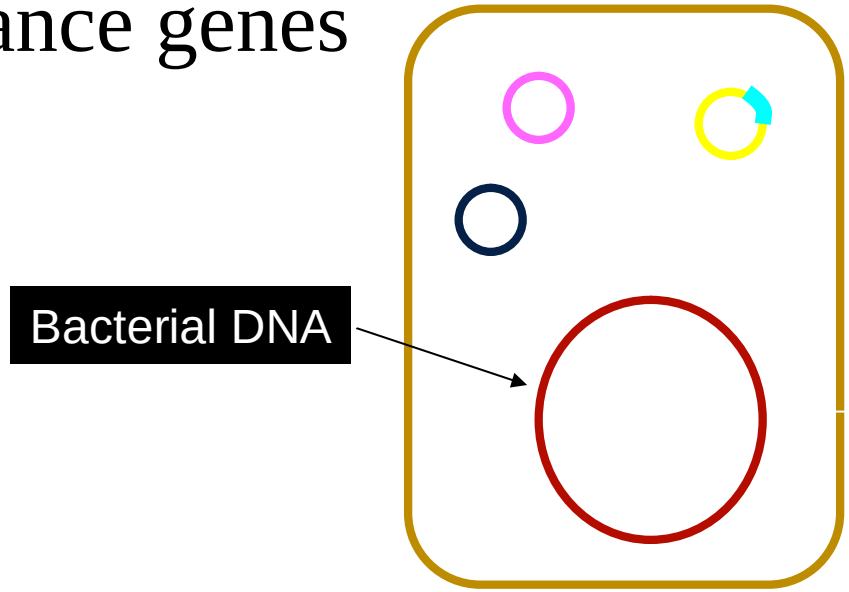
Plasmids

Self replicating, extra chromosomal DNA

- Semi-autonomous

Commonly carry ‘adaptation’ genes

Frequently harbor resistance genes



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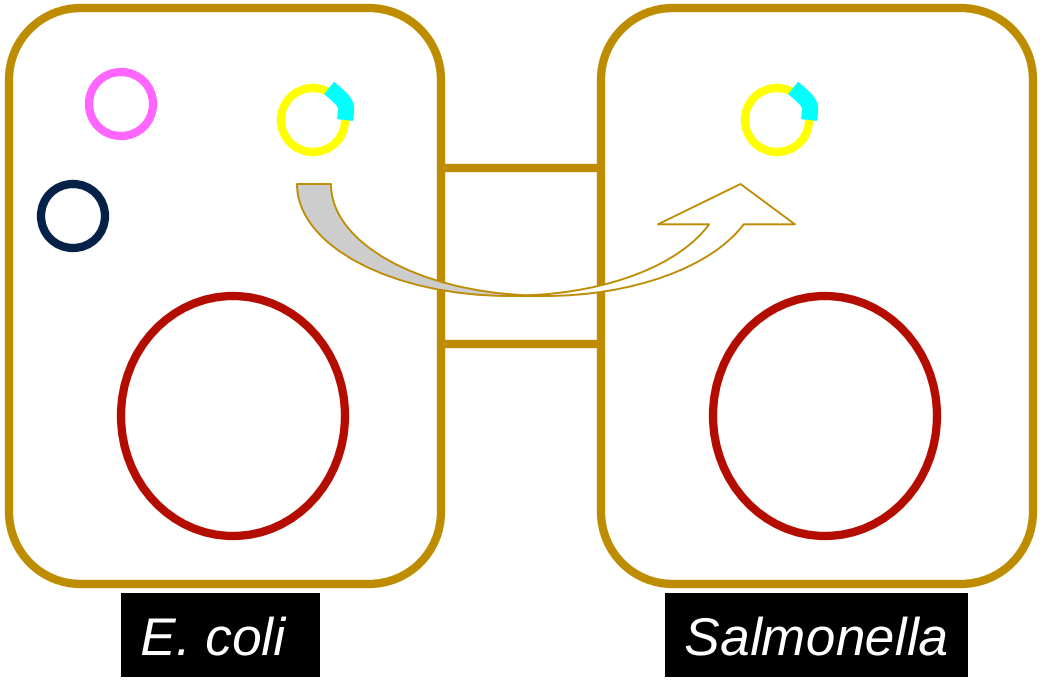


Plasmid-induced conjugation.

Active Transfer of DNA

Well-described for plasmids

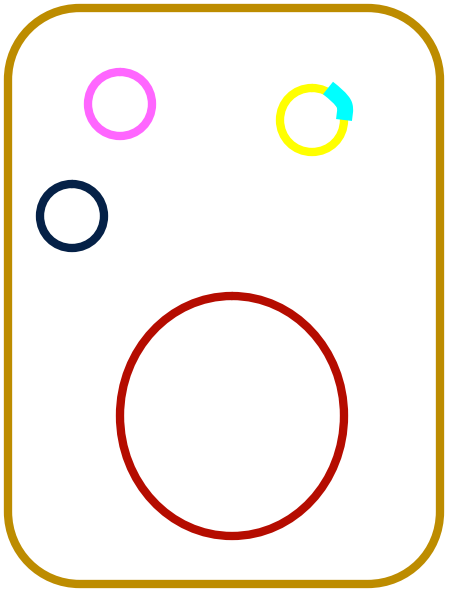
- Transposons (another mobile genetic element) can also undergo a kind of conjugation

MDR *Salmonella* Newport frequently reported to carry resistance genes on a plasmid

- $bla_{\text{CMY-2}}$ gene found on a number of plasmids

Transferable to *E. coli*

Some resistance genes are located on bacterial chromosomes while some on plasmids



Acquisition of MDR - Integrons

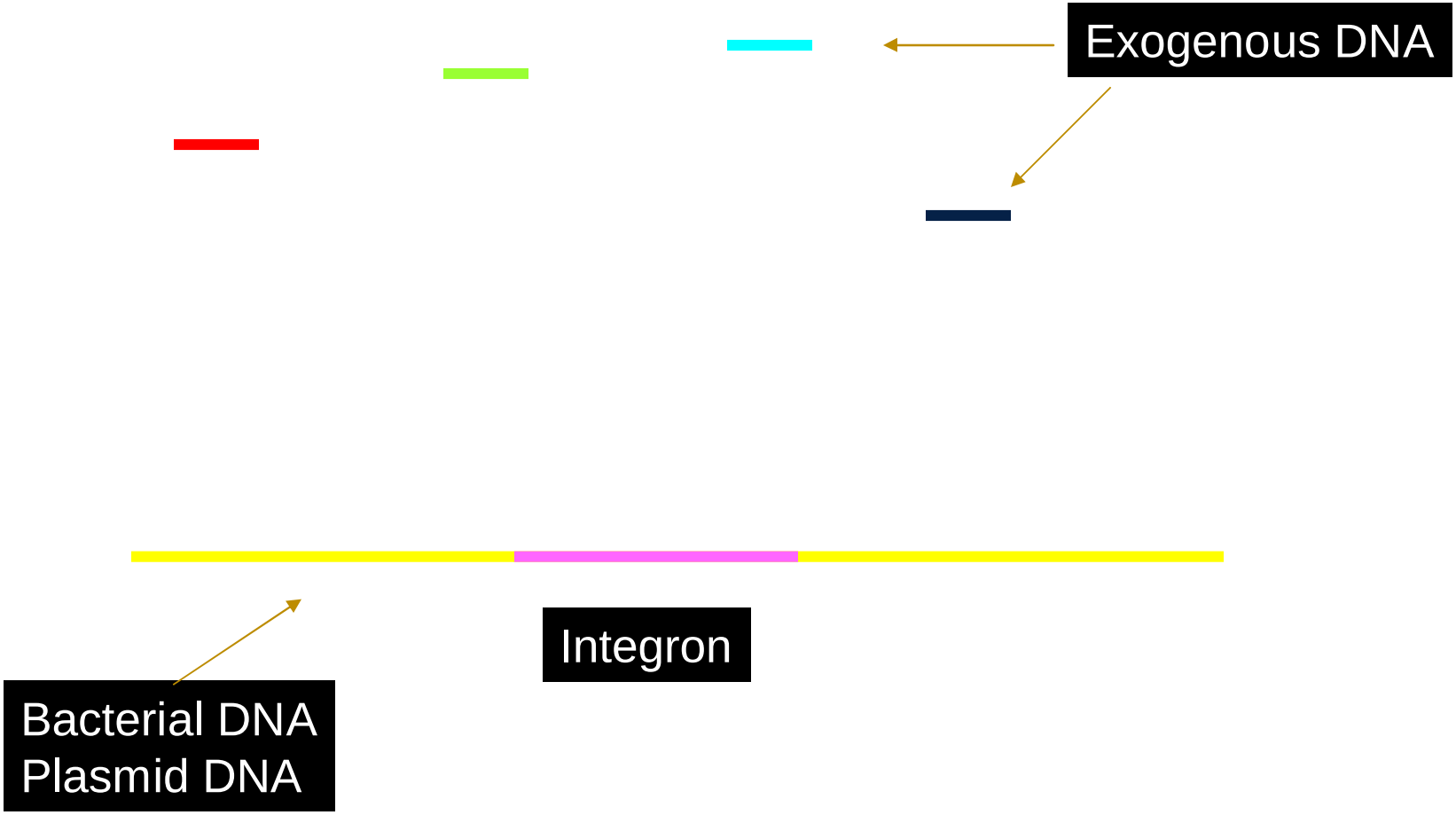
Act as scavengers of foreign DNA

- Allow rapid adaptation to hostile environments
- Have genetic machinery to insert and express genes

Can be located on plasmids, in transposons, or in chromosomal material

Frequently associated with development of multidrug resistance

Integrons



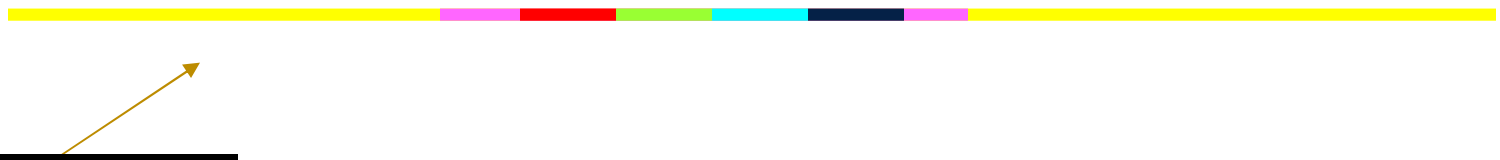
Integrons

Genes *integrated* into DNA

May store gene cassette for future needs

Assembled cassettes can be shared between bacteria

- Conjugation i.e., plasmids, transposons
- Transduction through bacteriophages



Bacterial DNA
Plasmid DNA

Example of Integrons

Salmonella Typhimurium DT104

Harbors its resistance genes on 2 main integrons

- *Salmonella* genomic island 1 (SGI1)
- Acquired through bacteriophage transduction.

Integrons incorporated into chromosomal DNA

SGI1 is also found in other MDR *Salmonella* Newport

Genetic propagation

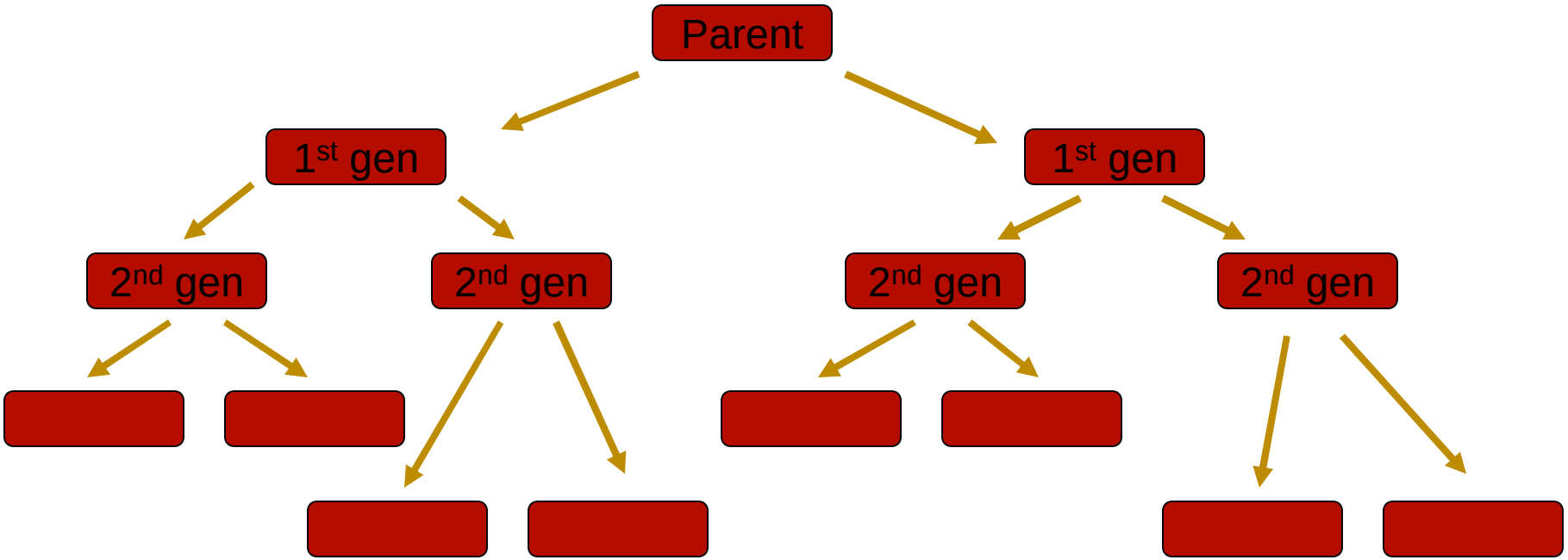
Clonal expansion

Horizontal transfer

- Expansion of genes in unrelated bacteria secondary to genetic sharing

Clonal dissemination

Selection pressure applied to diverse population of bacteria



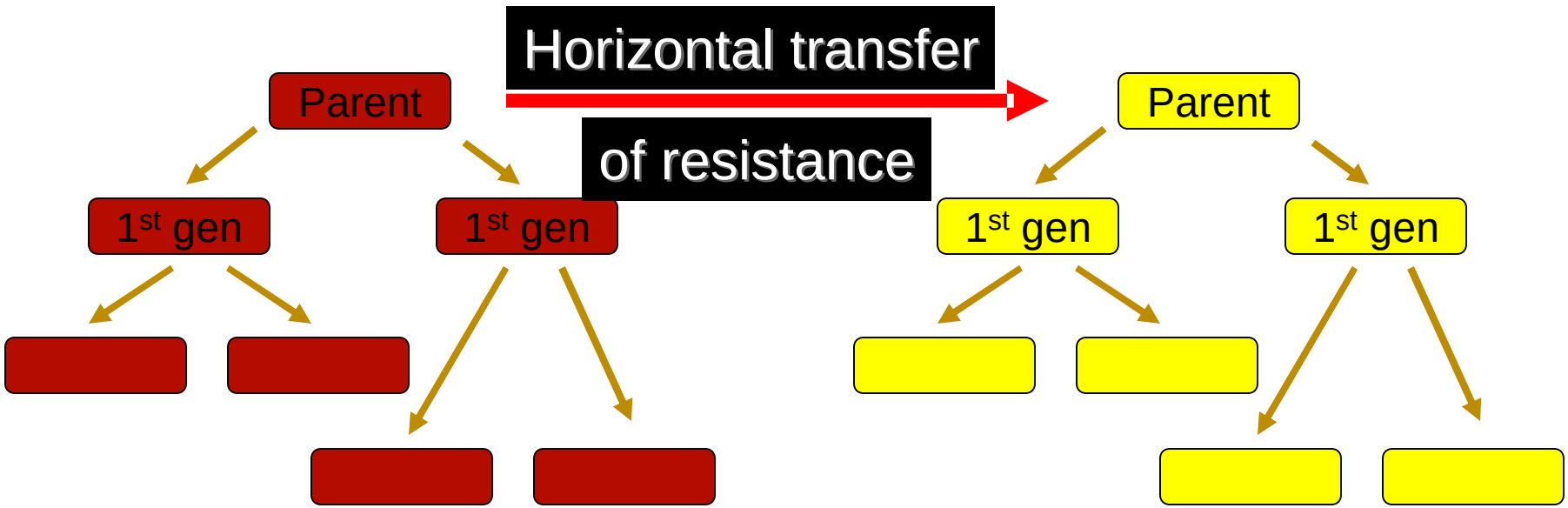
Mobile DNA dissemination

Selection pressure applied to diverse population of bacteria

Parent → Horizontal transfer of resistance → Parent

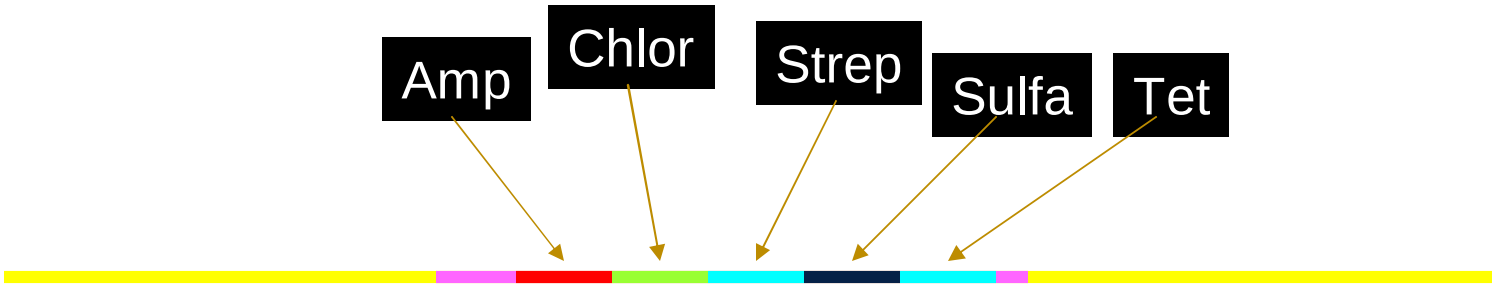
Mobile DNA dissemination

Selection pressure applied to diverse population of bacteria



Importance of MDR

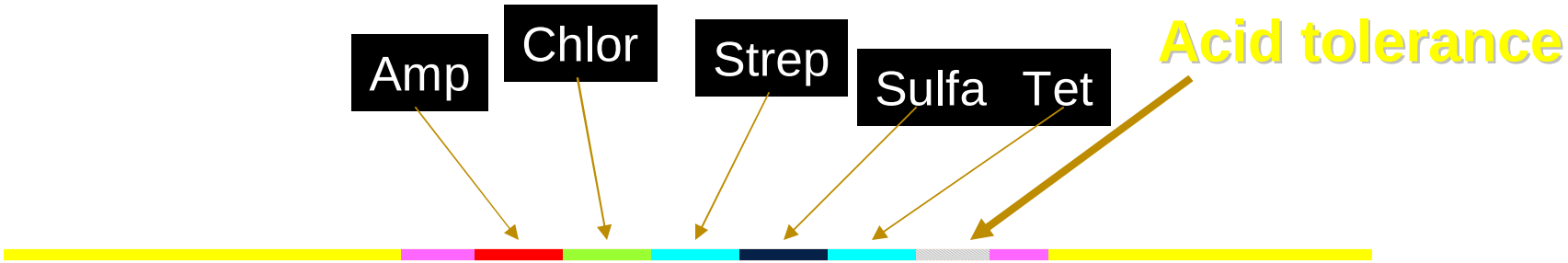
Genes can be genetically linked



If use ampicillin, then select for ampicillin, streptomycin, sulfamethoxazole, and tetracycline resistance.

Potential Complexities

Genes genetically linked to other adaptive characteristics e.g., pathogenicity, acid survival, heavy metal/disinfectant resist.



If exposed to high acid, then favors survival of those with ACSSuT R types.

Public Health Perceptions

Salmonella serotype Newport MDR-AmpC was commonly identified… [in] humans and food animals. These findings support the possibility of transmission of this organism to humans through the food chain (Zhao et al *JCM* 2003)

Antibiotic-resistant strains of salmonella in the United States evolve primarily in livestock (Fey et al., *NEJM* 2000)

Antimicrobial resistance in Salmonella and other foodborne bacteria is largely a consequence of the use of antimicrobial agents in food-producing animals (CDC, RFP 2003)

Public Health Perceptions

Continued efforts to reduce unnecessary use of antimicrobial agents in food animals… [is] critical to prevent further development of resistance to quinolones and cephalosporins (Devasia et al. *MDR* 2005)

Broad-sweeping statements paint clear picture but not necessarily true

- E.g., *Salmonella* Typhimurium DT104

Emergence, Disappearance of DT104

DT104 was subsequently replaced by other strain types (sometimes less resistant)

No associations were made between DT104 outbreaks and antimicrobial drug use

- No antimicrobial resistance advantage over previous bovine Typhimurium clones
- Susceptible strains have emerged similarly, e.g., Typhimurium PT10 (Khakhria, *Can J Micro*, 1983)

DT104 disseminated very effectively in Norway, despite very strict controls on on-farm antimicrobial use. (*Epi Inf* 2000)

Antimicrobial Drug Use in Cattle

Subtherapeutic doses

- Prevent Liver abscesses and Improve Feed Efficiency

Therapeutic Doses

- Arrival – Prevent Infectious Diseases
- Treatment of Infectious Diseases

THEORY – Could select for Resistant bacteria that could be transferred to Humans

Little Understanding of the Link between Human MDR *Salmonella* and Animal Isolates

Sub therapeutic Drugs Given to Cattle

Monensin

- Ionophore
- 96.5% Cattle
- Improve Feed Efficiency

Tylosin

- Macrolide
- 42.3% Cattle
- Prevent Liver Abscesses

Others

- Chlortetracycline, oxytetracycline and chl and sulfamethazine
- Virginiamycin also approved – Not commonly used

Therapeutic Drugs given to Cattle

Injectable

- Tilmicosin (macrolide)
- Prevent BRD or bacterial diseases – metaphylaxis - 10.4% upon arrival

Treatment – 14.4% treated for BRD

- Tilmicosin, florfenicol, enrofloxacin, oxytetracycline and ceftiofur

On-going Research

Packing plants (hide samples)

- 2 fed beef plants
- 2 cull plants
  - *Cull dairy cows*
  - *Cull beef cows*

Feedlots (6 feedlots; fecal samples)

- Healthy harvest-ready cattle
- Cull animals (realizers)

Samples collected during each of 4 seasons

- 2 seasons completed to date

On-going Research

Outcomes:

- *Salmonella* detection and quantification
- Tested against 16 USDA:NARMS defined ABX
  - *Provides us ABX concentration required to inhibit bacteria. Then categorized as susceptible or not.*
- Serotyping performed by USDA:NVSL

Created a bank of *Salmonella* isolates for further characterization

Preliminary Results

Source	Type	Prevalence (%)
	Fall 2005	
Packing plant	Fed Beef	63.3
Packing plant	Cull dairy cows	75.6
Packing plant	Cull beef cows	71.1
	Winter 2006	
Packing plant	Fed Beef	92.2
Packing plant	Cull dairy cows	66.7
Packing plant	Cull beef cows	50.0

Preliminary Results

Source	Type	Prevalence (%)
	Fall 2005	
Feedlot	Healthy harvest-ready	63.3
Feedlot	Cull animals (realizers)	23.3
	Winter 2006	
Feedlot	Healthy harvest-ready	20.0
Feedlot	Cull animals (realizers)	4.4

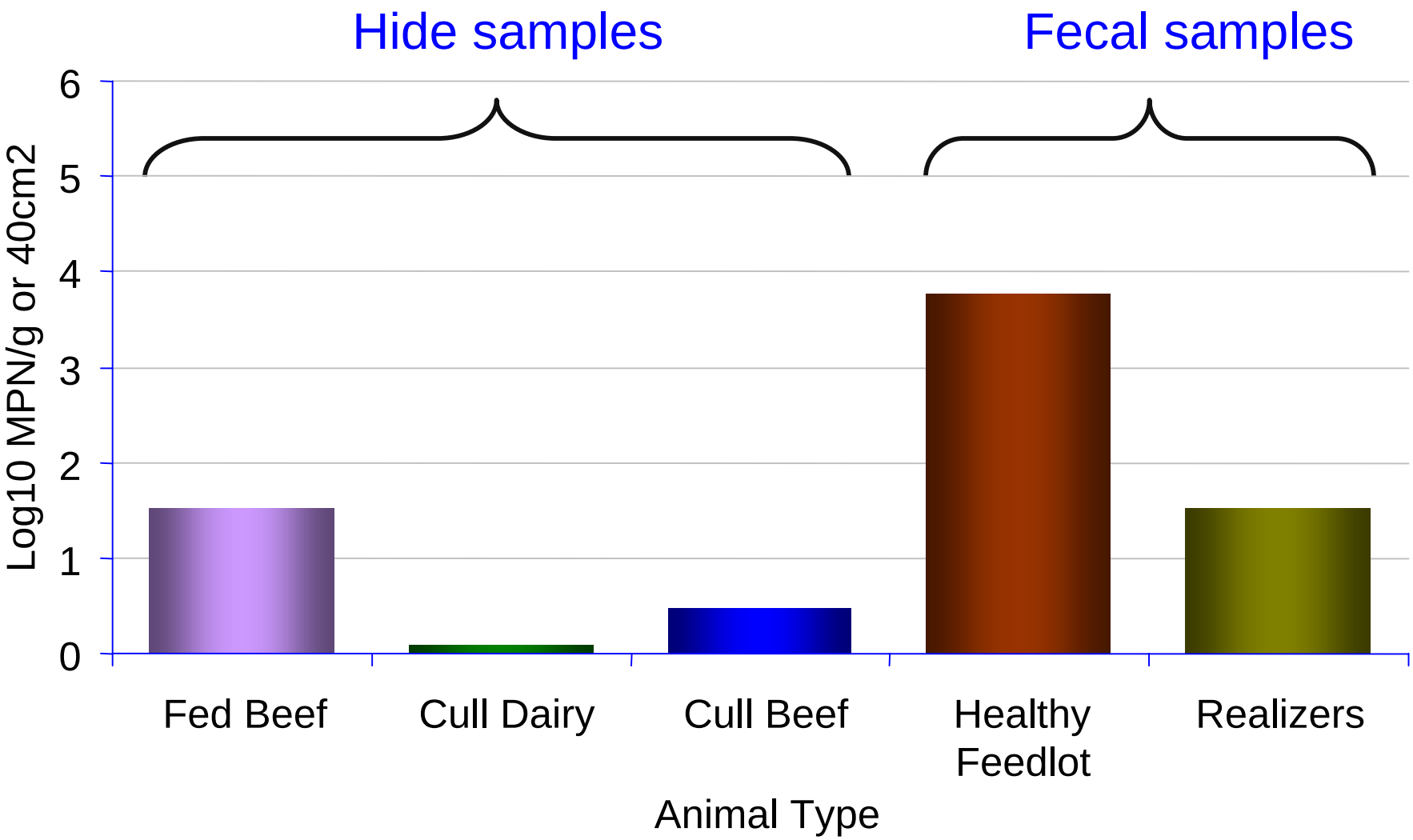
MDR *Salmonella*

Source	Type	Prevalence	# drugs
Fall 2005			
Packing plant	Fed Beef	3.3	2.3
Packing plant	Cull dairy	2.2	3.5
Packing plant	Cull beef	3.3	3.0
Winter 2006			
Packing plant	Fed Beef	8.1	2.2
Packing plant	Cull dairy	10.0	3.7
Packing plant	Cull beef	36.6	2.5

Quantitification Data

Source	Type	Log MPN/g or $40cm^2$
Packing plant	Fed Beef	1.52 (±0.2)
Packing plant	Cull dairy cows	0.09 (±0.1)
Packing plant	Cull beef cows	0.47 (±0.1)
Feedlot	Healthy cattle	3.77 (±0.8)
Feedlot	Cull cattle	1.52 (±1.0)

Quantitification Data



Salmonella Serotypes Recovered

Rank	Fall 2005 (94%)	Human 2004 (60%)
1	Montevideo (25.5)	Typhimurium (19.2)
2	Mbandaka (19.7)	Enteritidis (14.1)
3	Cerro (15.9)	Newport (9.3)
4	Anatum (15.9)	Javiana (5.0)
5	Kentucky (5.1)	Heidelberg (4.9)
6	Muenster (4.5)	Montevideo (2.4)
7	Fresno (3.8)	I 4,[5],12:i:- (2.1)
8	Havana (3.2)	Muenchen (2.1)
	No Typhimurium, Newport	From CDC

Preliminary Findings

Salmonella widely prevalent in cattle populations of the Texas High-Plains

- Frequently recovered from healthy cattle

MDR (particularly high-level) is rare in all groups.

- 68% suscept to all; 1.7% resist 4 or more
- Most common in cull animals
  - *Preliminary identification of a population at risk…*

Serotypes recovered unlike those associated with human disease

Bottom line

Acquisition, emergence, maintenance of antibiotic drug-resistance is very complex

- It is not fully understood

We do know:

- Resistance is in bacteria of livestock is uncommon
- ABX usage selects for resistance
- May be maintained without continued use of the antimicrobial drug or may disappear

Antimicrobial drug resistance is a complex and controversial issue

- Many unknowns
- Rationalization that on-farm antimicrobial drug use is the cause of epidemic dissemination of MDR *Salmonella* is not supported by data

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