Antibiotic Resistance

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The 61st Reciprocal Meat Conference

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FAILURE

When Your Best Just Isn't Good Enough.

Acknowledgments

- David G. White, PhD
  - Director National Antibiotic Resistance Monitoring System (NARMS) FDA, CVM
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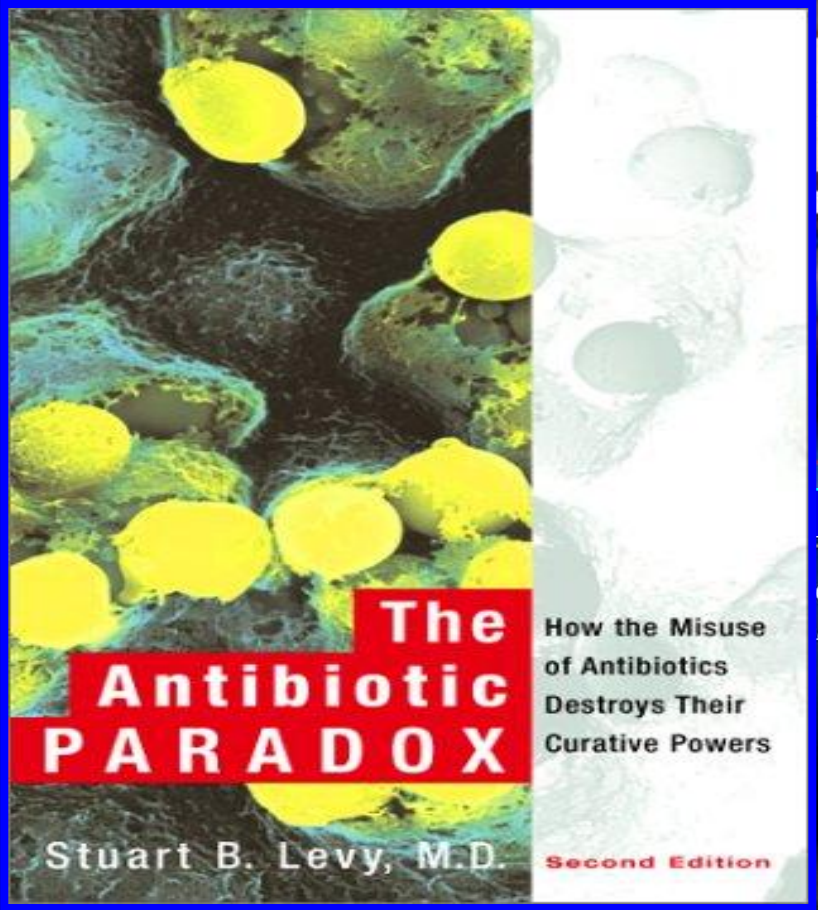
  - Research Leader, Bacterial Epidemiology and Antimicrobial Resistance (BEAR) Research Unit, USDA, ARS
- H. Morgan Scott, DVM, PhD
  - Associate Professor, Epidemiology, TAMU


Thanks to PENICILLIN
...He Will Come Home!


WHITEWATER: ANGUISH INSIDE THE WHITE HOUSE
Newsweek


BUY BONDS


The Antibiotic PARADOX
How the Misuse of Antibiotics Destroys Their Curative Powers
Stuart B. Levy, M.D.
Second Edition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WARNING
NO LONGER EFFECTIVE AGAINST KILLER BUGS


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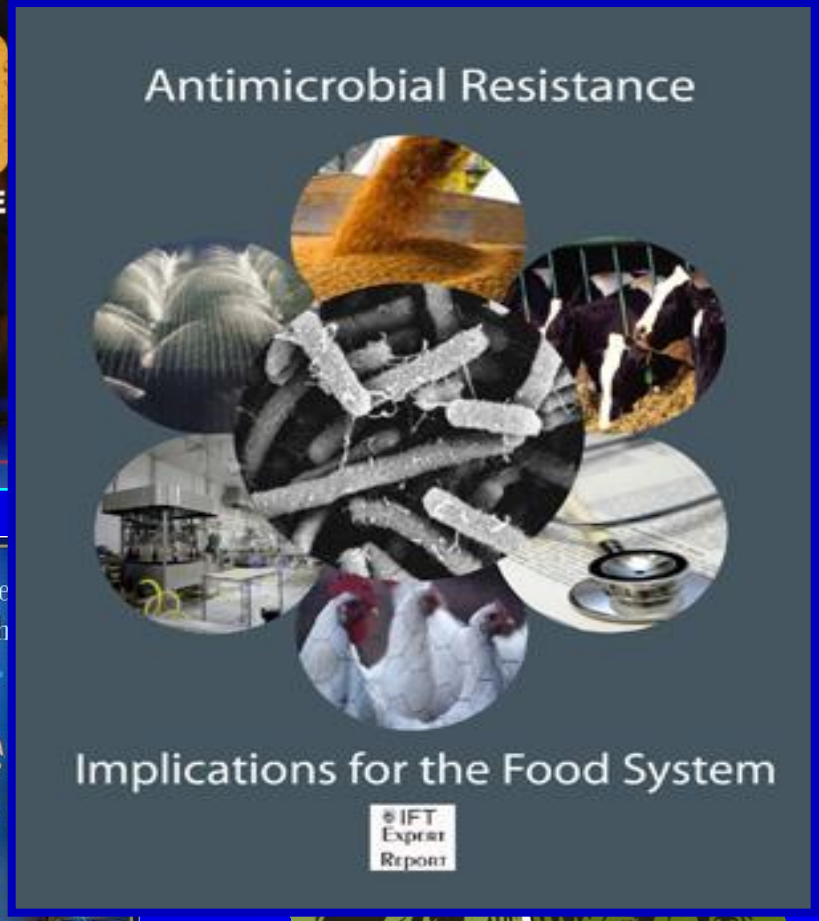
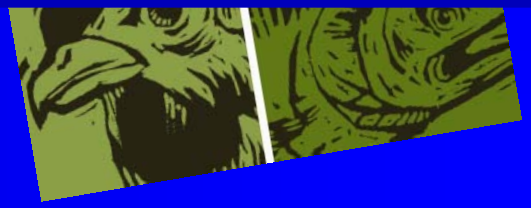
Antibiotic Th
CMR


Antimicrobial Resistance
Implications for the Food System
IFT Expert Report

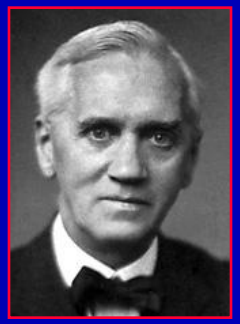

war against infectious diseases?



Words of Caution

The Nobel Prize in Physiology or Medicine 1945

"for the discovery of penicillin and its curative effect in various infectious diseases"



Sir Alexander Fleming

Sir Howard Florey

Ernst Chain

The sample of Penicillium can now be found in the Science Museum, London

Sir Alexander Fleming
Nobel Lecture,
December 11, 1945
Penicillin

"It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body."

There are > 100 antibacterial agents currently approved for use in clinical medicine

However, resistance has followed each new antibiotic, albeit with varying time and frequency

The Biology of Antimicrobial Resistance

- Resistance precedes the use, and in some instances even the discovery, of antimicrobial agents
- Historically, resistance emerged incrementally from low- to medium- to high-levels. Less true today, with MDR mobile DNA elements
- Resistance typically develops from single to multiple phenotypes
- Return to a susceptible microbial ecosystem following removal of drug is unpredictable

Exceptions to the "Rule"

PENICILLIN USE
[Nearly 60 Years]

YEAR 2002

S. aureus
(n=144,531; 92% Resistant)
S. pneumoniae
(n=8,632; 18% Resistant)

YEAR 2002

S. pyogenes
(n=868; 0% Resistant)
E. faecalis
(n=22,202; 1.7% Resistant)

Thornsberry, C. 2004. ASM.

Why are bacteria resistant to antimicrobials that perhaps have not been used?

- Intrinsic resistance
- Linkage of antibiotic resistance genes
- Selection of resistance determinant with broad specificity
- Other selection pressures present
  - e.g. disinfectants, heavy metals

Are these chemicals perturbing the cell membrane?

- Chlorine – up to 50 ppm
- Cecure – cetylpyridium chloride (CPC)
- Inspexx – peroxyacetic acid
- $Safe_2O$ – acidified calcium sulfate
- Sanova – acidified sodium chlorite
- TomCO – CO_2 / chlorine system
- TSP – trisodium phosphate

Selection pressure: conflicting theories

Charles Darwin

"Resistance is Inevitable"
19th Century

The Borg

"Resistance is Futile"
24th Century

The Origin of Species by Means of Natural Selection, 1859

Charles Darwin

His theory of evolutionary selection holds, simply, that variation within species occurs randomly and that the survival or extinction of each organism is determined by that organism's ability to adapt to its environment

"Survival of the Fittest"

Drug Resistance Equation

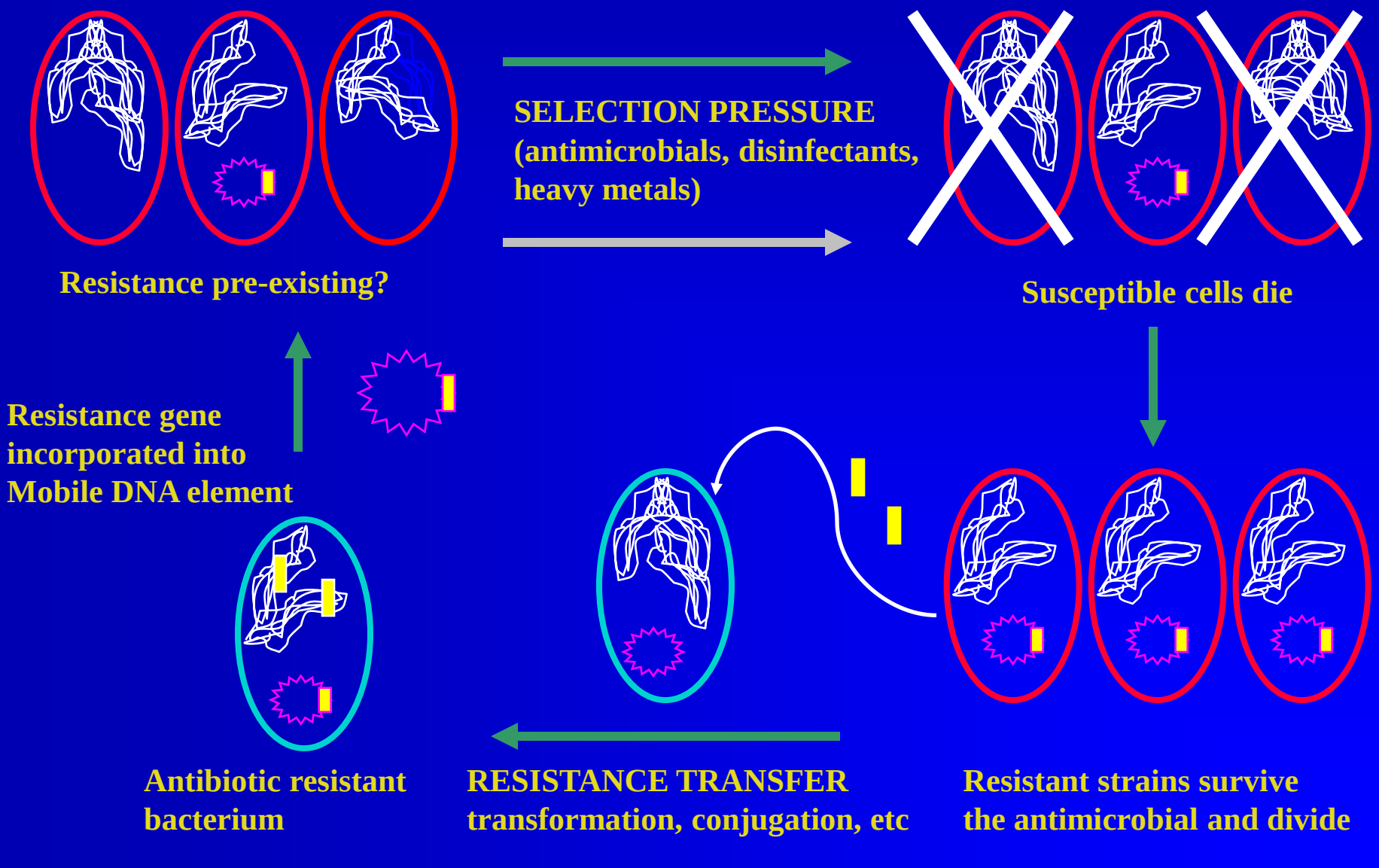
Resistance gene(s) in bacteria

+

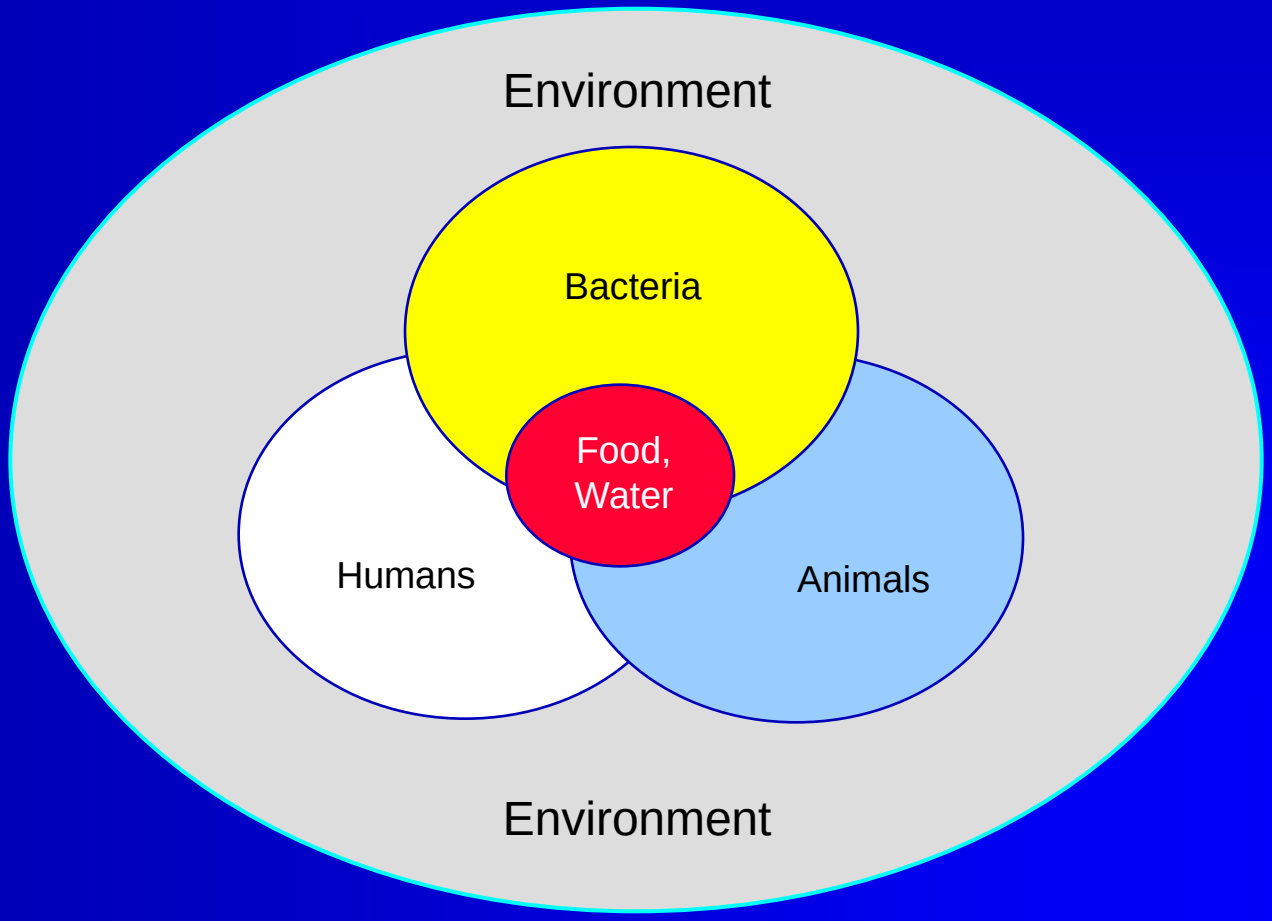
Antimicrobial (selective agent)

Antimicrobial-resistant bacteria

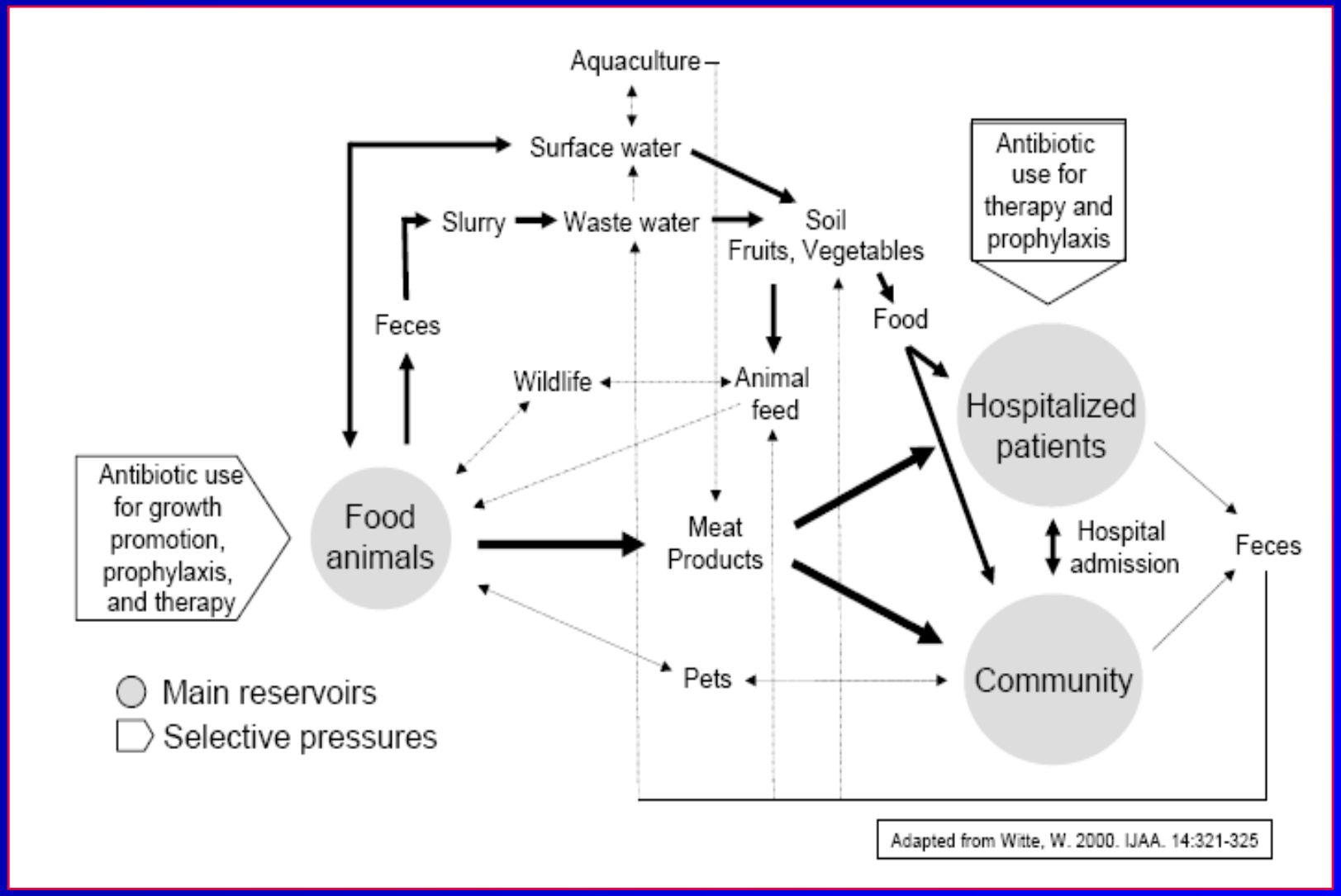
The Cycle of Resistance



Dissemination of Antimicrobial Resistance



Dissemination of Antimicrobial Resistance



Use of Antimicrobials in Food Animal Production

- To treat diseased animals
- To control outbreaks of disease
- To prevent infections
- To promote weight gain and improve feed efficiency
  - most controversial, since drugs can be administered continuously to healthy animals

Dissemination of Streptothricin Resistance in *E. coli*

Nourseothricin introduced in animal feed in 1983 in former East Germany

Origin	1982	1983	1984	1985	1986	1987
Pigs	-	+	+	+	+	+
Farm Personnel	-	-	+	+	+	+
Farm Families	-	-	+	+	+	+
Community	-	-	-	+	+	+
Community UTI's	-	-	-	+	+	+
S. sonnei	-	-	-	-	-	+

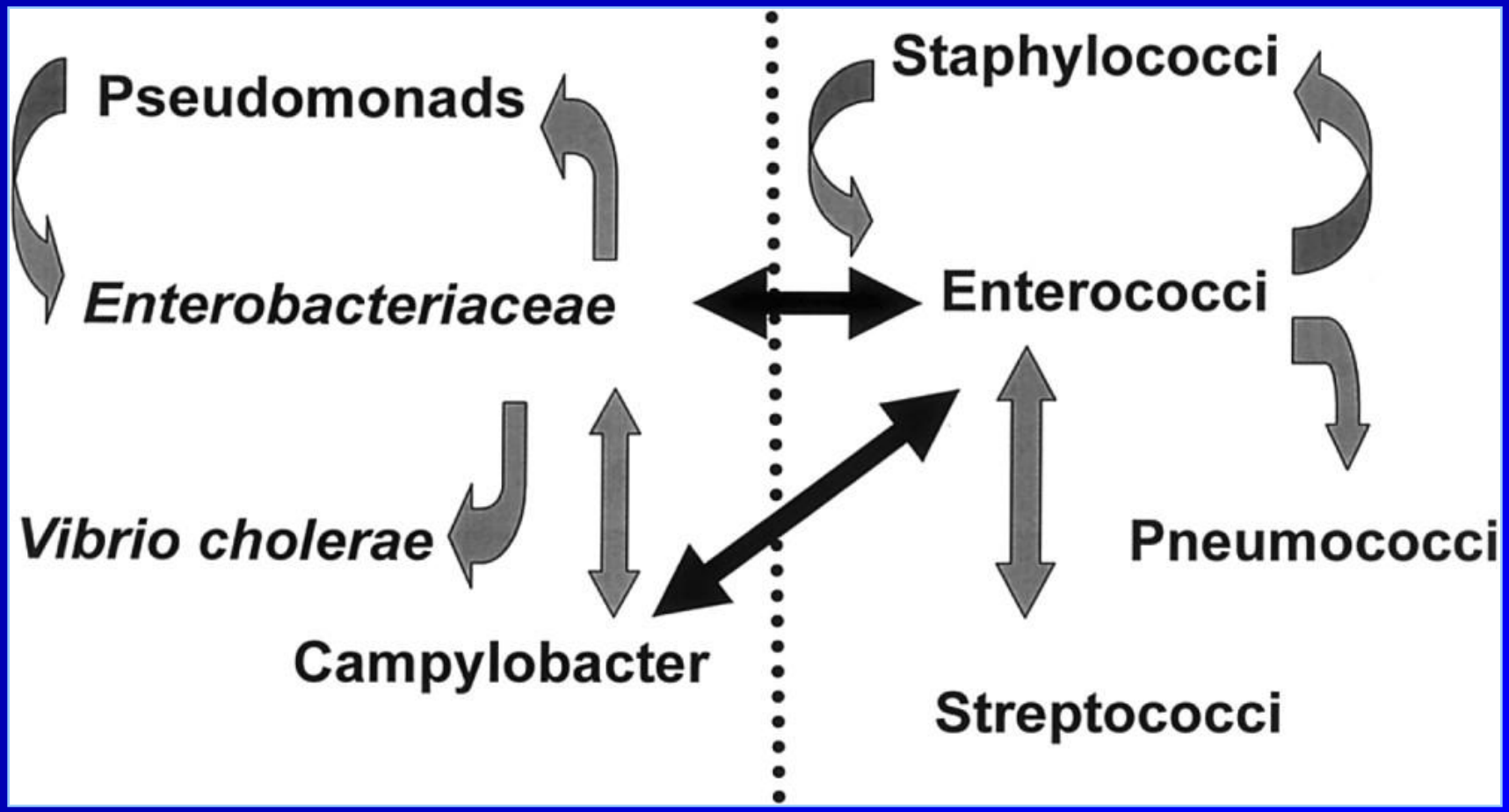
From: Witte, W. 1997. Antibiotic resistance, Ciba Foundation Symposium 207

Who is at Risk?

- Three year longitudinal study for AR *E. coli*
- 4048 human, 3429 swine samples
- Swine had higher rates of overall AR than humans
- Significant differences in AR between humans and swine
- Purchased boars > nursery piglets > breeding boars highest rates
- Slaughter workers > non-swine workers > swine workers

Scott et al, 2008 Appl Env Micro

Microbial Gene Exchange



Use of Antimicrobials in Food Animal Production and Potential Human Health Implications

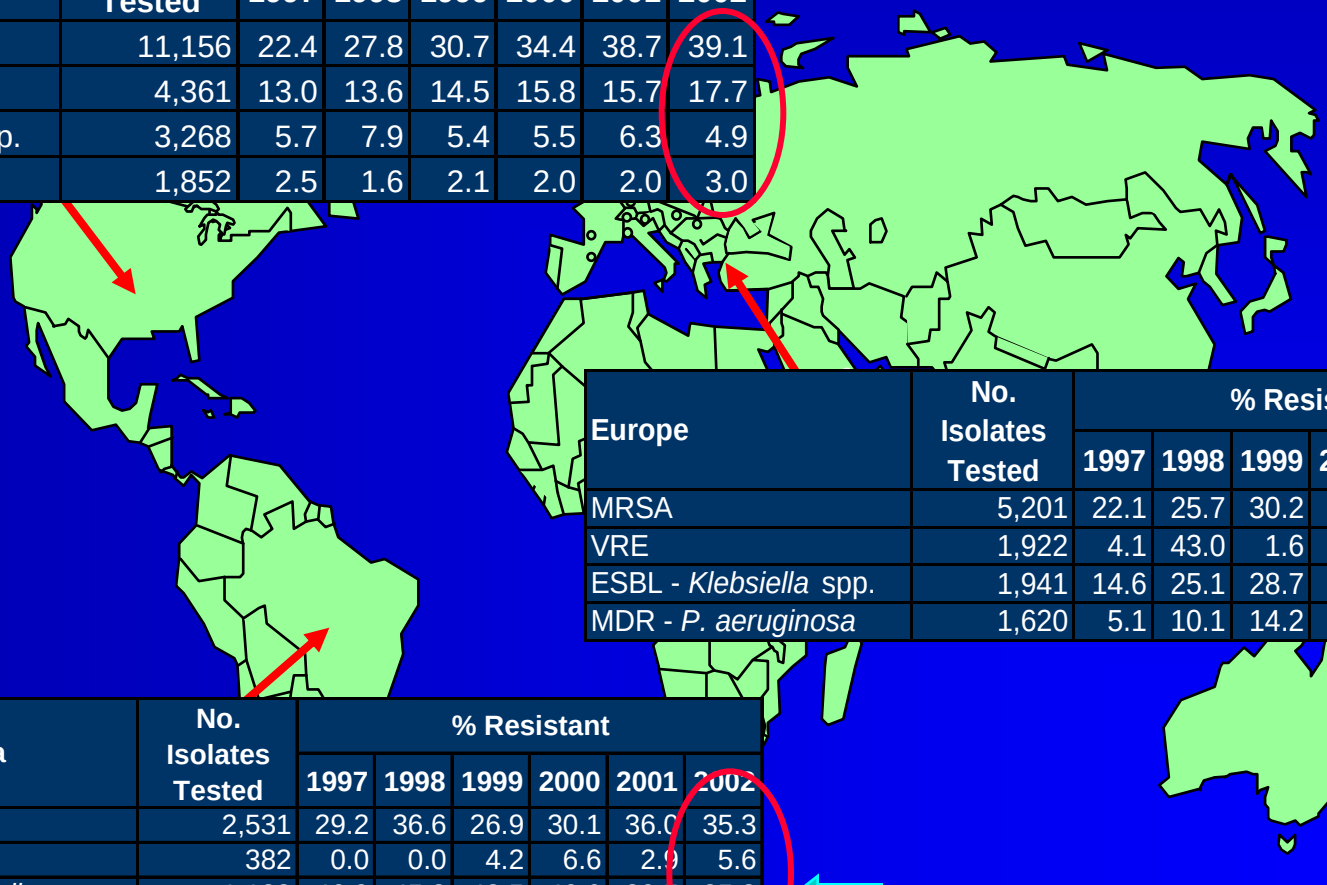
- Antimicrobial-resistant zoonotic bacterial pathogens are selected, and food is contaminated during slaughter and/or preparation
  - After consumption of contaminated food, pathogen causes an infection that requires antibiotic treatment and therapy is compromised
- Antimicrobial-resistant bacteria non-pathogenic to humans are selected in the animal
  - After consumption of contaminated food, bacteria transfer resistance determinants to other bacteria in the human gut
  - Including both commensal and potential pathogens
- Antimicrobials remain as residues in food products
  - Which allows for selection of antimicrobial-resistant bacteria after the food has been consumed

Potential Consequences of Infections Caused by Antibiotic Resistant Pathogens

- Increased likelihood of treatment with inappropriate antimicrobial
- Increased morbidity and mortality
- Change of empiric therapy
  - potentially less effective, more expensive and more toxic
- Increased length of illness in the hospital
  - increased length of hospital stay
  - increased laboratory costs
  - increased infection control costs

Worldwide Resistance by Select Bacterial Pathogens

North America	No. Isolates Tested	% Resistant					
		1997	1998	1999	2000	2001	2002
MRSA	11,156	22.4	27.8	30.7	34.4	38.7	39.1
VRE	4,361	13.0	13.6	14.5	15.8	15.7	17.7
ESBL - *Klebsiella* spp.	3,268	5.7	7.9	5.4	5.5	6.3	4.9
MDR - *P. aeruginosa*	1,852	2.5	1.6	2.1	2.0	2.0	3.0



Europe	No. Isolates Tested	% Resistant					
		1997	1998	1999	2000	2001	2002
MRSA	5,201	22.1	25.7	30.2	30.8	30.0	28.5
VRE	1,922	4.1	43.0	1.6	5.3	1.9	4.4
ESBL - *Klebsiella* spp.	1,941	14.6	25.1	28.7	31.5	20.5	17.3
MDR - *P. aeruginosa*	1,620	5.1	10.1	14.2	9.6	11.7	11.5

Latin America	No. Isolates Tested	% Resistant					
		1997	1998	1999	2000	2001	2002
MRSA	2,531	29.2	36.6	26.9	30.1	36.0	35.3
VRE	382	0.0	0.0	4.2	6.6	2.9	5.6
ESBL - *Klebsiella* spp.	1,183	46.9	45.8	43.5	46.6	39.5	35.8
MDR - *P. aeruginosa*	762	12.0	14.3	16.0	14.5	17.8	18.7

Source: Fritsche, T. R., et al., Antibiotics in Laboratory Medicine, 5th Ed., 2005

What is Responsible for the Widespread Dissemination and Diversity of Antimicrobial Resistance Phenotypes Among Bacteria?

- Plasmids
- Transposons
- Integrons

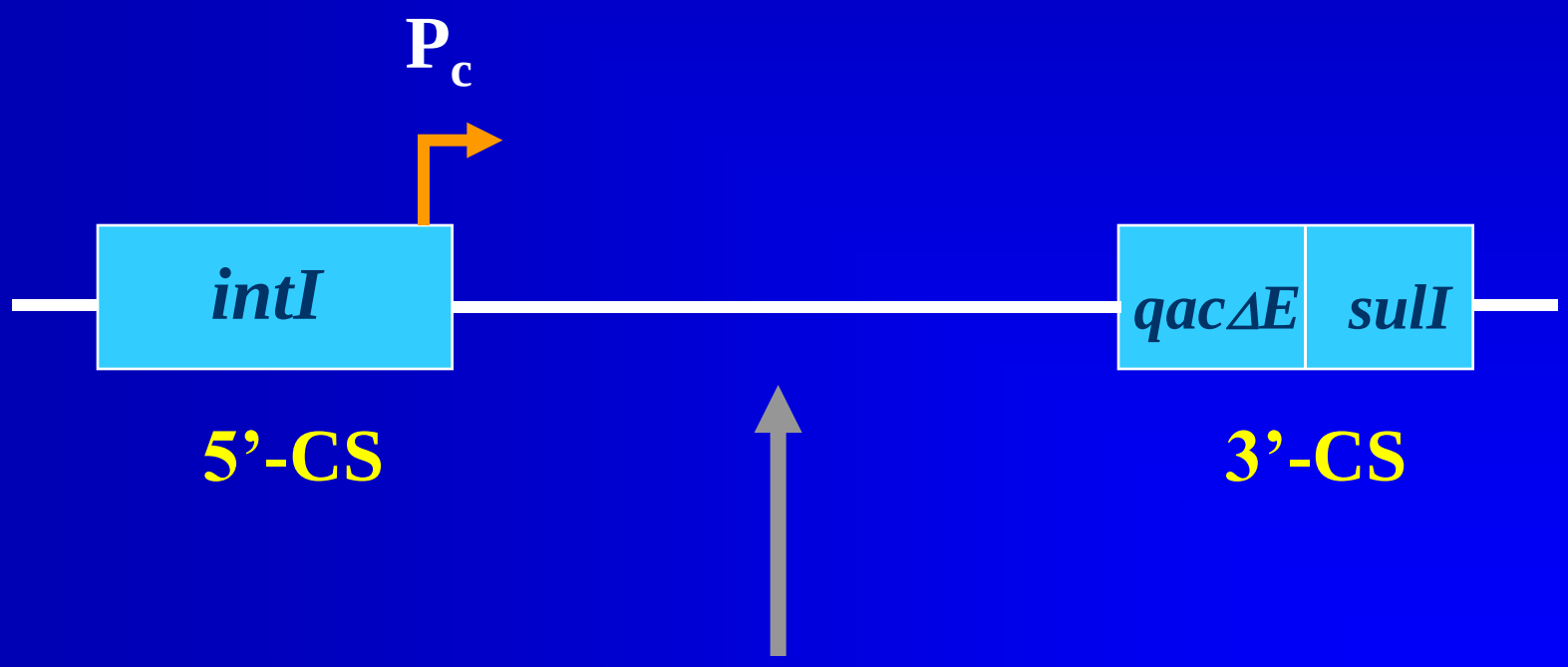
Transfer of Antibiotic Resistance

- **Plasmids**
  - **R factors**
  - **Can possess multiple antimicrobial resistance genes, including other elements**
  - **Conjugative (mediate their own transfer)**
- **Transposons**
  - **"Jumping genes"**
  - **Can possess multiple resistance genes**
  - **Can move freely from chromosome to plasmid and vice versa**
  - **May be conjugative**

Transfer of Antibiotic Resistance

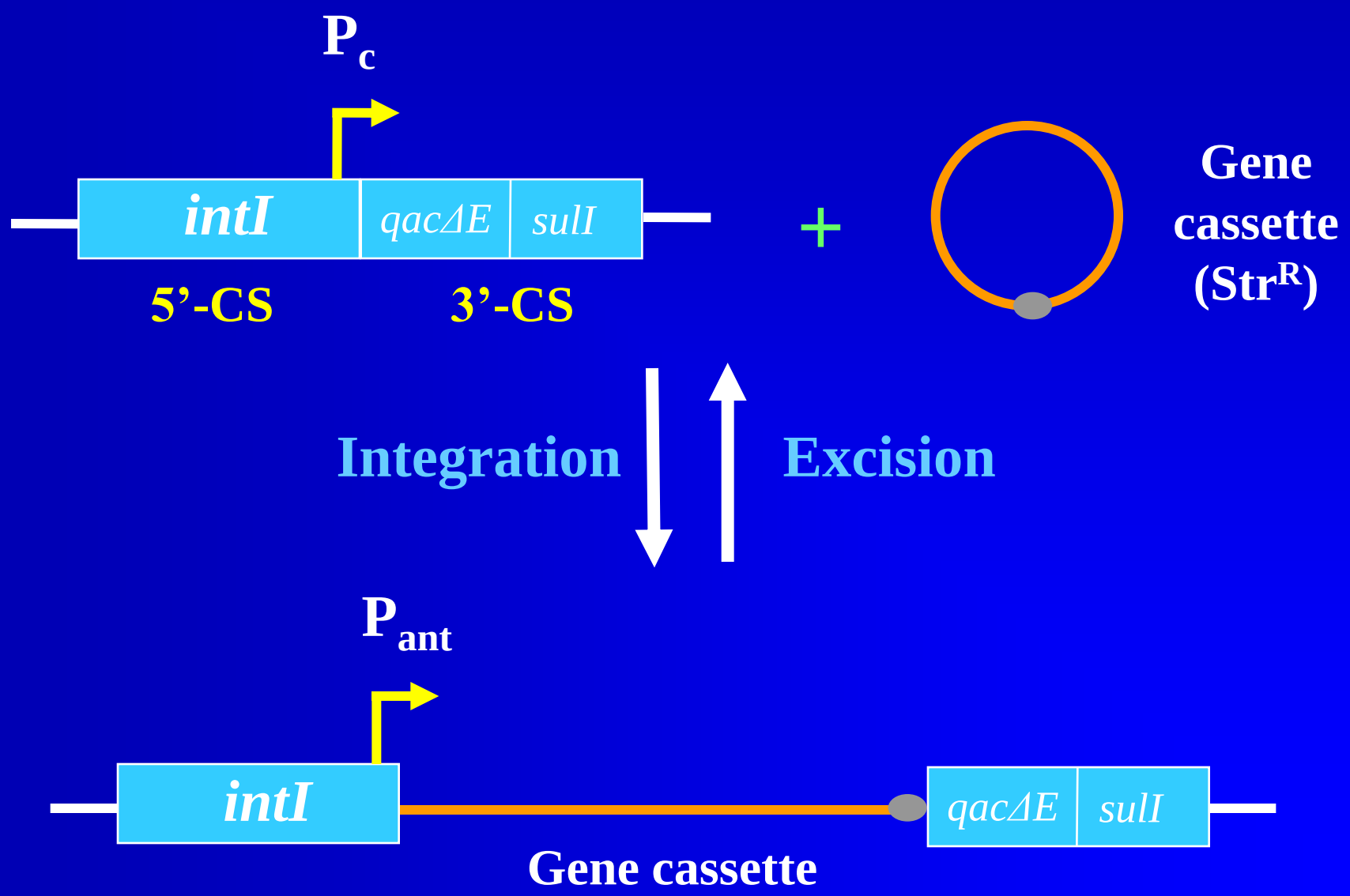
- **Integrons**
  - **"Gene catchers" Novel group of mobile DNA elements**
  - **Possess single or groups of antibiotic resistance genes in tandem (mobile gene cassettes)**
  - **Genes include β-lactamases, aminoglycoside modifying enzymes, sulfa/trimethoprim resistance, quinolone and chloramphenicol resistance**
  - **Can be plasmid mediated or chromosomal**
  - **4 classes currently recognized**
- **Common regions (CRs)**
  - **Found close to 3' conserved sequences of class 1 integrons**
  - **Resemble an atypical class of insertion sequences, IS*91*-like**
  - **Closely associated with many antimicrobial resistance genes (CTXs)**

General Structure of a Class 1 Integron

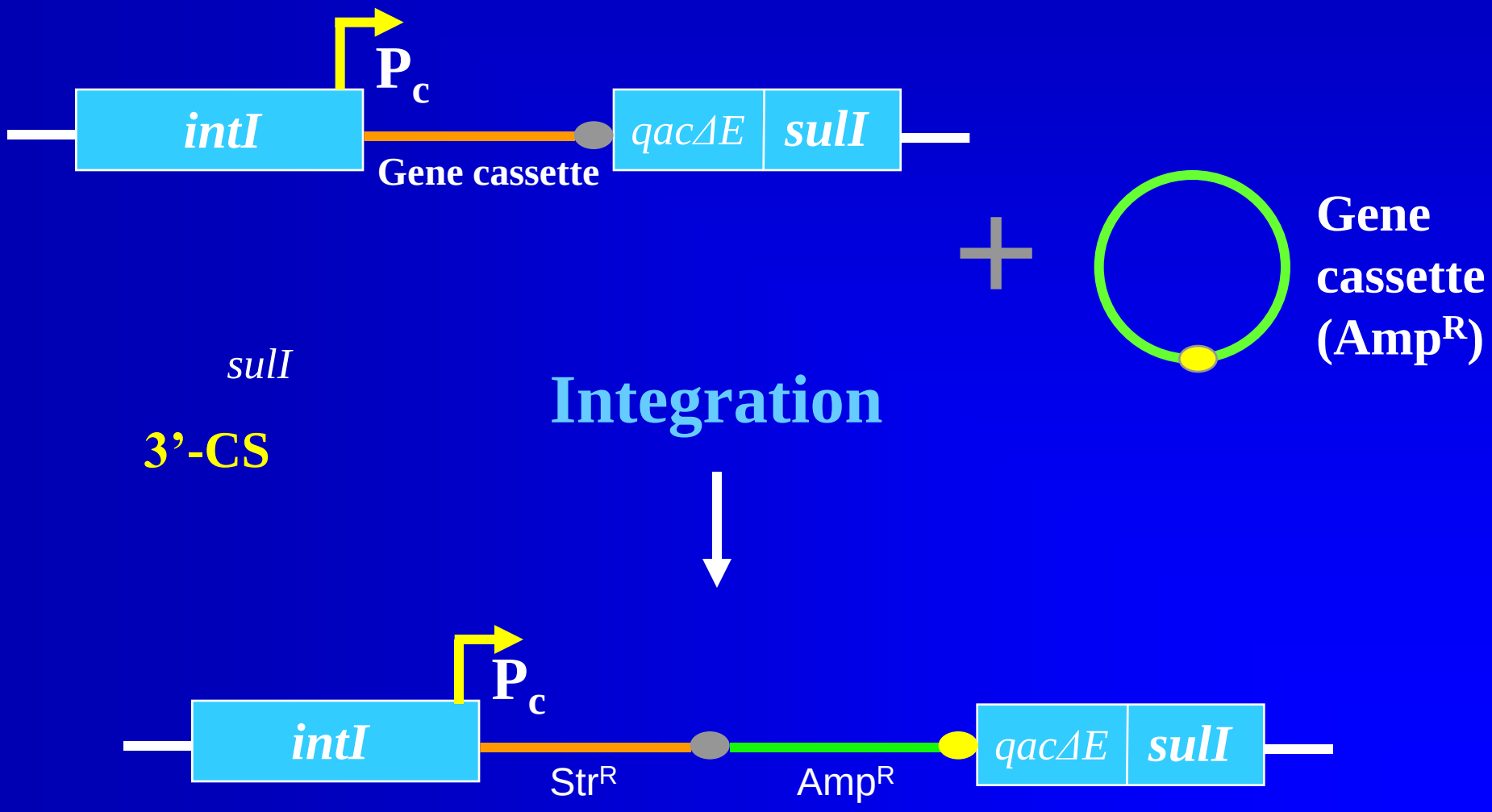


Insertion of antibiotic resistance genes

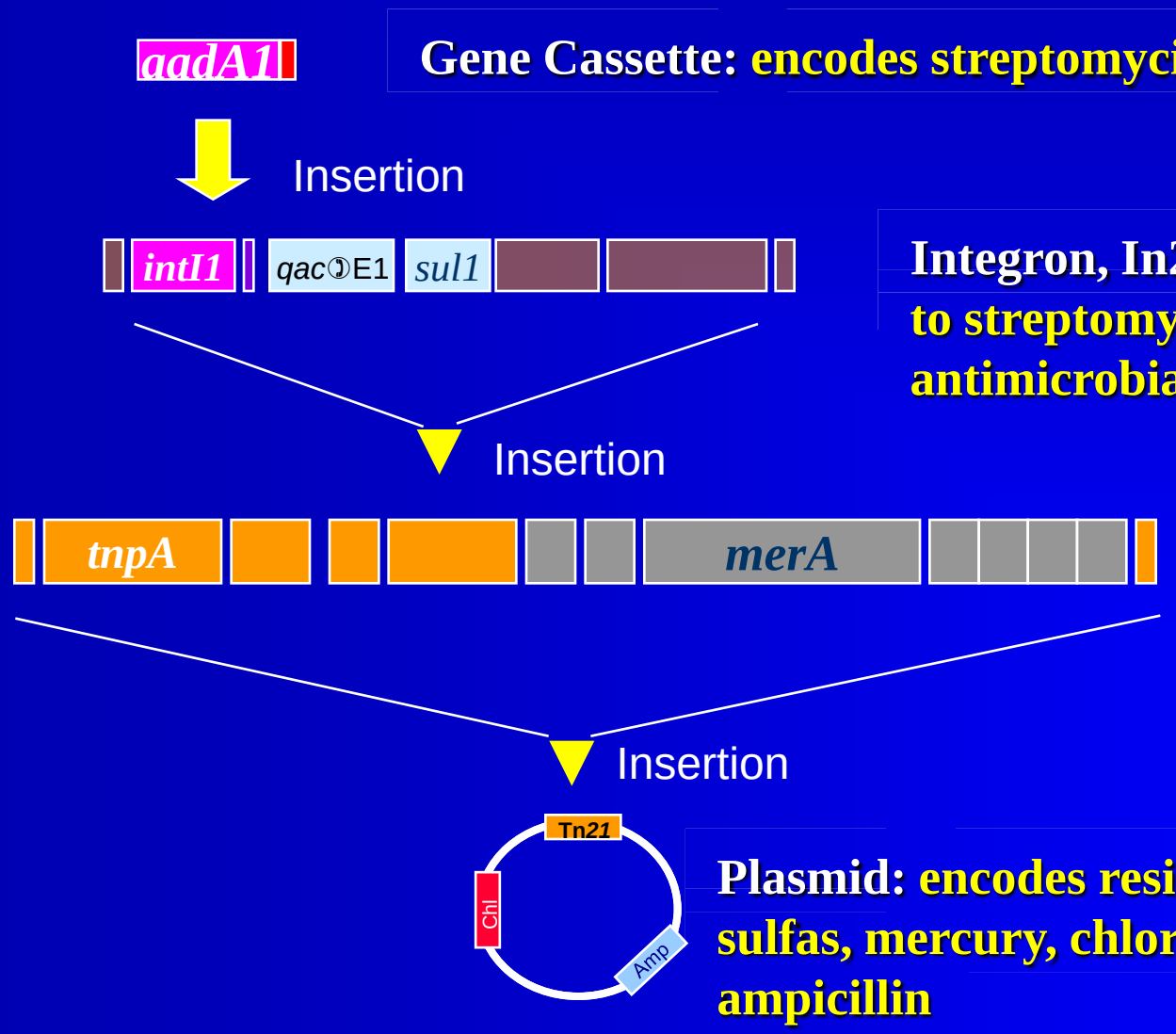
Insertion/Excision of Gene Cassettes into Integrons



Insertion/Excision of Gene Cassettes into Integrons



Transposon Tn*21*
The Biological Matryoshka effect



Gene Cassette: encodes streptomycin resistance

Integron, In2: encodes resistance to streptomycin and sulfa antimicrobials

Tn*21*: encodes Mercury resistance

Plasmid: encodes resistance to streptomycin, sulfas, mercury, chloramphenicol and ampicillin

Matryoshka (Russian doll) effect

Transferable Drug Resistances

- Beta-lactams
- Chloramphenicol; (Florfenicol)
- Tetracycline
- Macrolides; Lincosamides
- Sulfa drugs
- Aminoglycosides
- Quinolones?

Plasmid Mediated Quinolone Resistance

- **F**irst plasmid-mediated quinolone resistance determinant, Qnr (later termed QnrA), was described in a *K. pneumoniae* strain from the U.S. in 1998

- **T**hree major groups, QnrA, QnrB, and QnrS, have been identified in various enterobacteriaceae
  - At least five *qnrB* and two *qnrS* variants have been described
  - *qnrA* can be located in complex *sul1*-type class 1 integrons

- May protect DNA gyrase directly from quinolone inhibition, leading to an 8- to 32-fold increase in MICs

Global distribution of Qnr

Robicsek et al. 2006. Lancet Infect. Dis. 6:629-640.

Aquatic environment the reservoir???

Widespread *Salmonella* MDR-AmpC Plasmid Present in MDR *Yersinia pestis*

- *Y. pestis* plasmid pIP1202 has a IncA/C plasmid backbone that is shared by MDR plasmids isolated from *S.* Newport and the fish pathogen *Y. ruckeri*
- This plasmid backbone was detected in numerous MDR enteric bacteria from retail meats collected between 2002 and 2005 in the US
- The high degree of sequence identity and gene synteny of this shared plasmid backbone suggests recent acquisition of these plasmids from a common ancestor

	Backbone	*Y. pestis* (pIP1202)	*S. enterica* (pSN254)	*Y. ruckeri* (pYR1)
Resistance phenotypes*	Su	ACKSSpTMSul	ACSSulTCfAxG	STTmSul
β-lactams	-	bla_{SHV-1}	$bla_{CMY-2}1$, $bla_{CMY-2}2$	-
Aminoglycosides/Aminocyclitols	-	*strAB, aadA, aphA*	*strAB, aacC, aadA*	*strAB*
Tetracyclines	-	$tetRA_{class\ D}$	$tetRA_{class\ A}$	$tetRA_{class\ B}$
Phenicols	-	*cat*	*floR*	-
Quaternary ammonium compounds	-	*qacEdelta1*	*qacEdelta1, sugE1, sugE2*	-
Sulfonamides	*sul2*	*sul1, sul2*	*sul1, sul2*	*sul2*
Trimethoprim	-	-	-	*dhfrI*

*Abbreviations used for antibiotics: A, ampicillin; C, chloramphenicol; S, streptomycin; Sul, sulfonamide; T, tetracycline; Cf, cephalothin, Ax, ceftriaxone; G, gentamicin; Sp, spectinomycin; M, minocycline; Tm, trimethoprim.

doi:10.1371/journal.pone.0000309.t001

Welch et al. Multiple antimicrobial resistance in plague: an emerging public health risk. PLoS ONE. 2007 Mar 21;2:e309

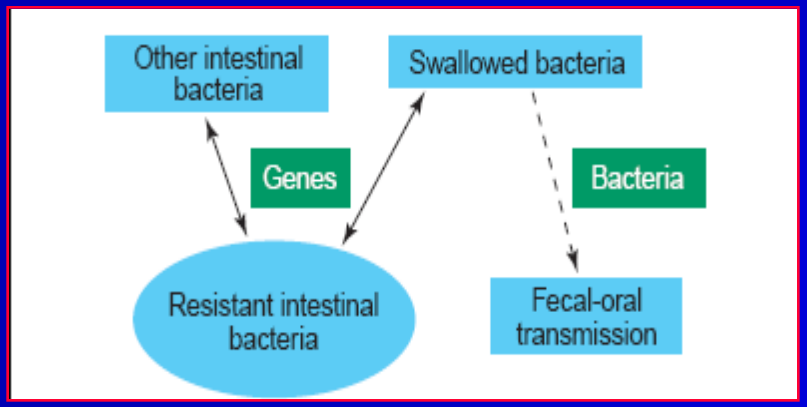
Gene Synteny

The condition of two or more genes being located on the same chromosome whether or not there is demonstrable linkage between them.

Wright, G. 2007. Nature Reviews Microbiol. 5:175-186.

Commensal Bacteria as a Reservoir?

Resistance gene reservoir hypothesis



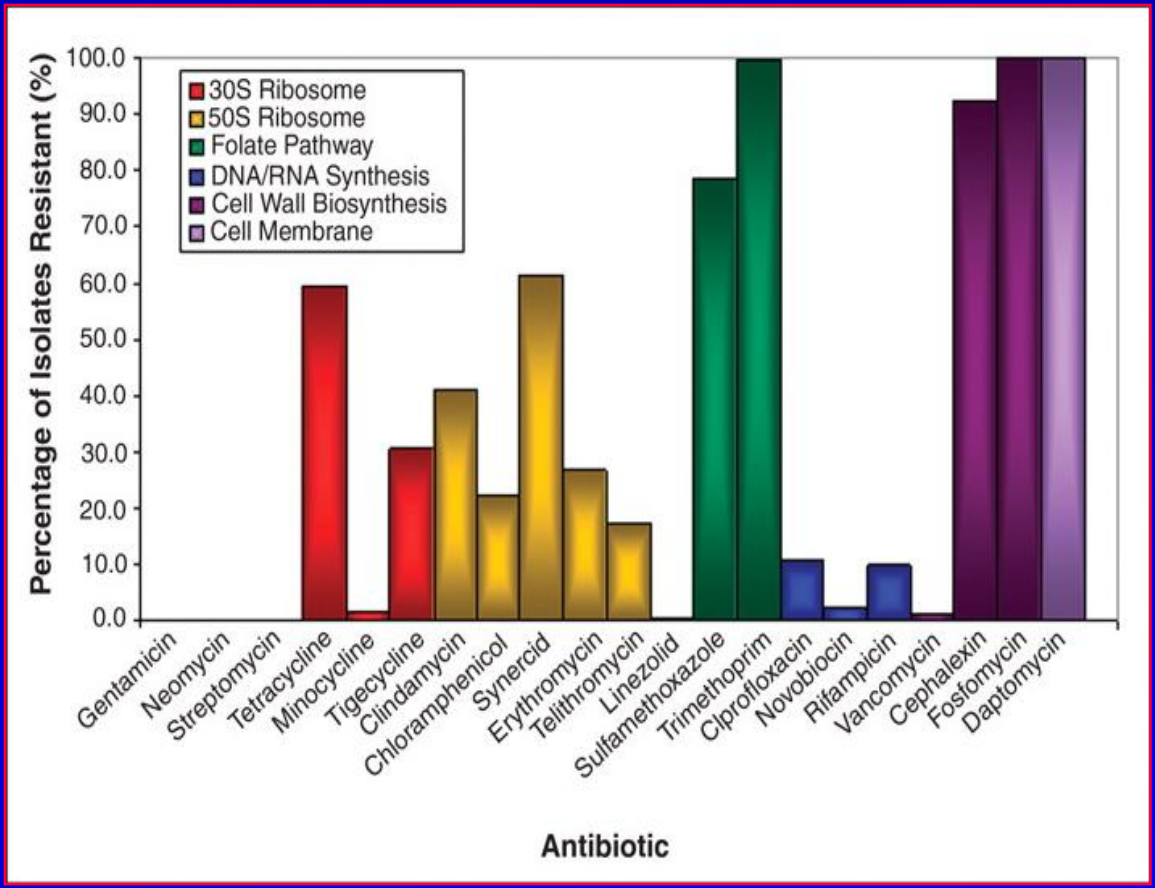
http://roarproject.org/

Possible resistance gene transfers

Gram-positive		Gram-negative
Bacillus spp. *Clostridium* spp. *Staphylococcus* spp.	ermG	*Bacteroides* spp.
Enterococcus spp. *Staphylococcus* spp. *Streptococcus* spp. *Clostridia* spp.	ermB	*Bacteroides* spp.
Enterococcus spp. *Staphylococcus* spp. *Streptococcus* spp. *Actinomyces* spp. *Bifidobacterium* spp. *Clostridia* spp.	tetM	*Campylobacter* spp. *Fusobacterium nucleatum* *Gardenella vaginalis* *Haemophilus* spp. *Neisseria* spp. *Veillonella* spp.
Firmicute *Eubacterium*	ermF tetQ	*Bacteroides* spp. *Prevotella* spp. *Porphorymonas* spp.

Soil as a Reservoir of Resistance

- D'Costa et al. 2006. Science 311:374-377
- Screened 480 soil bacteria against a panel of 21 antimicrobials
- Multidrug resistance was extensive
- No evidence of direct transfer of resistance elements to pathogens
- However, shows the environmental resistome is much more significant then previously thought



The Antimicrobial Resistome

The resistome comprises all the antimicrobial resistance genes and their precursers in pathogenic and non-pathogenic bacteria (including antibiotic-producing bacteria)

Wright, G. 2007. Nature Reviews Microbiol. 5:175-186.

The Enemy Among Us

- Chromosomal resistance factors (DNA) found in contaminated antibiotics
- Produced from source antibiotic-producing organism
- Possible explanation for rapid rise in resistance factor dissemination

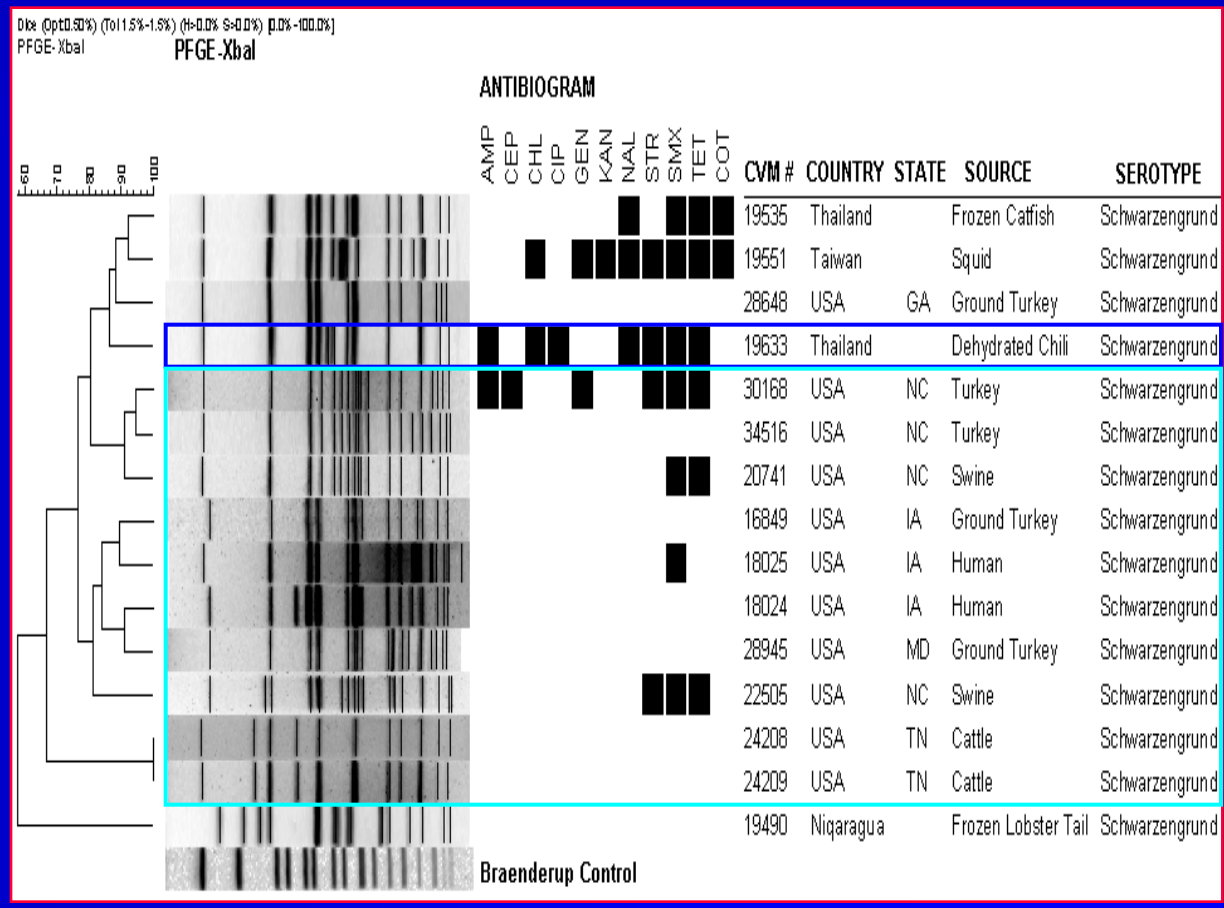
Webb V, Davies J. Antibiotic preparations contain DNA: a source of drug resistance genes? Antimicrob Agents Chemother 1993; 37 (11):2379-84.

The Global Village and Antimicrobial Resistance

- The rapid globalization of food production and trade has increased the potential for international incidents involving food contamination with microbial or chemical hazards
- Information on the magnitude of the public health burden due to resistant foodborne pathogens shows that the circumstances are complex and differ by region and country
- Can be influenced by a number of variables
  - Human and veterinary antimicrobial use practices
  - Process controls at animal slaughter
  - Food storage and distribution systems
  - Availability of clean water
  - Proper cooking and home hygiene methods, among others

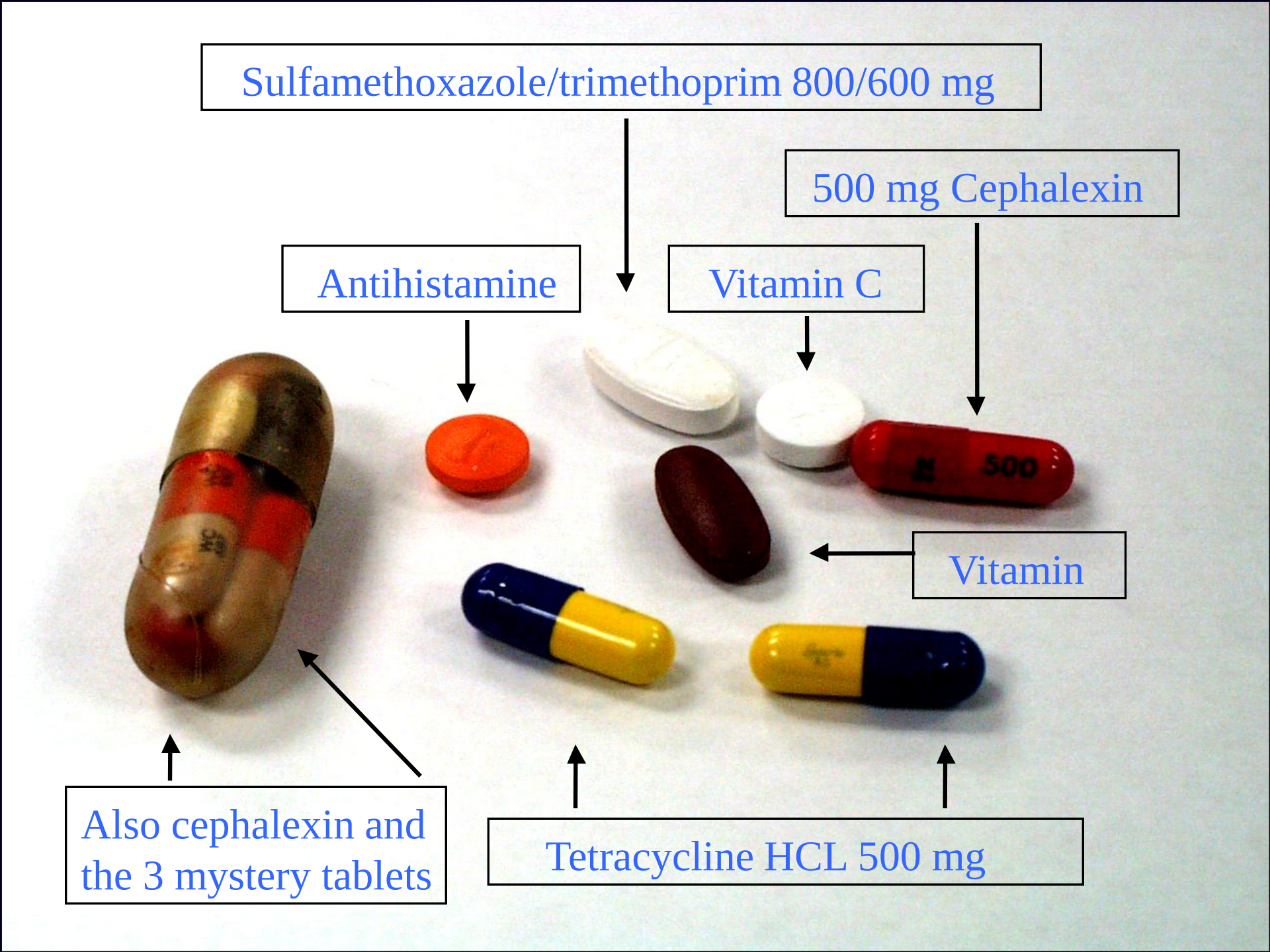
An International Outbreak Strain?

- Imported food studies revealed a multidrug resistant (including Cip^R) *S.* Schwarzengrund isolate from dehydrated chili's from Thailand
- Its PFGE pattern was indistinguishable from that observed among three Cip^R resistant *S.* Schwarzengrund isolates recovered from human clinical illness in both Denmark and Thailand
- This data indicates that global food trade could transmit foodborne pathogens from one country to another
  - No illness linked to this strain in the U.S.



Aarestrup, F.M. et al. Spread of multiple antimicrobial resistant *Salmonella* Schwarzengrund from Thailand to Denmark and the USA through the international trade of food products. Emerg. Infect. Dis 13:726-731.

Hurry, Sale ends today!!!


Sulfamethoxazole/trimethoprim 800/600 mg
500 mg Cephalexin
Antihistamine
Vitamin C
Vitamin
Also cephalexin and the 3 mystery tablets
Tetracycline HCL 500 mg

Resistance patterns for *Escherichia coli* isolates from large animal panels for the year 2001, NDSU VDL, March 2002.

Antibiotic	n	Percent Sensitive	Percent Intermediate	Percent Resistant
Amikacin	489	**99**	-	1
Ampicillin	503	36	-	**63**
Ceftiofur	534	**76**	6	16
Erythromycin	534	-	-	**100**
Florfenicol	489	-	1	**99**
Gentamicin	520	**76**	3	20
Penicillin	534	-	-	**100**
Spectinomycin	520	31	23	45
Tetracycline	534	12	1	**86**
Tiamulin	489	-	-	**100**
Tilmicosin	489	-	3	**97**
Tribrissen	489	56	-	43

NDSU VDL Antibiotic Resistance Patterns for E. coli, 2006

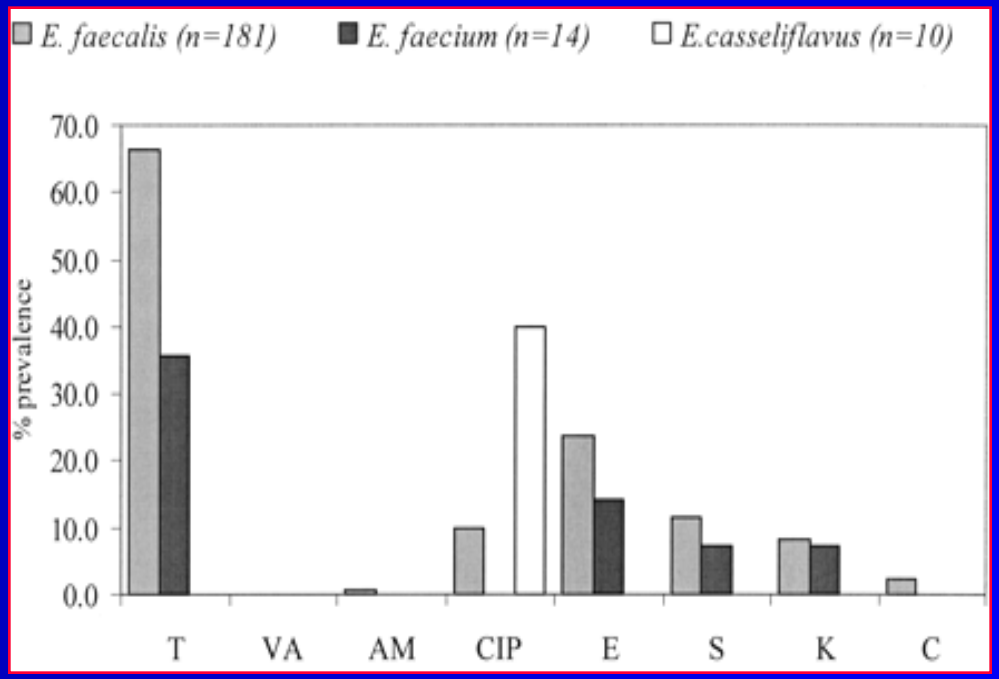
Drug	% Resistant	% Susceptible	% Intermediate
Polyflex	60.1	39.3	0.7
Naxcel/Excede	22.8	71.5	5.7
Baytril			
Nuflor	55.9	7.4	36.7
Micotil	100.00	0.0	0.0
Gentocin			
Terramycin	87.8	12.2	0.0
TMZ-SMZ	85.3	14.7	0.0
Penicillin	100.0	0.0	0.0
Amiglyde			

n=298

Food for Thought

- Macovei and Zurek, AEM, 2006, 72:4028-4035

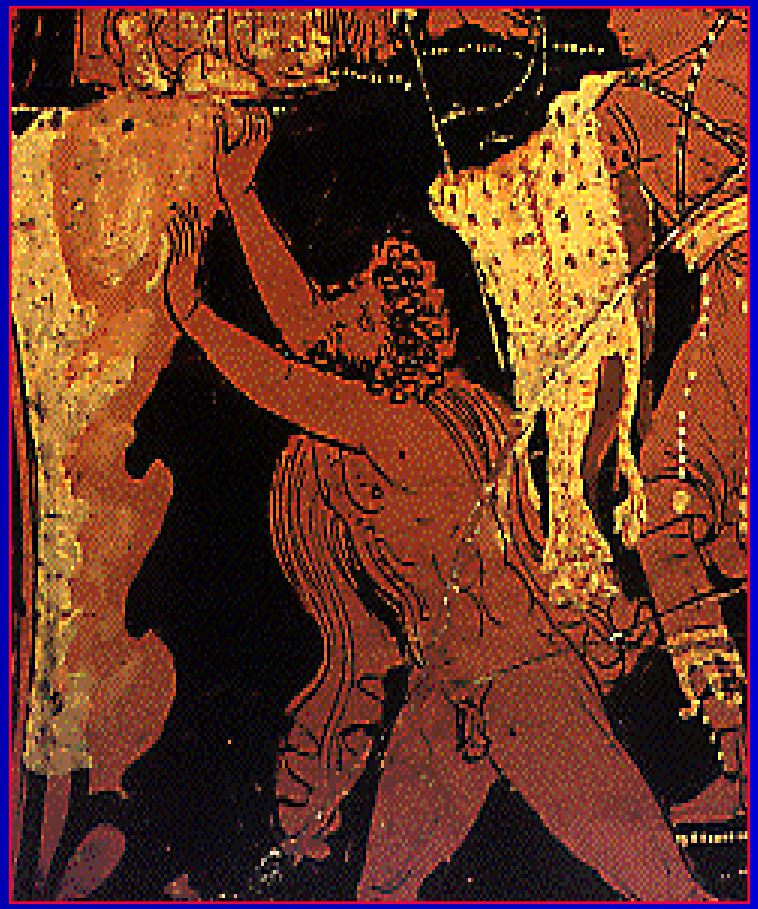
- Showed that houseflies in food-handling and -serving facilities carry antibiotic-resistant and potentially virulent enterococci



We still need to improve our understanding of -

- The contribution of the normal flora to antimicrobial resistance
- Factors that contribute to the selection of resistant microbes (dose, frequency of dose, duration, organism exposure)
- The frequency of selection for antimicrobial resistance bacteria
- Adaptation of resistance mechanisms
- Mechanisms of linkage and transmissibility of resistance determinants

Cycle of Antimicrobial Development and Emergence of Resistance



Sisyphus is best known for being punished in the <u>Underworld</u>

by rolling a stone with his hands and head

in an effort to heave it over the top of a hill;

but regardless of how much he pushes, the stone

always rolled down again and again

AGONY

NOT ALL PAIN IS GAIN.

Antimicrobial Resistance = Complex Phenomenon

- Antimicrobial resistance is a broad-based problem that involves not just patients and doctors/veterinarians in clinical settings, but industry, public health officials, farmers, producers and consumers

- The solutions involve a range of federal and state agencies, international governments and organizations, consumer, scientific and professional groups and individuals

Antimicrobial Resistance = Complex Phenomenon

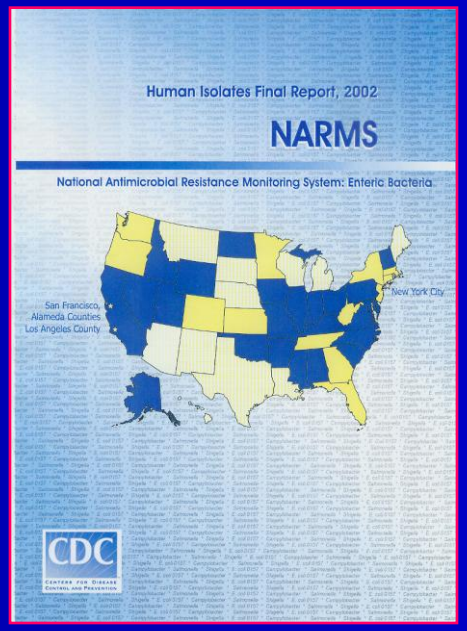
- It is most likely impossible to stop antimicrobial resistance from emerging
- Resistance is a natural consequence of biological adaptation, which is an inherent factor in the adaptation of organisms
- Therefore, efforts to tackle antimicrobial resistance should focus primarily on how we can better mitigate resistance development and transfer

NARMS Annual Reports

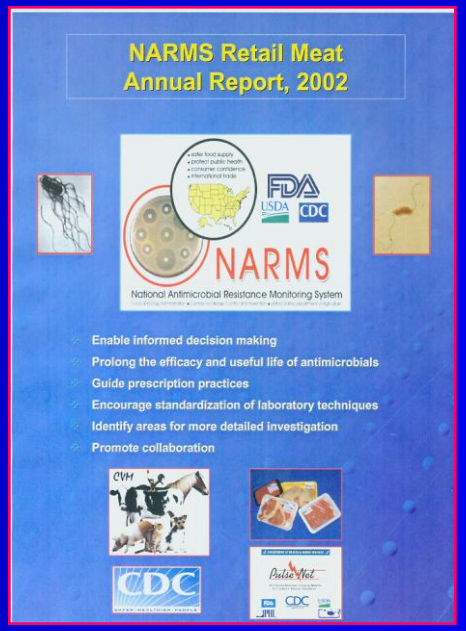
More details and data are available on the FDA-CVM webpage and links to CDC, USDA and CVM annual reports

http://www.fda.gov/cvm/narms_pg.html

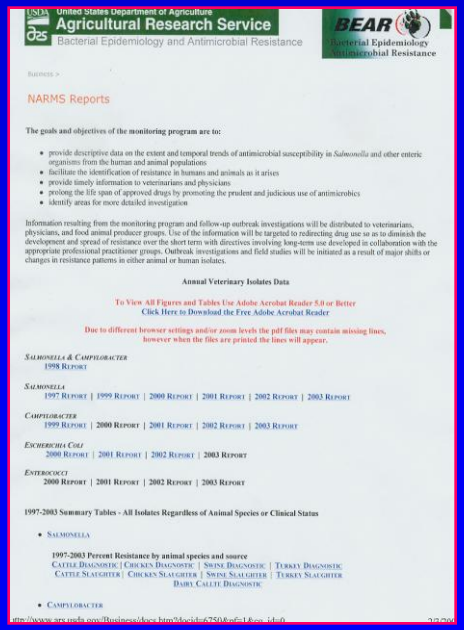
CDC – Human origin



FDA/CVM – Retail meats

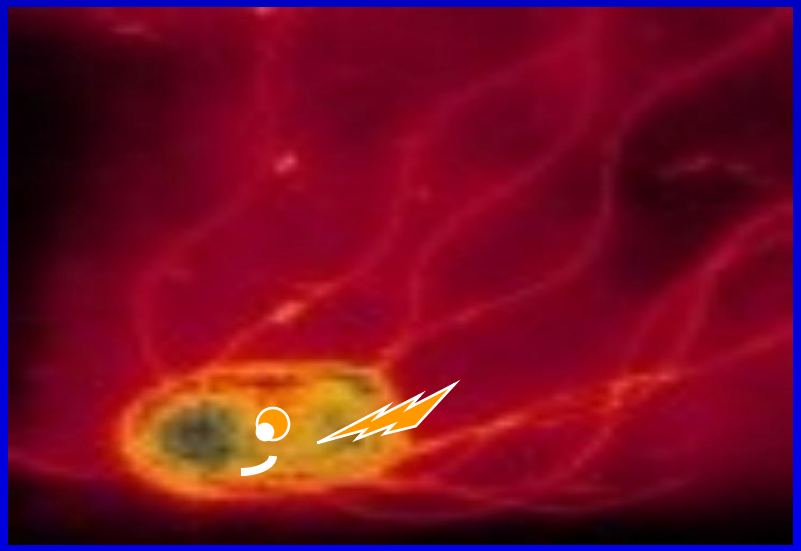


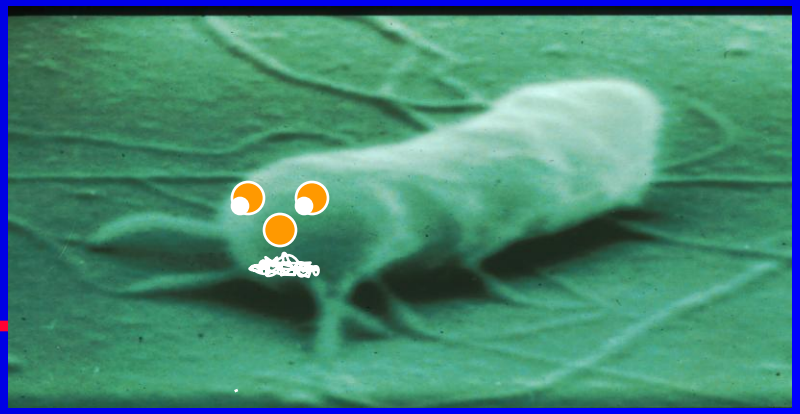
USDA – Animal origin



Salmonella - NARMS

- Sentinel organism
- ~ 51,000 isolates tested since 1997
- Serotypes
  - Vary over time
  - Vary by source





Summary reports and interactive feature on website

Percent Resistance Trends - Top Serotypes

Cattle at Slaughter

Antimicrobials

- Amikacin
- Gentamicin
- Kanamycin
- Streptomycin
- Ampicillin
- Amoxicillin/Clavulanic Acid
- Ceftiofur
- Ceftriaxone
- Cephalothin
- Cefoxitin
- Sulfonamides
- Trimethoprim/Sulfamethoxa
- Chloramphenicol
- Ciprofloxacin
- Nalidixic Acid
- Tetracycline

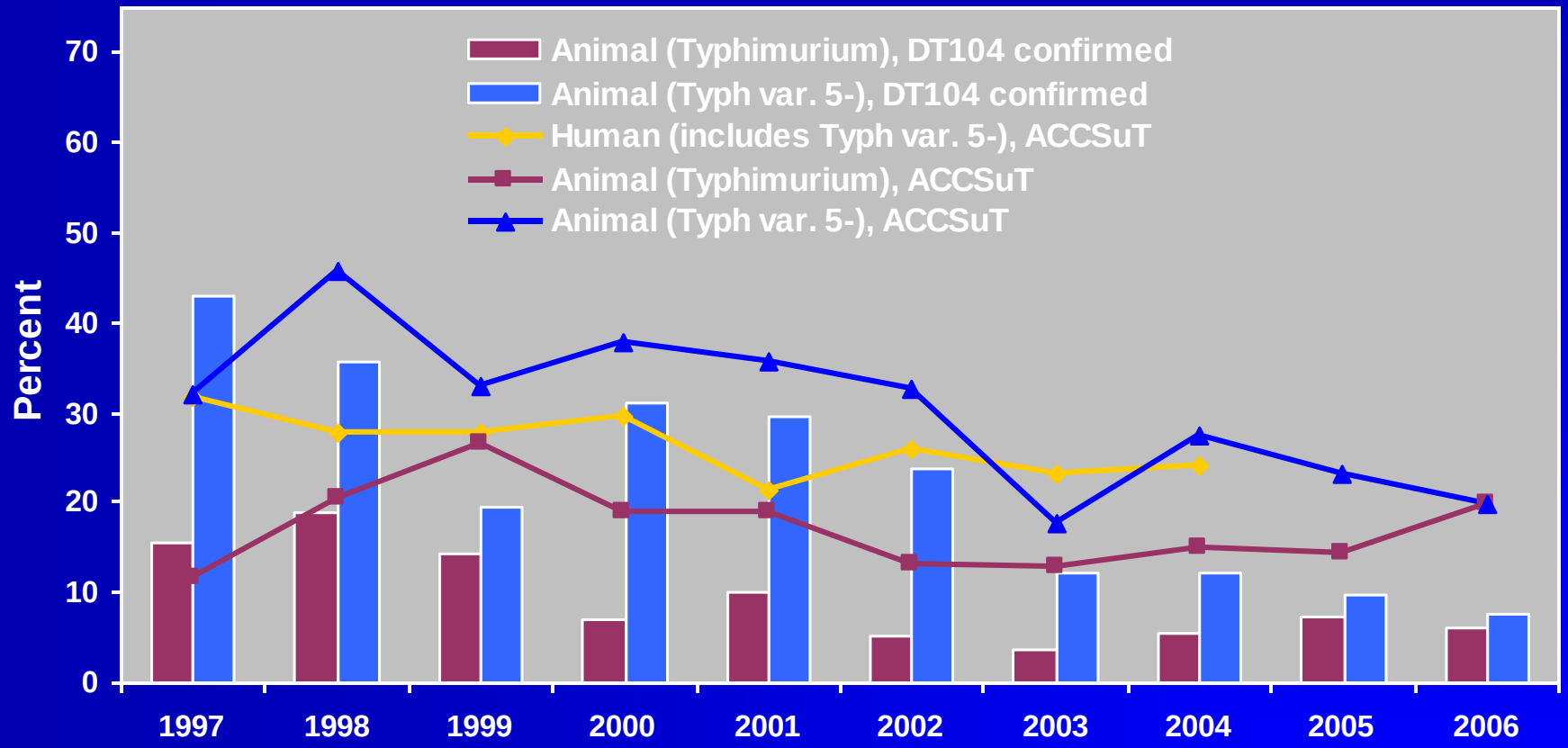
Serotypes

- Montevideo
- Anatum
- Newport
- Muenster
- Typhimurium
- Typhimurium var. 5-
- Kentucky
- Mbandaka
- Cerro
- Agona
- Meleagridis
- Reading
- Senftenberg
- Infantis
- Dublin

Number of Isolates Tested

Serotype	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Montevideo	1	29	312	193	117	107	64	82	43	63
Anatum	7	22	176	90	77	89	58	66	21	23
Newport	0	8	54	109	87	113	75	44	27	30
Muenster	3	19	113	80	63	83	44	54	24	38
Typhimurium	1	15	105	85	49	40	42	27	17	15
Typhimurium var. 5-	0	18	84	102	38	58	36	21	17	7
Kentucky	2	12	87	72	61	50	30	25	9	14
Mbandaka	1	11	53	54	43	49	31	19	15	15
Cerro	0	8	70	64	35	35	23	16	12	24

Percentage of Isolates of *S.* Typhimurium resistant to at least ACSSuT and percentage confirmed DT104, 1997-2006* (Slaughter isolates)

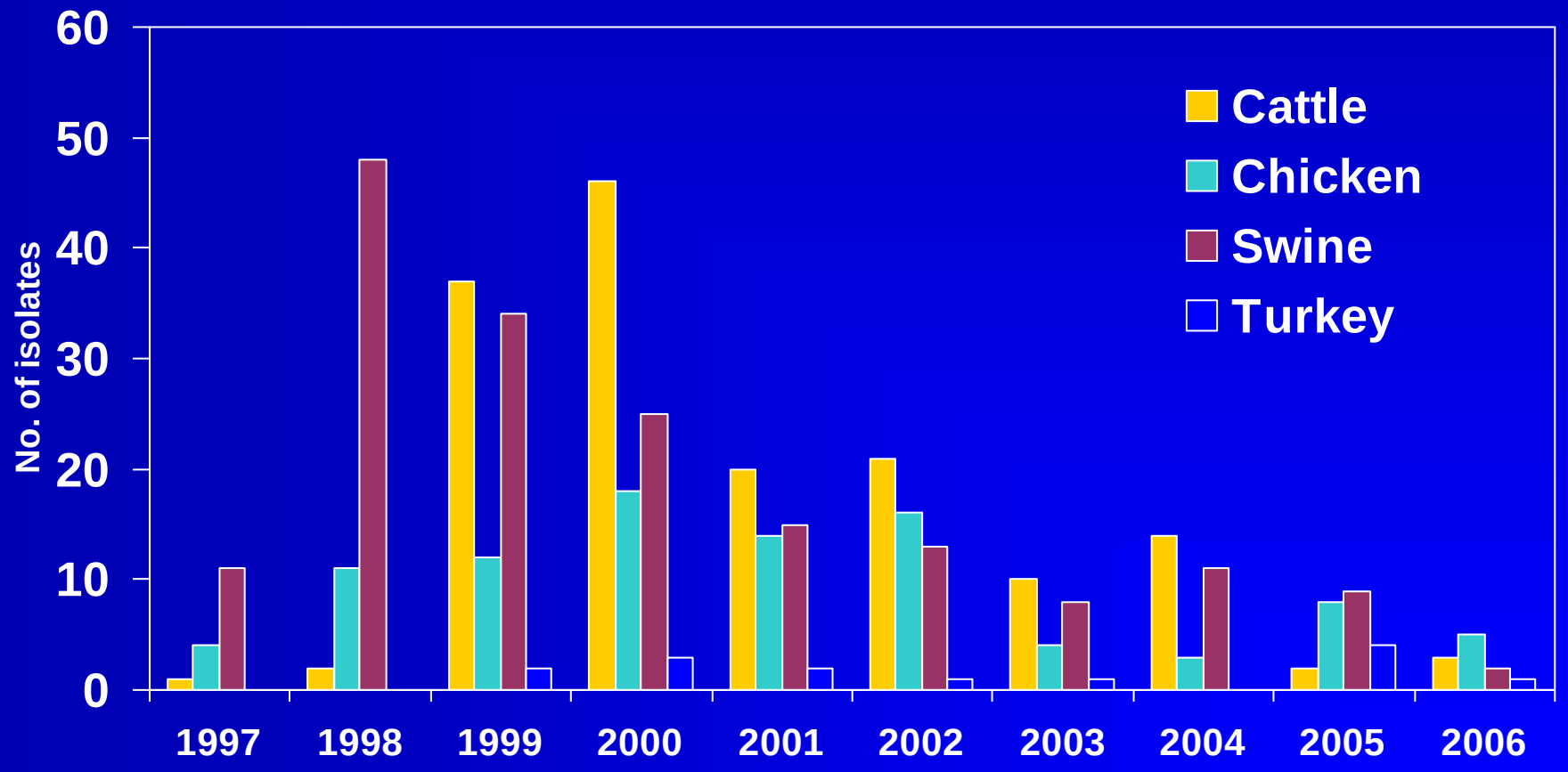


Number of Typhimurium/Typhimurium var. 5- isolates tested by year

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Typhimurium - Human (includes var. 5-)	328	377	362	303	325	393	403	382	NA	NA
Typhimurium, Animal	26	79	212	174	158	115	110	107	111	66
Typhimurium var. 5, Animal	28	129	282	257	118	190	157	179	155	91

* 2006 preliminary data

Distribution of DT104 isolates from slaughter by source, 1997-2006

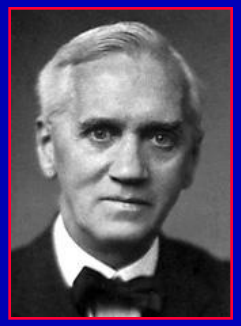


NDSU Animal Sciences

* 2006 preliminary data

Antimicrobial resistance is a multi-factorial problem that requires a multi-disciplinary and a multi-agency approach

Return to the pre-antibiotic era?



March 22, 1954, Sir Alexander Fleming was asked about a cure for colds

"A good gulp of hot whisky at bedtime -- it's not very scientific, but it helps."

APATHY

If We Don't Take Care of the Customer,
Maybe They'll Stop Bugging Us.