MOLECULAR EPIDEMIOLOGY TO REFINE DIAGNOSTICS IN BACTERIOLOGY: DOES IT HELP?

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Useful or only a toy for researchers ?

WHAT IS MOLECULAR EPIDEMIOLOGY ?

DEFINITIONS AND APPLICATIONS

Molecular Epidemiology: the study of distribution of molecular determinants of health and diseases in natural microorganisms populations

Applications:

- Tracing transmission events (single cases, outbreaks, hospital infections, new emerging pathogens)
- Identification of organisms or genetic elements with particular characteristics
- Identification of potential new threats
- Monitoring of infectious agents

DETERMINANTS OF HEALTH AND DISEASE

- Bacterial strain transmitted between patients within particular settings (Nosocomial transmission, foodborne outbreak, others…)
- Bacterial strains with particular characteristics (virulence, antimicrobial resistance, others…)
  - Strain or group of related strains (clonal lineage) with particular characteristics
  - Genetic element or groups of related elements associated with particular disease, virulence, antimicrobial resistance, others…)

GENETIC DIVERSITY AND TYPING

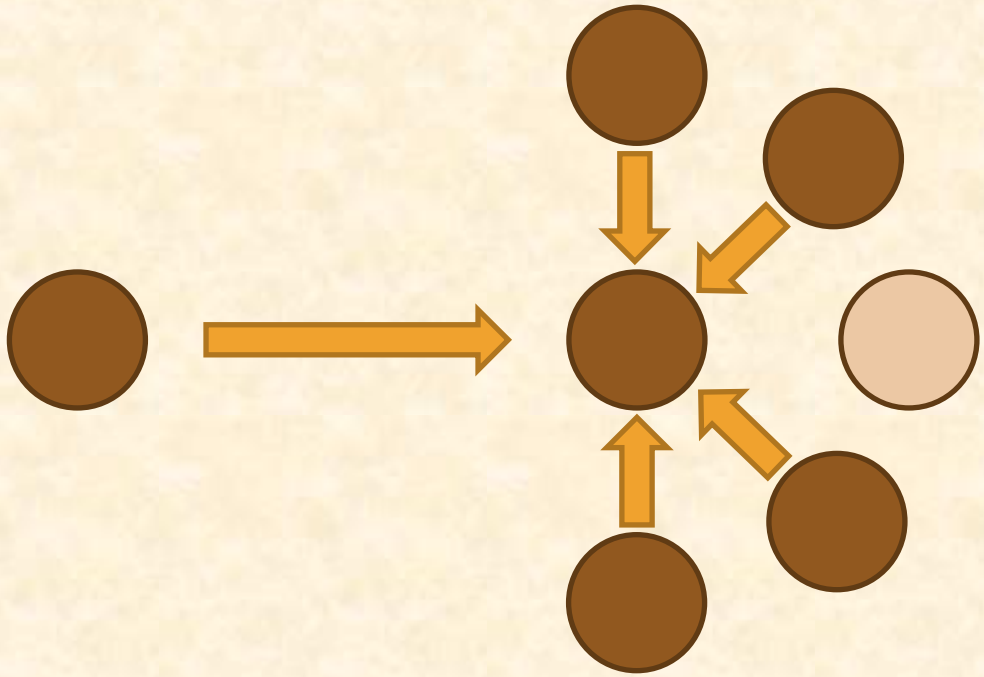
Type: A set of characteristics found in a specific strain or a genetic element

Basis for molecular typing:

- Strain diversity
- Presence/absence of genetic element(s) or of specific genetic marker
- DNA sequence diversity of gene(s) or genetic element(s)

CONTROLS, CONTROLS, CONTROLS, …

Similarity ≠ causality !



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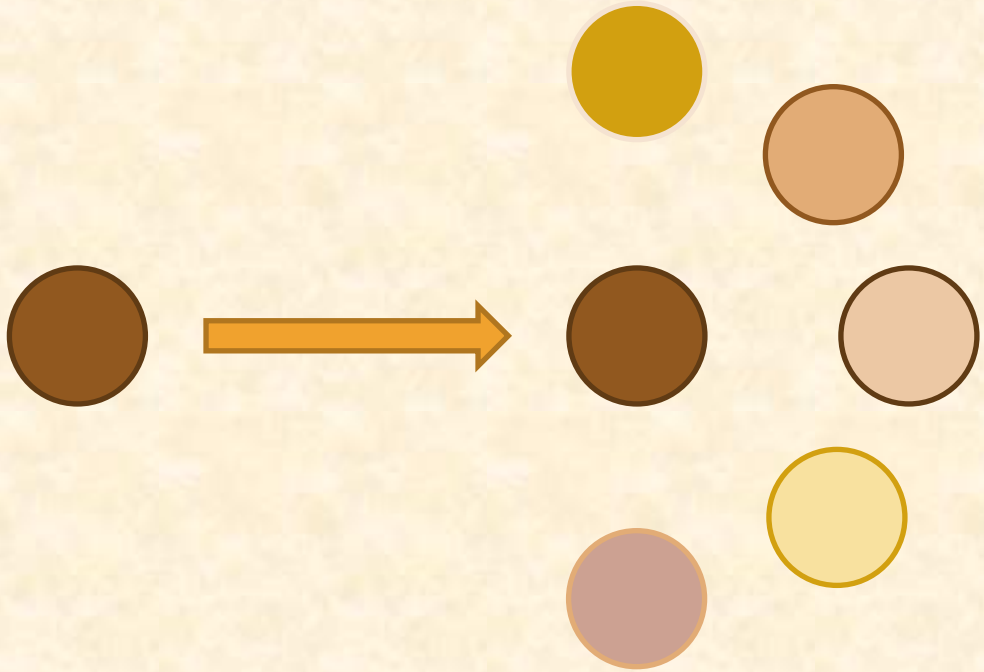

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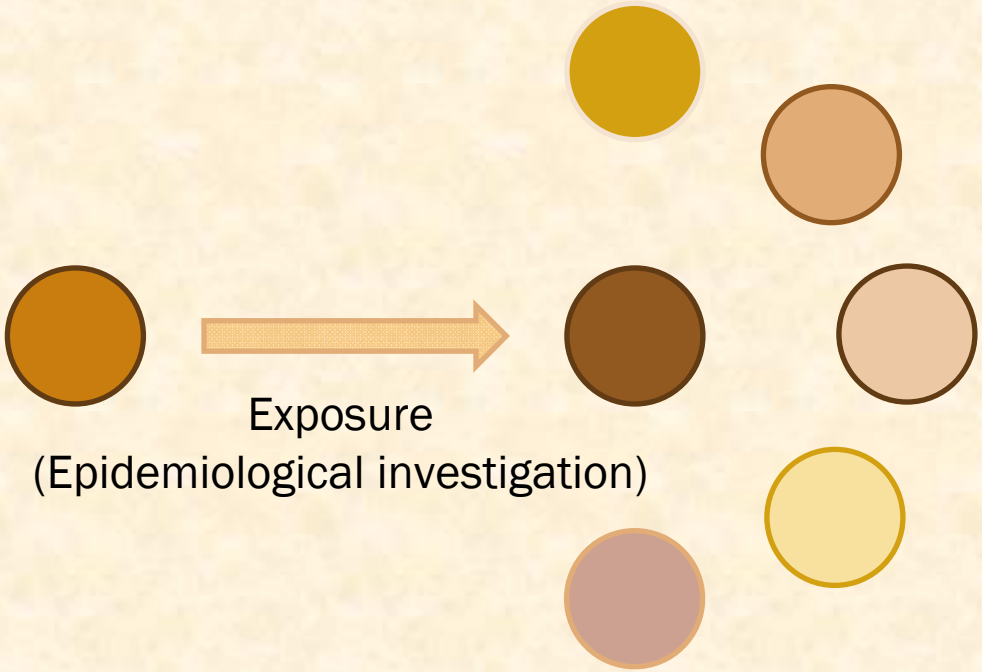
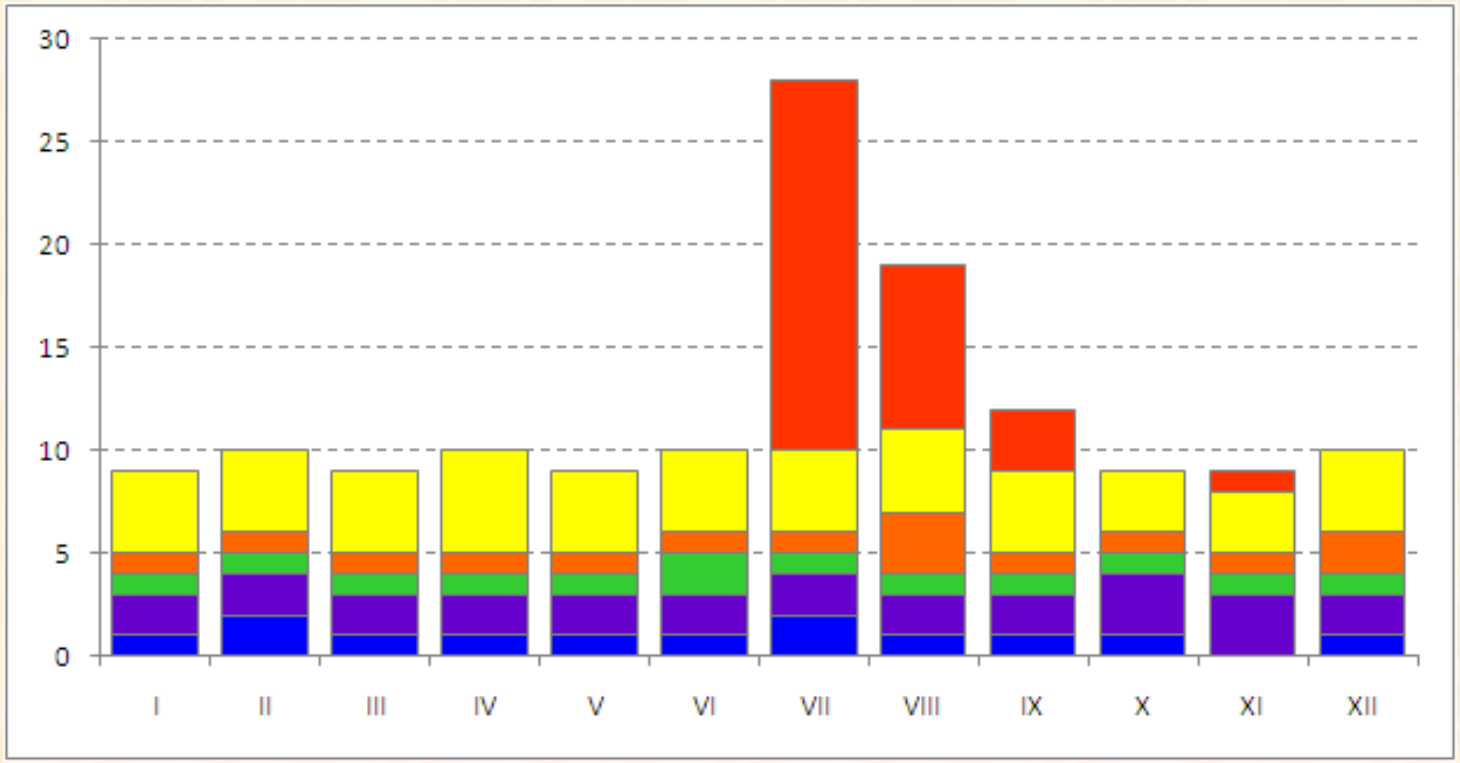


CONTROLS, CONTROLS, CONTROLS, …

Comparison (control) group(s) or knowledge of "background noise" necessary



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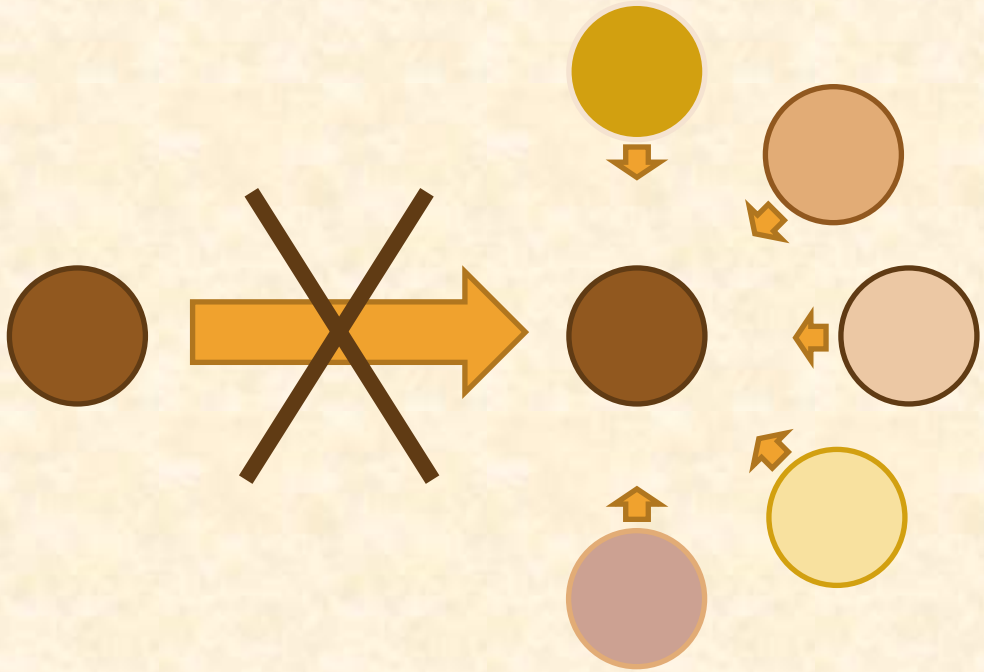

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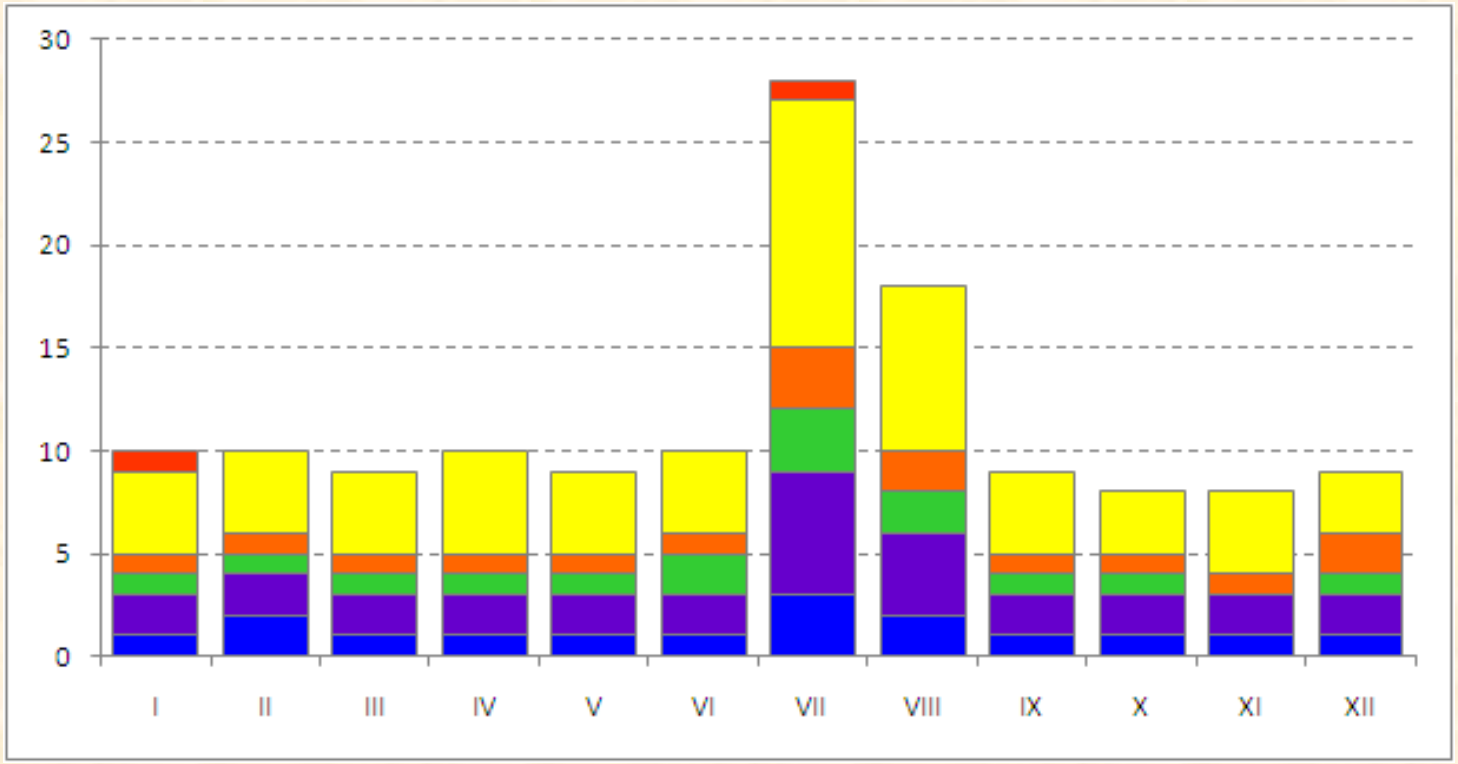

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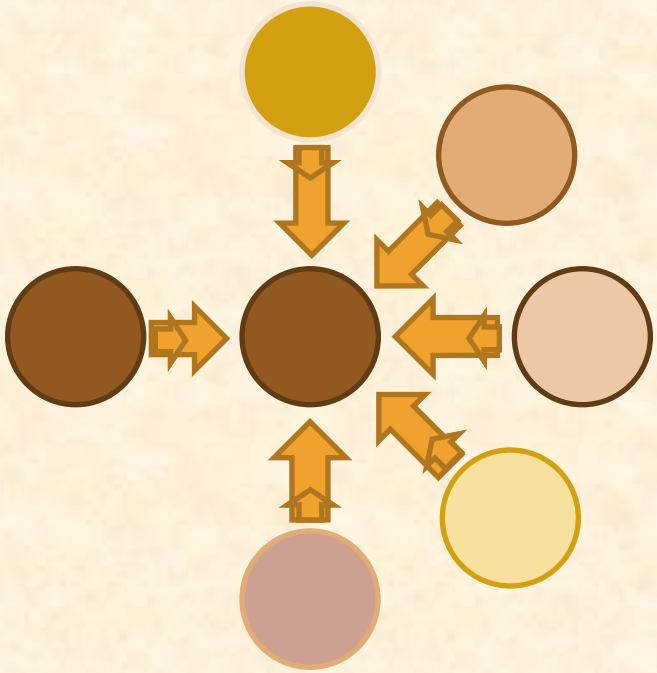


CONCLUSIONS

- Know endemic type diversity and distribution before concluding anything
- Know your method and its characteristics (discrimination/reproducibility/stability, etc.)
- Usefulness of systematic representative collections, or of molecular epidemiology monitoring of major pathogens
- Always support conclusions by epidemiological field investigations

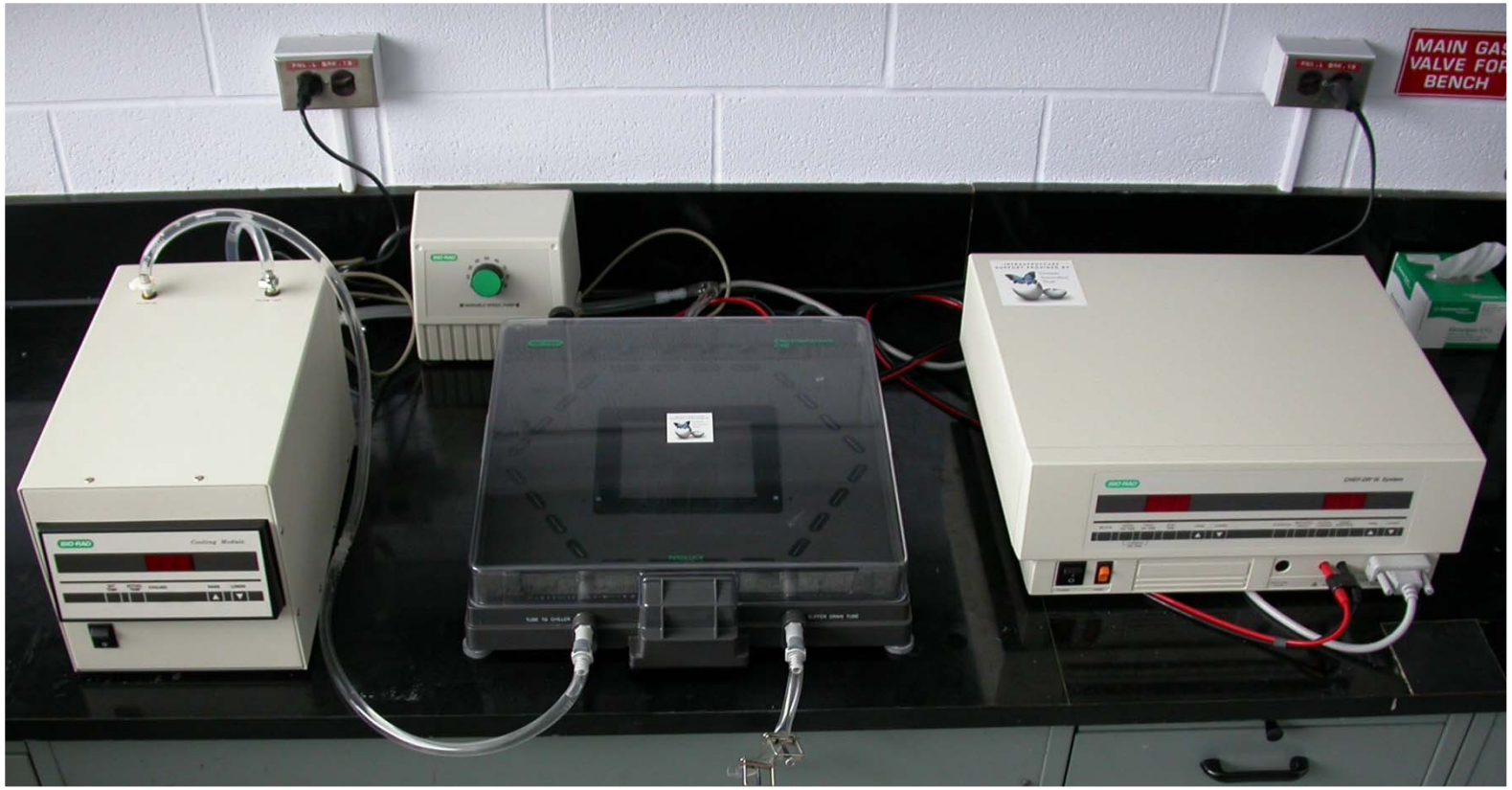
The good old gold standard of typing

PULSED-FIELD GEL ELECTROPHORESIS

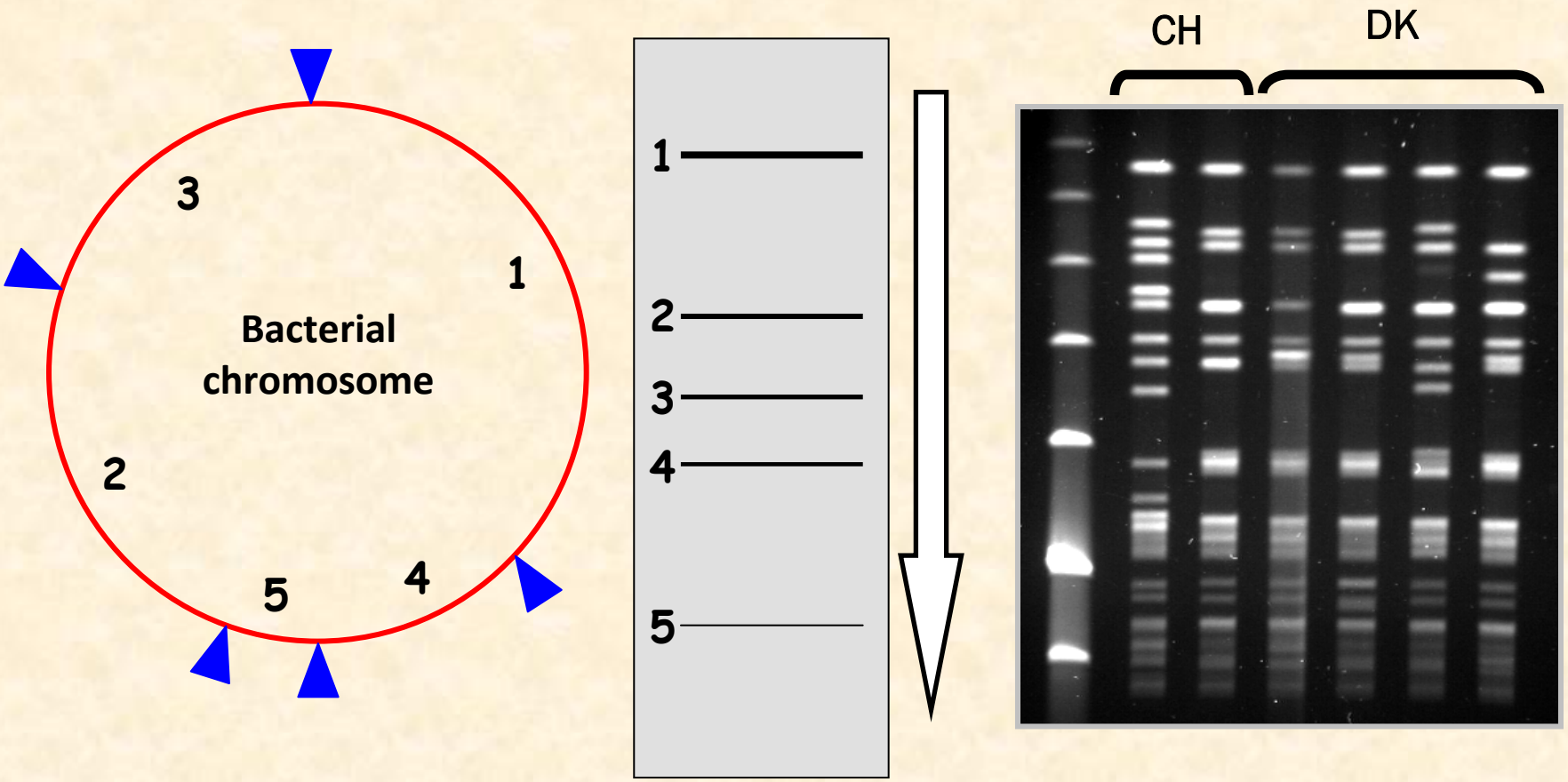
PFGE

- Basis for variation:
  - Variation in genome sequence (point mutations at restriction sites and recombinations between restriction sites)
- Genetic elements:
  - Large fragments of unknown nature, whole genome
- Procedure:
  - DNA extraction/purification
  - Digestion with “rare cutting” restriction enzyme
  - Gel Electrophoresis (pulsed-field gel electrophoresis)
- Not for cladistic !

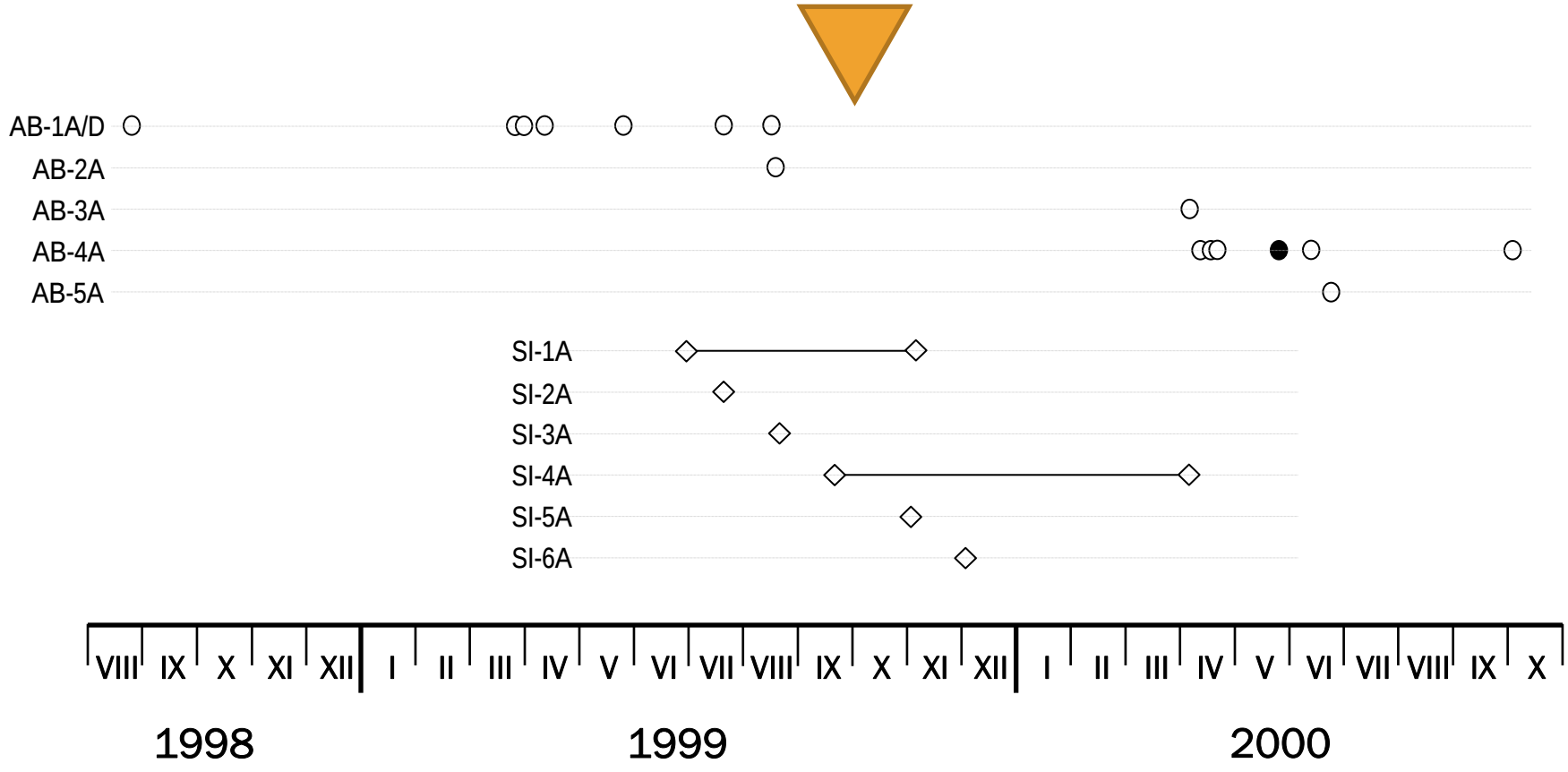
PFGE



PFGE



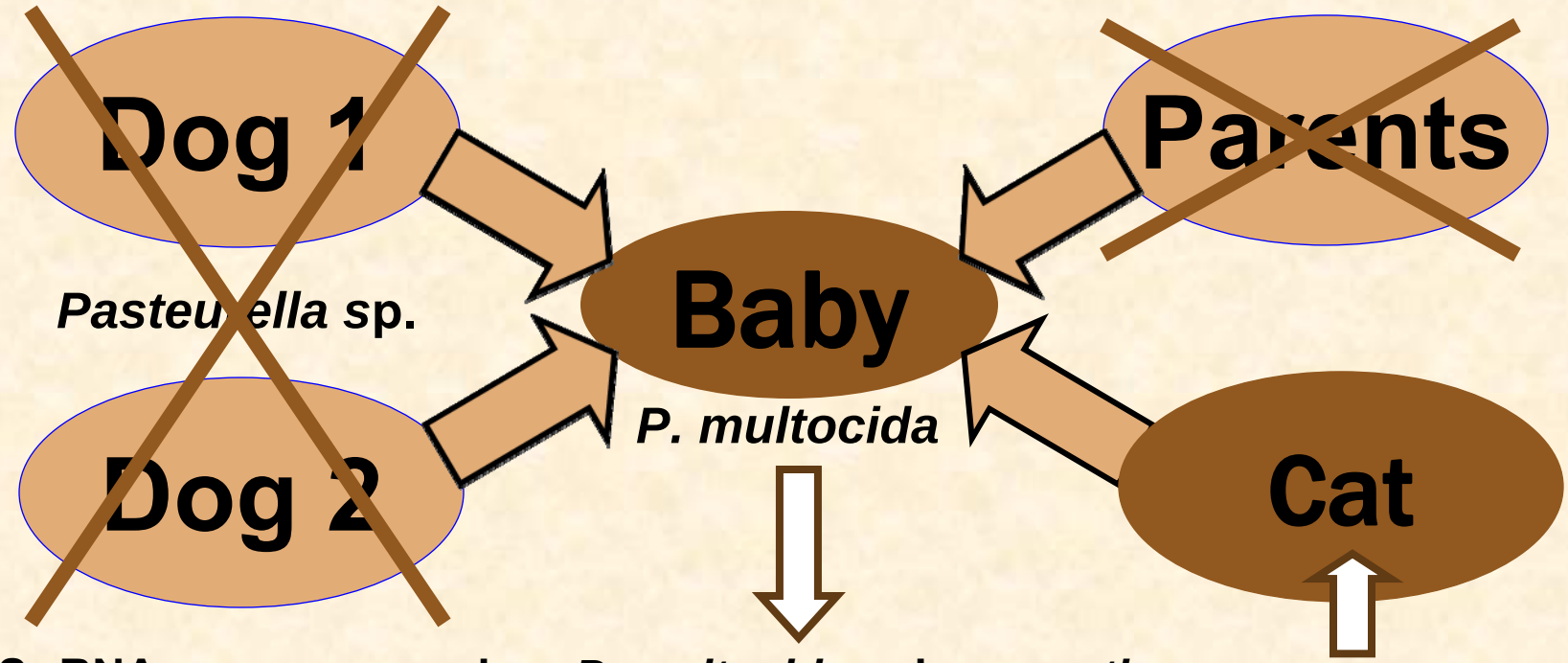
PFGE: NOSOCOMIAL INFECTIONS



S. pseudintermedius from different animals had different PFGE-profiles

PFGE: SOURCE TRACKING

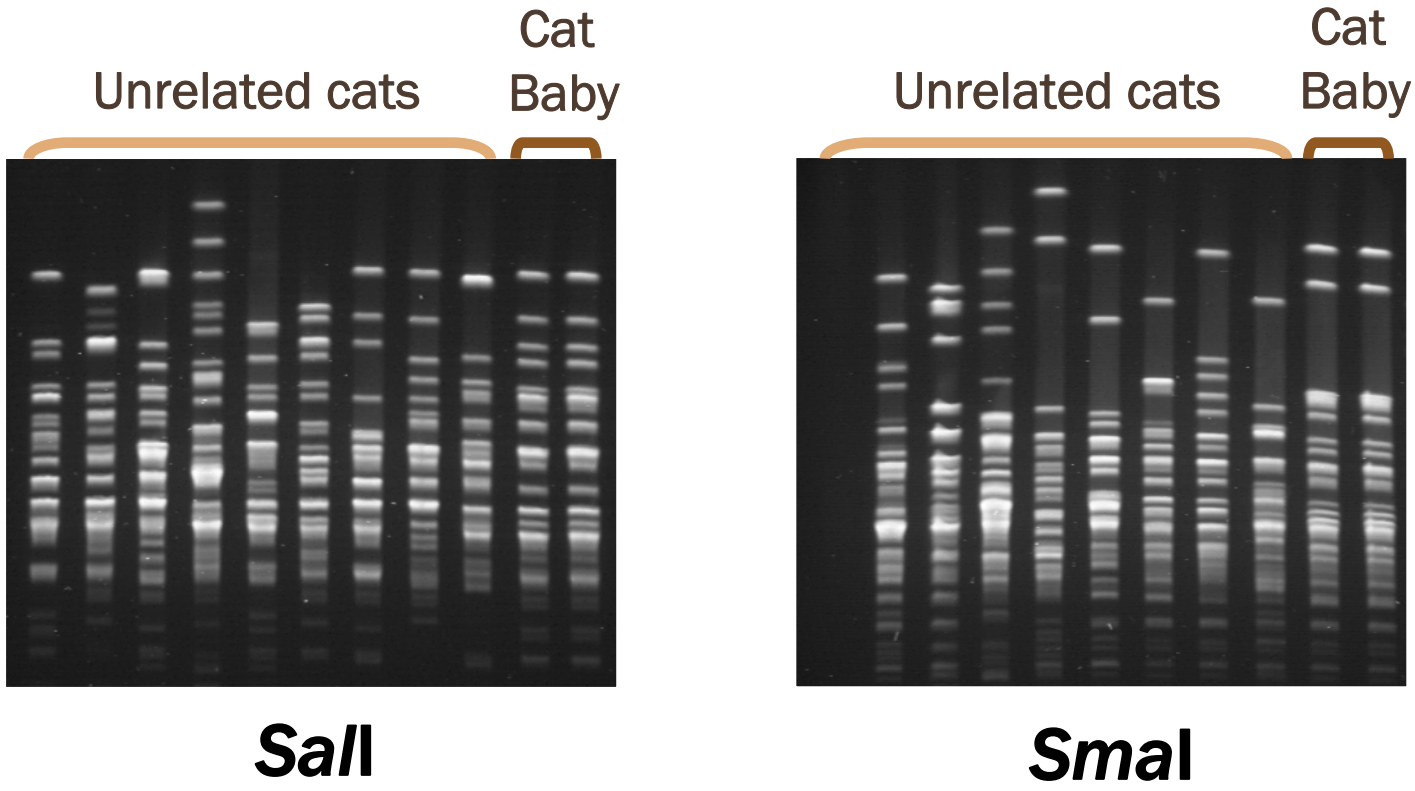
- A one-month-old baby hospitalized with a bacterial meningitis
- *Pasteurella multocida* isolated from CSF



16S rRNA gene sequencing: *P. multocida* subsp. *septica*

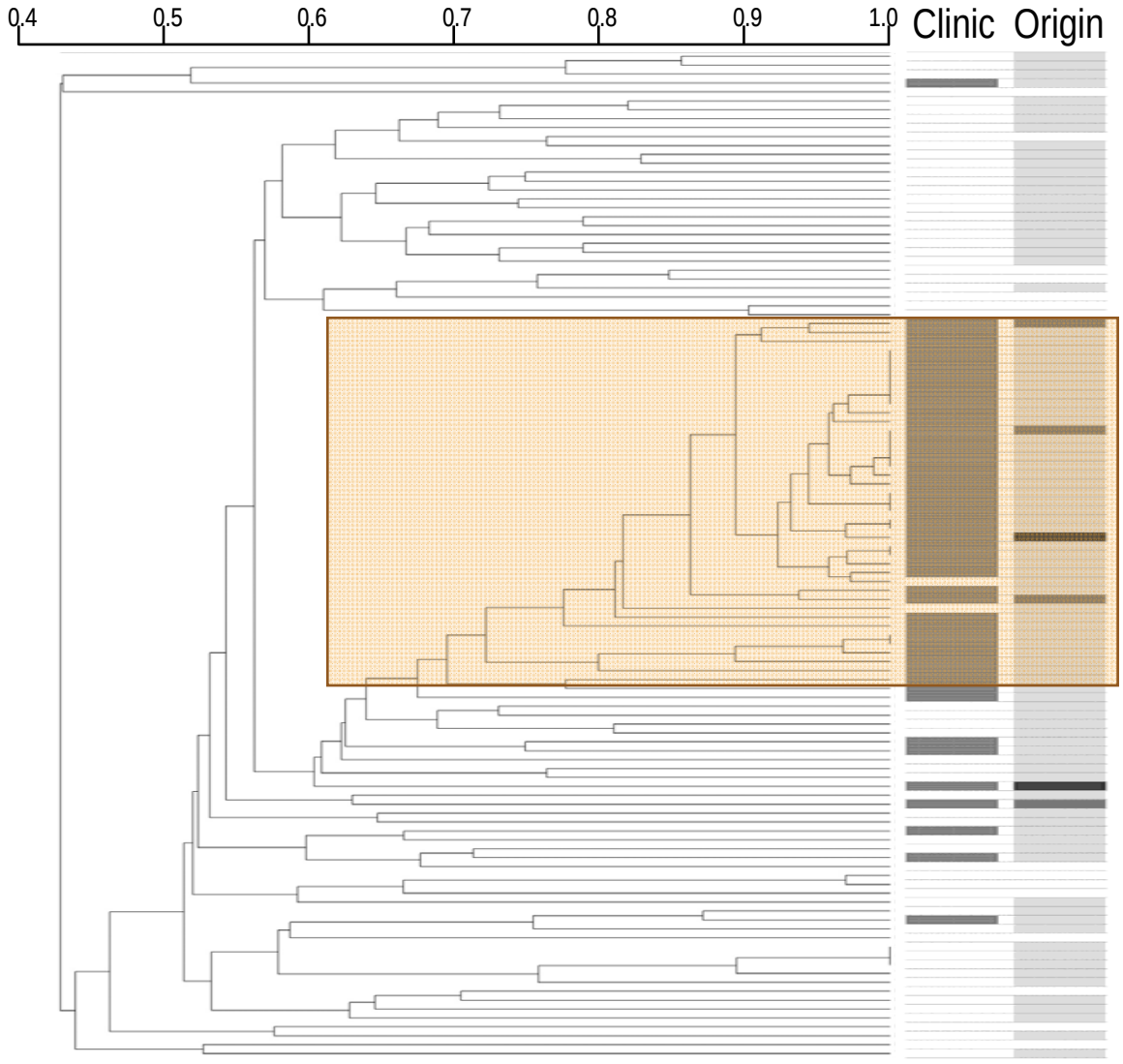
PFGE: SOURCE TRACKING

P. *multocida* subsp. *septica* typing



***Sal*I** ***Sma*I**

PFGE: A NEW *ENTEROCOCCUS CECORUM*



Sample

50 clinical isolates

50 birds

39 farms

ON, NFL, AB

63 intestinal isolates

39 birds

21 farms

ON, Belgium

Controls (cecal)

Multiple types per farm

(median pairwise similarity: 59%)

Multiple types per bird (median pairwise similarity: 62%

A NEW *ENTEROCOCCUS CECORUM*

Clinical isolates are different from cecal isolates from healthy birds with regards to antimicrobial susceptibility

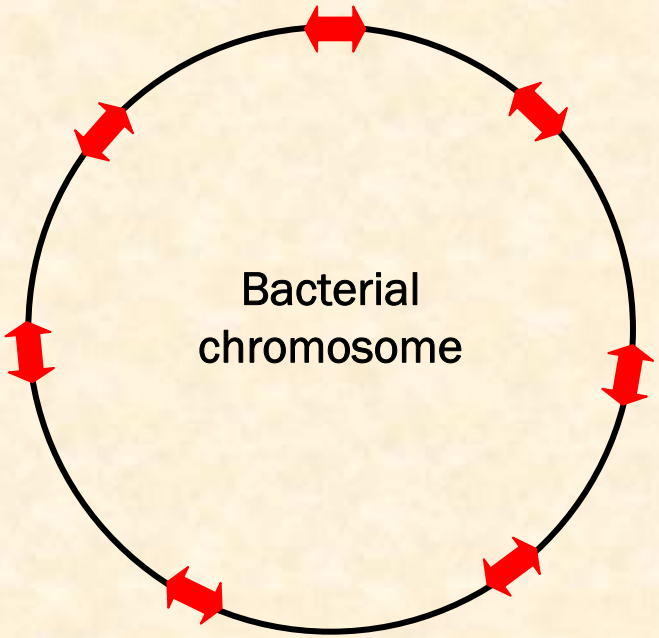
	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	MIC_{50}/MIC_{90}	%R	P	OR (95% CI)
PEN		11	12	11	16	12	8	1	2								0.5 / 2	≤3%	NS	
ERY			8			1		64									≥4 / ≥4	89%	0.031	11.3 (1.2-103.7)
ENR			4	15	25	4	25										0.5 / ≥2		<0.001	9.3 (2.8-30.5)
FFN						61	5	4	3								1 / 2		NS	
HL-STR									1	3	6	2	3	4	13	41	≥1024 / ≥1024	53%	<0.001	9.6 (3.0-31.2)
GEN							14	32	27								4 / ≥8		<0.001	12.2 (3.3-45.2)
TET								1	72								≥8 / ≥8	99%	NS	

	MIC_{50}/MIC_{90}	<1.5	1.5	2	…	3	…	96	…	≥256	%R	P	OR (95% CI)
BAC	≥ 256/ ≥ 256		3	1		1		1		67	93%	NS	

The new ones

MLST AND MLVA

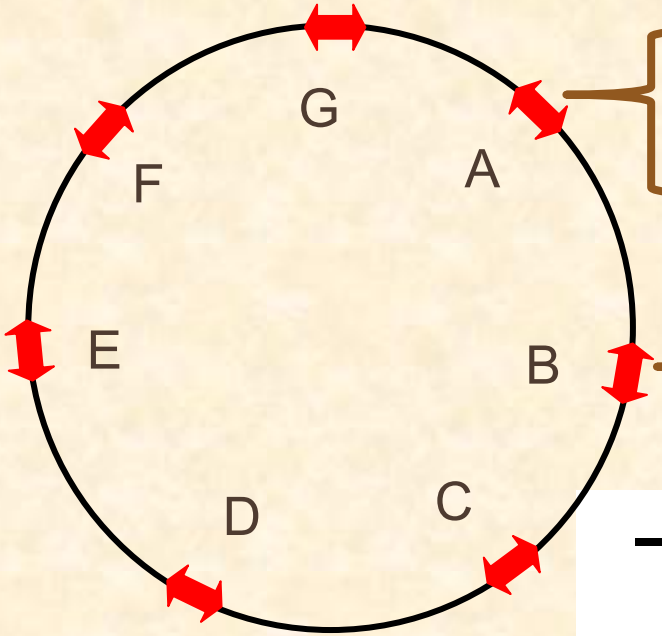
PFGE REPLACEMENT ?



MLST
Loci within house keeping genes
Sequencing PCR products
Allele identification at each locus
Allelic make up of strains determined

MLVA
Loci with variable number of tandem repeats
Sizing PCR fragments (occasional sequencing)
Allele identification at each locus
Allelic make up of strains determined

MLST: HOW IT WORKS

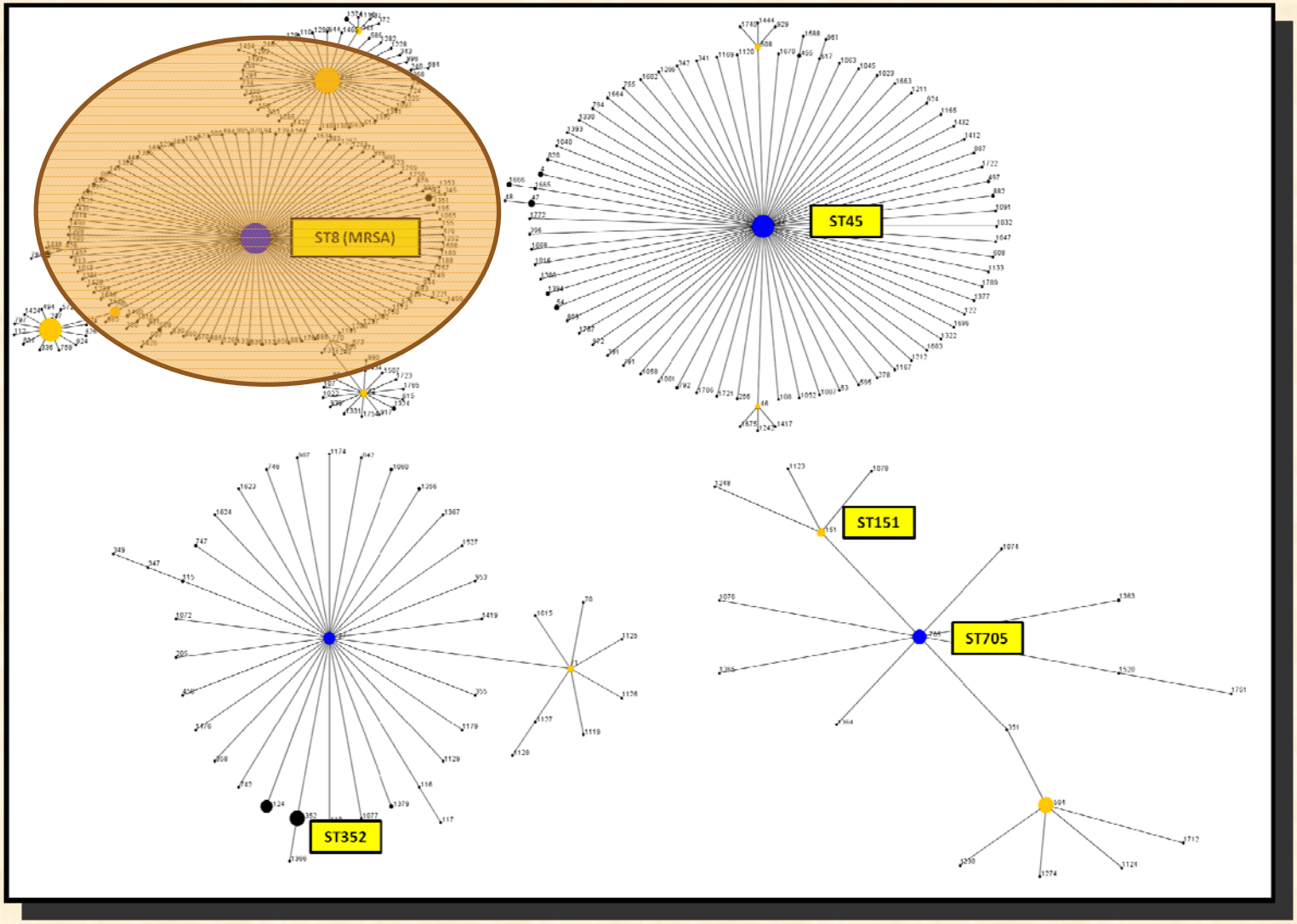


1 . . GGCAT**T**ACGTT**A**ACAA**C**CTCTGAG**A**GCCG . .
2 . . GGCAT**A**ACGTT**A**ACAA**G**CTCTGAG**A**GCCG . .
3 . . GGCAT**T**ACGTT**C**ACAA**G**CTCTGAG**G**GCCG . .

1 . . CAATATAC**G**GATTTCTCACAGGATAAAGT . .
2 . . CAATATAC**G**GATTTCTCACAGGATAAAGT . .
3 . . CAATATAC**A**GATTTCTCACAGGATAAAGT . .

	Locus						
	A	B	C	D	E	F	G
Strain 1	1	1	1	1	1	1	1
Strain 2	2	1	2	1	2	1	2
Strain 3	3	2	1	1	2	2	3

MLST: FOR A MORE GLOBAL PICTURE



MLST: FOR A MORE GLOBAL PICTURE

bla$_{CTX-M}$ positive *E. coli* isolates from canine UTIs

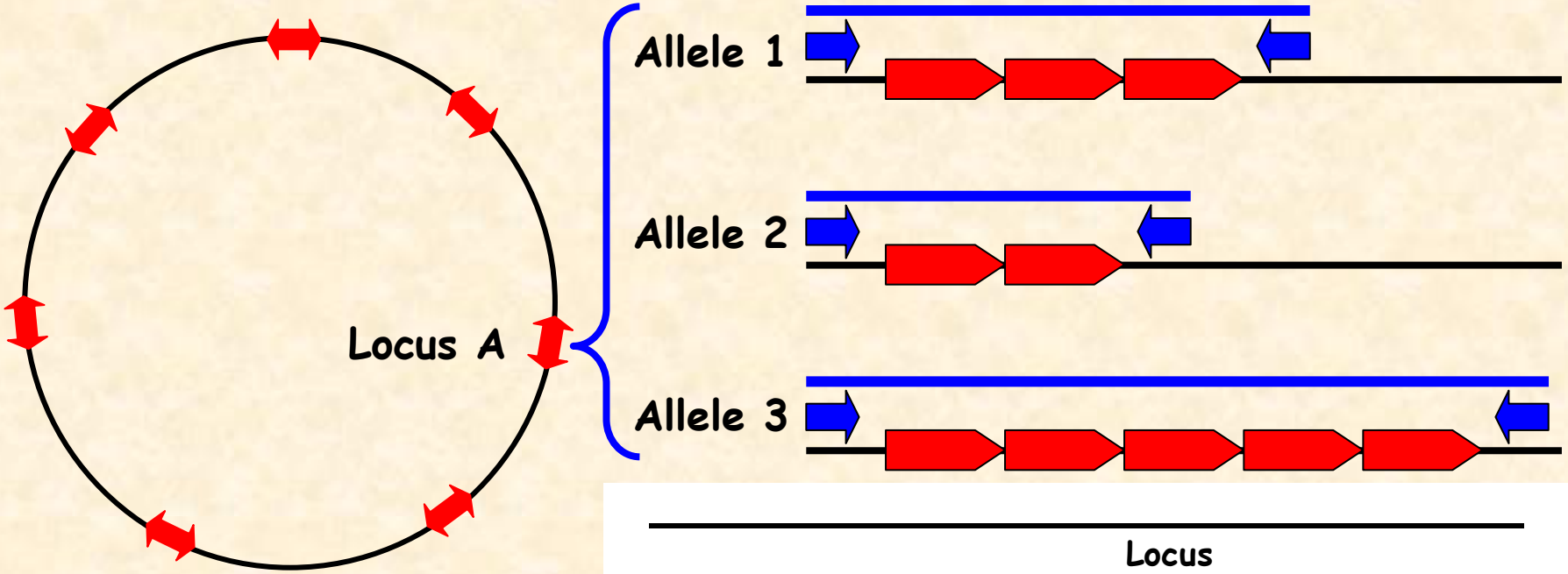
Patient	Isolate name	Serotype	Source	CTX-M group	CTX-M location
					Chromosome
					Plasmid
					Plasmid
C*		01:H16	(Convenience)	CTX-M-15	Plasmid
D	Sur 314	025:H4	Ontario (Convenience)	CTX-M-15	Plasmid
E	Sur 255	025:H4	Ontario (Convenience)	CTX-M-15	Plasmid
F	115459	O153:H34	Ontario (Convenience)	CTX-M-15	Plasmid

Human pandemic ST131:

ESBL + fluoroquinolone resistance

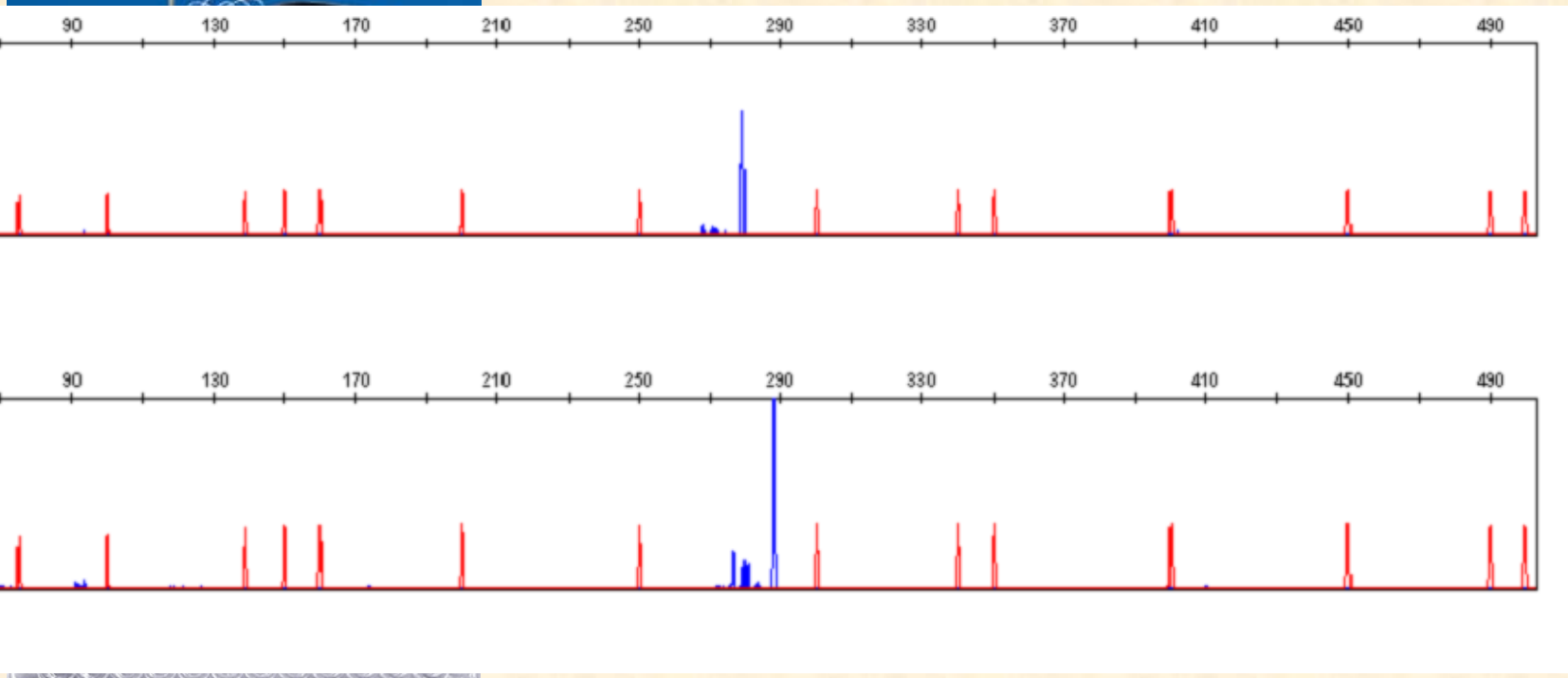
*** These two samples were recovered from the same patient at more than a month interval**

MLVA: HOW IT WORKS



	Locus						
	A	B	C	D	E	F	G
Strain 1	3	10	1	11	17	1	5
Strain 2	2	10	3	11	20	1	2
Strain 3	5	20	1	11	20	2	8

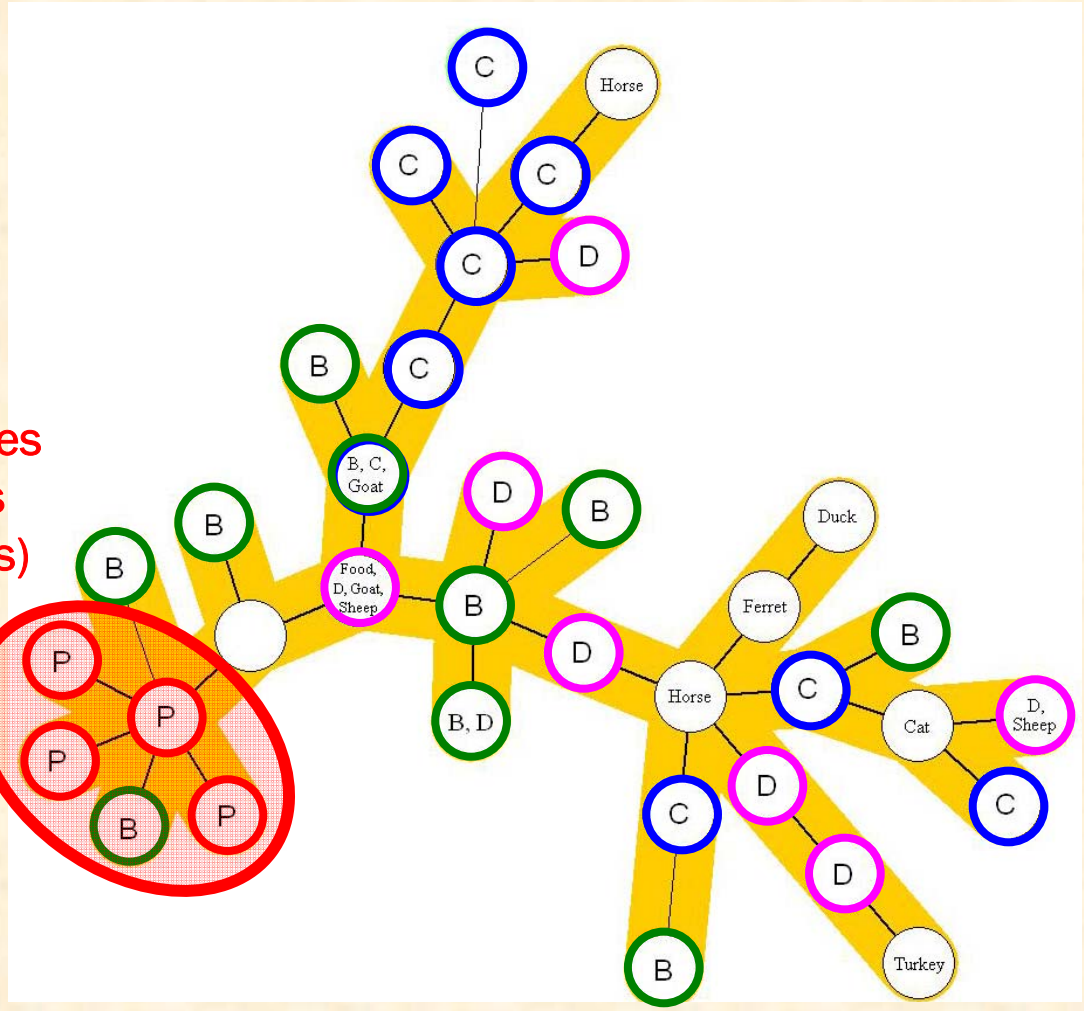
MLVA: *C. PERFRINGENS*



ROBOTICS

PFGE

10 isolates from pigs (10 farms)



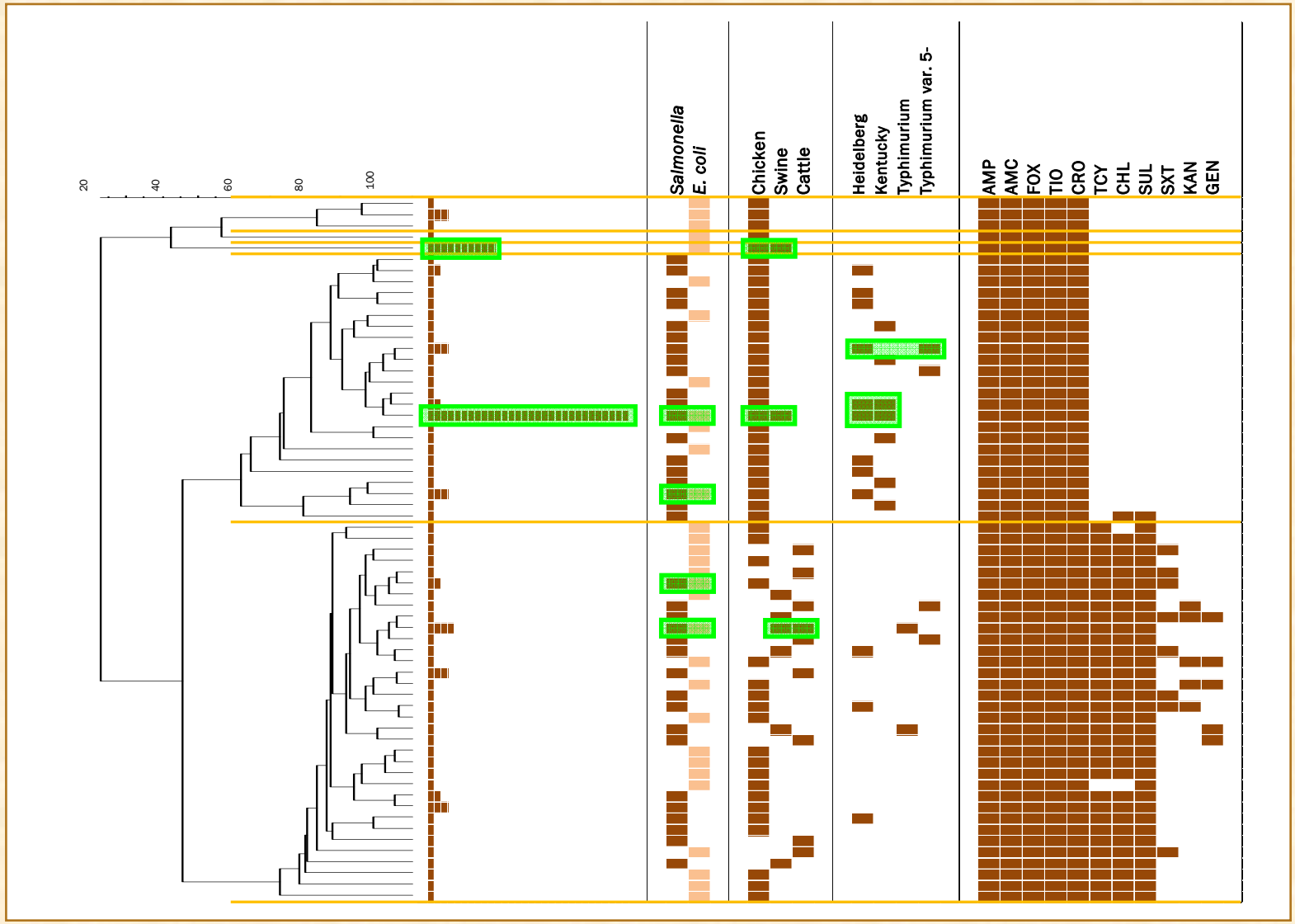
<u>54 epidemiologically unrelated isolates</u>

12 chicken
11 cattle
10 swine
10 dogs
3 goats
2 horses
1 sheep
1 turkey
1 duck
1 cat
1 ferret
1 foodborne outbreak

Why not only susceptibility testing ?

ANTIMICROBIAL SUSCEPTIBILITY

CEPHAMYCINASES: PROMISCUITY

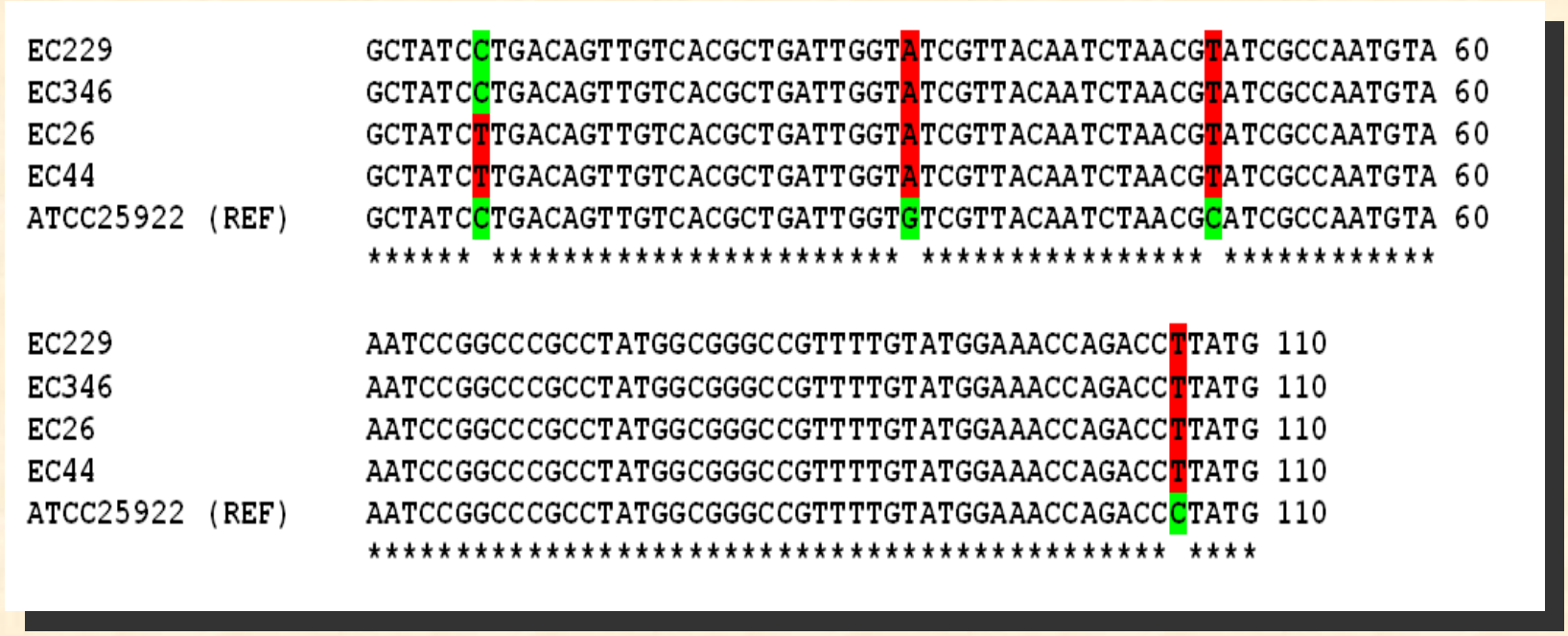


CEPHAMYCINASES: NOT SO MOBILE ONES

Chromosomal AmpC in *E. coli*

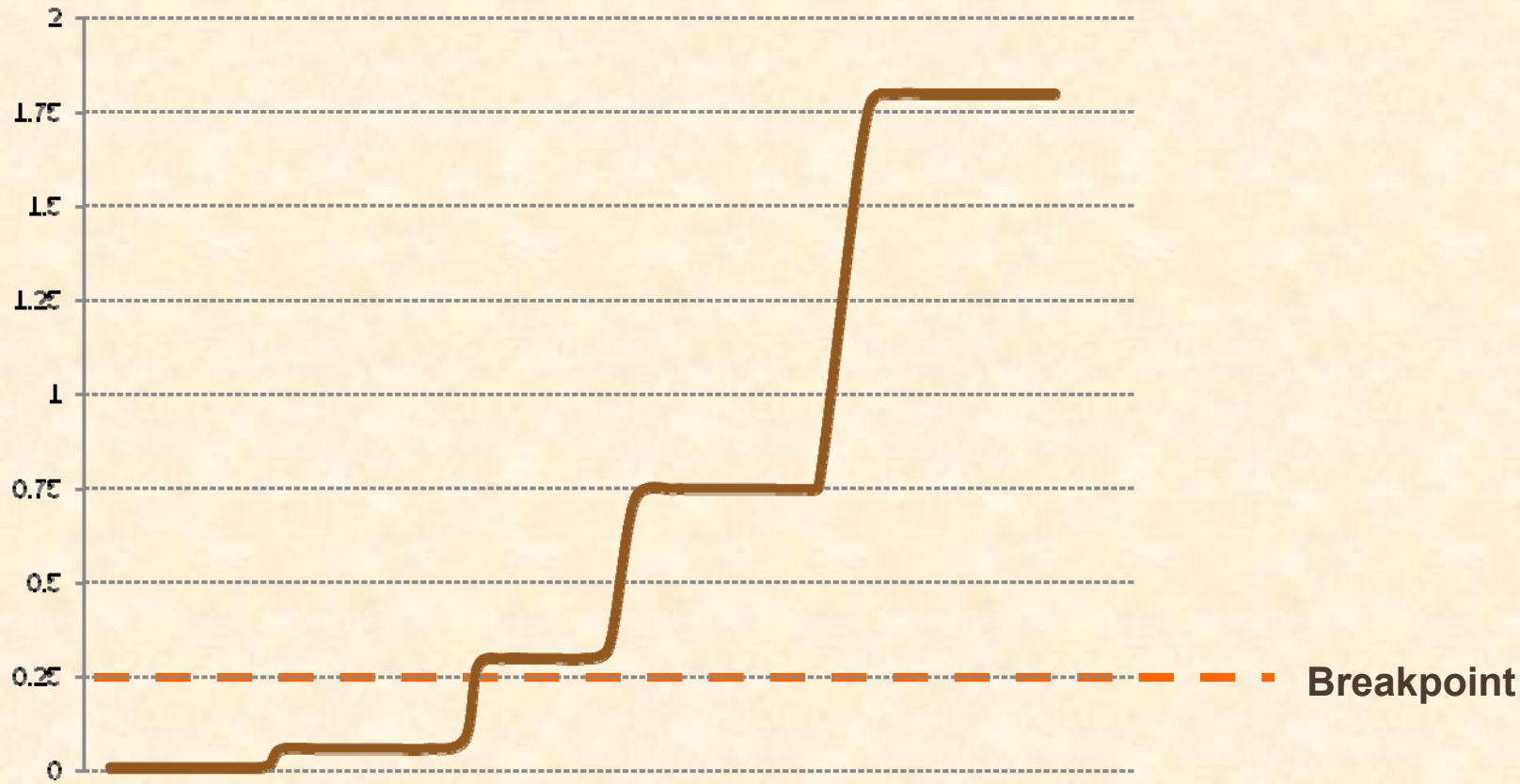
Mutations in AmpC gene promoter region

Non-transferable



FLUOROQUINOLONES

HGT Helping mutants



FLUOROQUINOLONES: QNR GENES ARE HERE

- *qnrS* and *qnrB* present in *E. coli* and *Salmonella*
- Partial sequence of *qnrB* plasmid: S. Heidelberg from horses
  - 371 ORFs identified
    - 119 ORFs known but unassigned or unclassified
    - 37 ORFs plasmid **transfer**, replication (RepHI2), maintenance, partitioning, copy number, **addiction**
    - **10 ORFs recombinases, transposases, integrases**
    - 10 ORFs restriction, modification, methylation, UV protection, DNA-binding proteins
    - 10 ORFs **exported proteins**
    - 9 ORFs membrane proteins
    - 4 ORFs regulation and modulation of expresssion
  - Resistances (ORFs >2000bp): Tellurite, arsenate, mercury & heavy metals, quinolones (*qnrB*), aminoglycosides (*strA/strB*, *aph(3')-I*, *aac(3')*), β-lactams (bla_{TEM}), macrolides (*ereA*, erythromycin esterase), rifampin (ADP-ribosyl transferase)
  - Additional resistances by PCR: Florfenicol (*floR*), sulfonamides (*sul1*, *sul2*), aminoglycosides (*aadA*), β-lactams (bla_{SHV})

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