



An Integrated View on *Listeria* Genomics and Virulence

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Institut Pasteur



EMBO
Conference

The programme for this event
was reviewed and approved by the
EMBO Course Committee.

Problems of Listeriosis ISOPOL XIX

14 – 17 June 2016 | Institut Pasteur, Paris, France

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REGISTRATION

Registration deadline

31 May 2016

Abstract submission deadline

15 May 2016

Student 250 EUR

Academic 400 EUR

Industry 600 EUR

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ISOPOL2016@PASTEUR.FR

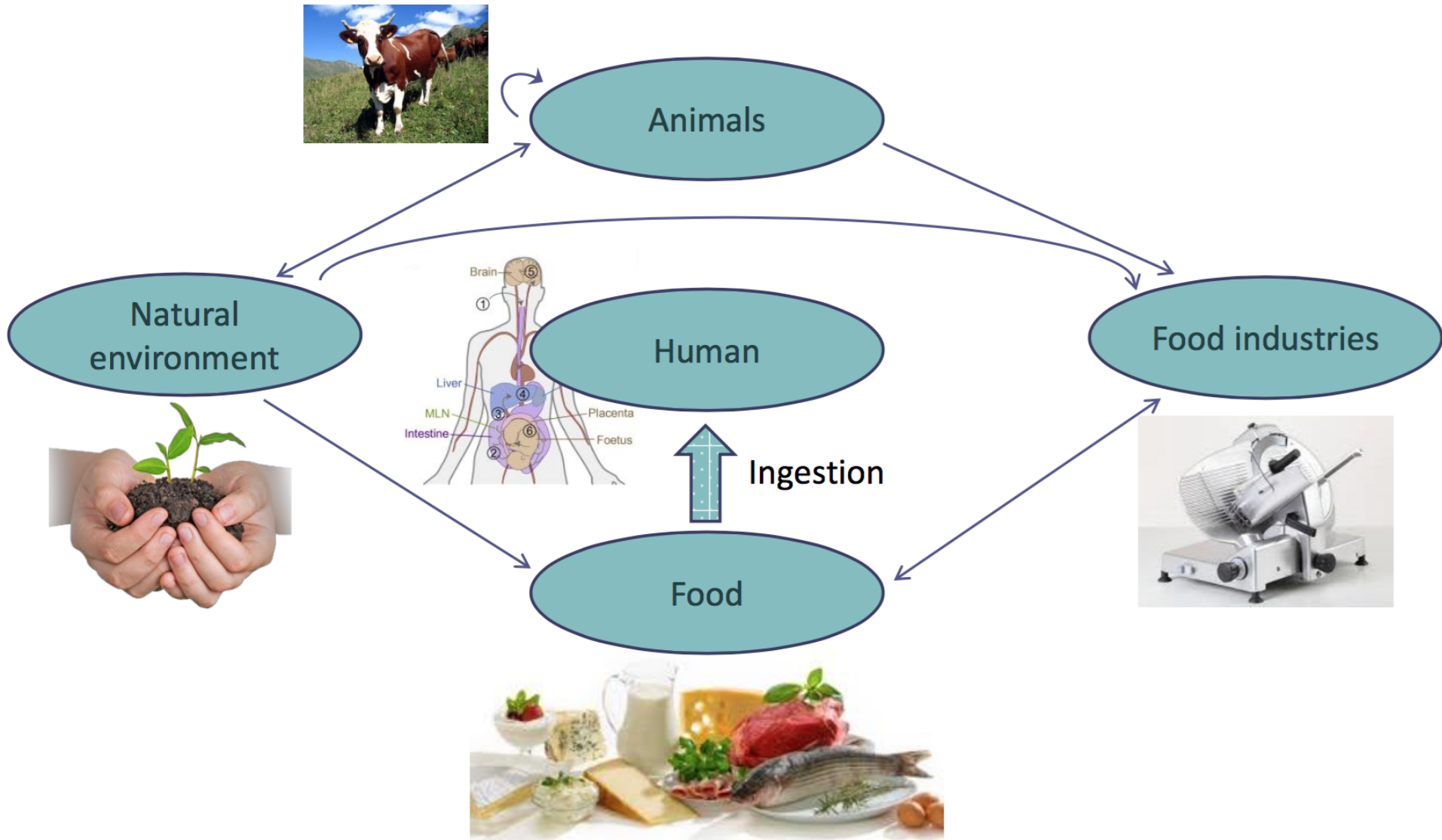
<http://www.isopol2016.org>



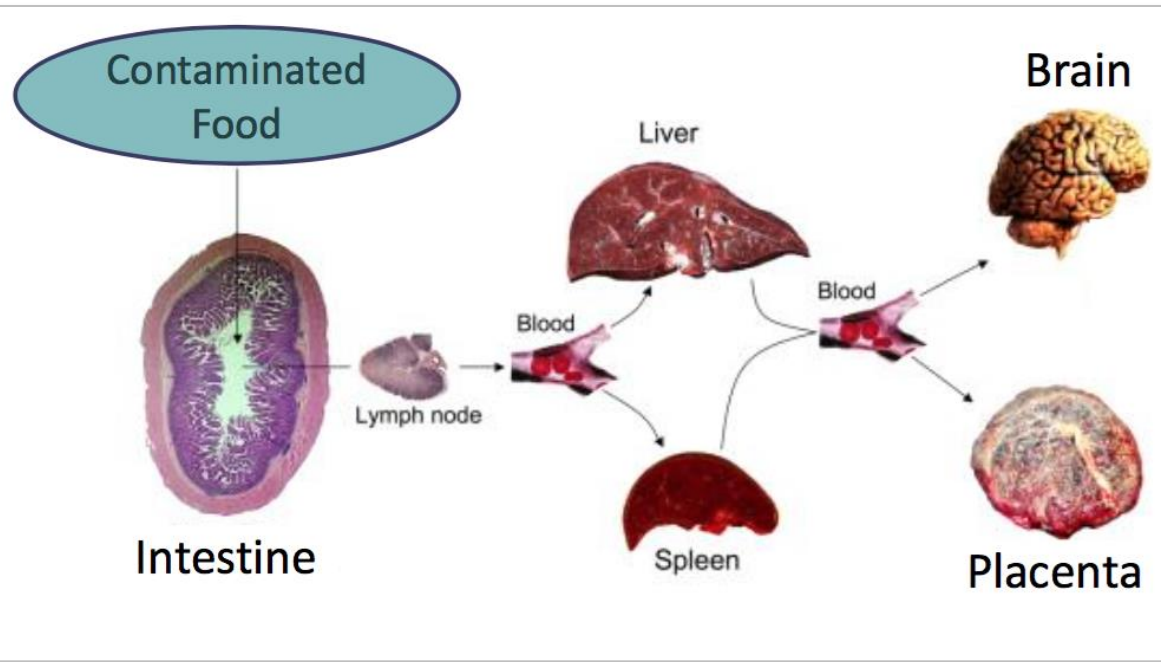
Institut Pasteur, Paris, June 14-17, 2016



Listeria monocytogenes is everywhere, and infects humans through food



Listeria monocytogenes invasive infections



- CNS infection
- Bacteremia
- Materno-neonatal infection

- ~ 300 human cases/year in France (1,600 in the USA)
- Severity of invasive forms (hospitalization rate around 100%)
- Mortality rate of 20 to 30 % (up to 45% in case of CNS infection)
- Strict control procedures in food industry

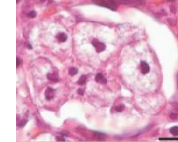
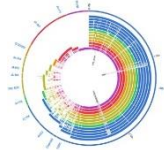
Objectives

GENOTYPE

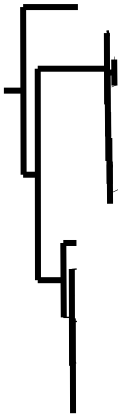


PHENOTYPE

2



1



Population structure
Strain nomenclatures
Databases
Genome dynamics

Phenotypic diversity
Link genotype-phenotype

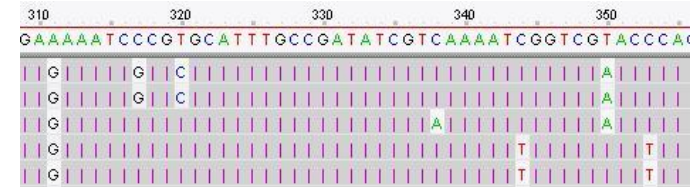
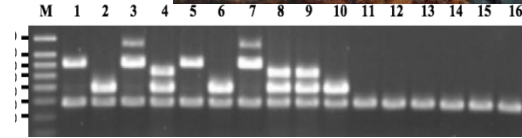
3



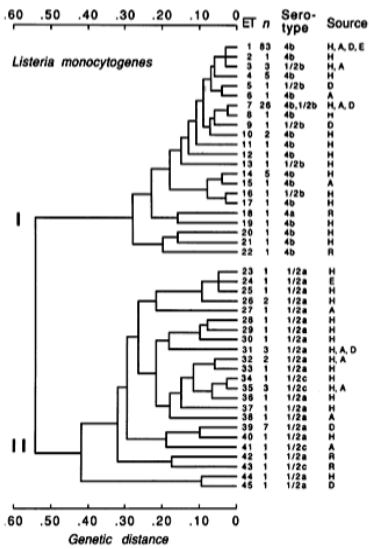
Applications: diagnostic, epidemiology, risk assessment...

L. monocytogenes strain typing methods

- Serotyping
- Pulsed-field gel electrophoresis (PFGE)
- Ribotyping
- Amplified Fragment Length Polymorphism (AFLP)
- Multilocus sequence typing (MLST)
- Multi-virulence sequence typing (MvLST)
- Multilocus VNTR Analysis (MLVA)
- Single Nucleotide Polymorphisms (SNP)
- Genome sequencing



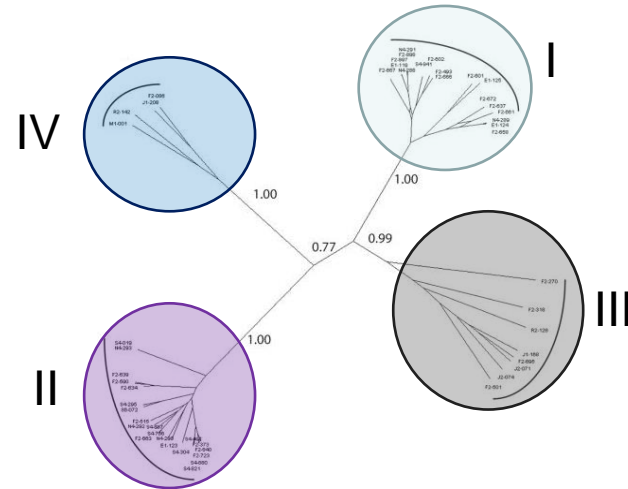
L. monocytogenes: four phylogenetic lineages, of which two are epidemiologically important



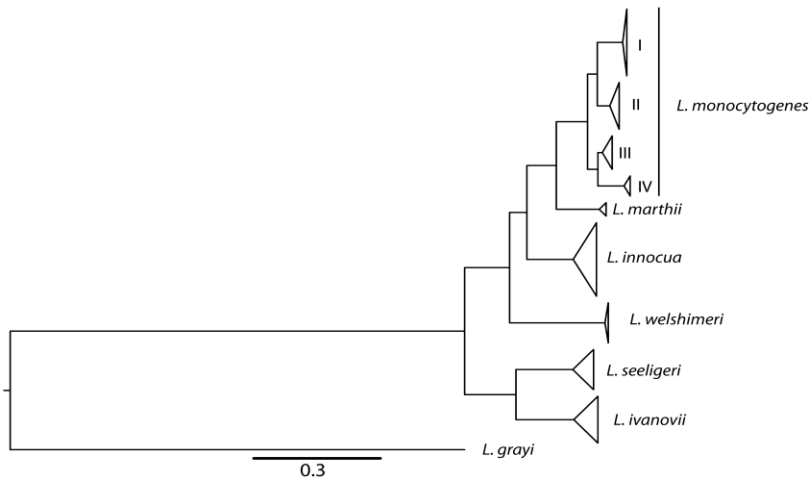
4b, 1/2b

1/2a, 1/2c

Piffaretti et al., 1989



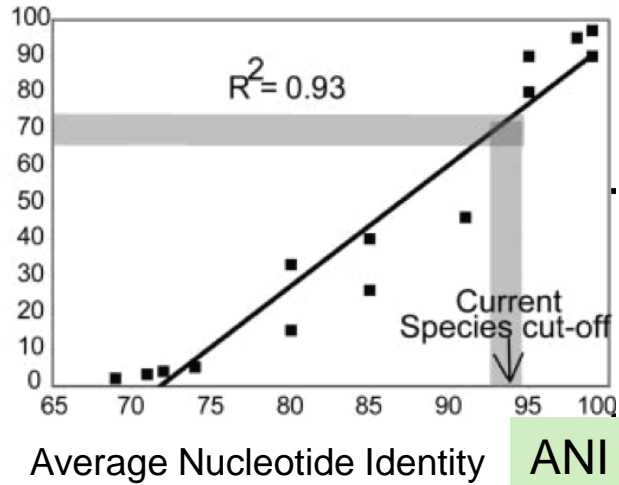
Tsai et al., 2011



Den Bakker et al., 2010

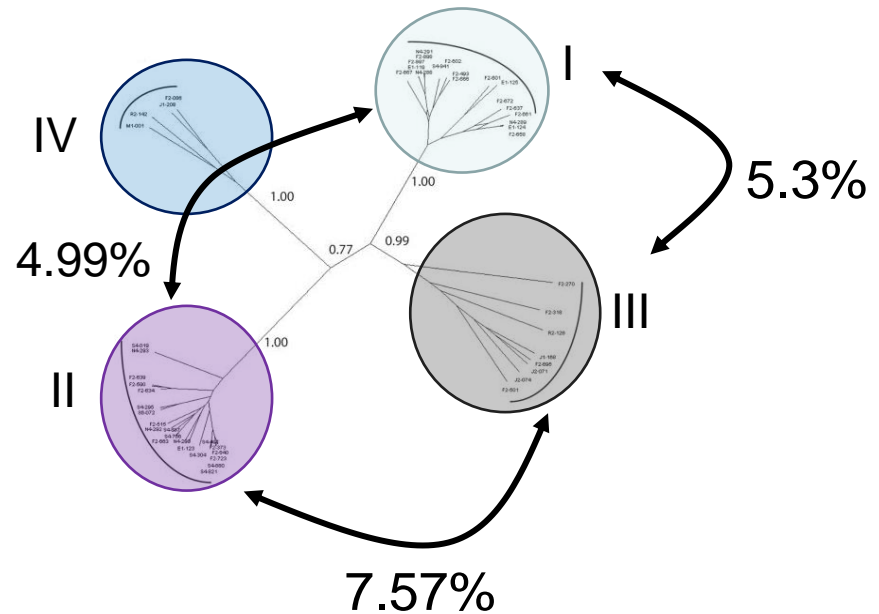
L. monocytogenes lineages ≈ distinct genomic species

DNA-DNA reassociation



Konstantinidis & Tiedje 2005

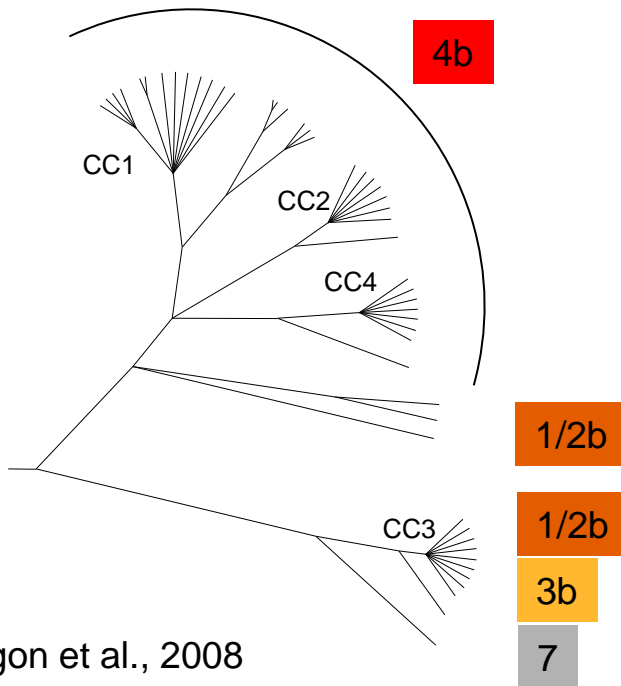
ANI among *L. monocytogenes* lineages:



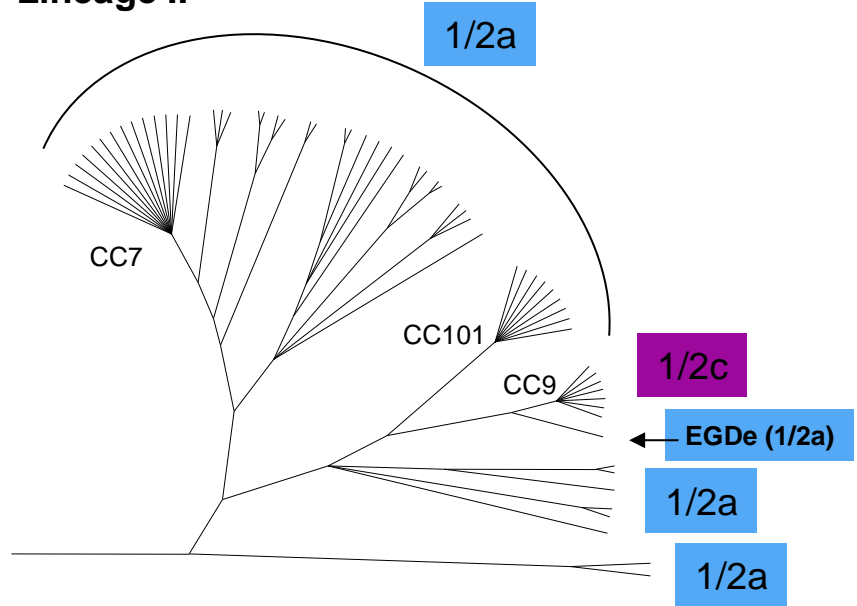
Major *L. monocytogenes* serotypes ≈ lineages

Lineage	Serotypes
I	1/2b, 4b, 4d, 4e, 3b, 7
II	1/2a, 1/2c, 3a
III	4a, 4b, 4c
IV	4b

Lineage I



Lineage II

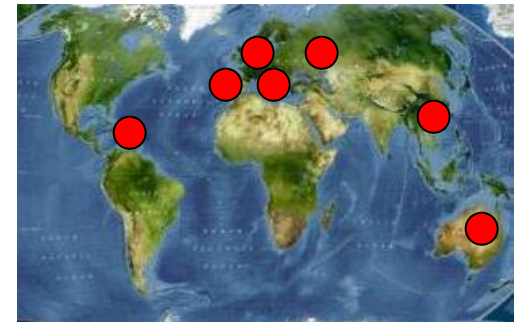


Listeria monocytogenes Multilocus Sequence Typing

www.pasteur.fr/mlst

Institut Pasteur MLST Databases

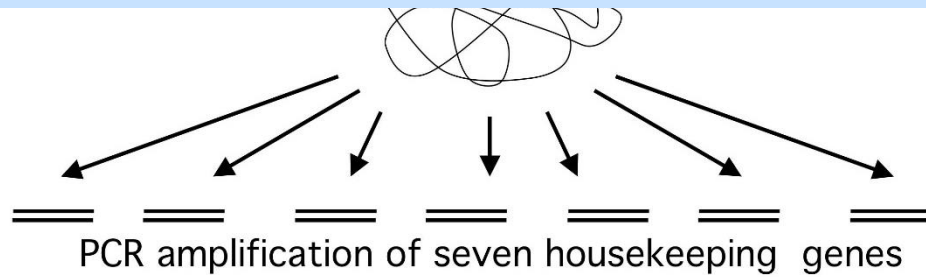
ST	abcZ	bglA	cat	dapE	dat	ldh	lhkA
1	3	1	1	1	3	1	3
2	1	1	11	11	2	1	5
3	4	4	4	3	2	1	5
4	1	2	12	3	2	5	3
5	2	1	11	3	3	1	7
6	3	9	9	3	3	1	5
7	5	8	5	7	6	2	1
8	5	6	2	9	5	3	1
9	6	5	6	4	1	4	1
10	3	1	20	1	3	1	3



~3,500 entries, > 1000 STs

> 60 labs

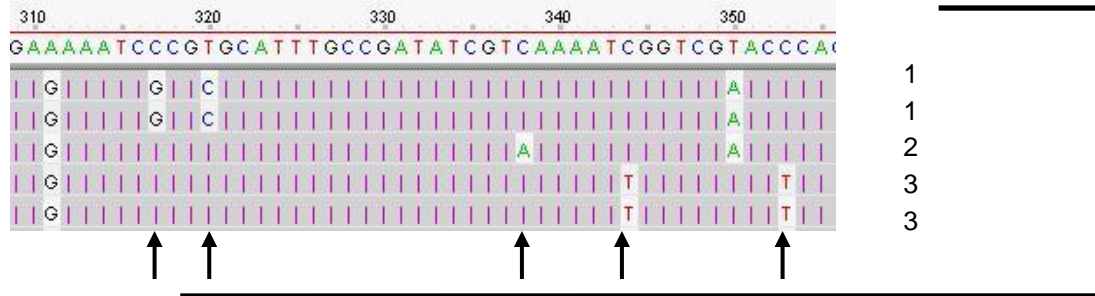
Common language / strains



Sequencing on both strands, approx. 500 bp

• Sequence of one gene (*lhkA*, 480 bp)

• Allele



<i>abcZ</i>	<i>bglA</i>	<i>cat</i>	<i>dapE</i>	<i>dat</i>	<i>ldh</i>	<i>lhkA</i>
3	1	1	1	3	1	3

Sequence type (ST)	Alleles
1	3 1 1 1 3 1 3
2	1 2 1 7 22 39 7
3	3 1 1 1 3 1 2

Salcedo et al. 2003

Ragon et al. 2008

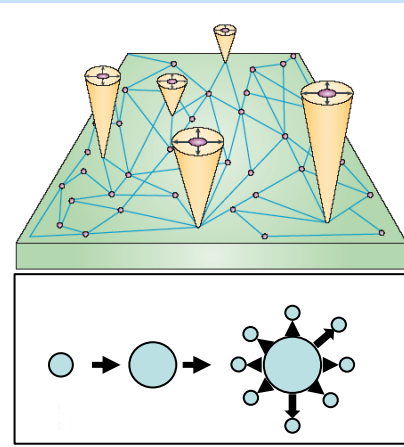
MLST: major *L. monocytogenes* clones

Ragon et al 2008

Chenal-Francisque et al 2011

Haase et al 2014

...



- CC2
- CC6
- CC4
- CC1
- CC5
- CC59
- CC87
- CC3

Lineage I

Lineage II

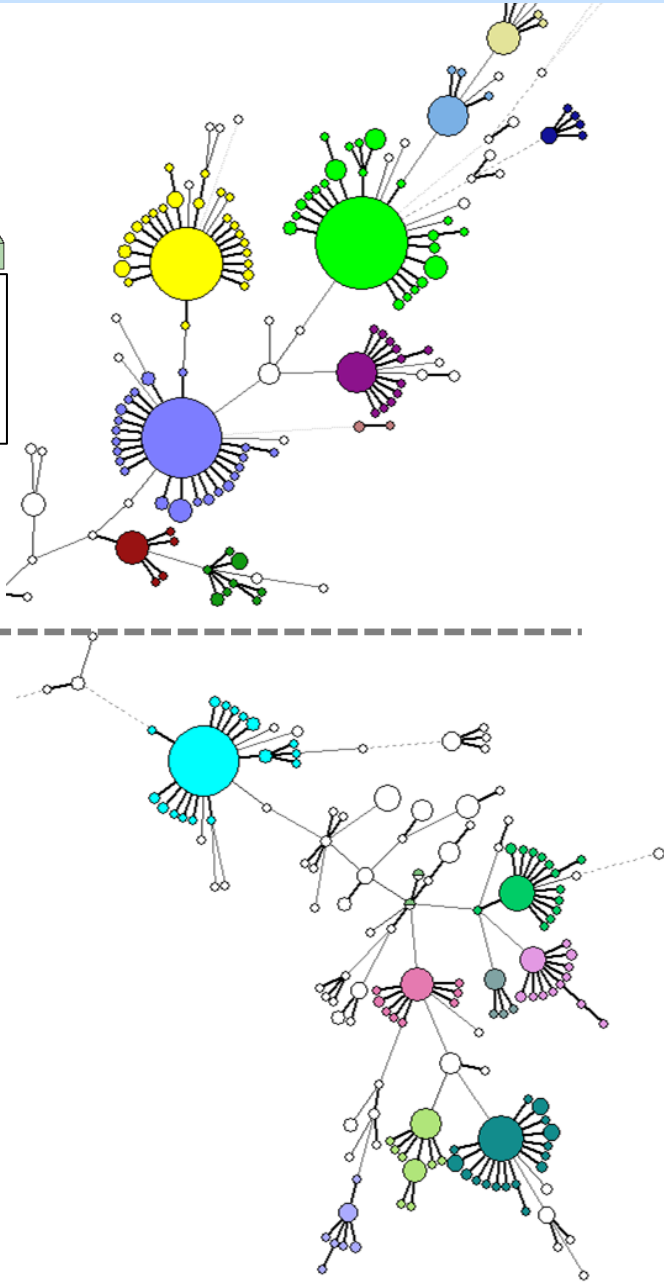
- CC89
- CC121
- CC11
- CC101
- CC193
- CC31
- CC13
- CC20
- CC7
- CC8
- CC155
- CC18
- CC9

Lineage III

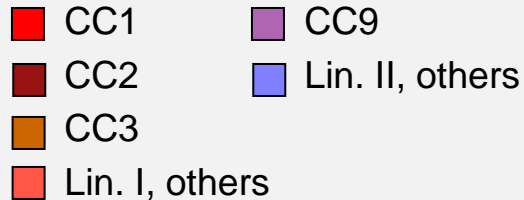
Lineage IV *L. innocua*



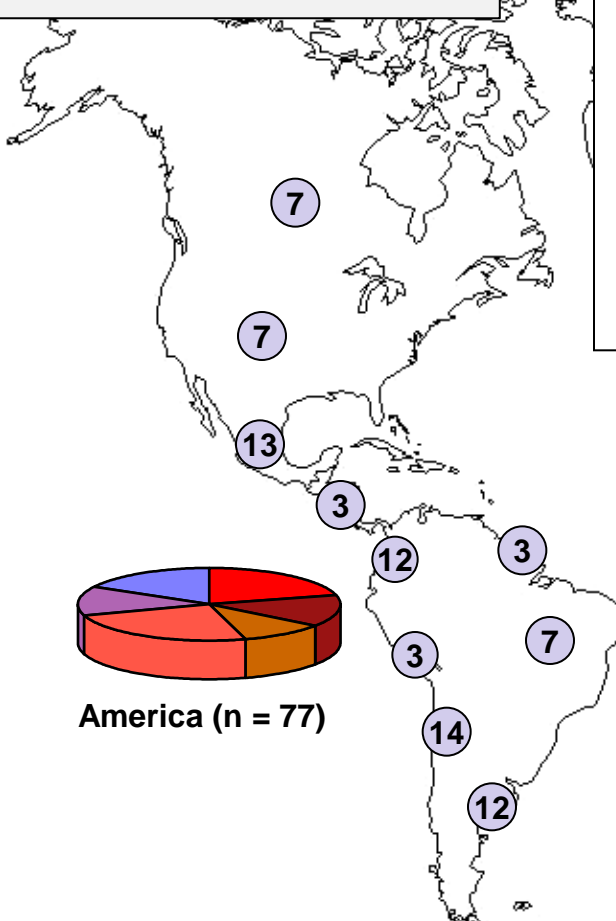
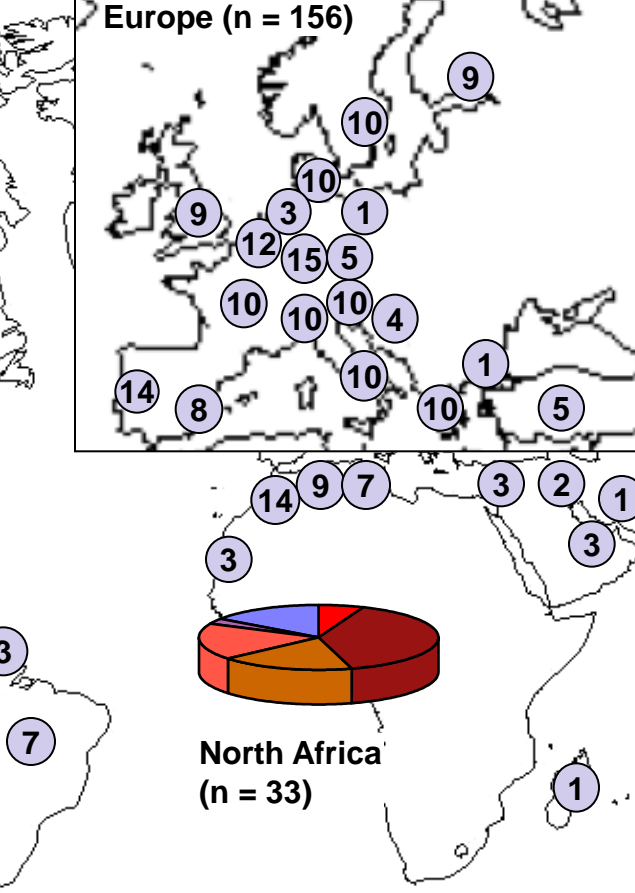
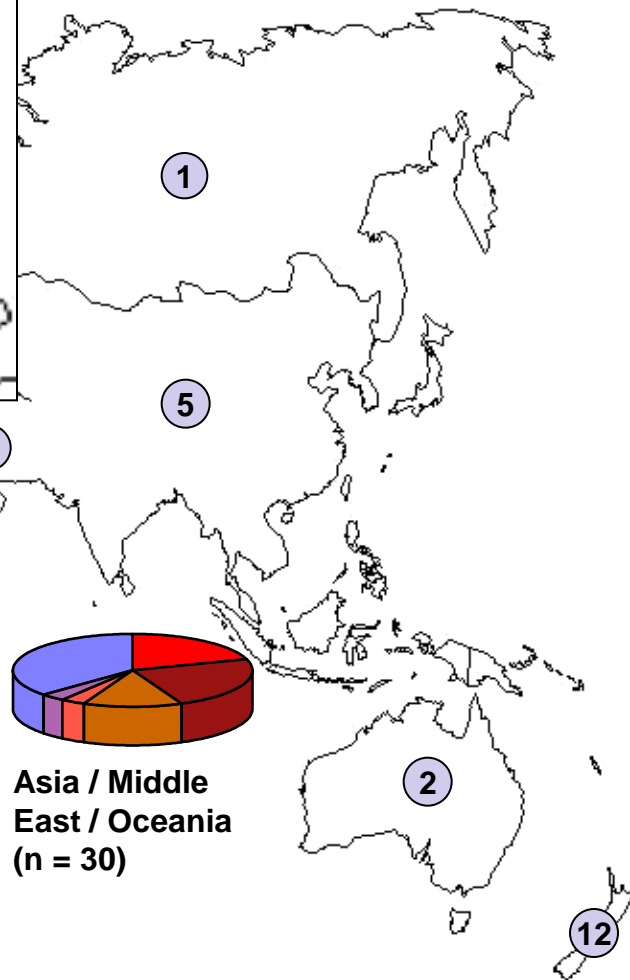
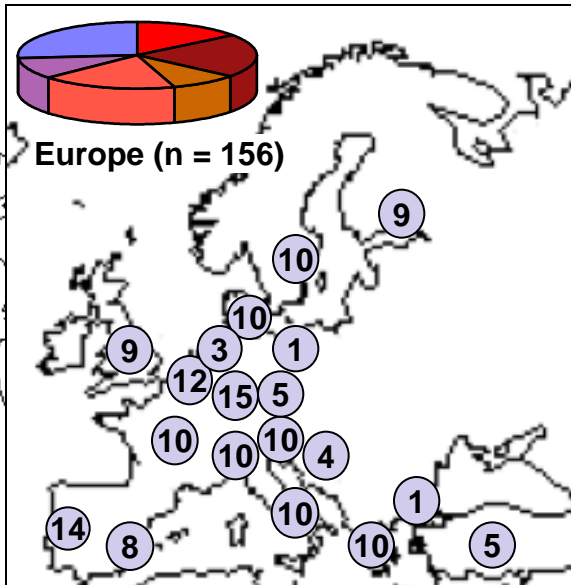
0.03



MLST clones: Everything is Everywhere



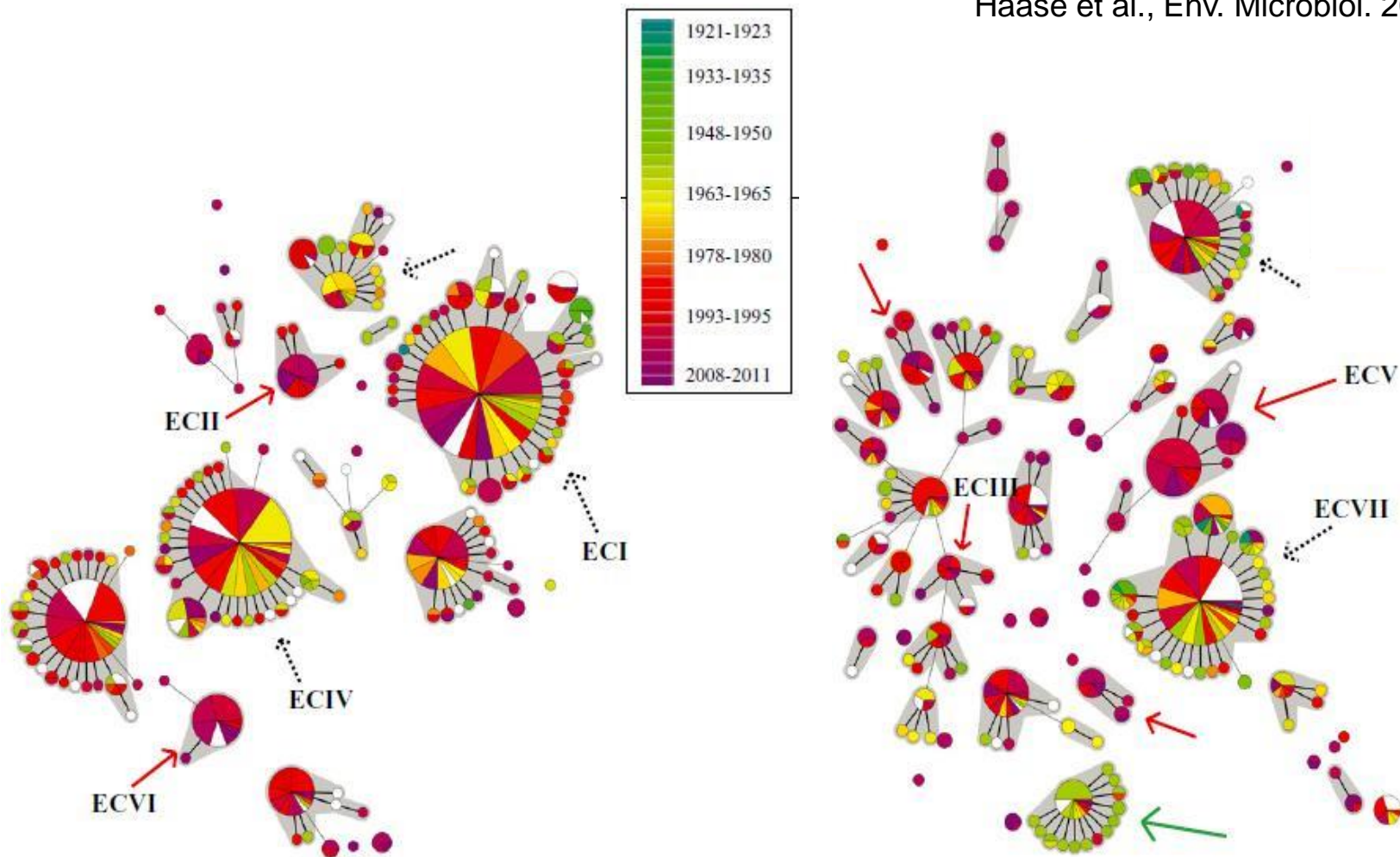
Chenal-Francisque et al., 2011



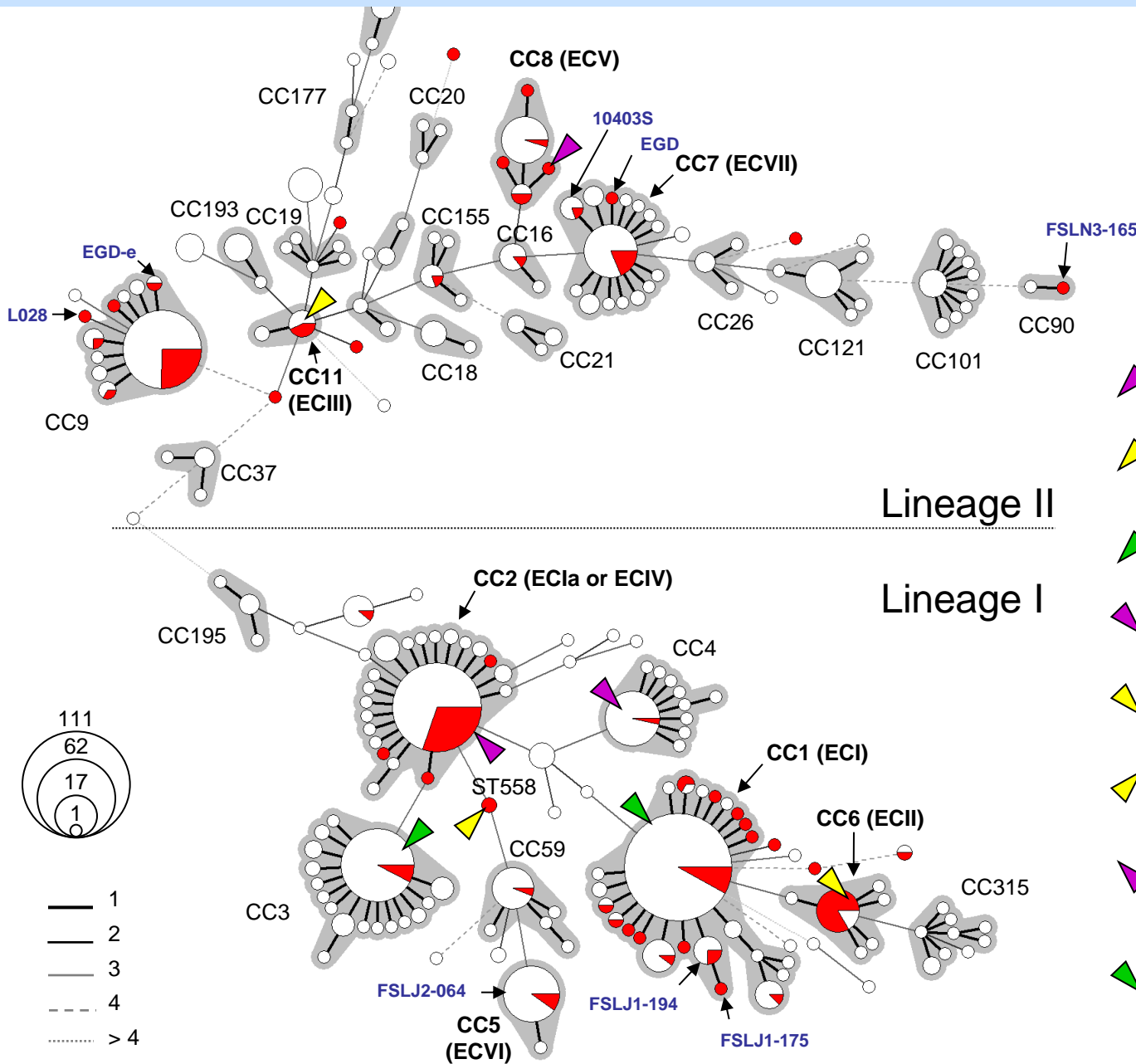
(No significant *Fst*)

L. monocytogenes MLST clones are old

Haase et al., Env. Microbiol. 2014











'Epidemic clones' ≈ CCs



Cantinelli et al., 2013

See also:
Kathariou & coll.
Knabel & coll.

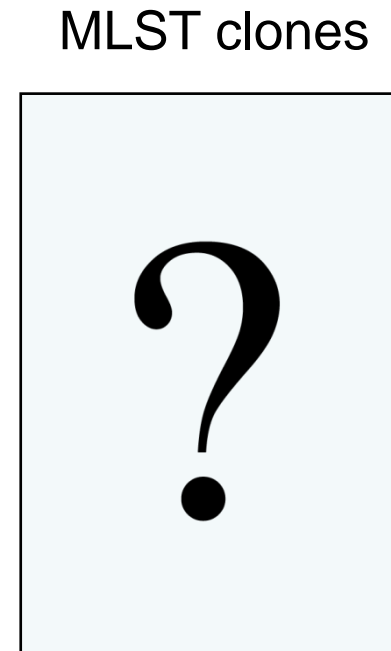
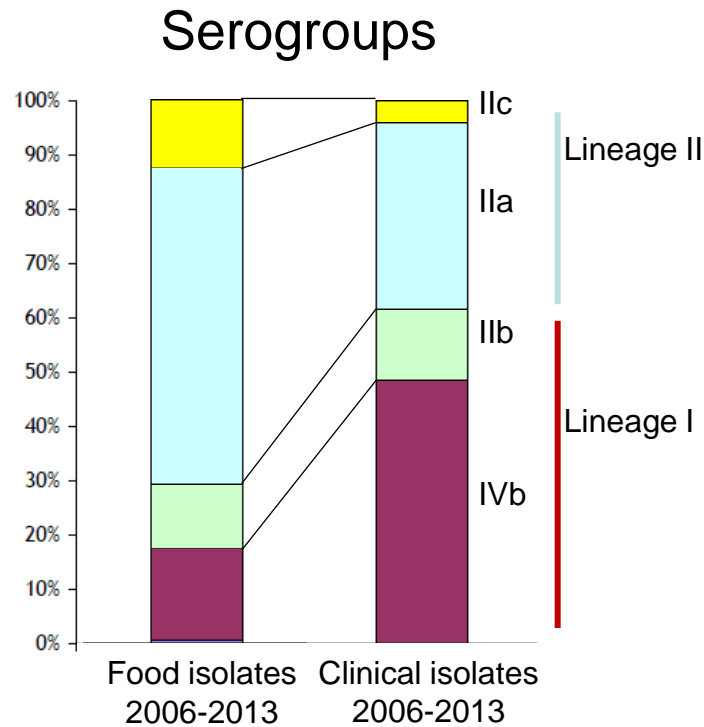
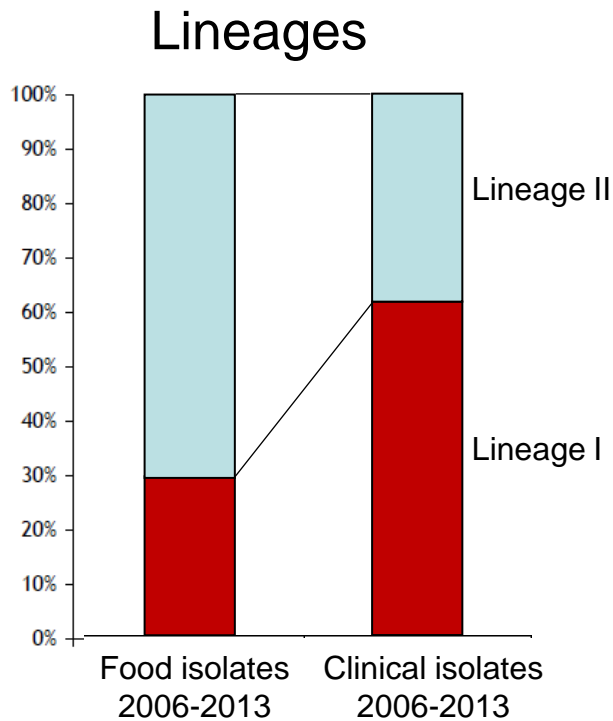
Outbreak strains:

-  08-5578, Canada, 2008
-  TS4/F.6854, USA 1989
J0161, USA 2000
-  G6054, USA 1994
-  CLIP 80459, France 1999
-  H7550, USA 1998-99
J1735, USA, 2002
-  J0211, USA 2000-2001
-  Scott A, USA, 1983
Aureli97, Italy 1987
TS38/L.3306, UK & Ireland 1987-89
-  TS27/L.4738, Canada 1981
TS60/L.4486b, Switzerland, 1983-87
F2365, California, 1986-1987

Lineage II

Lineage I

Distribution in food vs. clinical samples

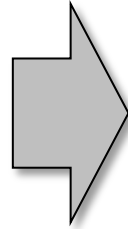


Data from French National Reference Center

Quantification of MLST clones in sources



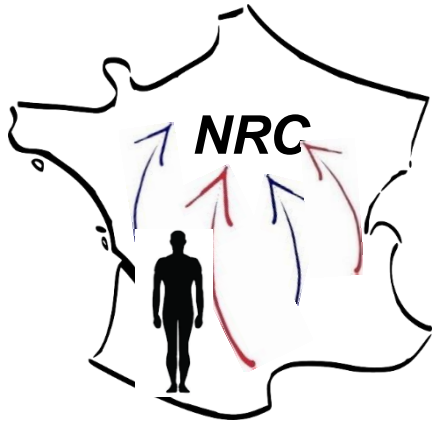
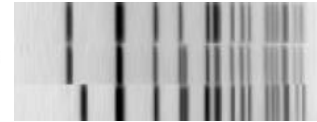
NRC + NRL isolates



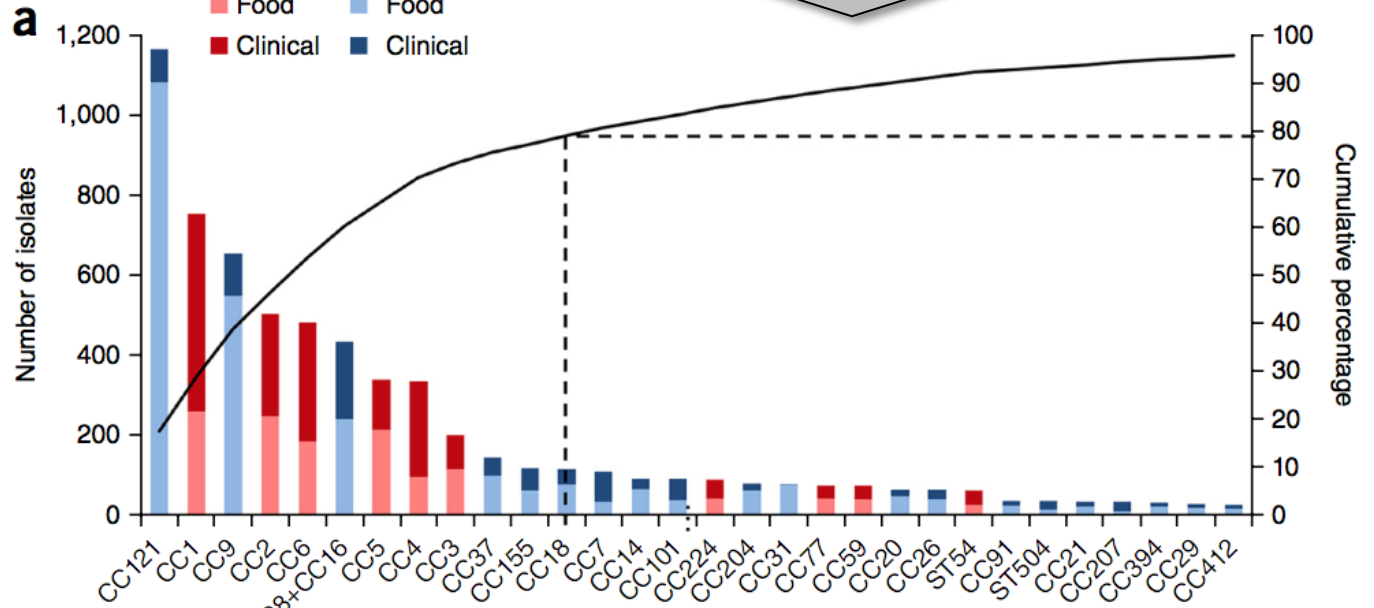
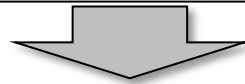
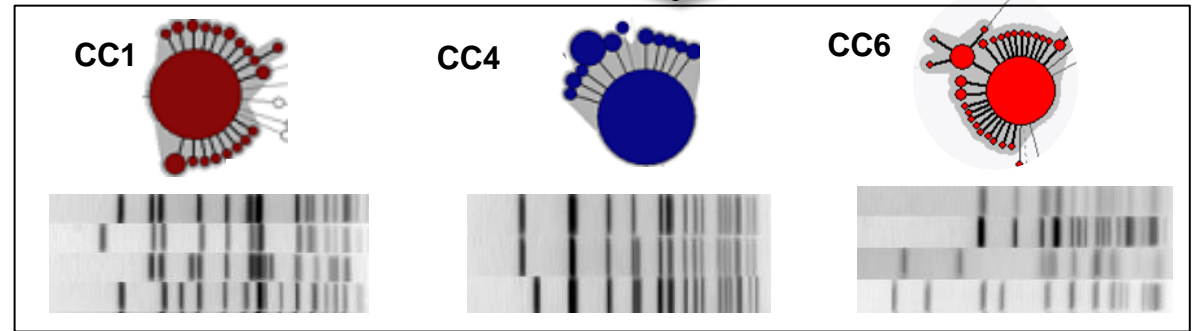
2584 clinical
4049 food



6633 isolates
PFGE Apal, AscI



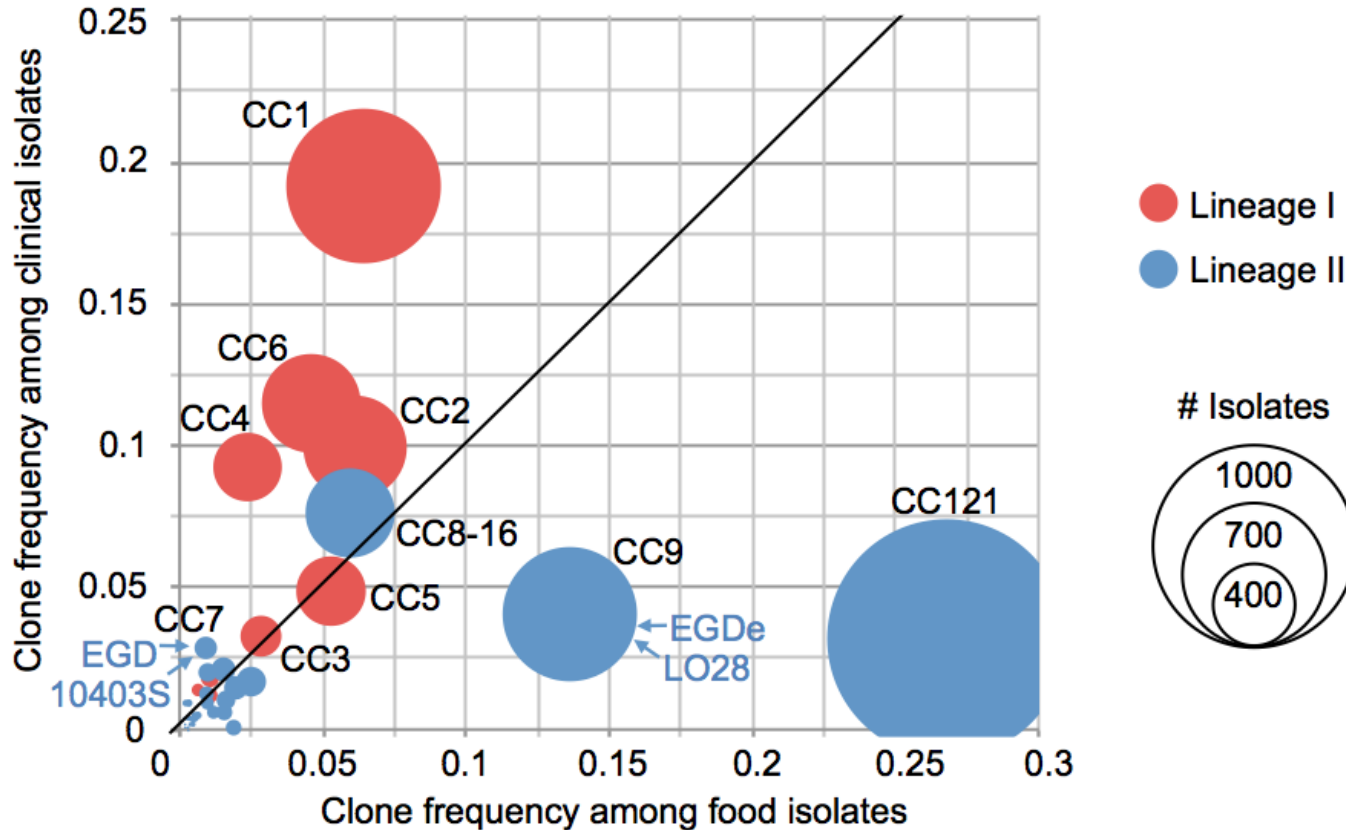
2005 – 2013



M. Maury

Unequal distribution of clones in food and clinical sources

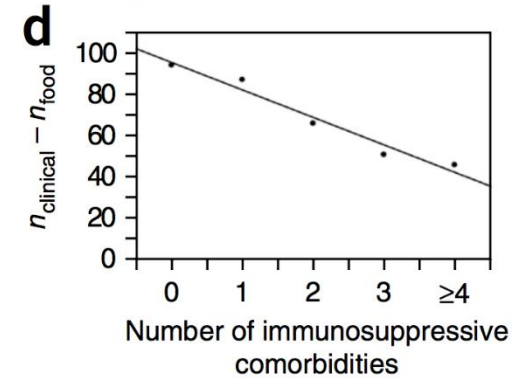
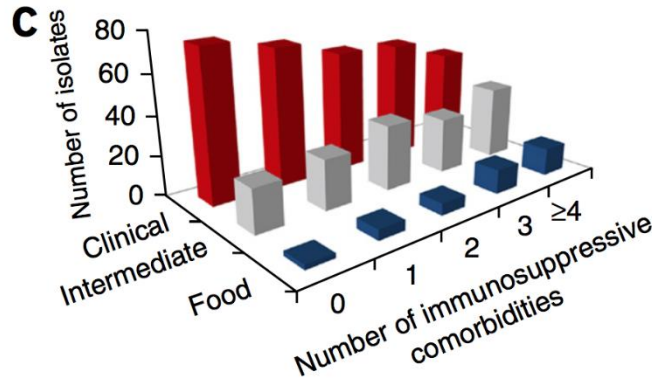
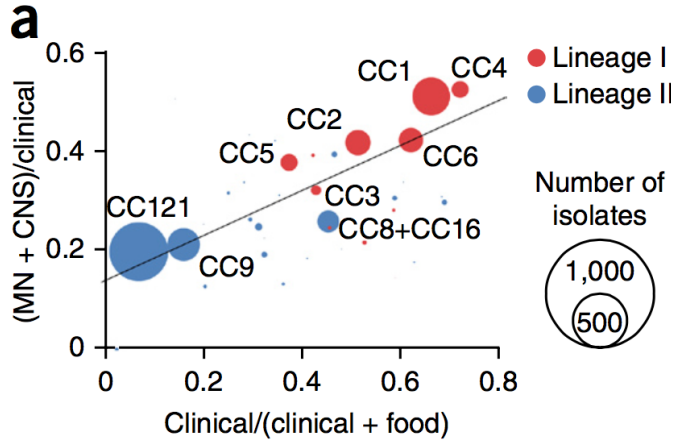
Maury, Tsai et al. Nat Genet 2016



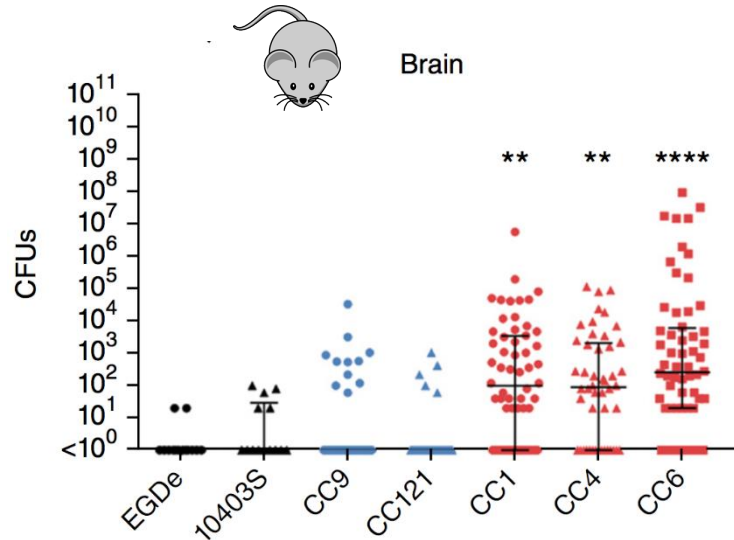
Clinical-associated clones are hypervirulent

❖ 6 633 isolates, invasive infections

❖ 811 infected patients, immunosuppressive comorbidities
(MonaLisa study, C. Charlier)



R2 = 0.9995, P < 0.03

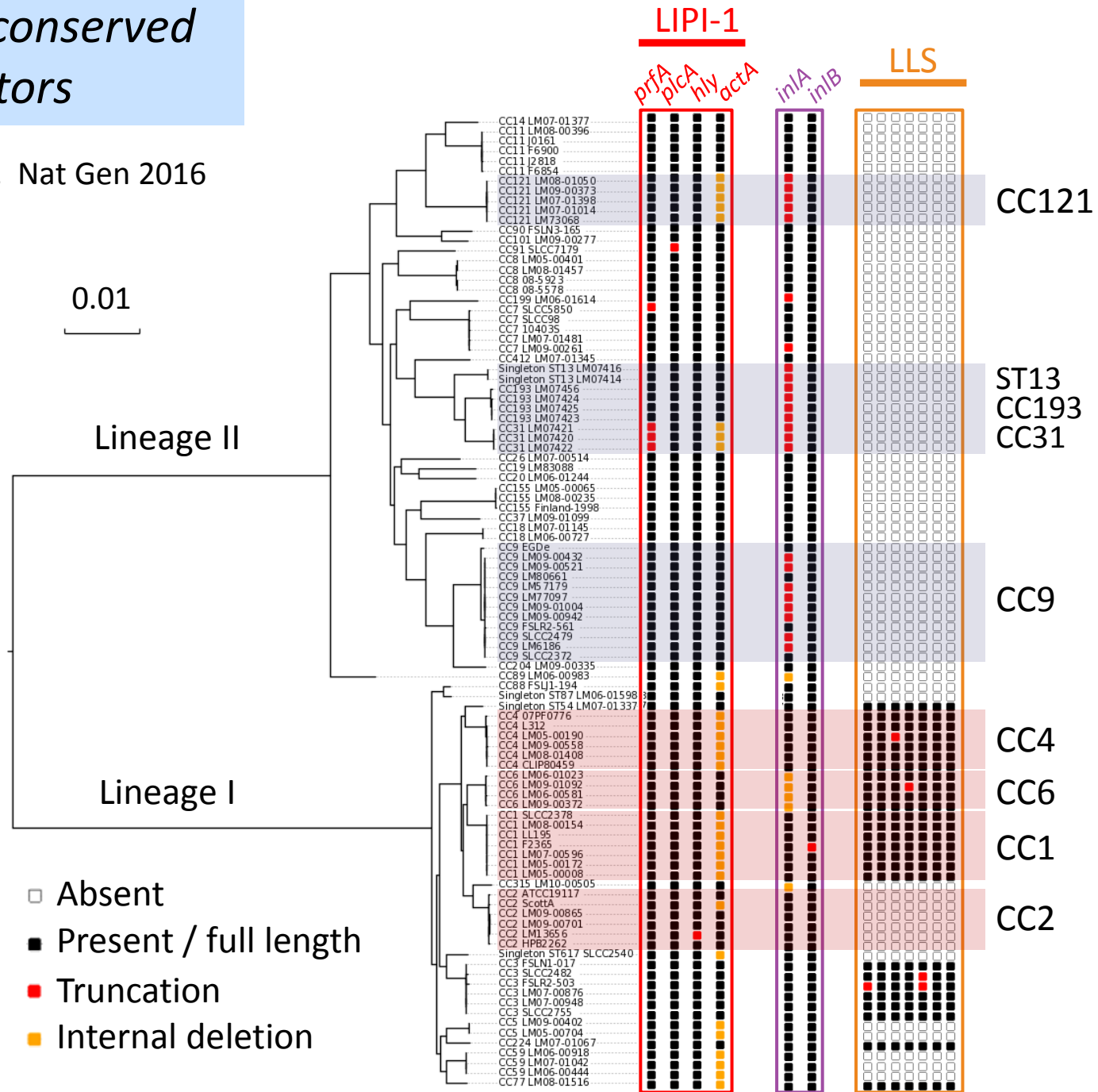


Coll. lab
M. Lecuit

Maury*, Tsai* et al. Nat Gen 2016

Variation of conserved virulence factors

Maury*, Tsai* et al. Nat Gen 2016



Novel putative virulence factors

104 genomes

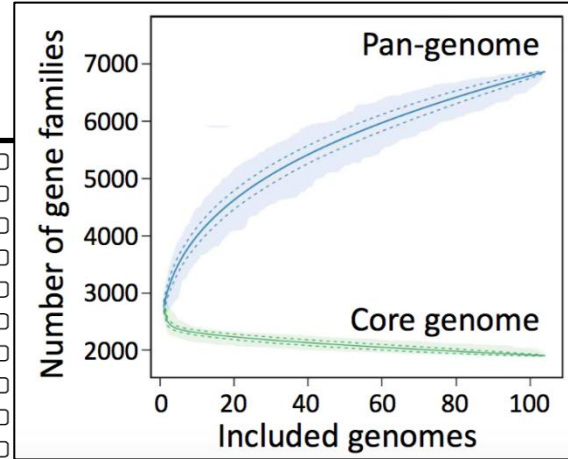
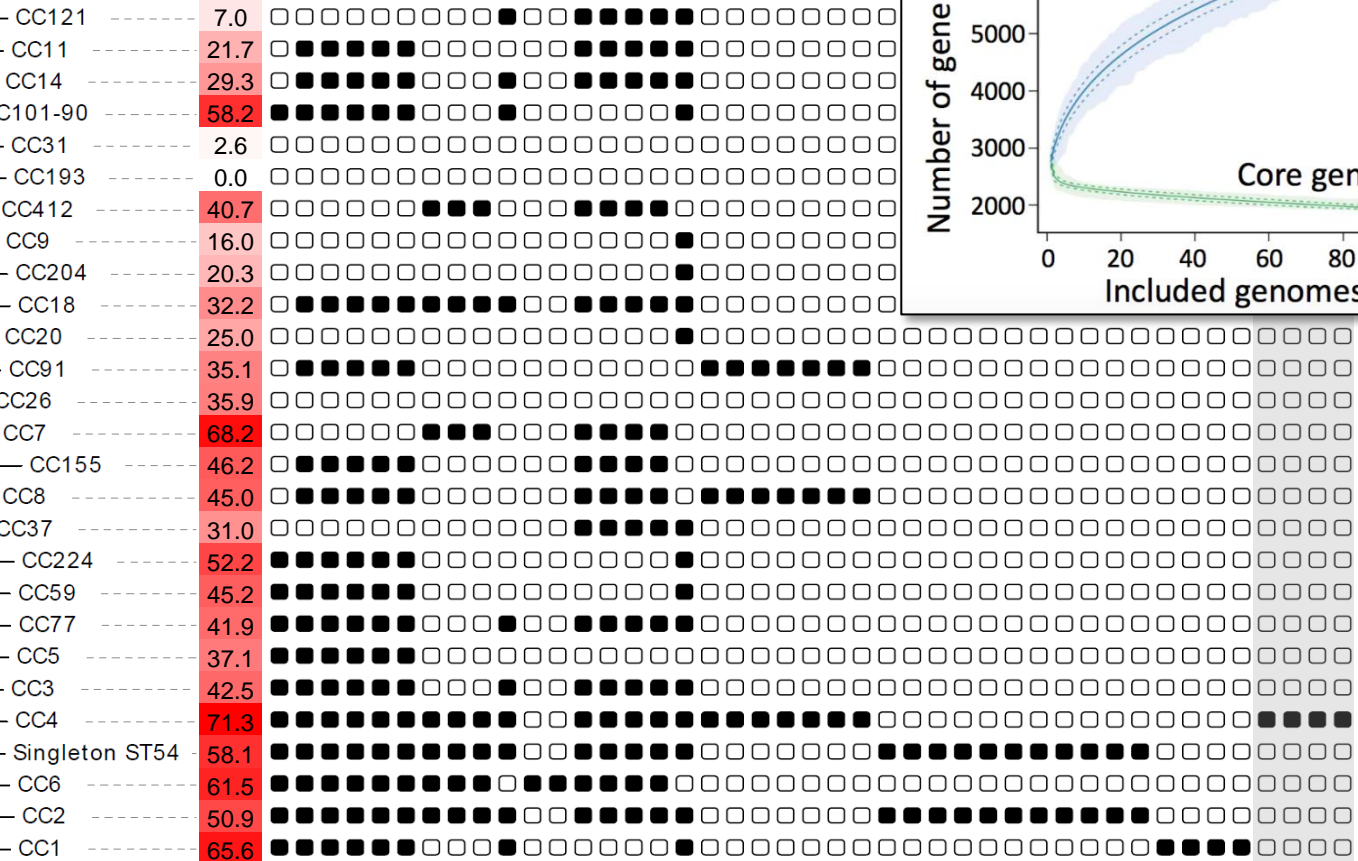
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Lineage II

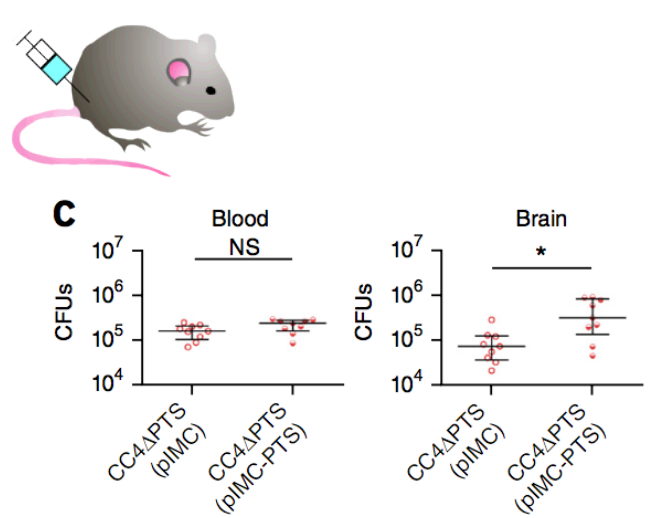
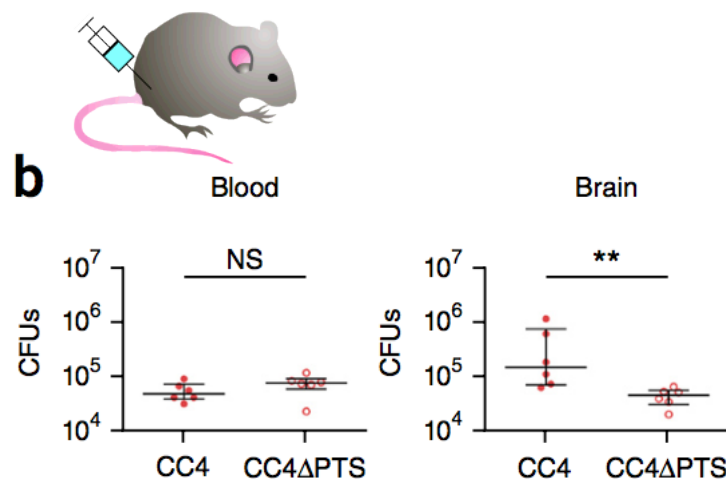
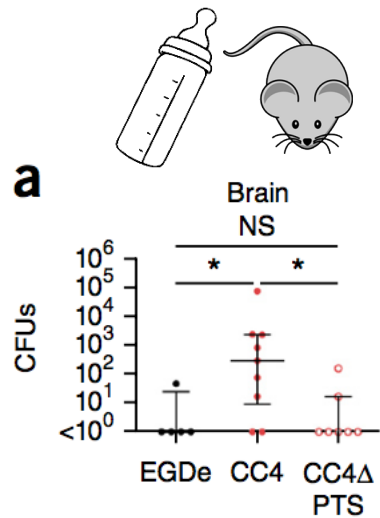
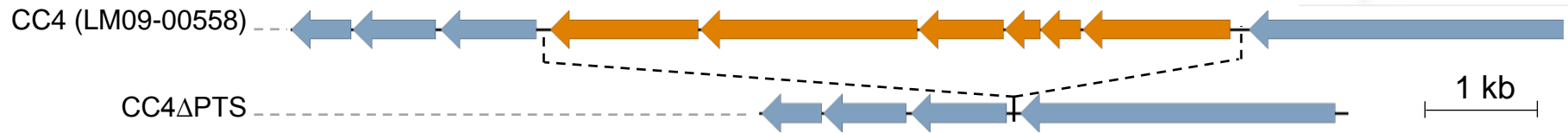
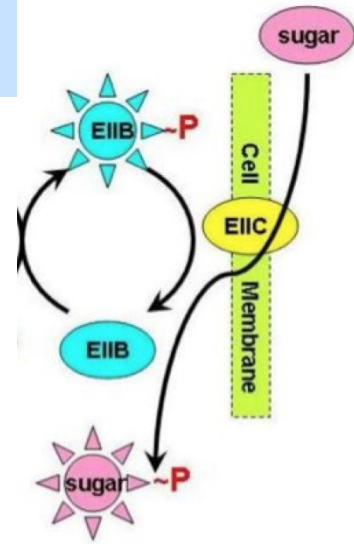
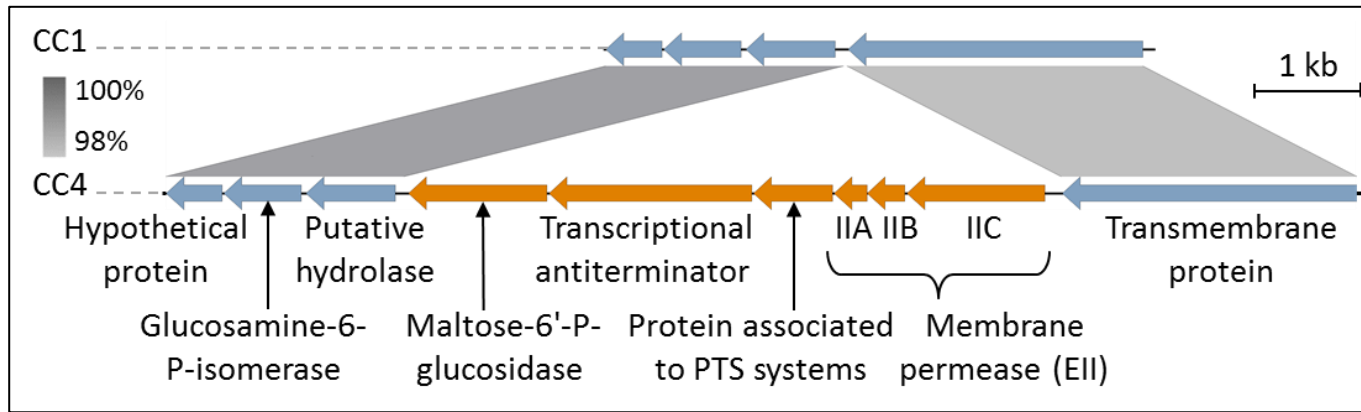
Lineage I

Clinical frequency

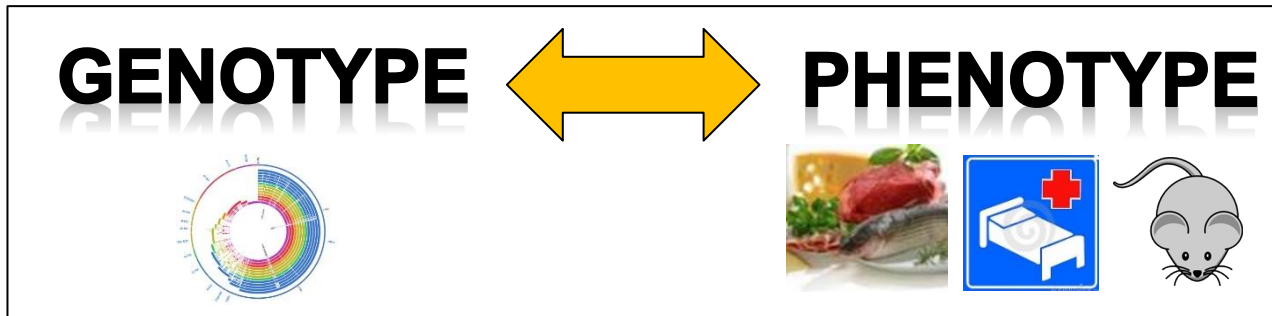
□ Absent
■ Present



Listeria pathogenicity island 4 (LIPI-4)



Biodiversity of *Listeria monocytogenes*



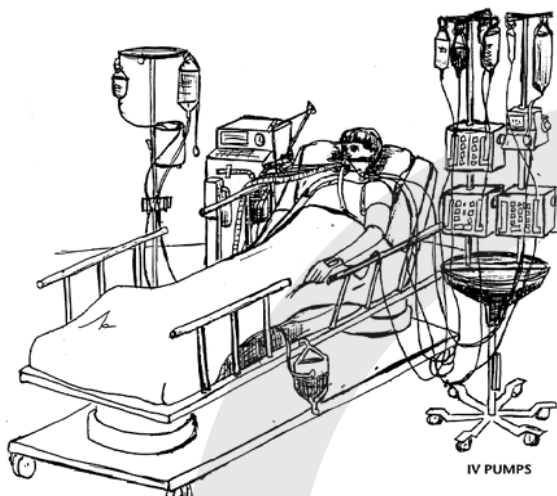
- Hypervirulent clones
- Novel putative virulence factors
- First CNS-associated virulence factor

Nat Genet. March 2016

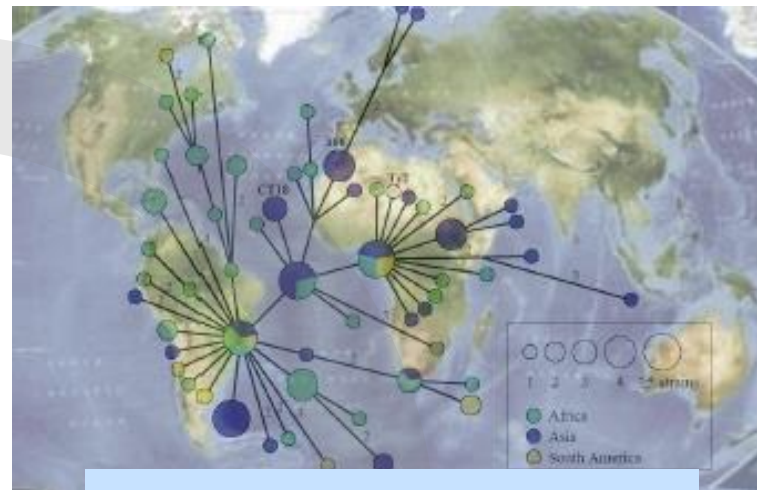
Uncovering *Listeria monocytogenes* hypervirulence
by harnessing its biodiversity

Mylène M Maury^{1-3,11}, Yu-Huan Tsai^{4,5,11}, Caroline Charlier⁴⁻⁸, Marie Touchon^{1,2}, Viviane Chenal-Francisque^{4,6,7}, Alexandre Leclercq^{4,6,7}, Alexis Criscuolo⁹, Charlotte Gaultier^{4,5}, Sophie Roussel¹⁰, Anne Brisabois¹⁰, Olivier Disson^{4,5}, Eduardo P C Rocha^{1,2}, Sylvain Brisse^{1,2,12} & Marc Lecuit^{4-8,12}

Integration of public health and biological research



Epidemiology
Diagnostics



Global diversity
Population structure

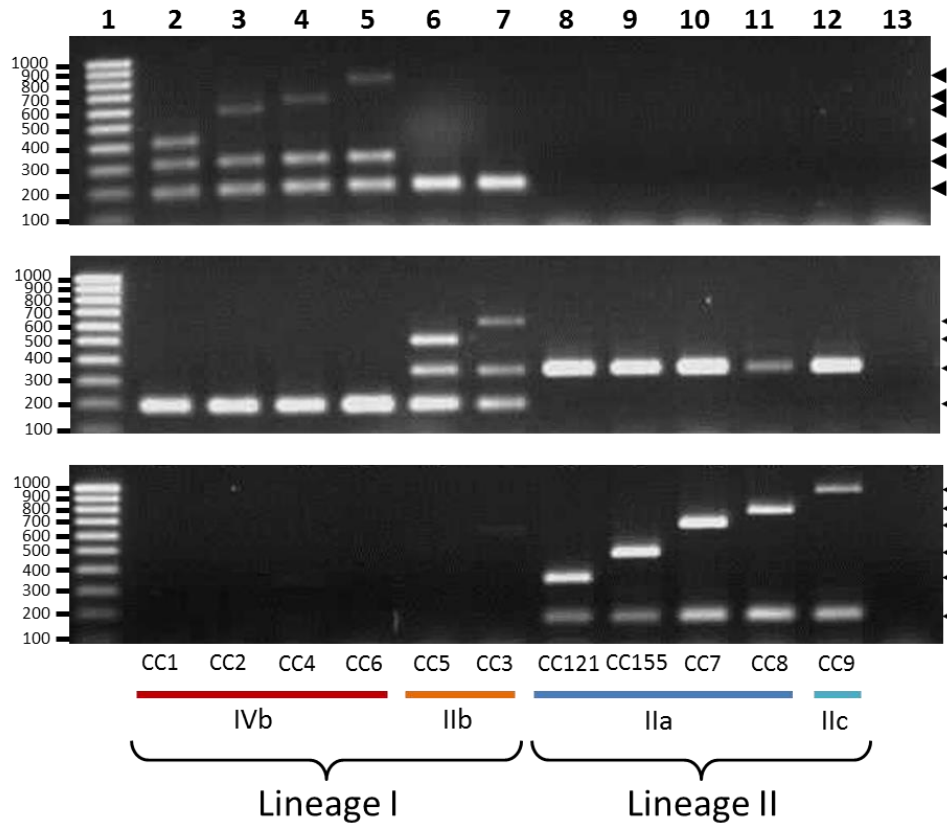


Ecology



(patho)physiology

Clonogrouping: rapid multiplex PCR assay to identify major clones of *L. monocytogenes*



➔ 95.6% of correct identification at clone level

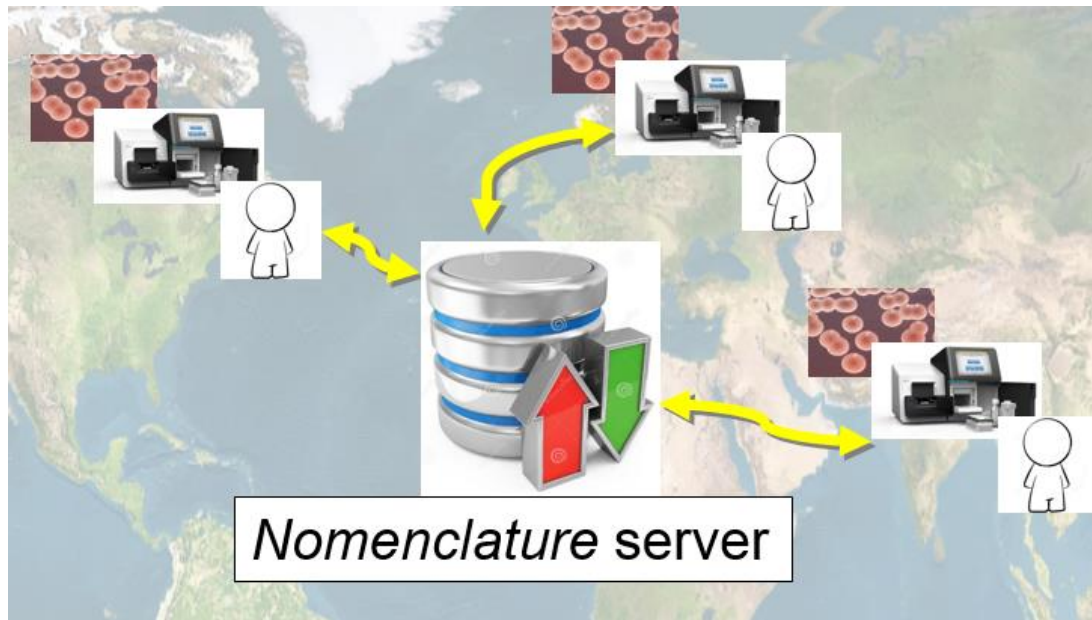
Talking the same language: How can we share nomenclatures of strains?

From: foodNetwork@ecdc.org

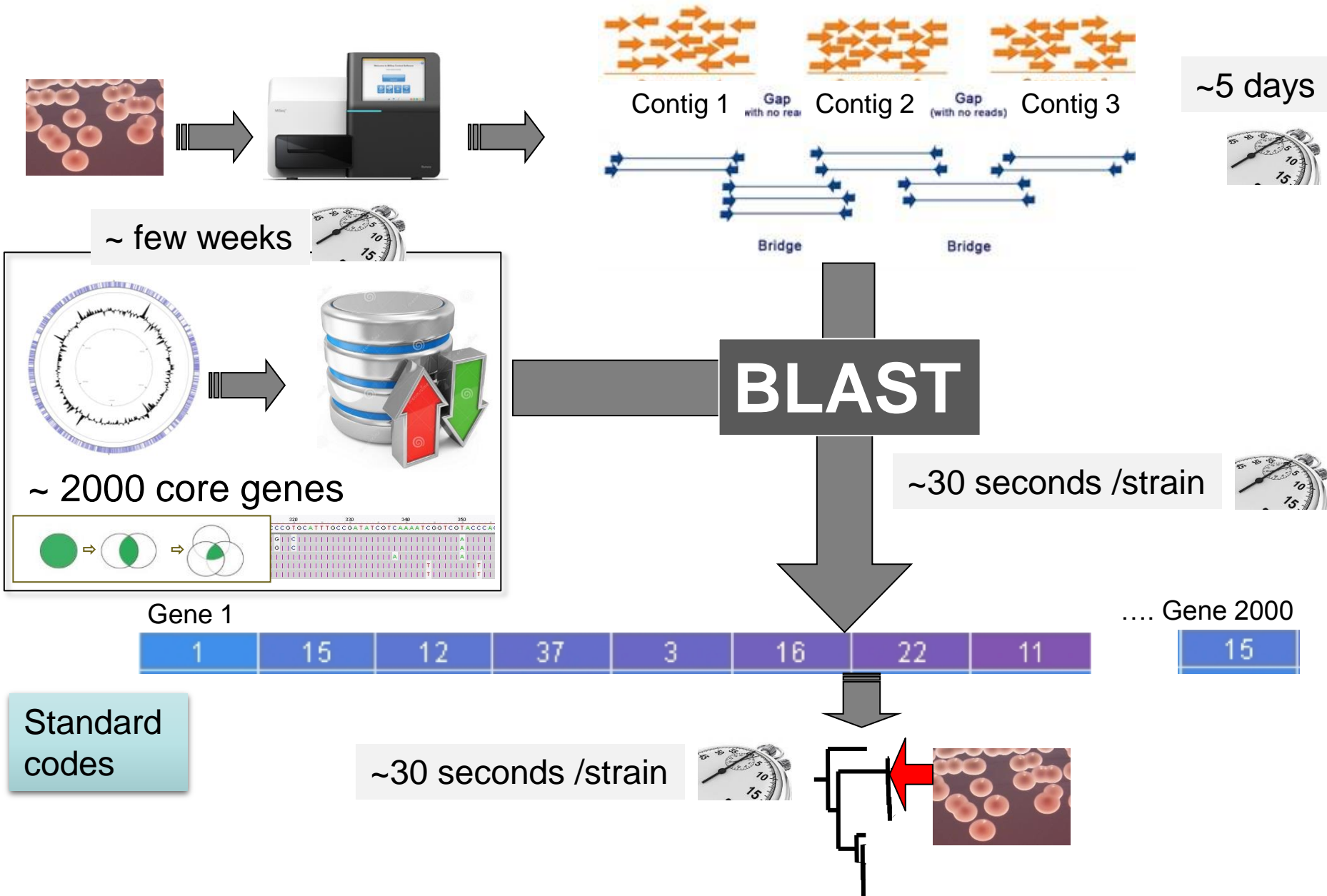
Subject: type CT131 Denmark outbreak

Body: Have you observed this type in your country?

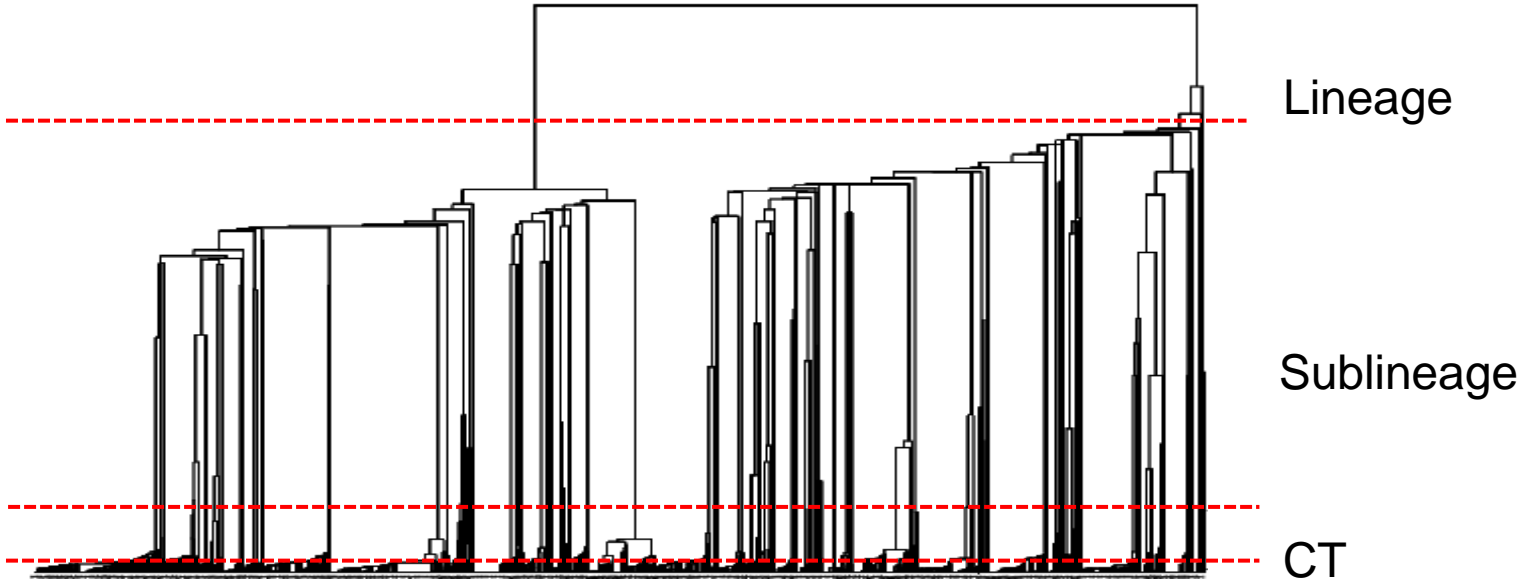
1. Define genotypic groups and nomenclature designations
2. Develop a nomenclature database
3. Provide on-line genotyping tools



Core-genome genotyping (cgMLST)



A nomenclature proposal for *L. monocytogenes* strains



Lineage - Sublineage - ST - cgMLST type (CT)

LII - SL7 - ST7 - CT932

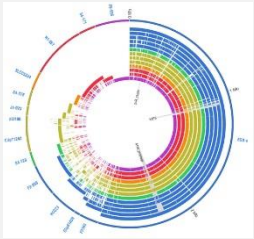
Share *nomenclature*, not genomes or provenance data

Isolates
databases

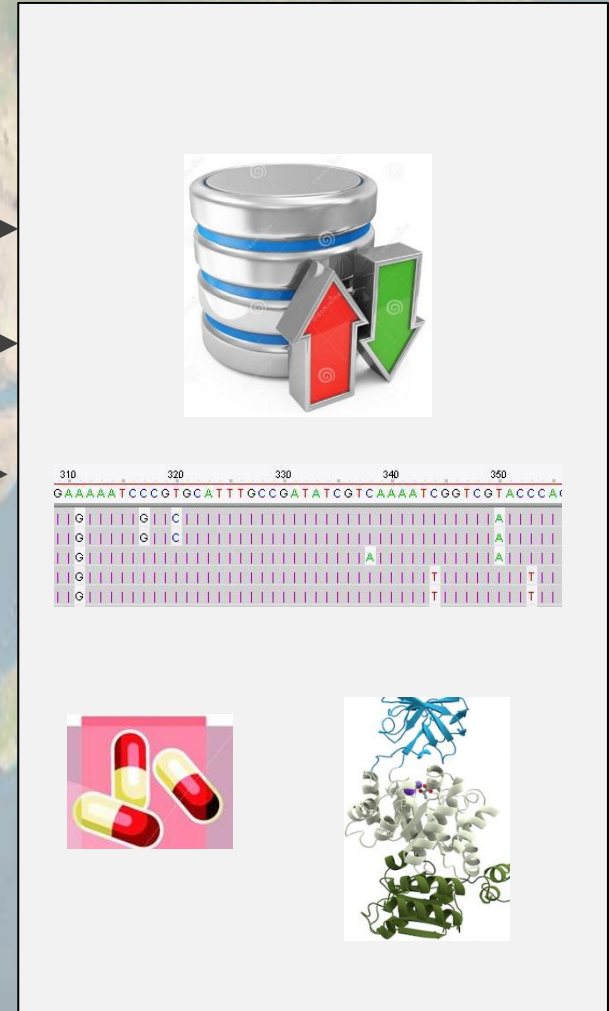
Nomenclature
database



Provenance data
& genomes



1	15	12
1	5	4
27	28	9



Standard nomenclature

Confidentiality of data

Standard nomenclature

<http://bigsdb.web.pasteur.fr/listeria>

Sequence query - *Listeria* locus/sequence definitions

Please paste in your sequence to query against the database. Query sequences will be checked first for an exact match. Query sequences do not need to be trimmed. The nearest partial matches will be identified if an exact match is not found. You can

[i](#)

Please select locus/scheme

Order results by

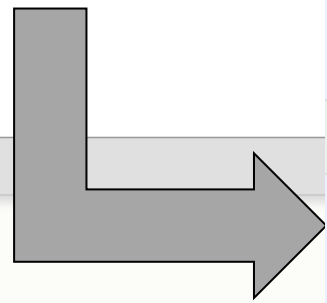
All loci

locus

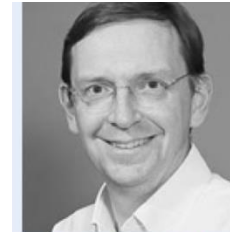
All loci

MLST
cgMLST1748
PCR-serotyping
Virulence
abcZ
actA (lmo0204)
agrA (lmo0051)
agrC (lmo0050)
ami (lmo2558)
aut (lmo1076)
bglA
bsh (lmo2067)
cat
cheA (lmo0692)
cheY (lmo0691)
cwhA (lmo0582)

(single or multiple contigs up to whole genome in size)



phylogenetic lineage	serotype	clonal complex	sublineage	cgmlst type
II	1/2a	CC9	SL9	CT637
I	IVb; 4b	CC1	SL1	CT304
I	7	CC3	SL3	CT83
I	IVb; 4b	CC4	SL4	CT188



Martin C.J. Maiden



Keith Jolley

BIGSdb
Jolley & Maiden 2010

Conclusions

- *L. monocytogenes* genotypic categories can be defined at different phylogenetic depths
- Integration of epidemiological surveillance and fundamental biology research provides novel insights on pathophysiology, ecology
- Standard genotyping strategy and public database for strain nomenclature: to be agreed upon
- *L. monocytogenes* clones are everywhere; International transmission both at evolutionary and at epidemiological timescales
- Need for internationally coordinated surveillance of *L. monocytogenes*

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