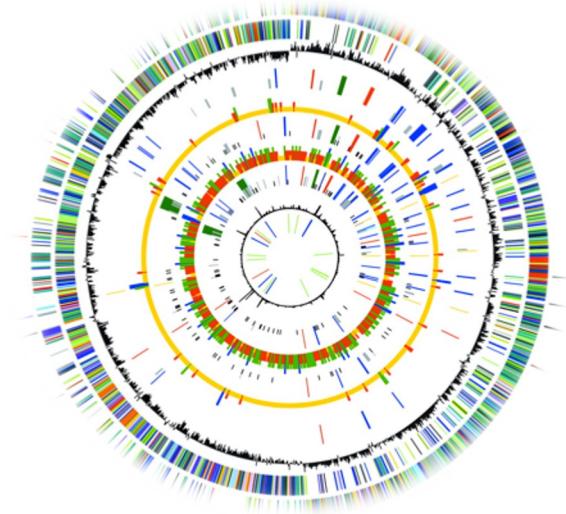


*Department of Food Science & Technology,
NGS Workshop,
Stellenbosch University
South Africa*

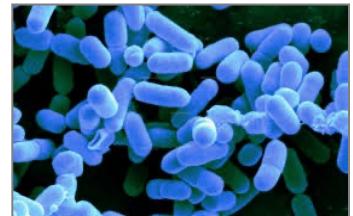
Monday 22nd January, 2024



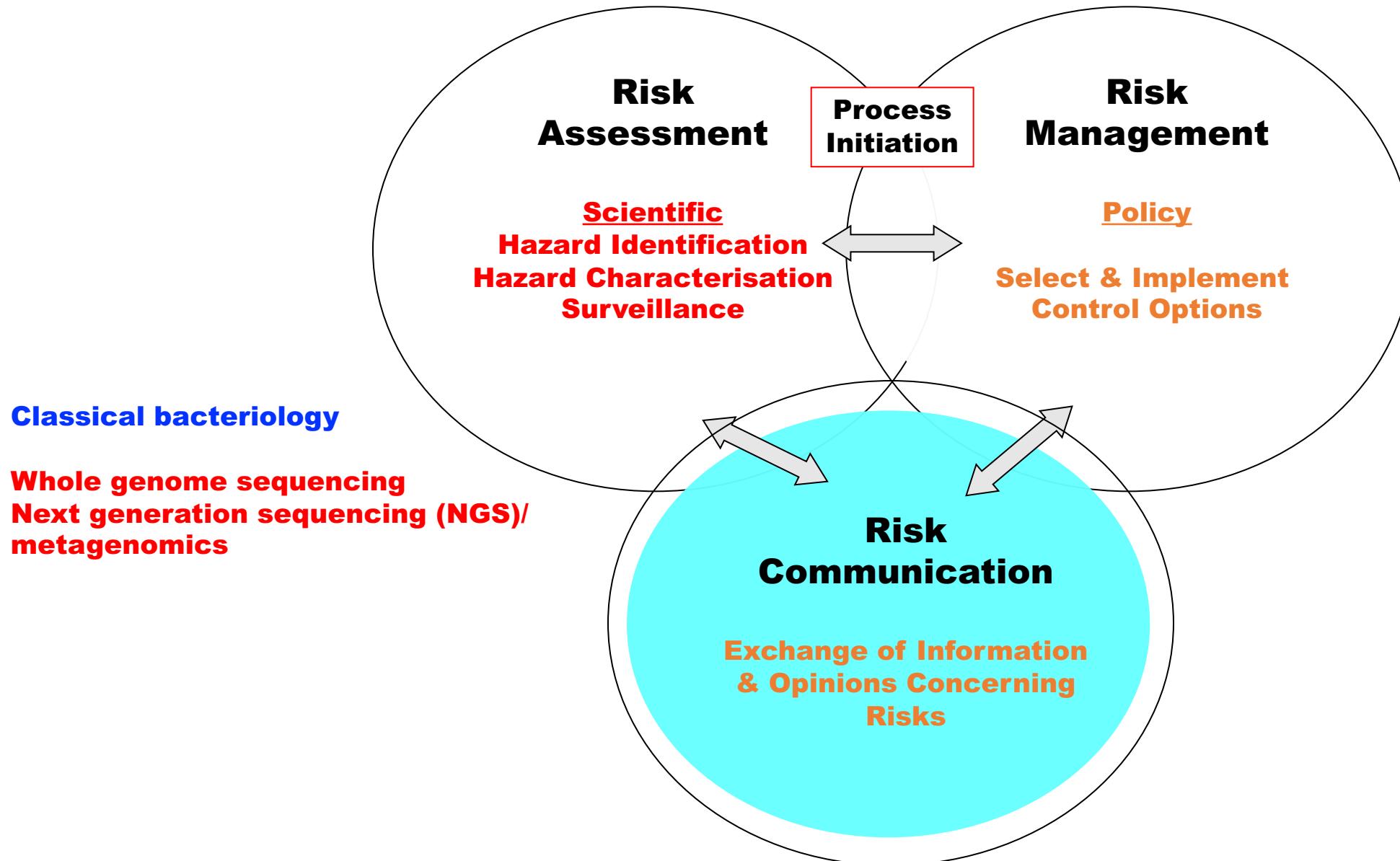
Next generation sequencing (NGS) technologies *-in the context of risk assessment & food safety*



Professor Séamus Fanning,
UCD-Centre for Food Safety,
School of Public Health, Physiotherapy & Sports Science,
University College Dublin, Ireland.

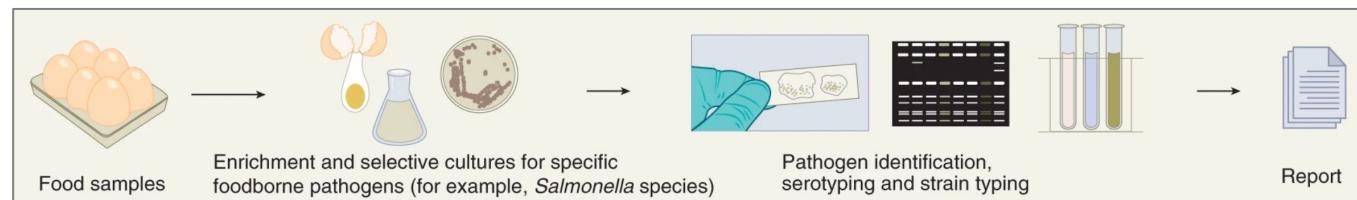


Risk analysis is the foundation on which food safety stands -



Risk assessment transitioning between classical bacteriology and *big data* -

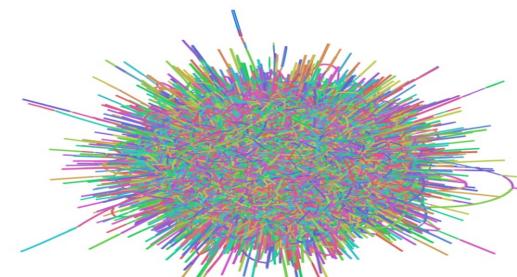
- microbiological analysis has traditionally been monitored by making use of ***culture-based phenotypic methods***; detection characterisation & identification **-*bed rock of diagnostic bacteriology***
- bacterial pathogens of importance to public health/brand protection are primarily studied at the species/isolate level and with no regard to the context of the microbial communities from which they came **-*microbiome***
- ***high-throughput sequencing strategies have heralded a seismic shift*** -study these bacteria of importance to human health and provided the opportunity to ***re-write the risk assessment paradigm***
- ***big data-based analysis is now a reality upon which future microbiology based on- Precision Food Safety***



MacConkey agar

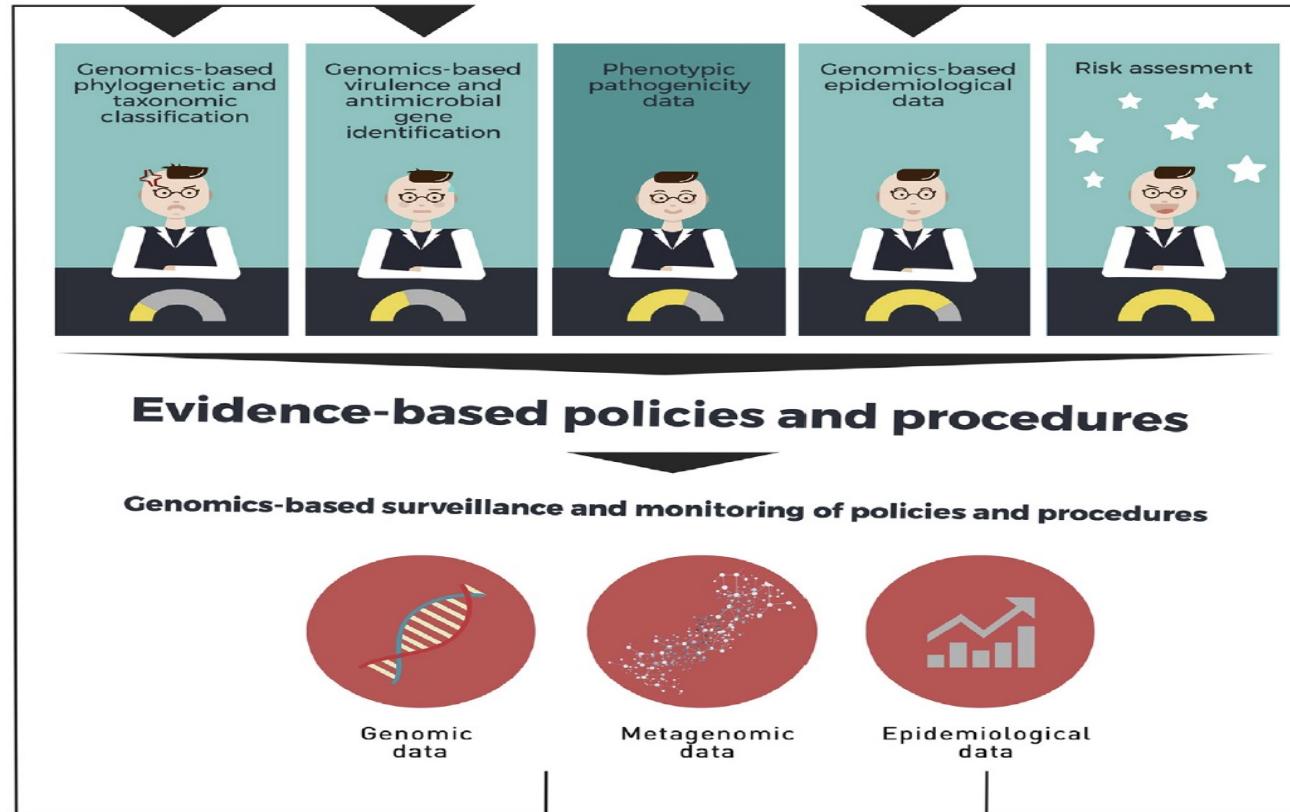


Blood agar



Whole genome sequencing

Precision food safety applied to food matrices & the processing environment; *can protect human health & brand reputation -*



identification
interpretation
phenotype
epidemiology
risk to health
& your brand

WGS-based analytics related to brand protection & public health-

□ *Limit food-borne contamination – pathogen source tracking*

- preventive controls;**
- improve industry practices by focusing interventional measures on vulnerable processes**
- monitor emergence of AMR/sanitizer tolerance**

□ *Identify source of food-borne outbreaks quickly – outbreak investigation*

- Whole-Genome Sequencing (WGS) surveillance of bacterial pathogens;**
- Environmental monitoring;**
- Tracking & tracing food-borne pathogens (worldwide)**

□ *Risk assessment – the holy grail!*

- Using *big (genome) data* to differentiate between a (regulatory controlled) pathogen and one that is *a true pathogen?***

Basic Data Flow for Global WGS Public Access Databases

DATA ACQUISITION

Sequence and upload genomic and geographic data



Other distributed sequencing networks



DATA ASSEMBLY, ANALYSIS, AND STORAGE

International Nucleotide Sequence Database Collaboration (INSDC)

Shared Public Access Databases

- NCBI – National Center for Biotechnology Information
- EMBL – European Molecular Biology Laboratory
- DDBJ – DNA Databank of Japan



PUBLIC HEALTH APPLICATION AND INTERPRETATION OF DATA

- Find clinical links
- Identify clusters
- Conduct traceback
- Develop rapid methods
- Develop culture independent tests
- Develop new analytical software

11/2014

State, Local, Federal, and Foreign Public Health Agencies

Academia/Industry



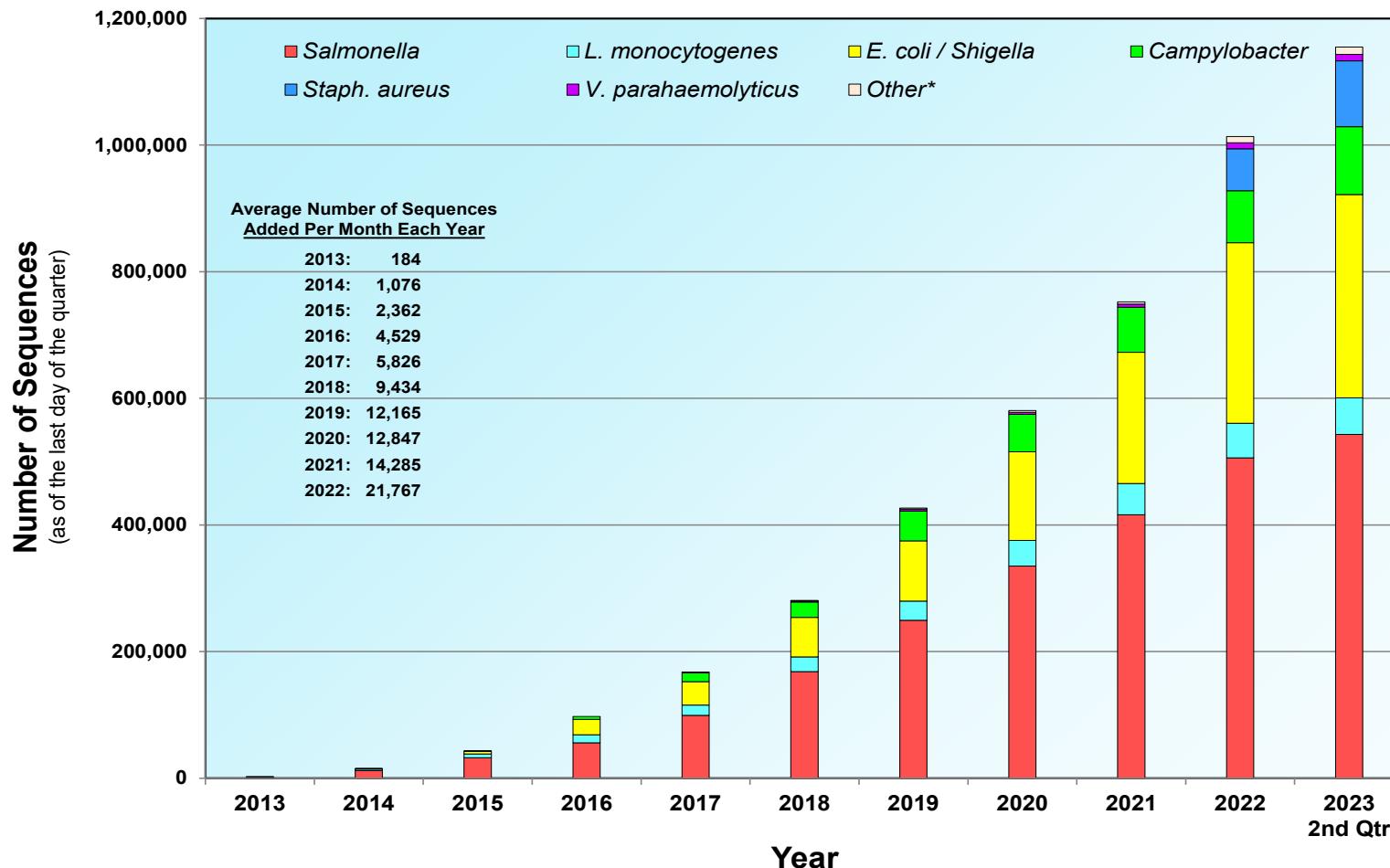
U.S. GenomeTrakr Labs



Labs Outside the U.S. Contributing to GenomeTrakr



Total Number of Sequences in the GenomeTrakr Database

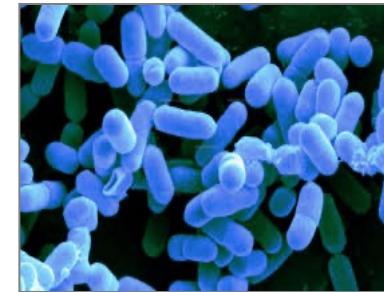
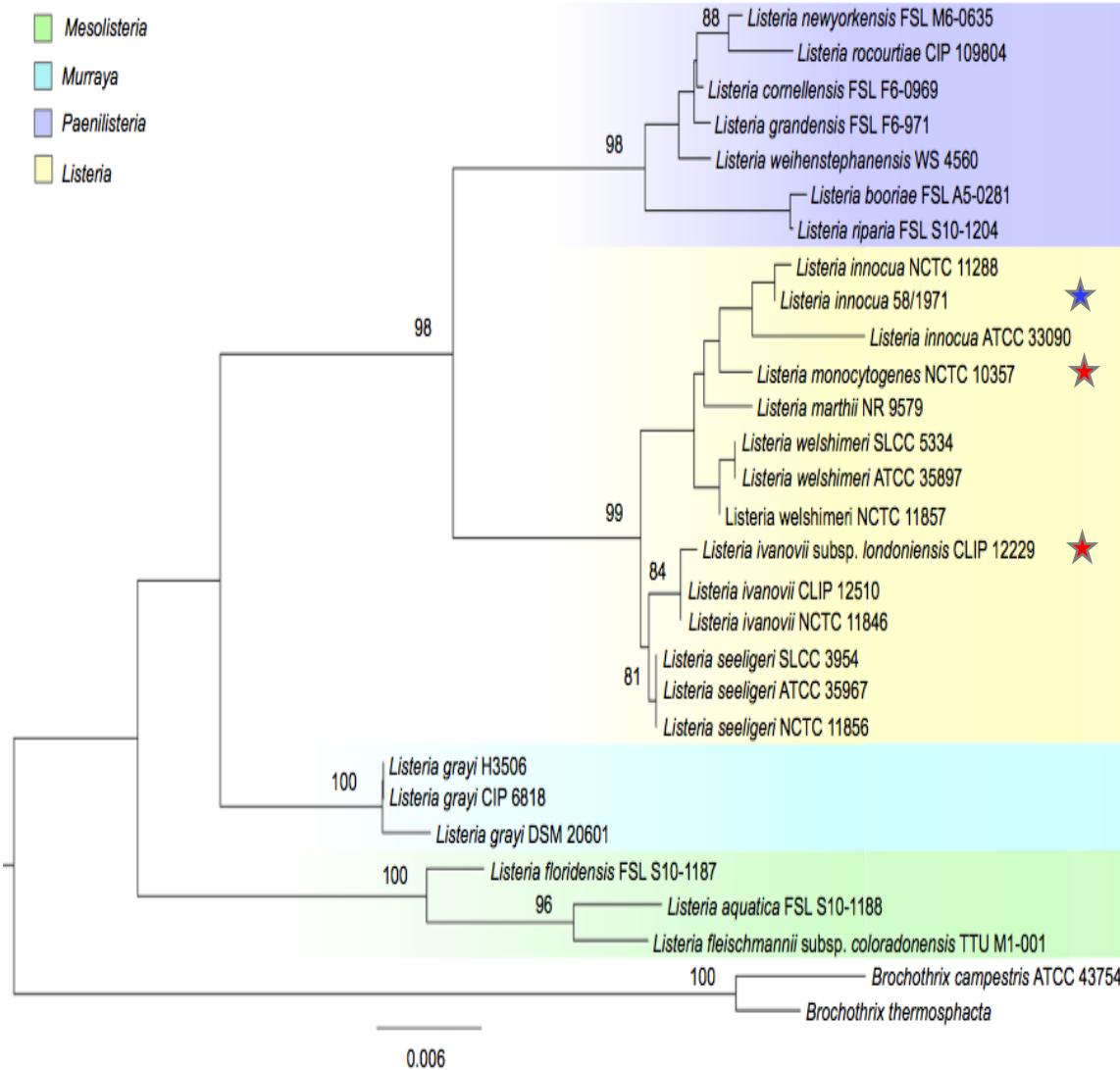


First sequences uploaded in February 2013

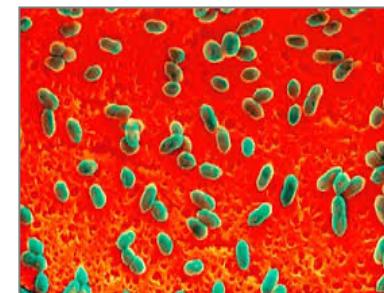
* Other pathogens: *Cronobacter*, *V. vulnificus*, *C. botulinum*, *C. perfringens*, and *Bacillus cereus* group



Taxonomy of the genus *Listeria* -



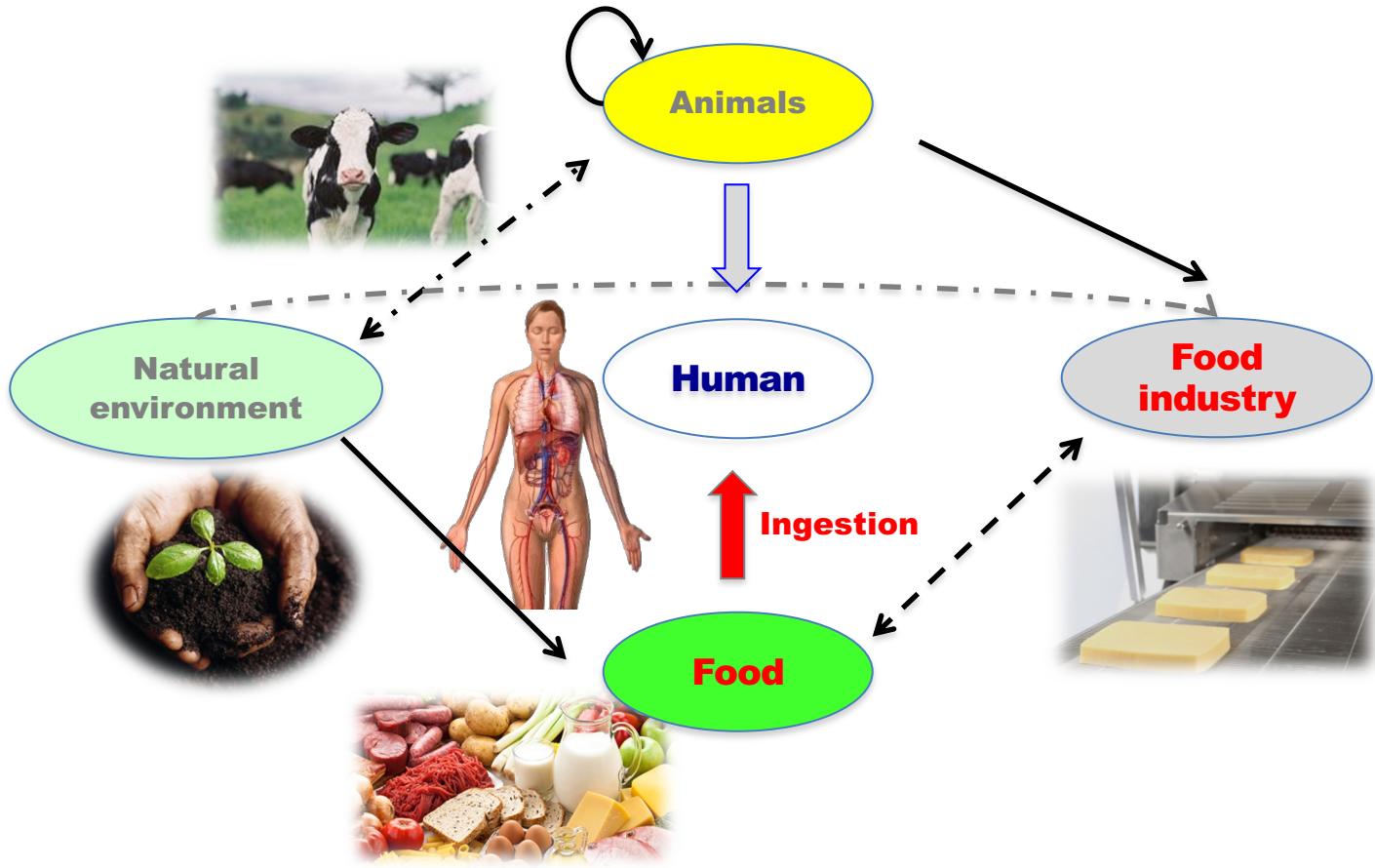
L. monocytogenes



L. ivanovii

- ***Listeria monocytogenes*** divides across **four evolutionary lineages**, that can be further sub-divided into **14 serotypes**
- **serotypes 1/2a** (Lineage II); **1/2b and 1/2c** (both Lineage I) cause **95% of human listeriosis cases**
- **clonal complexes (CC)** represent **sequence types (ST)** with a similar central allelic profile (**MLST**)

Life cycle of *Listeria monocytogenes* through the food chain -



saprophyte → facultative intracellular bacterial pathogen

Human cases

Notification rate
(per 100,000 population) **0.42**
Trend (2016–2020)
Increasing

1,876 Cases of illness

1,285 Infections acquired in the EU
5 Infections acquired outside the EU
586 Unknown travel status or unknown country of infection

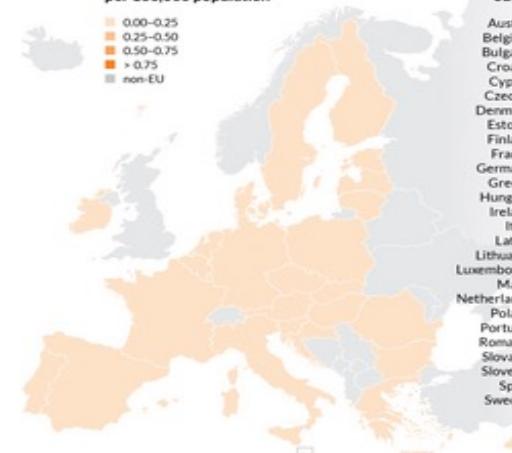
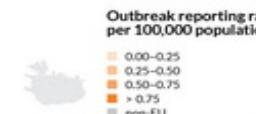
780 Hospitalisations
167 Deaths

Human cases in foodborne outbreaks

16 Foodborne outbreaks
9 Strong-evidence outbreaks
7 Weak-evidence outbreaks

120 Cases of illness
83 Hospitalisations
17 Deaths

Foodborne outbreaks in the EU



No. of listeriosis outbreaks
Top food vehicles causing strong-evidence outbreaks

- Fish and fish products: 6 Outbreaks
- Other or mixed red meat and products thereof: 2 Outbreaks
- Cheese: 1 Outbreak

ECDC data EFSA data

* Differences among countries shall be interpreted with caution as this indicator depends on several factors including the type of outbreaks under surveillance and does not necessarily reflects the level of food safety in each country.

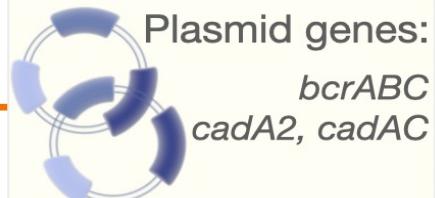
Stresses encountered by *Listeria* species in ecological niches

Detergent stress response

- Resistance to:
 - Benzalkonium chloride (*bcrABC*)
 - Cadmium (*cadA2-AC*)

Tolerance to cold, acid and salt stress

Stress responsive factor SigB



Plasmid genes:
bcrABC
cadA2, cadAC

Stress survival islands:
 SSI-1 and SSI-2

Osmotolerance

Acidic and oxidative environment survival

Stressosome

High osmolarity

Low pH

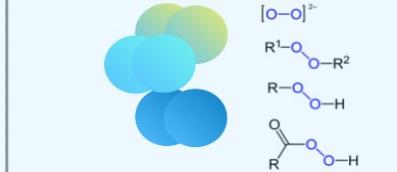
Heat shock

Competition

Preservatives

Nutrient deficiency

Peroxide resistance



Resistance to heat

Acid tolerance response

Adaptation and cross resistance to unrelated stresses

Tolerance to low pH exposures

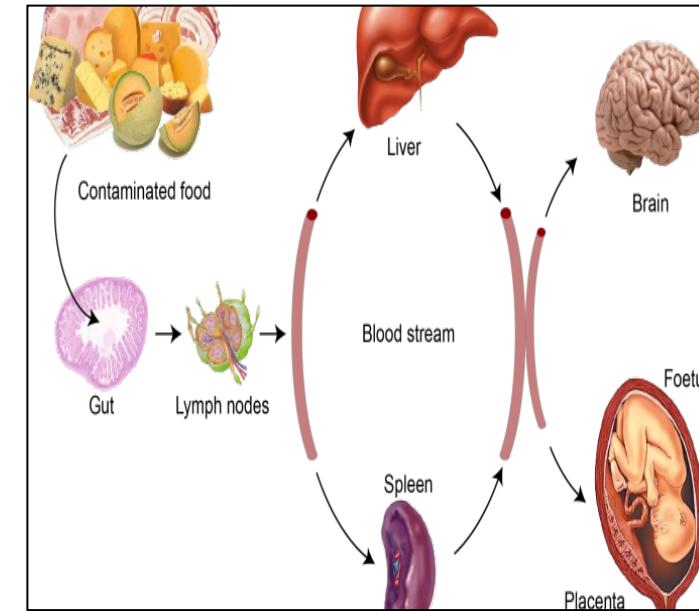
Reorganisation of cytoplasm and cell membrane composition

Survival to these stresses related to-

- serotype;
- clonal complex
- full length *inIA* (functions other than virulence) &
- plasmid profile

Listeriosis infection -

- Listeriosis risk groups (in humans):
 1. **Immunocompromised and cancer patients;**
 2. **Elderly;**
 3. **Pregnant women**
- ***Two main clinical forms of listeriosis-***
 1. **Adult listeriosis** – CNS disorders (meningitis/meningoencephalitis). Encephalitis/cerebrospinal fluid invasion more common in animals
 2. **Perinatal listeriosis** – occurs when *L. monocytogenes* crosses the placenta (abortion; stillbirth; infected neonate)
- ***Considerable heterogeneity in pathogenic potential has been reported***

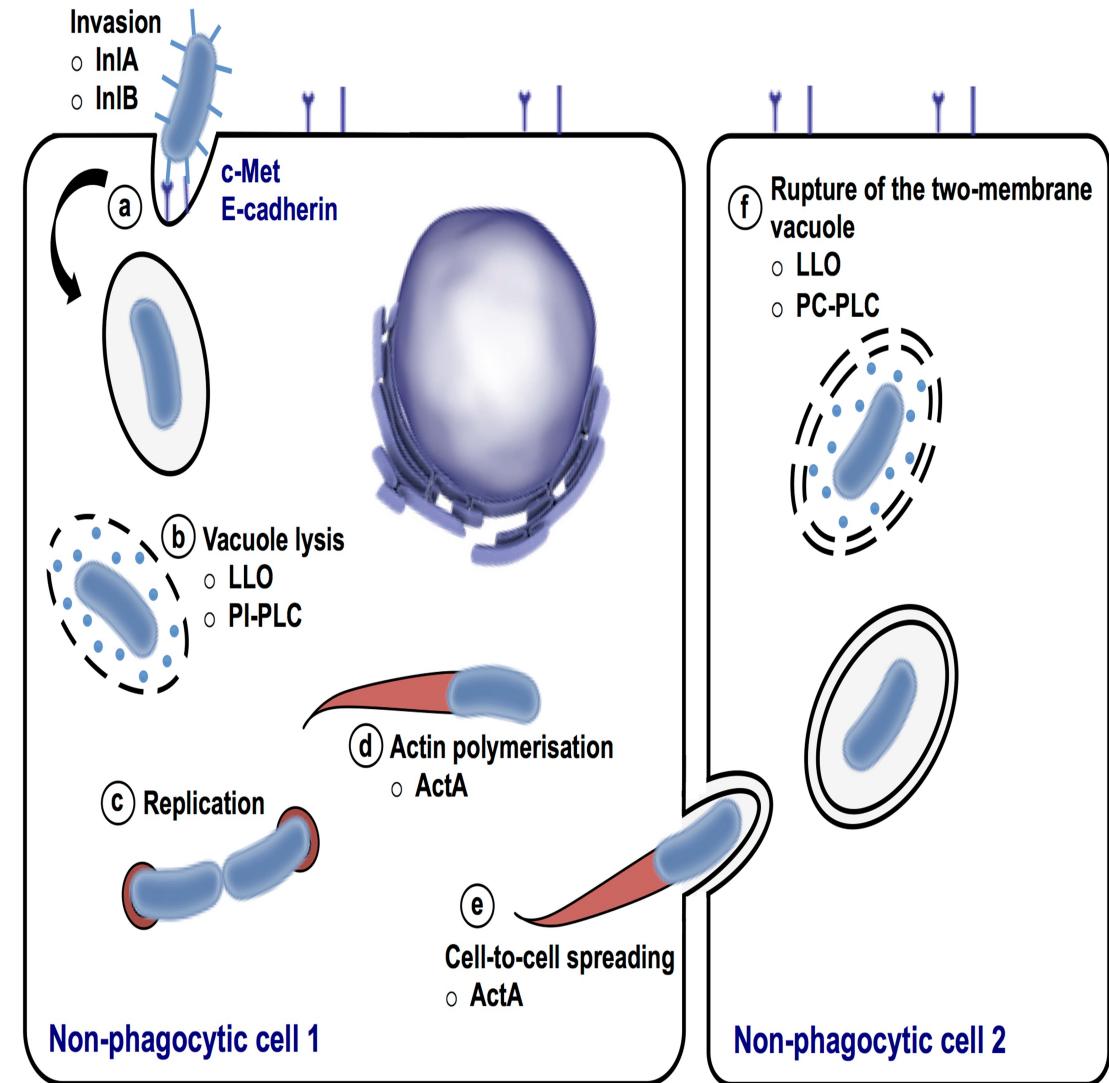


High mortality rates up to 30% reported

Listeriosis in humans is mainly associated with the consumption of contaminated food

Intracellular life cycle of *Listeria monocytogenes* -

- **Internalins** – Surface proteins responsible for triggering bacterial internalization through interactions with different **cell receptors**
 - *inlA* – E-cadherin
 - *inlB* – c-Met
- **Listeriolysin O (LLO)** – Hemolysin, pore forming toxin; allows for the escape from phagocytic endosome to cytosol; required for intracellular growth
 - *hly*
- **Phospholipases** – synergize with LLO for phagosomal escape
 - **PI-PLC** – *plcA*
 - **PC-PLC** – *plcB*
 - **Metalloprotease** – *mpl*
- **Actin A** – Surface protein involved in actin recruitment and polymerization; intracellular movement and cell-to-cell spread; autophagy evasion
 - *actA*



***Listeria monocytogenes* in food & the production environment -**

- Tolerant to an extended range of temperatures (-0.4 - 45°C)-**psychrophile**
- **Biofilm formation**
- **Resistant to biocides**
- **Resistant to wide range of stresses such as-**
 - **Low pH**
 - **High salt concentrations**
- *Listeria monocytogenes* is commonly **found in a wide range of food sources including-**
 - **Ready-to-eat** deli meats, raw meats
 - **Soft cheese**
 - **Seafood**



Nearly 30% of food processing drains
tested positive for listeria

Source: USDA

**How *Listeria monocytogenes* adapts to food processing environments
remains to be fully elucidated-**

Outbreak of listeriosis in South Africa -

Listeriosis outbreak in South Africa -

- January, 2017 until July, 2018 – 1,060 laboratory confirmed cases
- **216 deaths**
- **MLST analysis showed that 91% of cases were linked to a ST6, often associated with outbreaks**

*Outbreak traced to polony
Multiple plants implicated*



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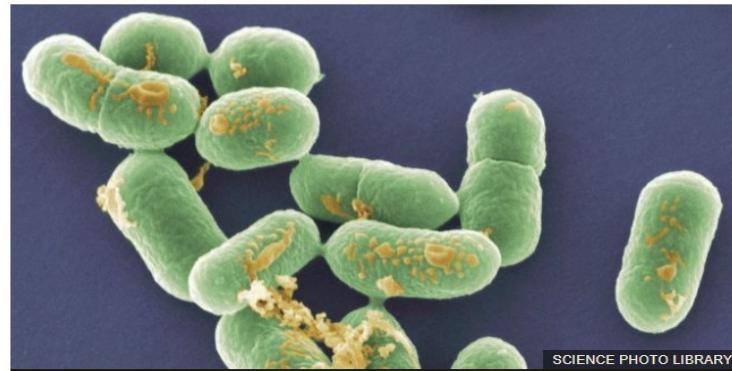
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South Africa listeria: Source of 'world's worst outbreak' found

4 March 2018

f t m Share



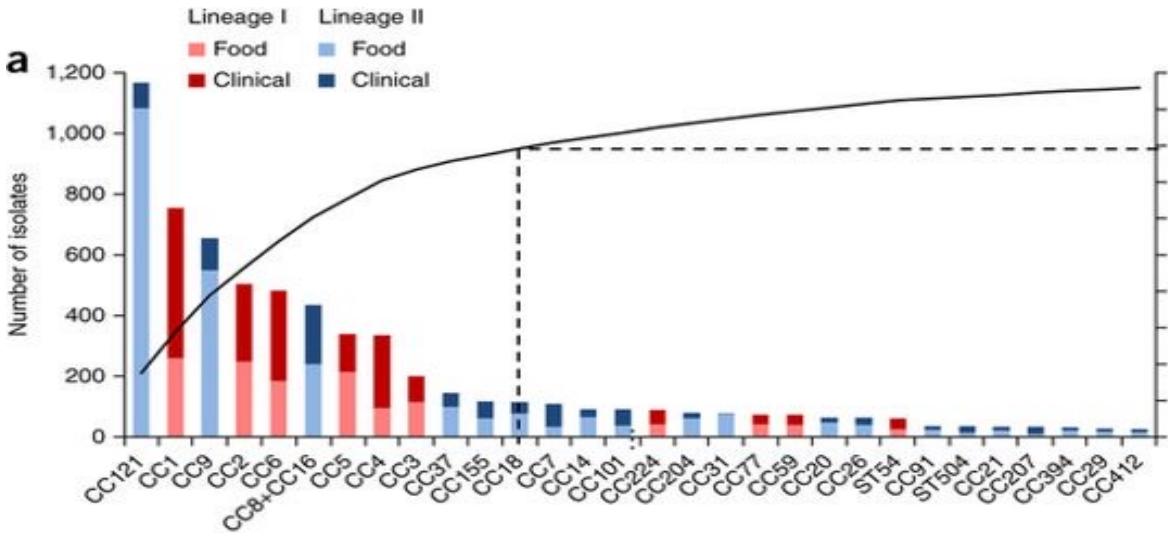
SCIENCE PHOTO LIBRARY

The strain of listeria found in the Polokwane factory that tested positive is particularly virulent

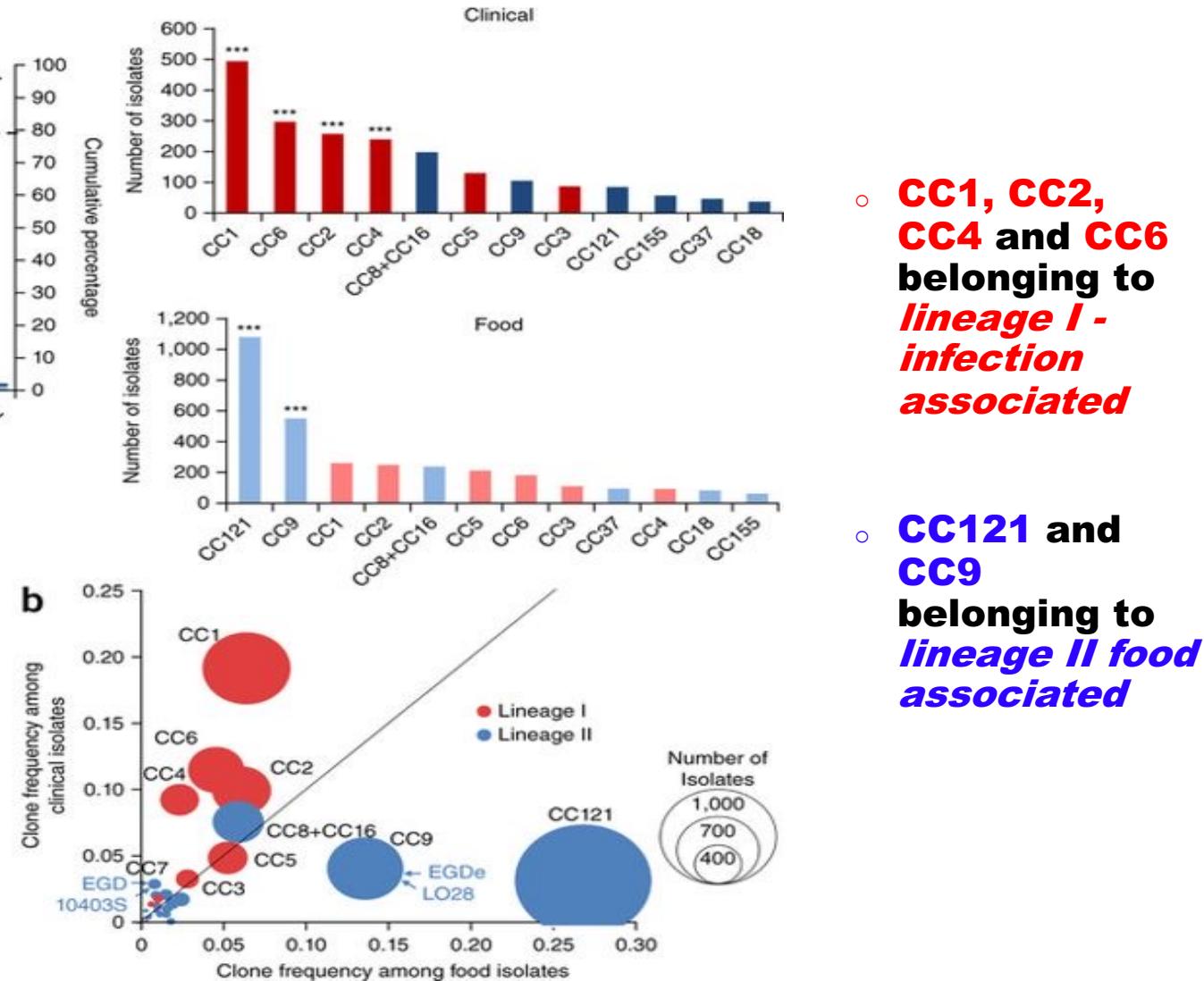
South Africa says it has finally traced the source of a listeria outbreak that has killed 180 people in the past year - said to make it the worst in history.

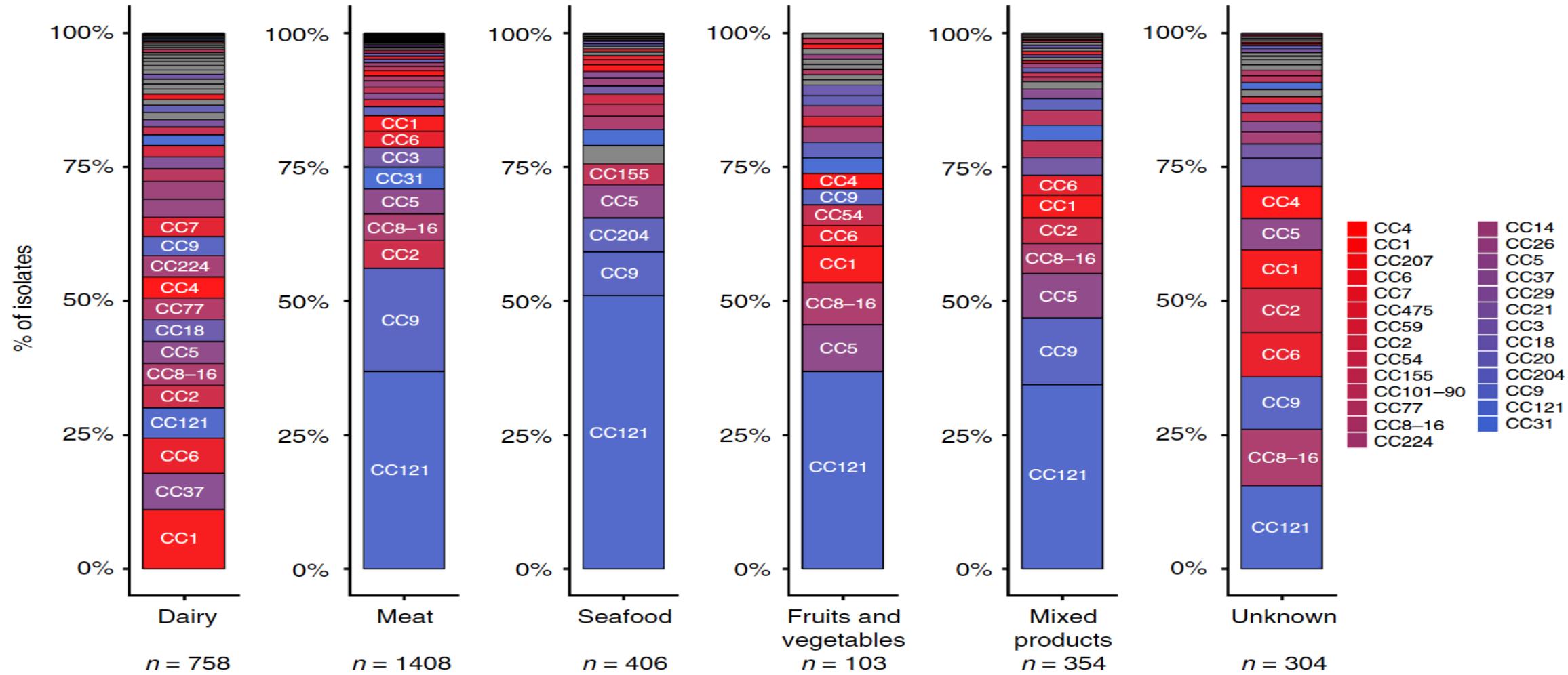
WGS has facilitated the detection of international outbreaks of listeriosis

Epidemiology of clonal complexes (CC) of *Listeria monocytogenes* in clinical and food sources -



- some 6,633 clinical & food isolates collected between the years 2005-2013
- these divided between 2,584 clinical and 4,049 food isolates
- [a] shows the unequal prevalence of *L. monocytogenes* MLST clones
- [b] frequencies of clones > 10 isolates from food (x-axis) and clinical (y-axis) sources

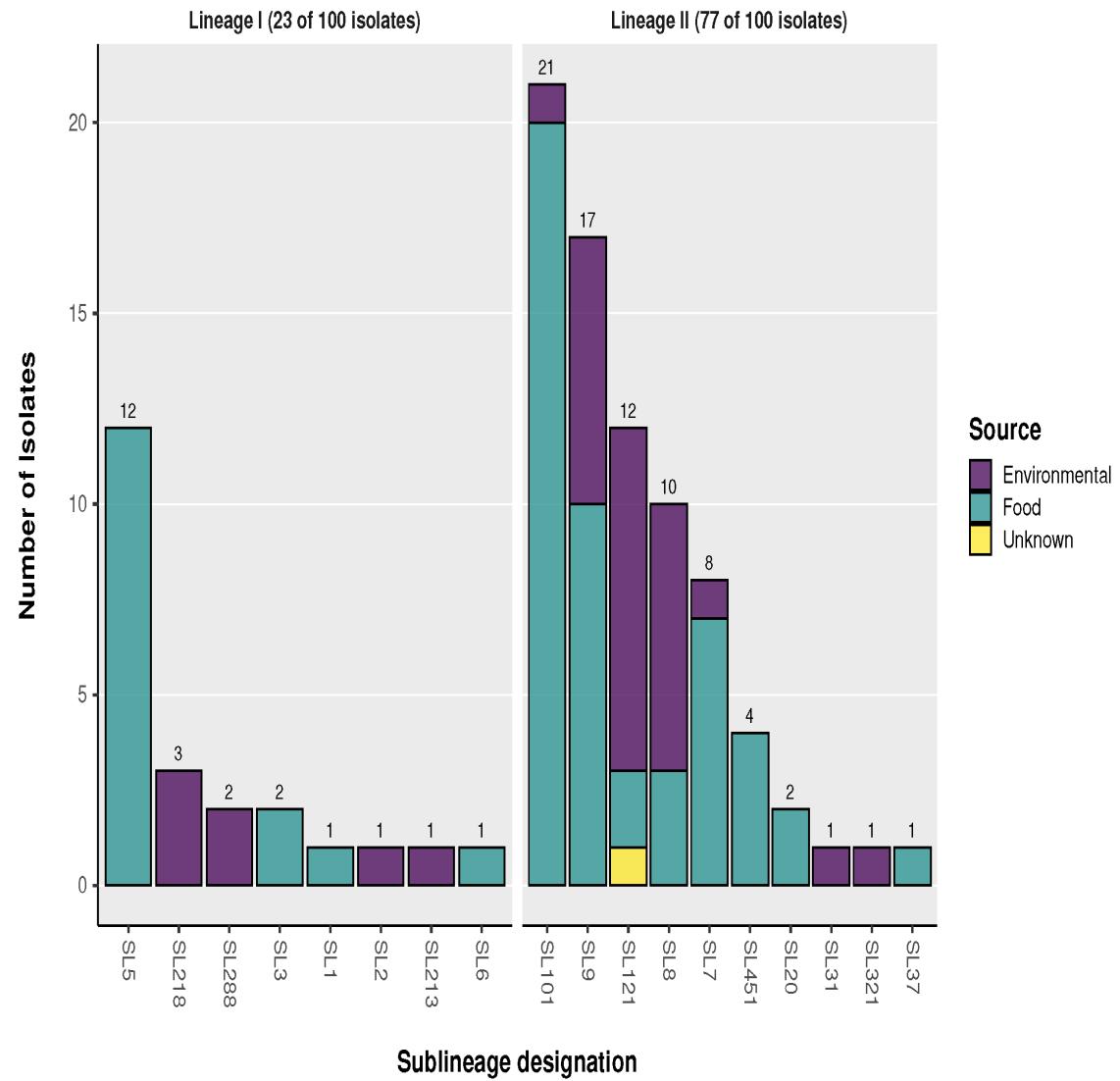




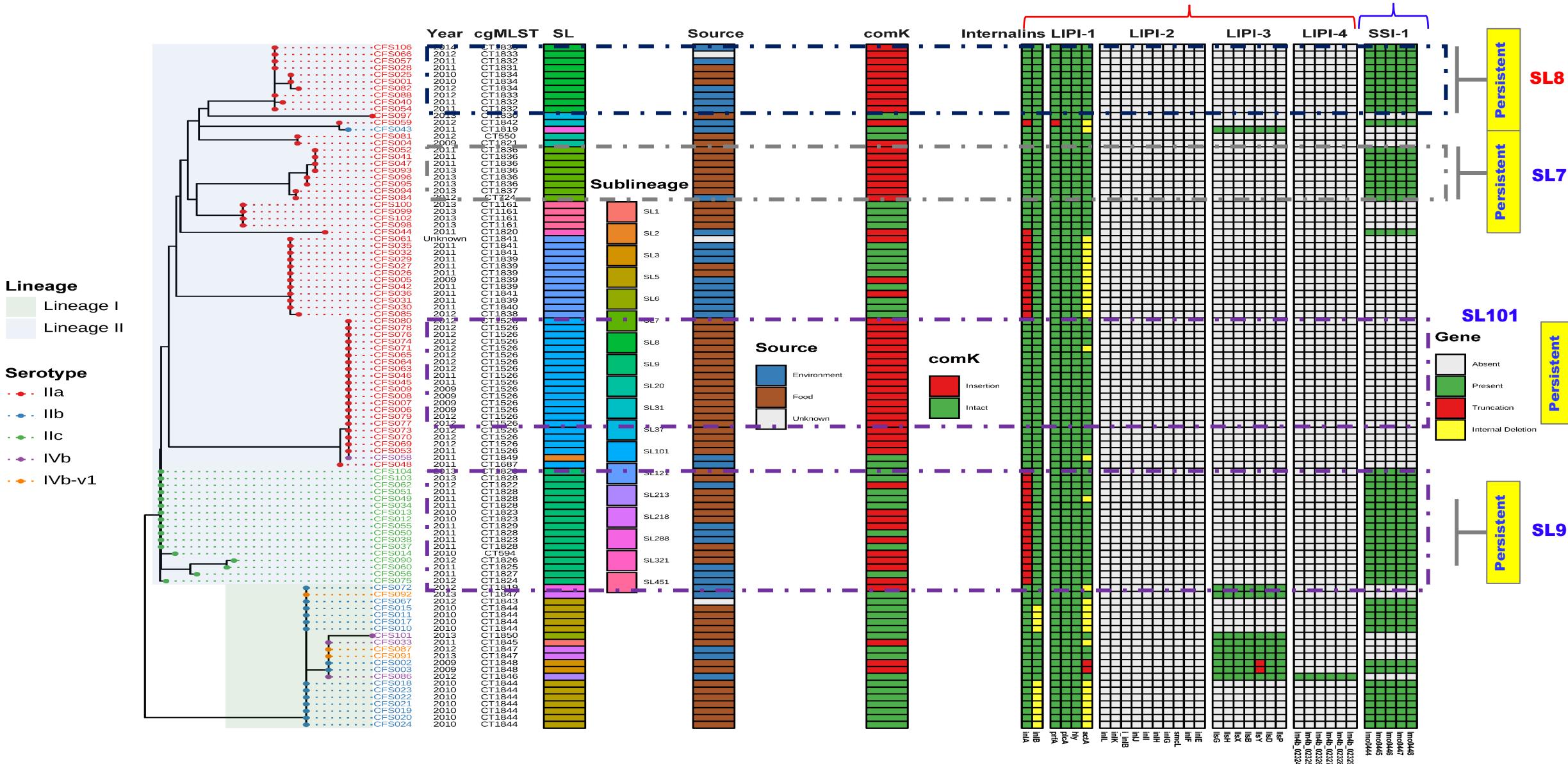
- Hypervirulent clones, such as CC1 are strongly associated with dairy products
- Hypovirulent *L. monocytogenes* clones, CC9 and CC121 are associated with meat products & the food processing environment, with higher prevalence of stress-resistance genes
- ***Clone adaptation to specific environmental niches, may explain these observations***

Listeria monocytogenes study in a cooked meat production site -

- **Food and environmental *L. monocytogenes*** recovered from a cooked meat processing company over a 5-year time period
- **Assess the genomic diversity** of 100 isolates recovered using whole genome sequencing
- Identify **genotypic profiles associated with persistent isolates**
- Identify **gene content differences between putative hyper- and hypovirulent isolates**
- **Inform risk to human health**

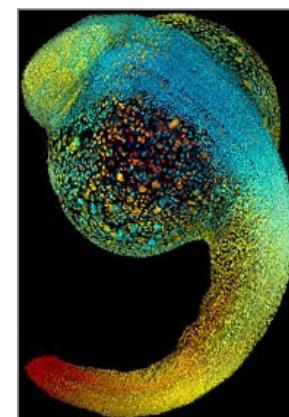
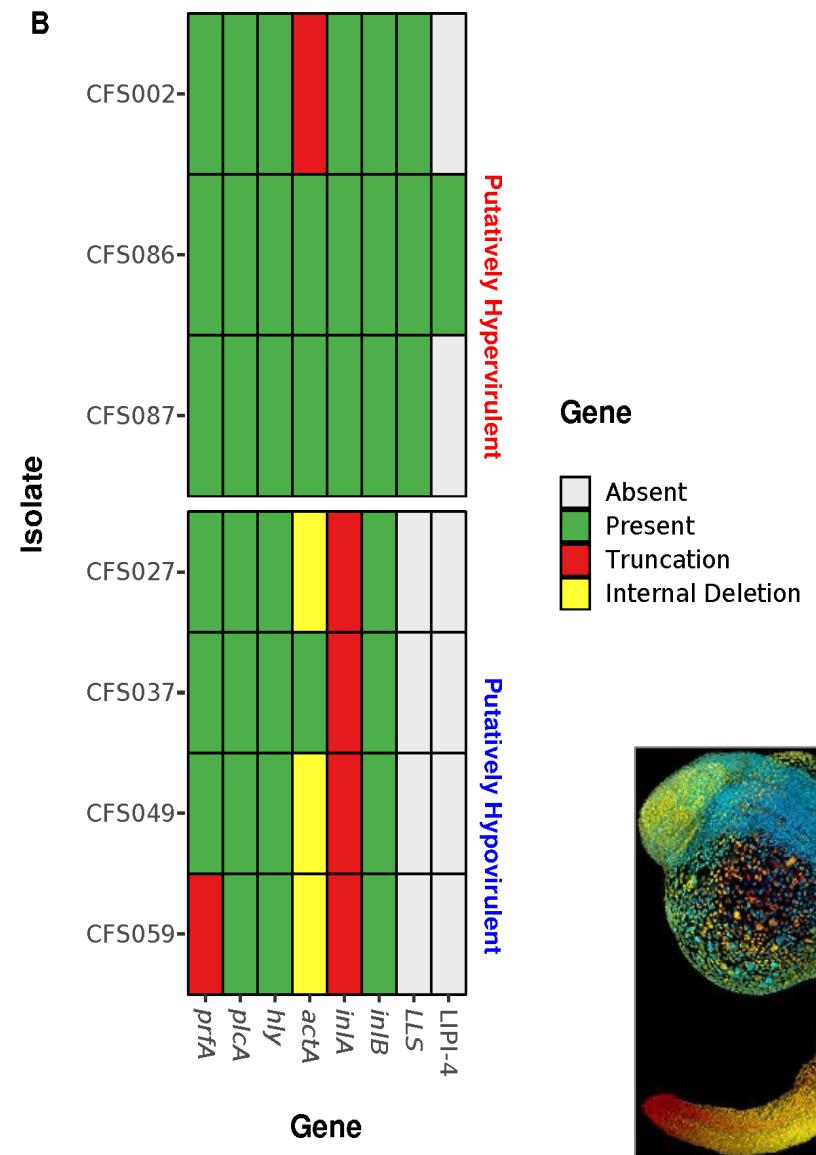
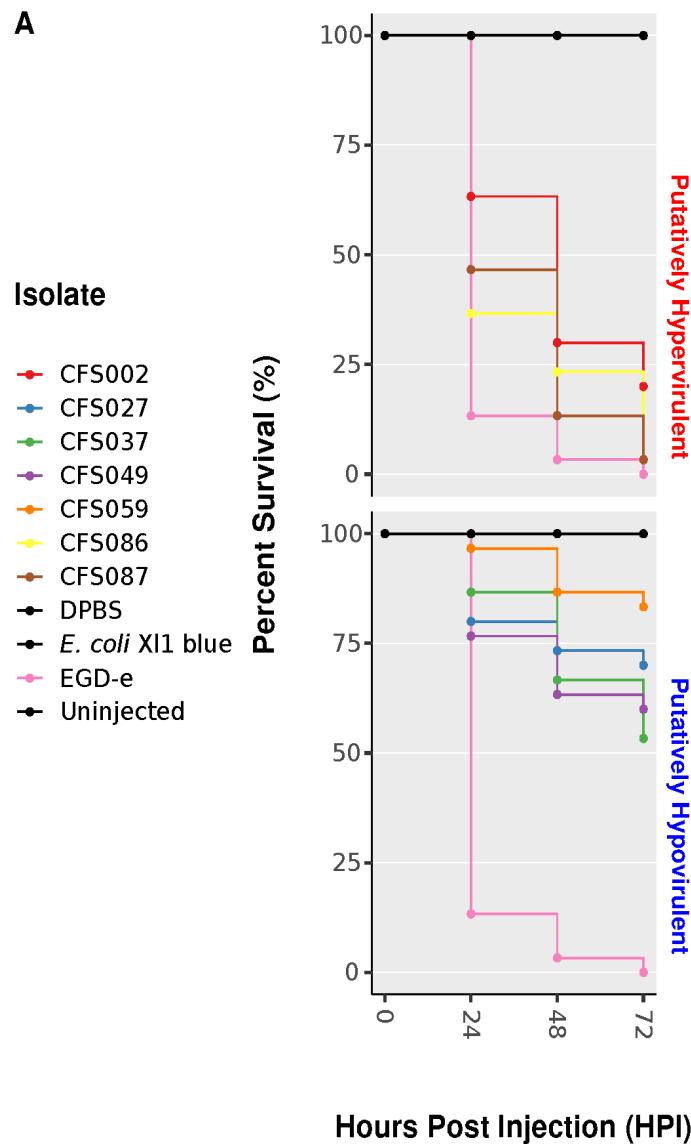


Identifying persistent *Listeria monocytogenes* -

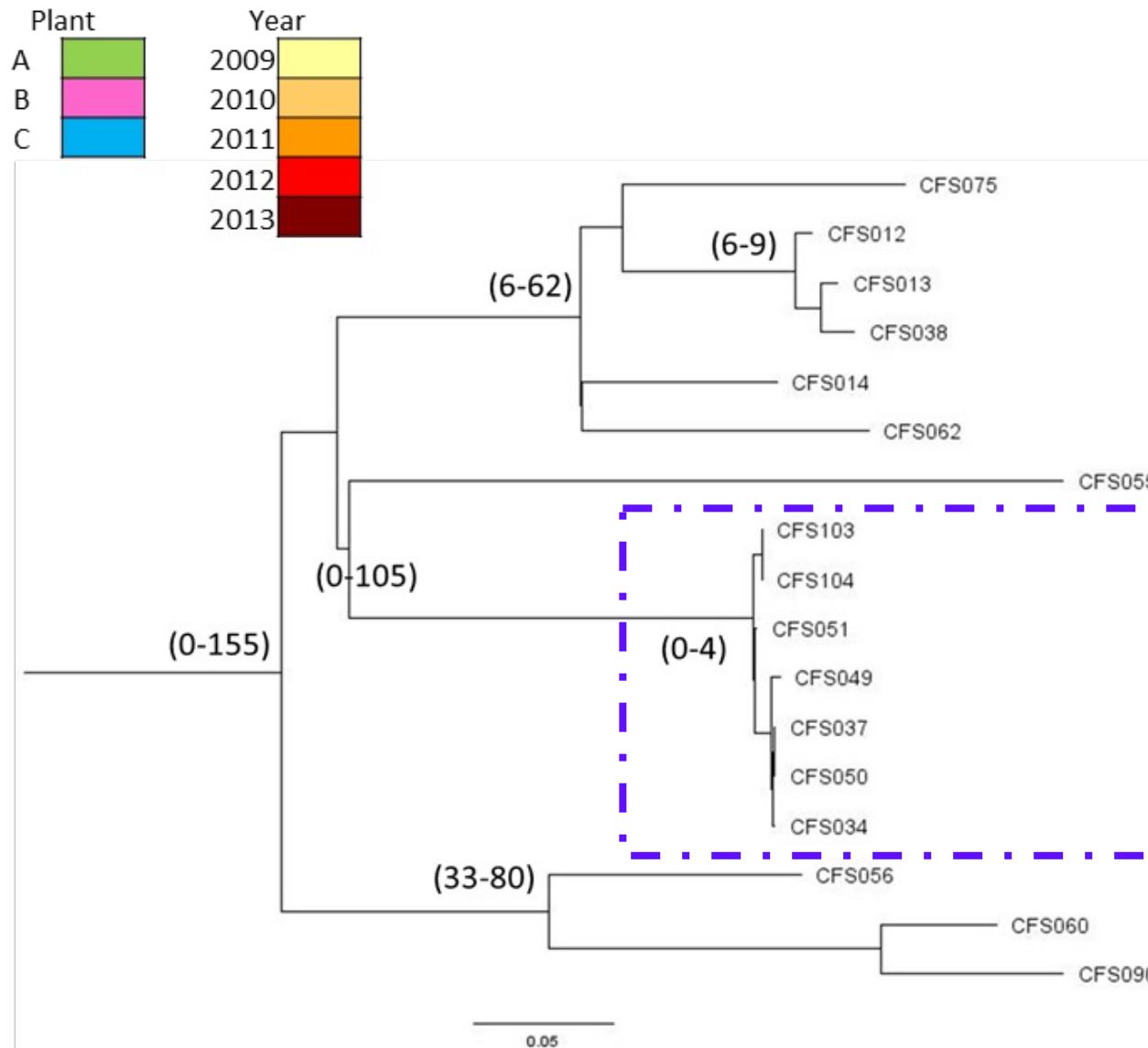


Listeria monocytogenes virulence modelled using a zebrafish embryo infection system -

- Phenotypic differences in virulence aligned with genetic profiles
- Mutations identified within known virulence genes, **including *actA* and *inlAB*** of CC9, CC31, CC121 and CC321 isolates
- ***These genomic insights begin to redefine risk assessment***

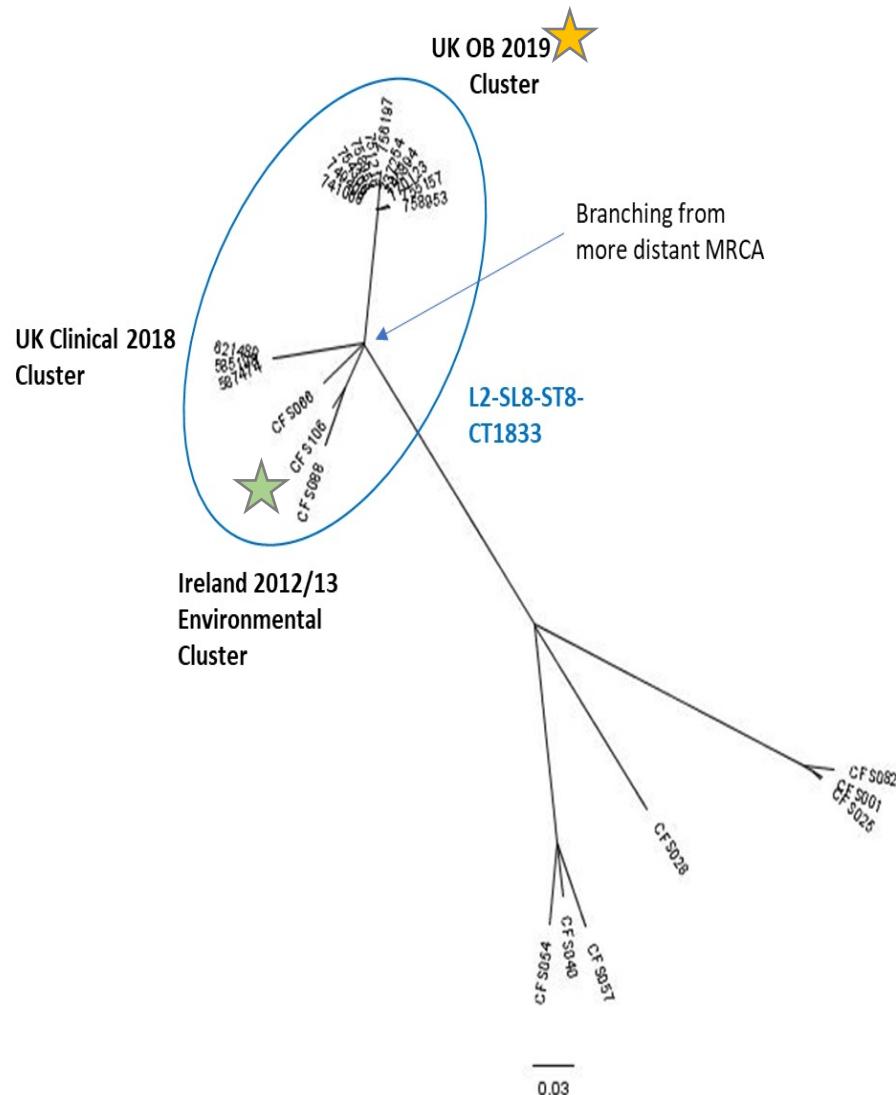


Tracking *Listeria monocytogenes* L2-SL9 using SNP analysis -



SL9	CT	Date	Location	Plant	Year
SL9	CT1824	17/04/12	Environmental swab - Floor		
SL9	CT1823	21/09/10	Food - Meat		
SL9	CT1823	21/09/10	Food - Meat		
SL9	CT1823	23/08/11	Environmental swab - Drain		
SL9	CT594	28/07/10	Food - Meat		
SL9	CT1822	10/01/12	Environmental swab - Floor		
SL9	CT1829	22/11/11	Environmental swab - Floor		
SL9	CT1828	10/12/13	Food - Meat		
SL9	CT1828	28/12/13	Food - Meat		
SL9	CT1828	18/10/11	Food - Meat		
SL9	CT1828	18/10/11	Environmental swab - Conveyor		
SL9	CT1828	08/08/11	Food - Vegetables		
SL9	CT1828	26/10/11	Environmental swab - Conveyor		
SL9	CT1828	08/07/11	Food - Vegetables		
SL9	CT1827	22/11/11	Environmental swab - Pallet		
SL9	CT1825	23/12/11	Environmental swab - Wall		
SL9	CT1826	30/10/12	Food - Raw Meat		

***Listeria monocytogenes* L2-SL8-ST8-CT1833 -the Manchester outbreak-**



On 14th June, 2019 FSA in the UK reported 9 human cases of listeriosis over a 2-month period, with 6 associated deaths

epidemiologically linked to The Good Food Chain (Staffordshire, UK) with meats supplied by North Country Cooked Meats (Salford, UK)-from which a matched isolate with the outbreak strain was cultured

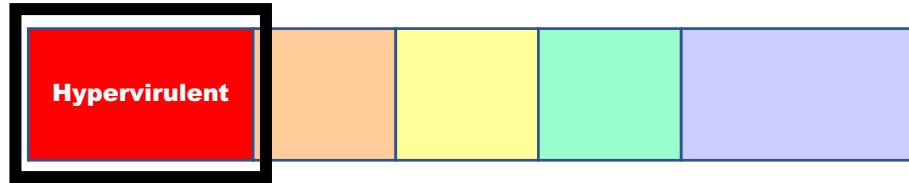
all outbreak isolates are highly related (1-2 SNP differences), suggesting a single point source

three Irish isolates matched the CT type, but when further analysed, SNP differences were 15-20 nucleotides -NOT A DIRECT MATCH

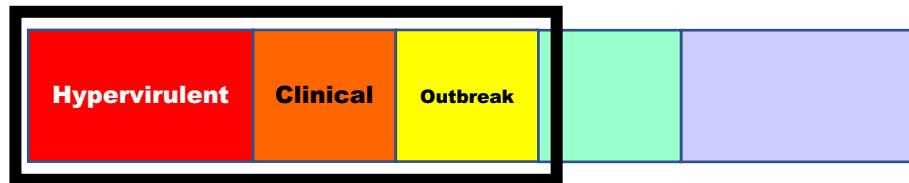
Can we use the bacterial genome sequence to risk characterize a *Listeria monocytogenes* isolate?

Tested the associations of genetic markers against clinical- and environmental adaptation phenotypes

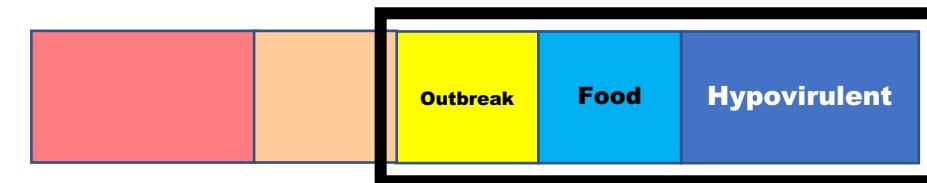
Several phenotypes tested to explore the potential overlap of virulence and environmental adaptation



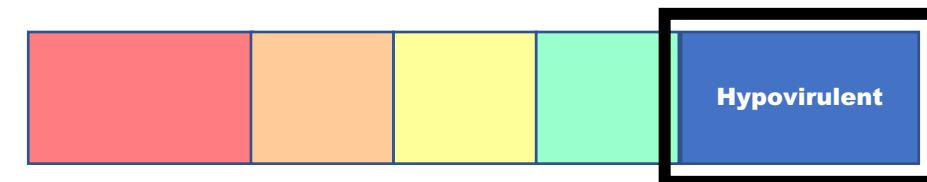
Hypervirulent phenotype –
CC1, CC2, CC3, CC4



More-associated-with-illness



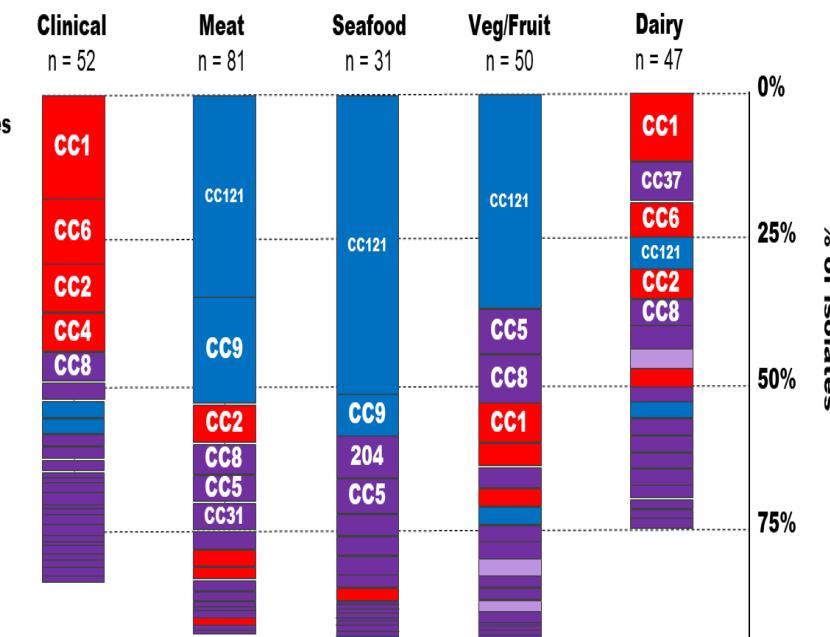
More-associated-with-food



276 isolates most frequently identified in
human infections and food matrices

- Infection associated clones
- Food associated clones
- Intermediate clones

After Maury et al, 2019



Hypovirulent phenotype –
CC121, CC9

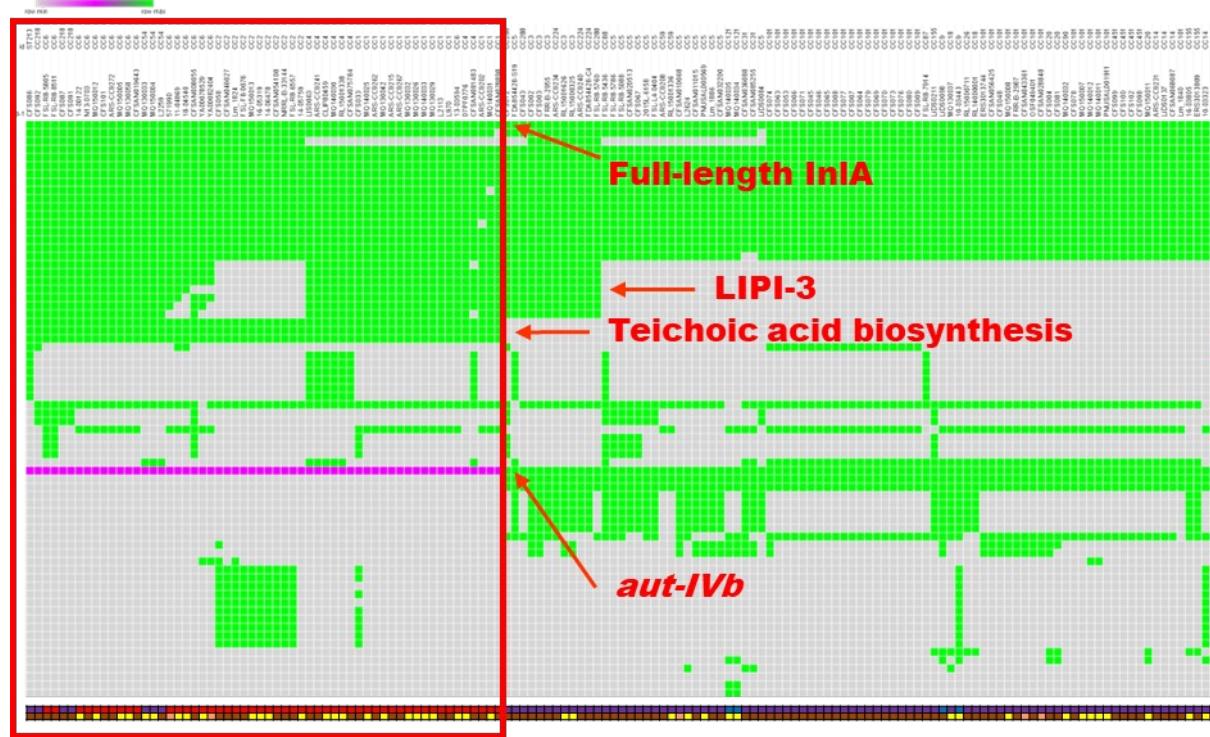
Associations tested using
heatmaps,
statistical tests, and GWAS

Hypervirulent and hypovirulent *Listeria monocytogenes*

cluster separately due to their virulence- and ecophysiology-encoding genetic features

Hypervirulent

CC6 CC2 CC4 CC1

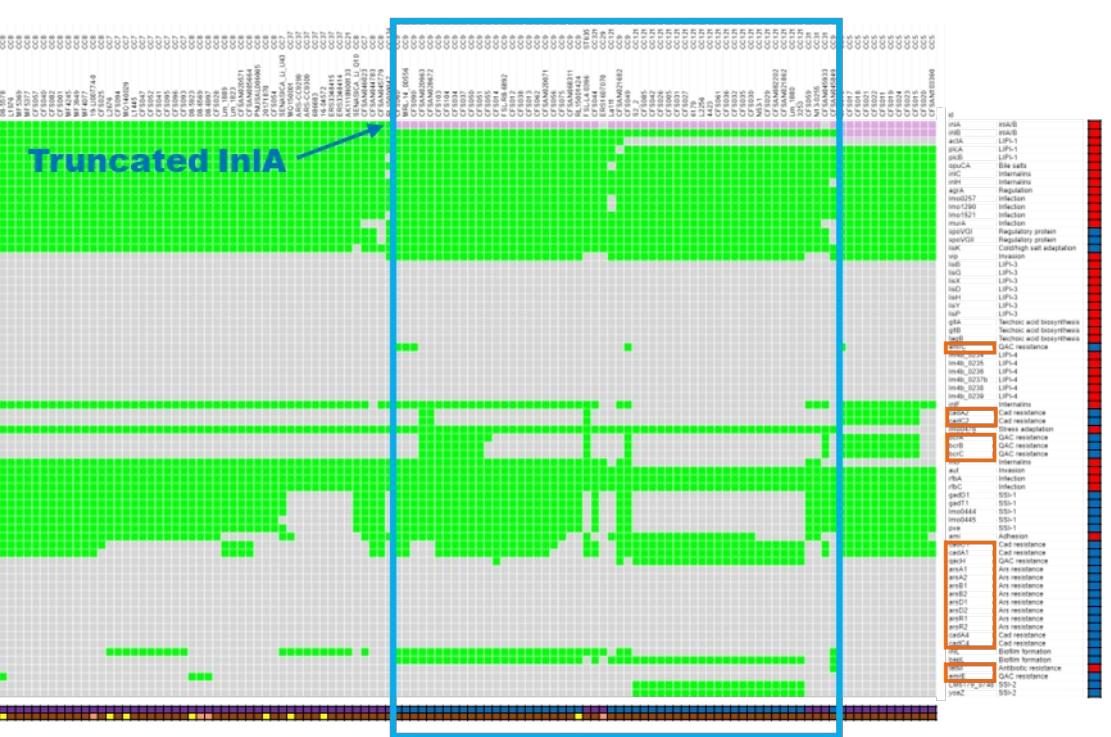


Cluster of infection-associated and the intermediate clones
CC54, CC218 and ST213

Heatmap Key	Isolate Source
Present	Clinical
Alternative Gene	Food (OB-assoc.)
Truncated	Food
Absent	

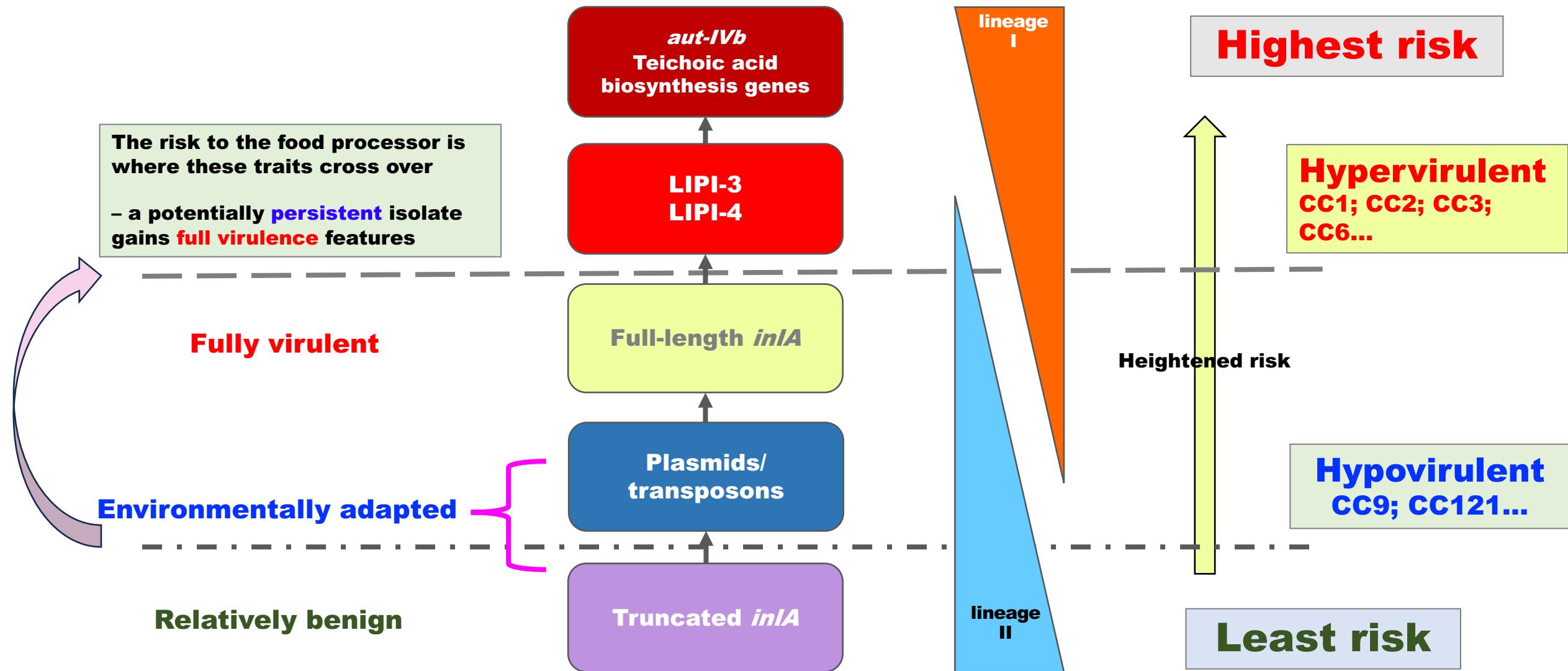
Hypovirulent

CC9 CC121



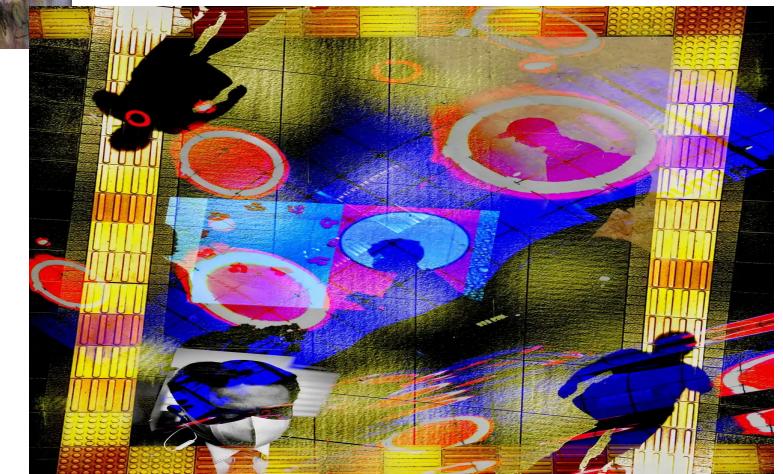
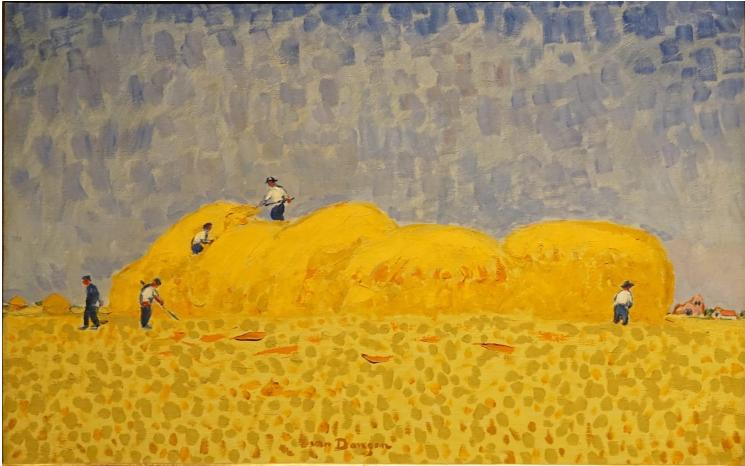
Cluster of food-associated and singleton intermediate clones

Qualitative framework for stratifying risk attributed to *L. monocytogenes* from the perspective of the food processor



Conclusions -

- **Precision food safety is now a reality and will contribute to redefine risk assessment**
- **Big data analyses provides** -unprecedented evidence-based decision making for both food safety and food quality
- **Tracking changes in the bacterial genome (SNP analyses) may in time hold the prospect of revealing the evolutionary pathway leading to contamination of food and the colonisation of its production environment -providing the ultimate food safety control tool**



Thank you