

# The use of Whole Genome Sequencing in Risk Assessment

## The cold smoked salmon-related listeriosis risk model

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Foodborne pathogens & whole genome sequencing  
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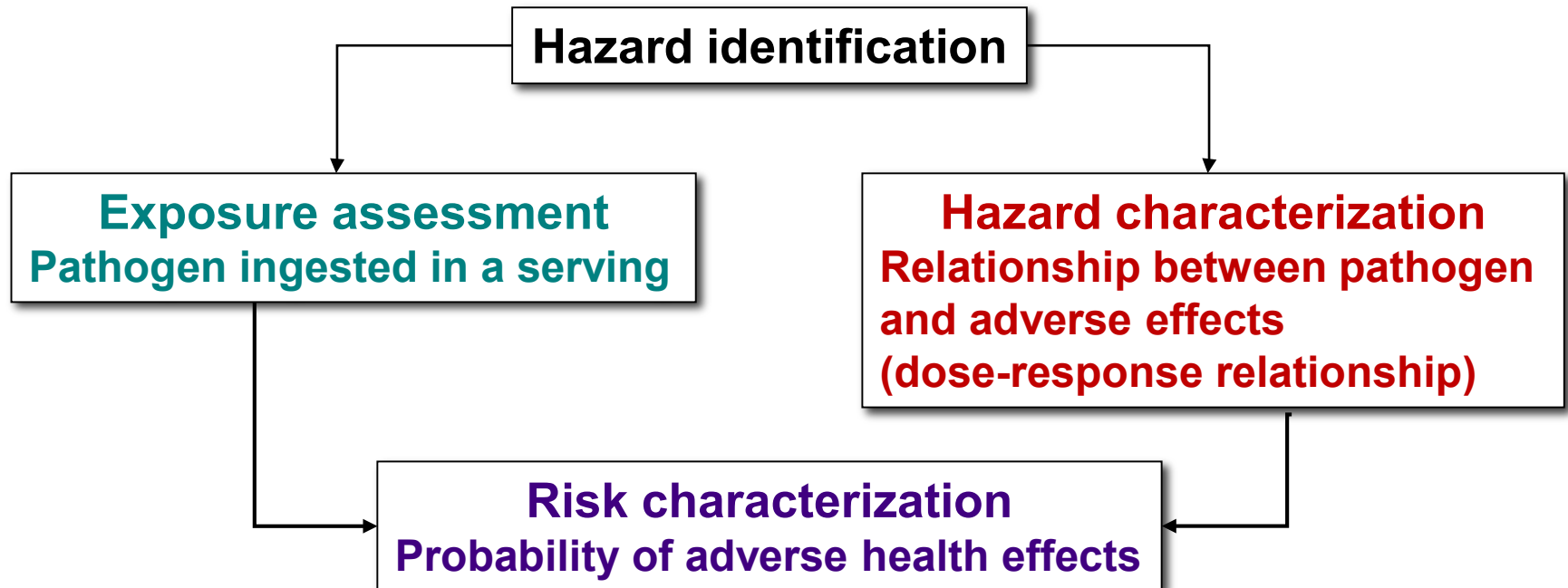
**Session 3 –**  
**Quantitative risk assessment and source attribution in the genomic era**  
**Thursday March 27<sup>th</sup> 2019**

1. Risk assessment: Definition and challenges
2. Genomics in risk assessment
3. Application to risk assessment of listeriosis in cold-smoked salmon
4. Conclusions and perspectives

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# QMRA – A definition

- Risk assessment = Scientific evaluation of the probability and severity of adverse health effects resulting from exposure to foodborne hazards



- Quantitative microbiological risk assessment (QMRA) = numerical estimates (*with their uncertainty*)

# QMRA – Context

- **Output** of QMRA = probability of illness per serving with its **VARIABILITY** = probability distribution

Ex. STEC infections  
(Smith et al., 2013)

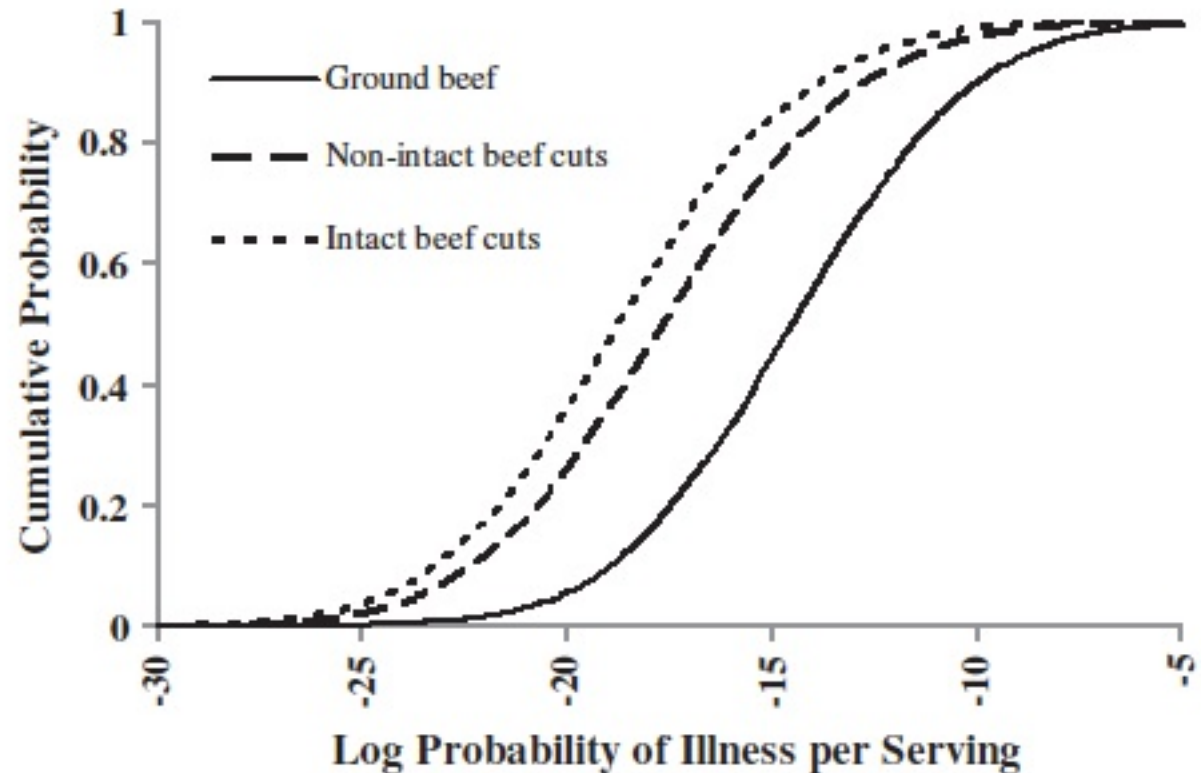
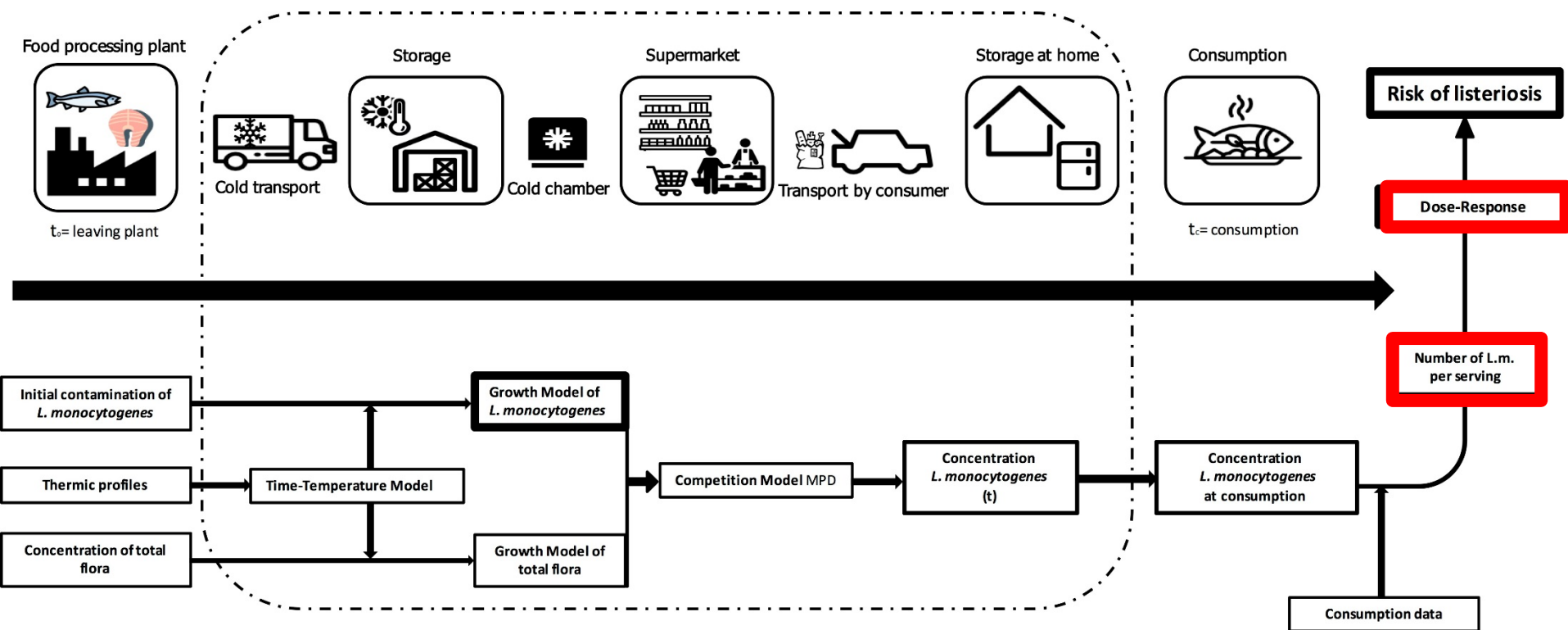


Fig. 2. Distribution of probability of illness for a single serving of cooked ground beef, non-intact beef cuts, and intact beef cuts reflective of current practices in Canada.

# QMRA – Sources of variability

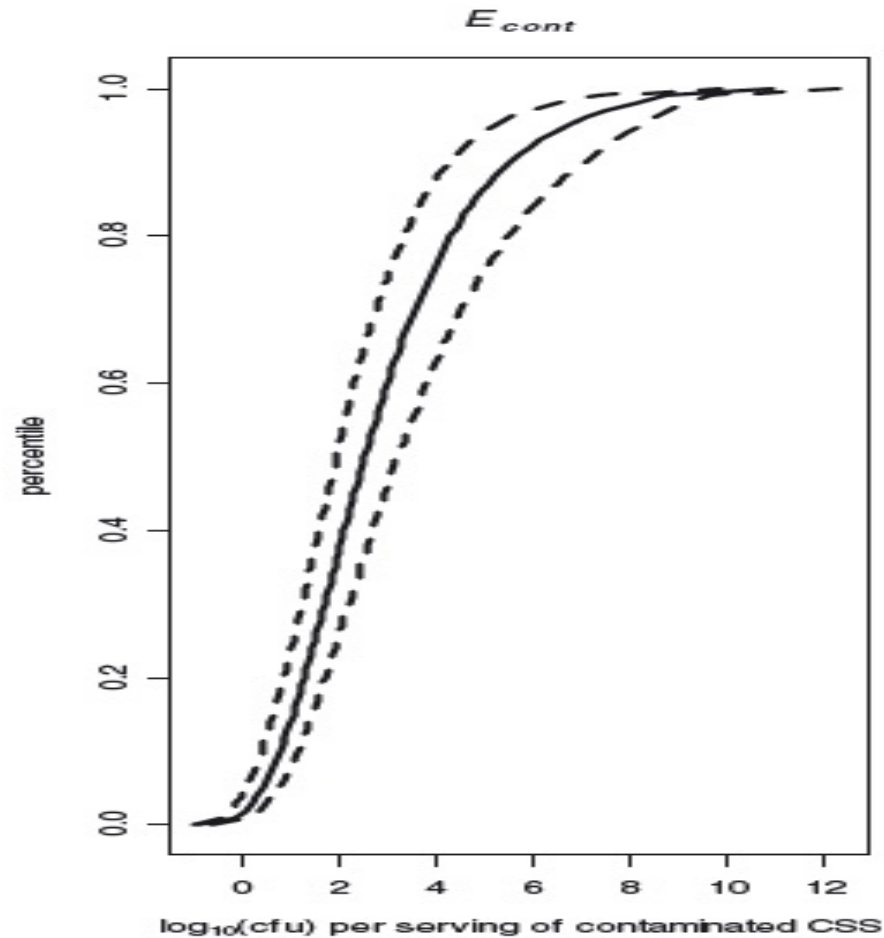
- Everything is variable!

Ex. cold smoked salmon-related listeriosis



# QMRA – Exposure variability

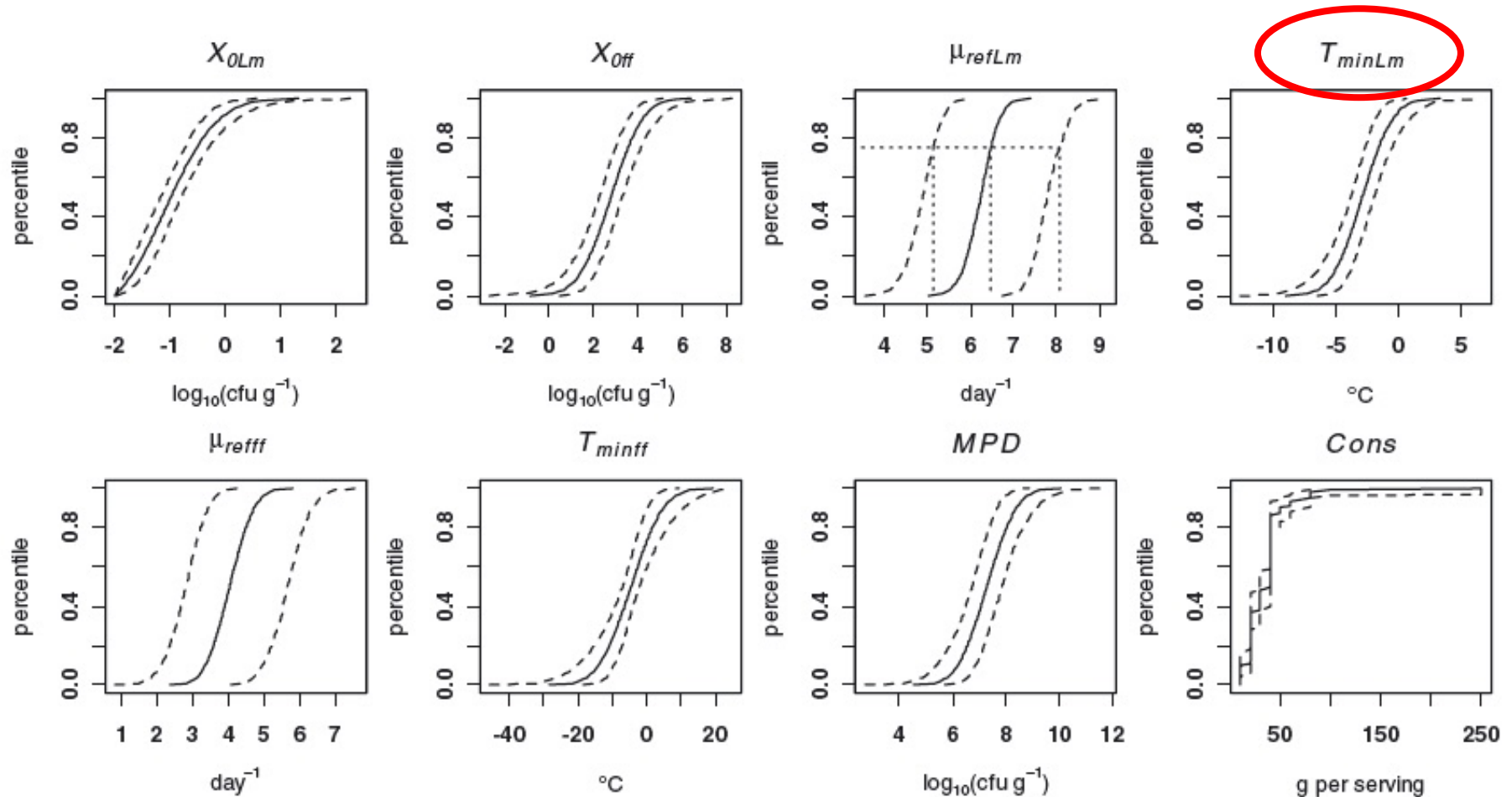
## Example of contaminated servings



(Pouillot et al., 2007)

# QMRA – Exposure variability

Sources of variability of exposure: variability of input parameters

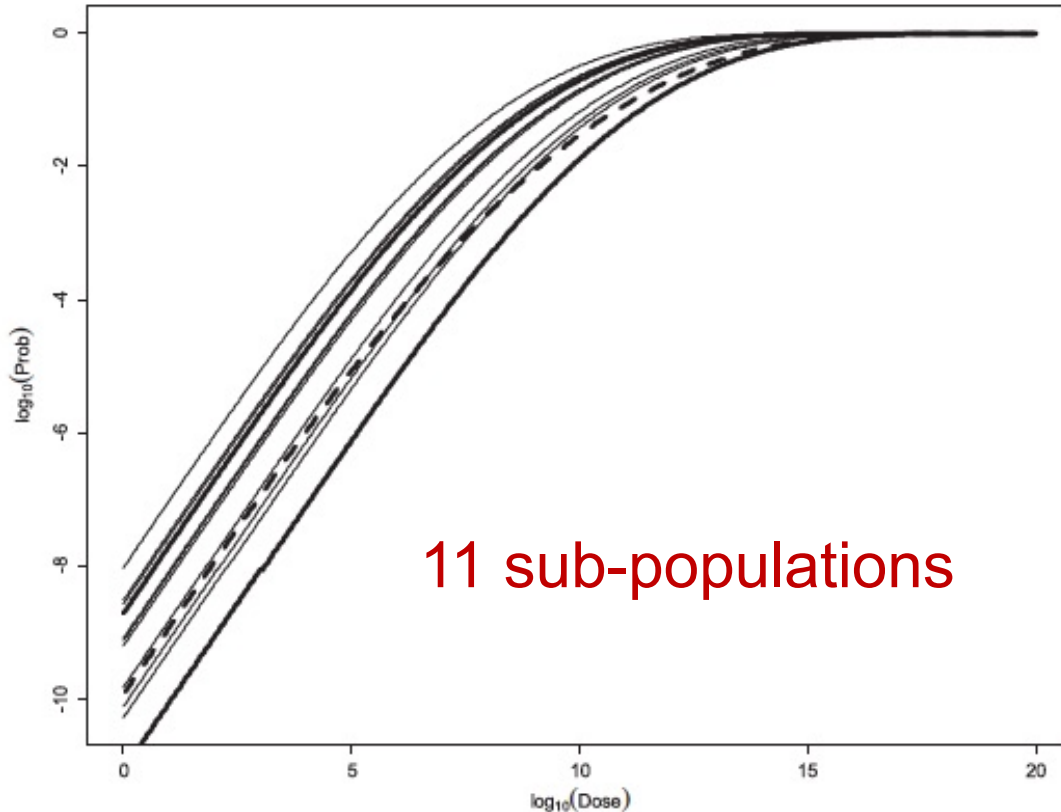
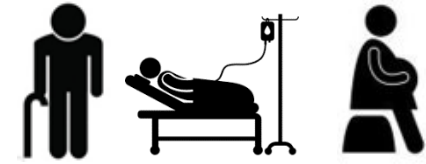


(Pouillot et al., 2007)



# QMRA – Dose response variability

Variability observed between individuals



Variability across population subgroups

Log( $r$ ) ranging from :

-14.1 for people less than 65 years old with no underlying condition (“healthy adults”) to

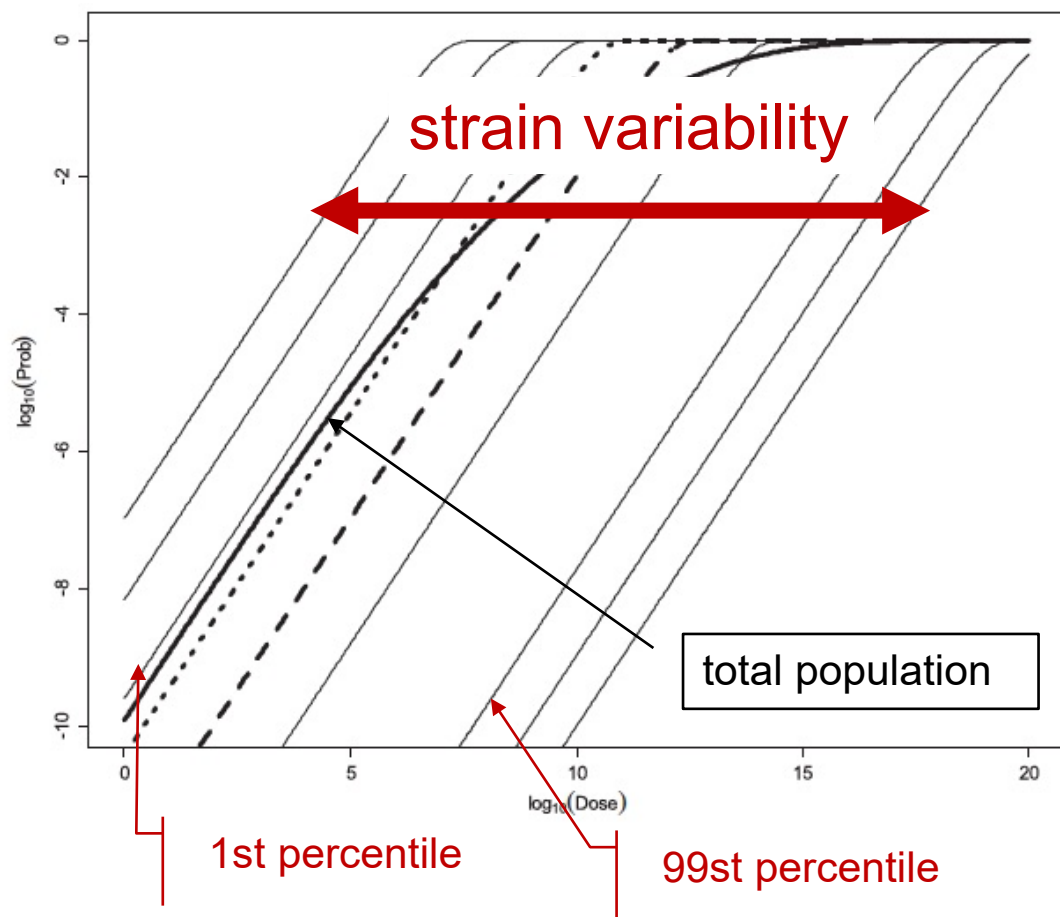
-11.0 for people with hematological cancer

= 3 log variability

(Pouillot et al., 2015)

# QMRA – Dose response variability

Variability observed between *Lm* strains

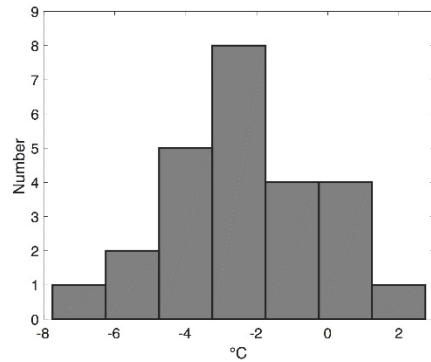


Log(*r*) increasing from more than 7 log between the 1<sup>st</sup> and 99<sup>th</sup> percentiles

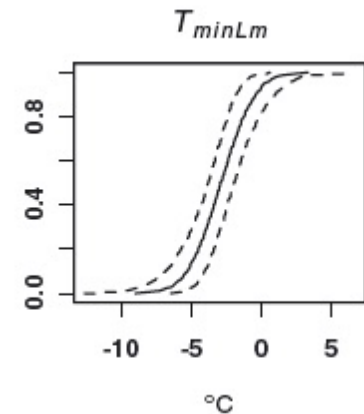
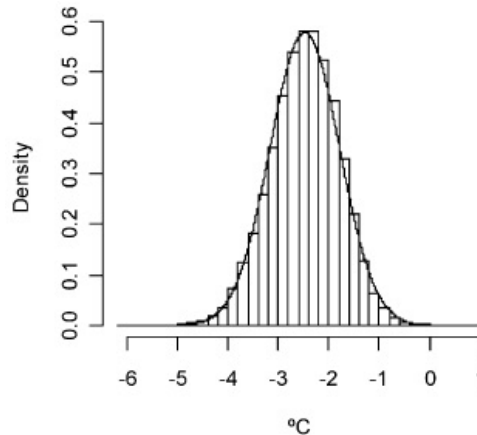
(Pouillot et al., 2015)

# QMRA – Considerations on variability

- Relevant probability distribution describing the variability of  $T_{\min}$ ?



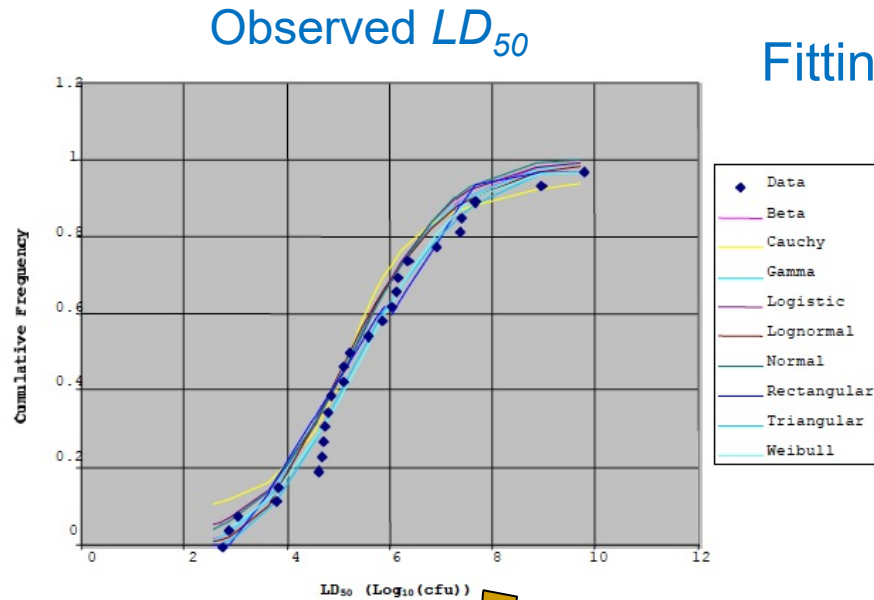
Fitting a Normal distribution



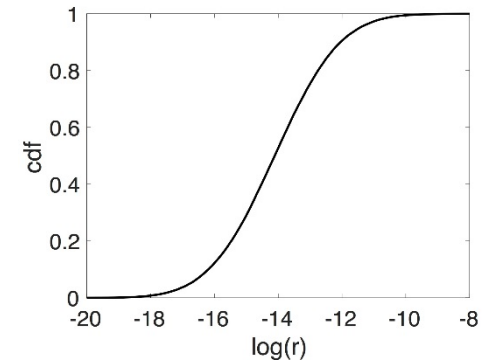
$$T_{\min} \sim N(-2.9^{\circ}\text{C}, 1.9^{\circ}\text{C})$$

# QMRA – Considerations on variability

- Relevant probability distribution describing the variability of  $r$ -values ?



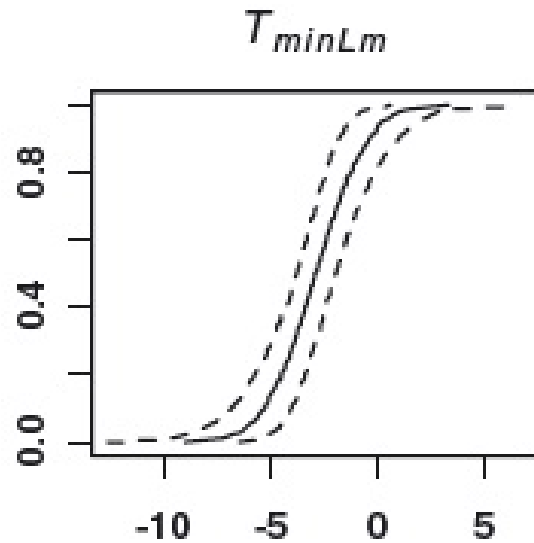
Fitting a Log-Normal distribution



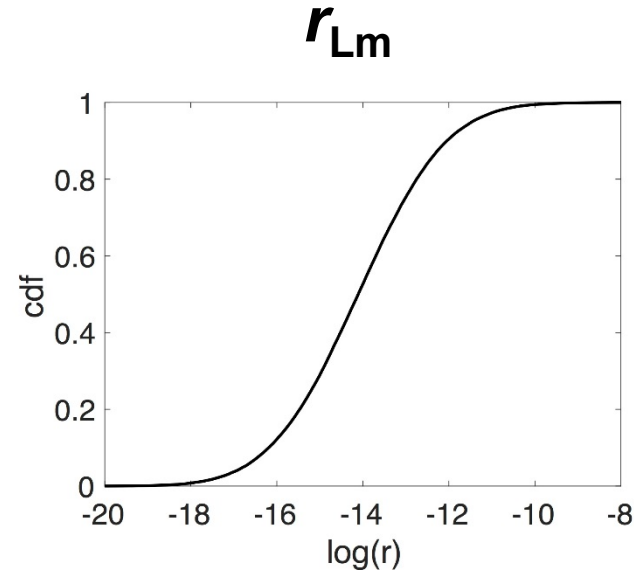
$$\text{Log}(r) \sim N(-14.1, 1.6)$$

# QMRA – Considerations on variability

- Probability distributions describing the variability = relevant



$$T_{min} \sim N(-2.9^{\circ}\text{C}, 1.9^{\circ}\text{C})$$



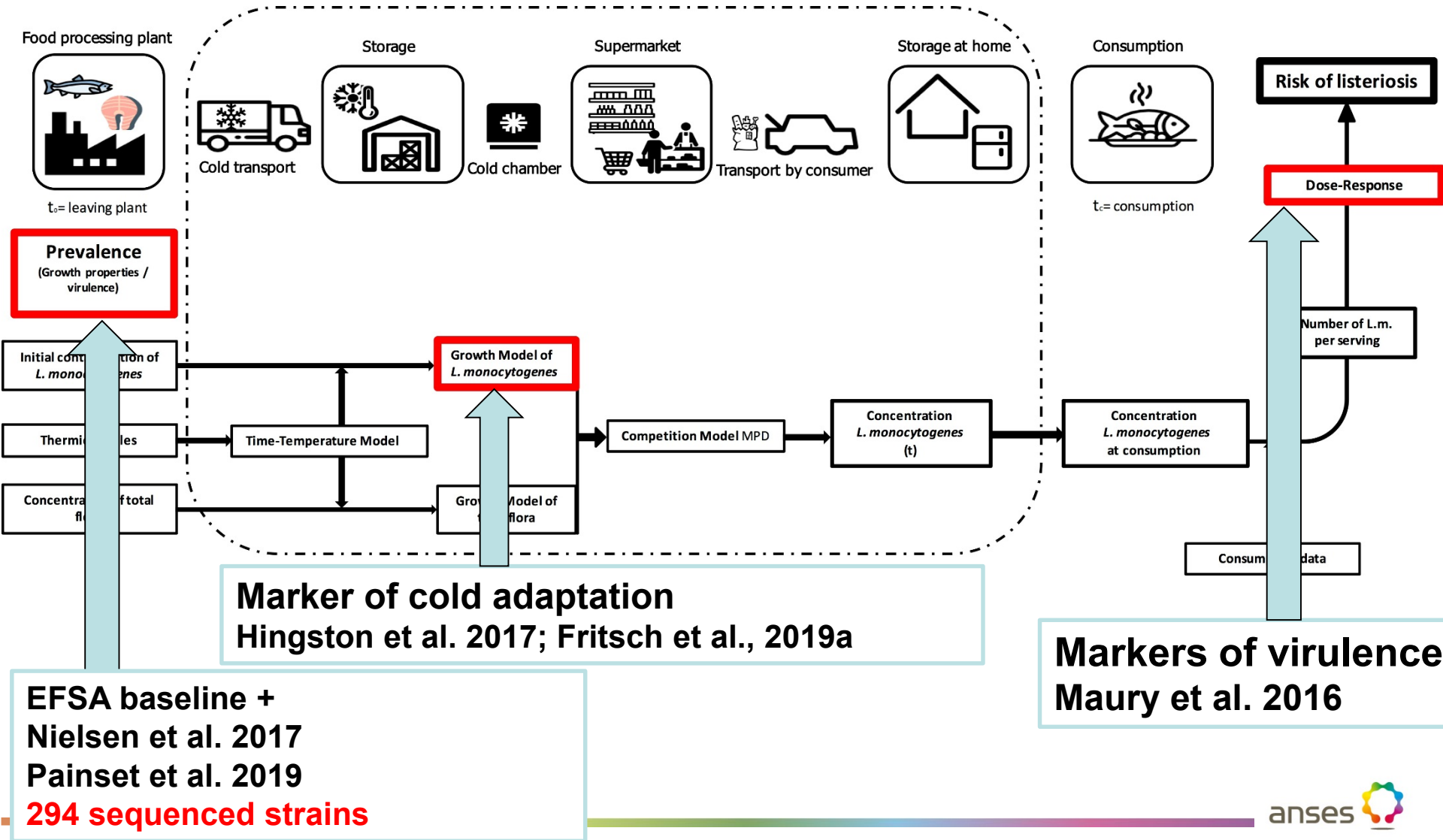
$$\text{Log}(r) \sim N(-14.1, 1.6)$$

**Are these strains representative of the food under consideration?**

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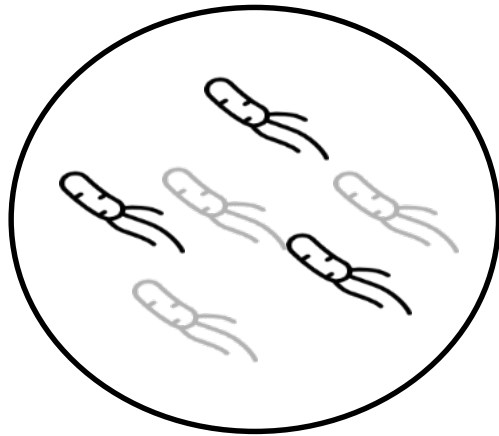
# Objective: Implementing genomics in QMRA

- Cold smoked salmon model (Pouillot et al., 2007, 2009)

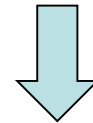
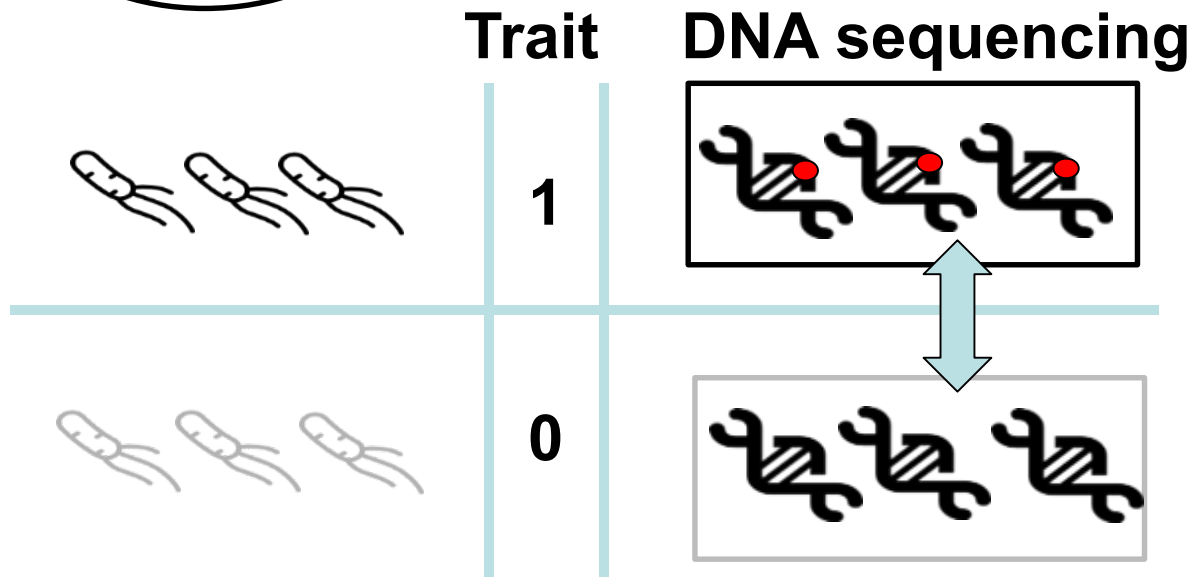


# Genomics and growth in cold conditions

Method: Genome wide association studies (GWAS)



Find associations  
between genetic  
variations and  
observable traits



**Biomarker « X »**

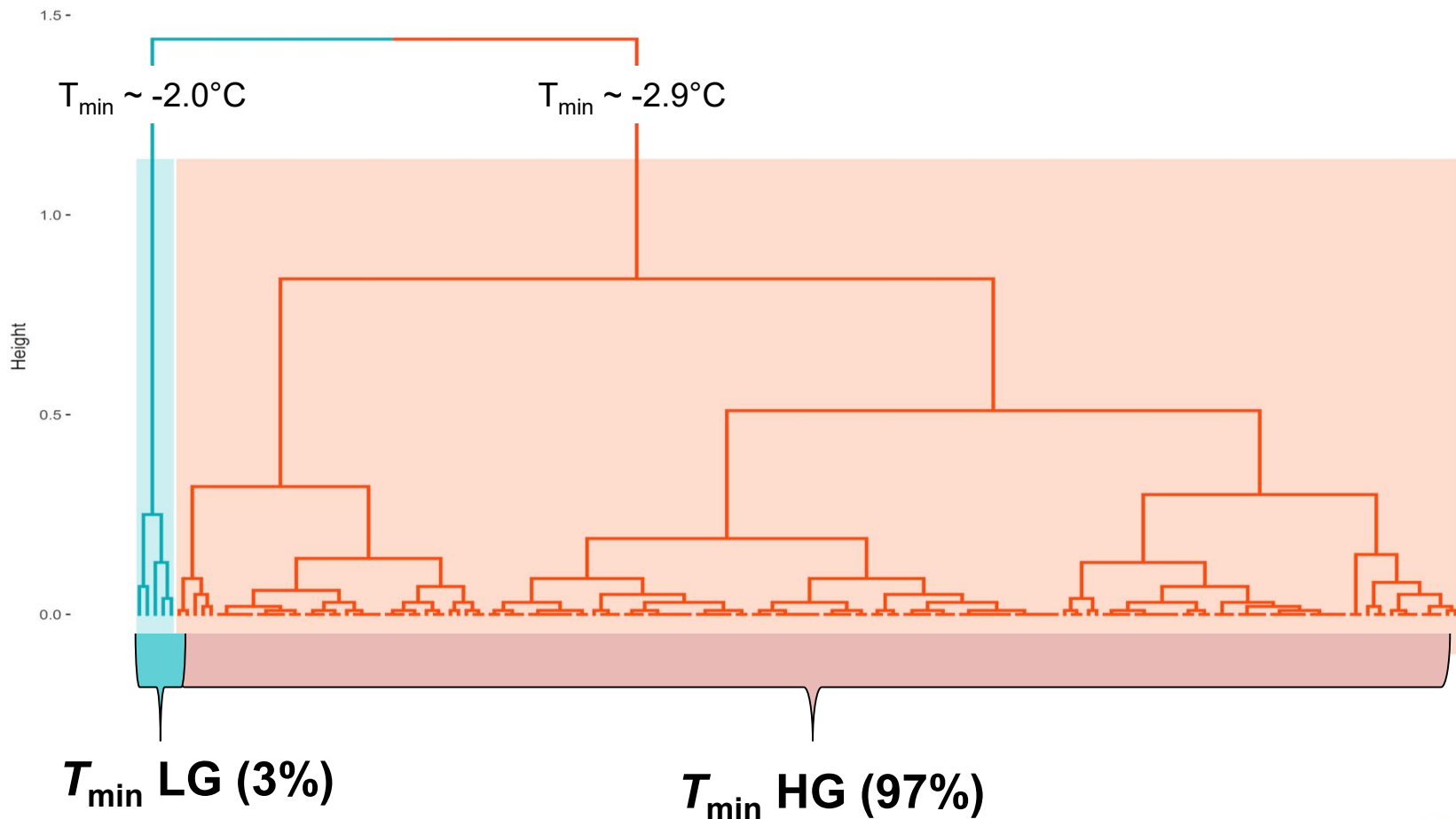
- Genes
- SNPs
- ....



# Genomics and growth in cold conditions

$T_{\min}$  – 166 strains (Hingston et al. 2017)

- Biomarker as Genes, SNPs, ...
- Biomarker full length inIA → associated with adaptation to cold temperature



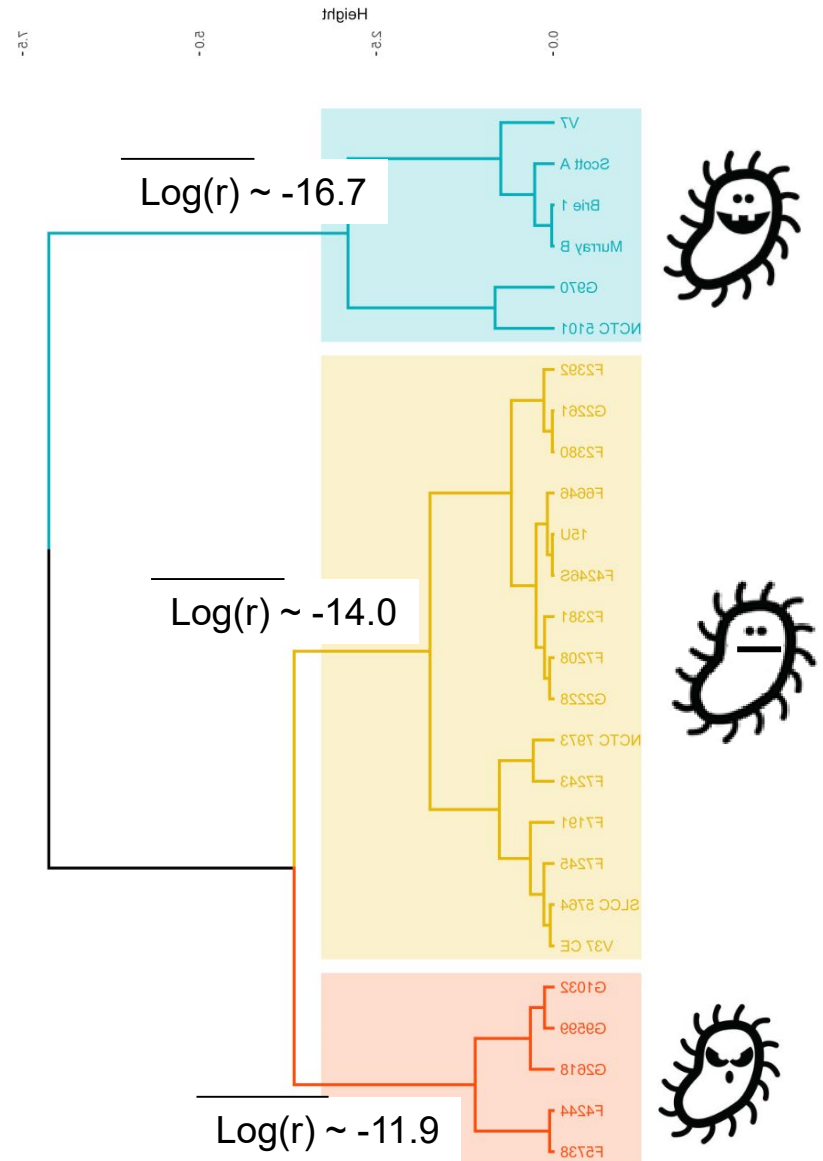
# Genomics and virulence

Virulence – 26 strains (FDA/FSIS 2003)

- $LD_{50}$  intraperitoneal infection route in mice
- $r$ -values deduced with a scaling factor
- $\text{Log}(r) = -\text{Log}(LD_{50}) - 8.79$

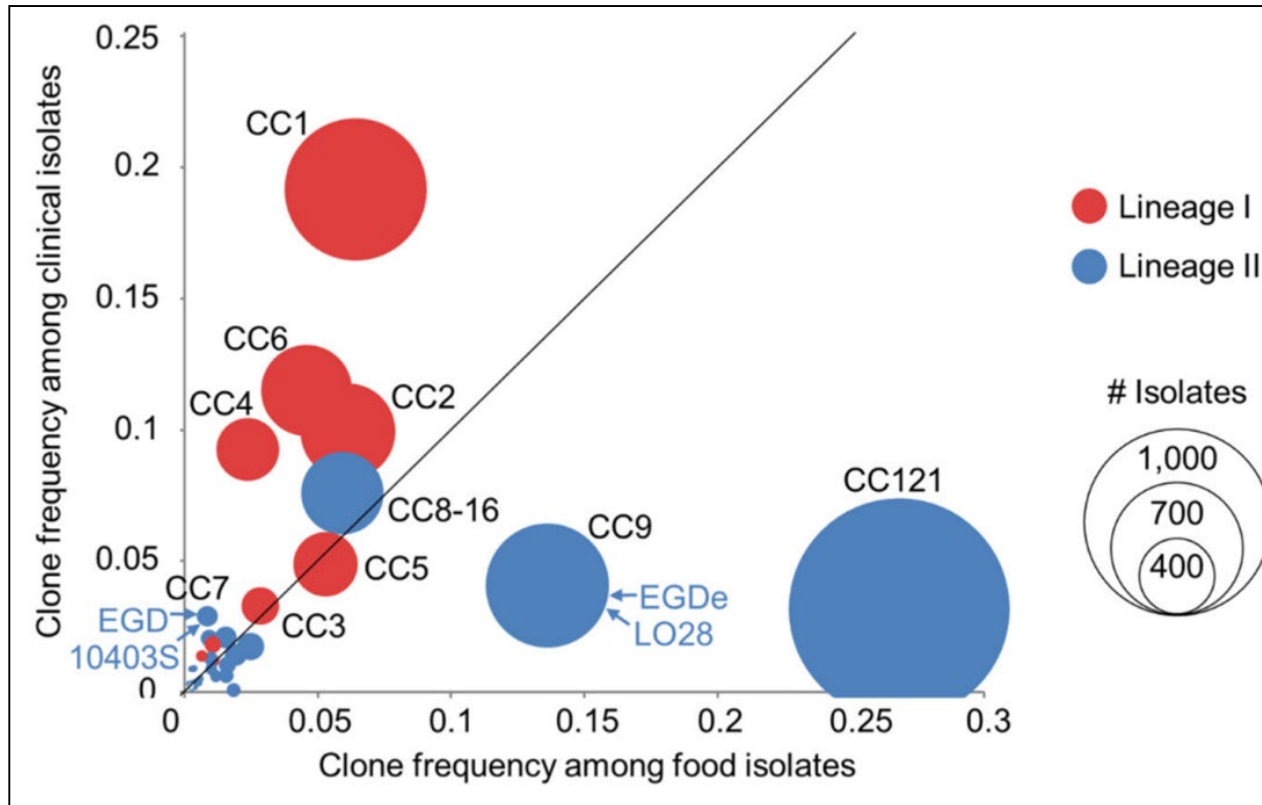
Question

- Links with genomic markers?



# Genomics and virulence

- Link based on epidemiological data: Hypo- and hypervirulent strains defined according to CC

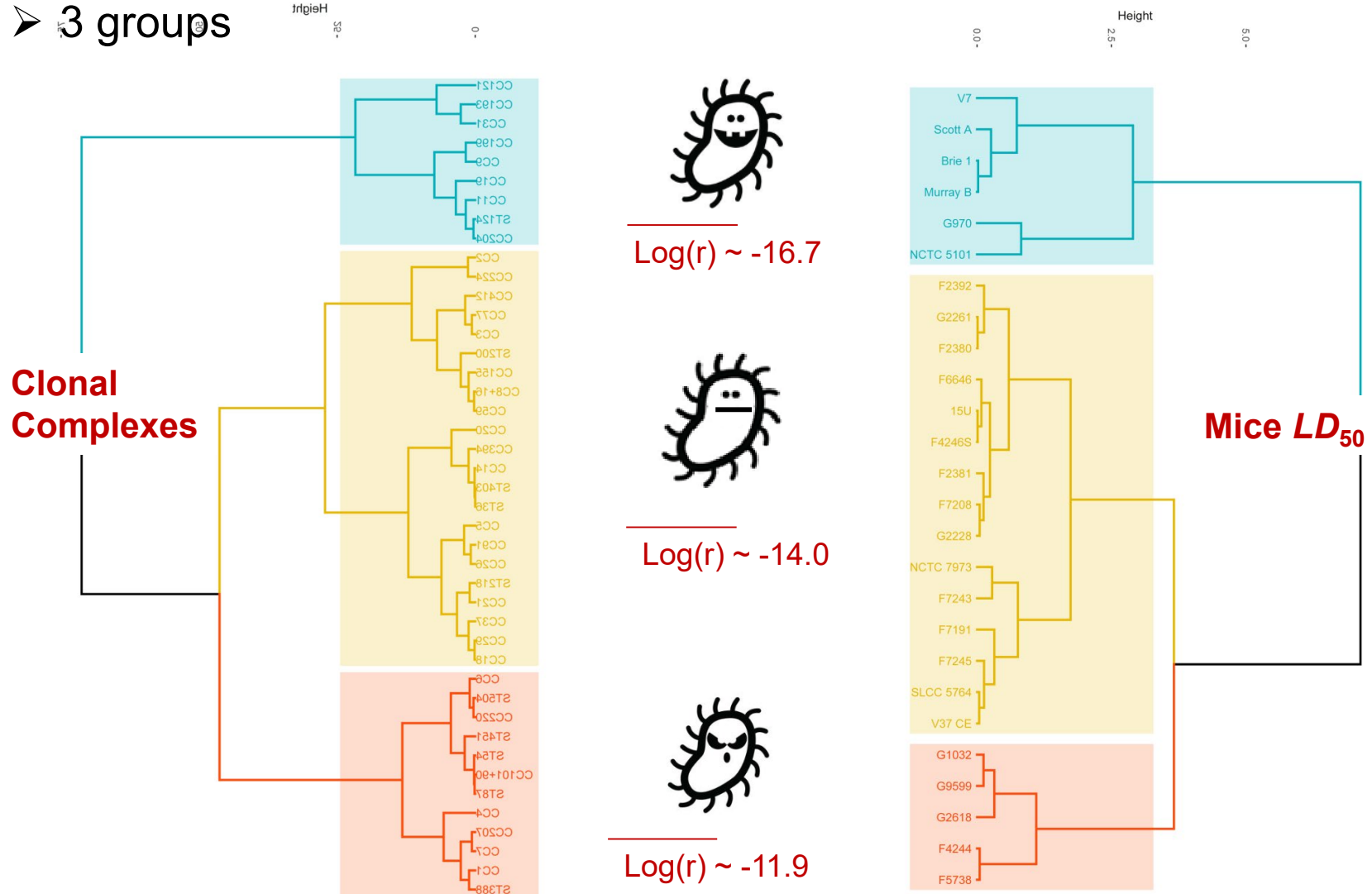


(Maury et al. 2016)

# Genomics and virulence

- **Relative clinical frequency** of CC assumed reflecting the virulence

➤ 3 groups

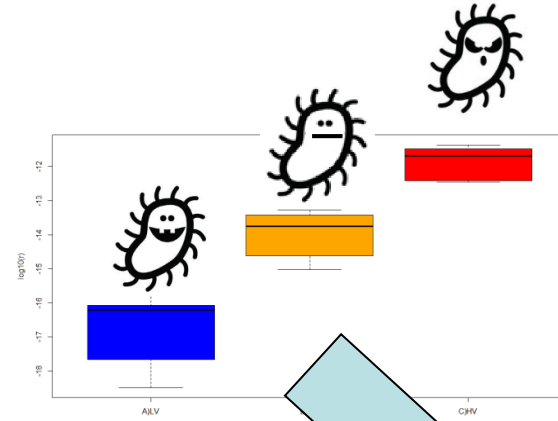
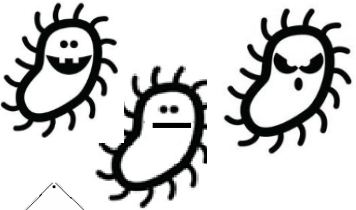


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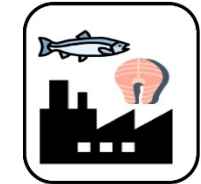
# A QMRA with genomics



Prevalence 10.4%  
(EFSA 2013)

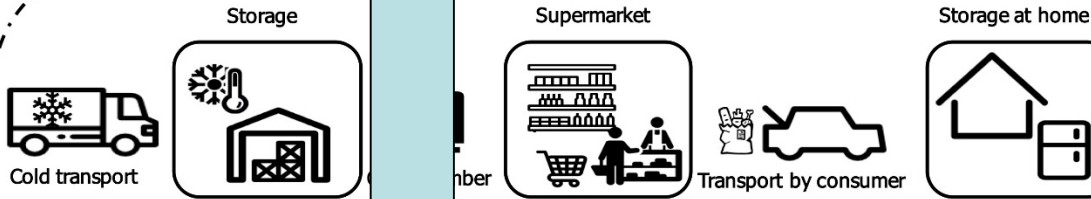


Food processing plant



$t_c$  = leaving plant

**Prevalence**  
(Growth properties / virulence)



$t_c$  = consumption

**Risk of listeriosis**

**Dose-Response**

Number of L.m. per serving

Consumption data

Initial contamination of *L. monocytogenes*

Thermic profiles

Concentration of total flora

Time-Temperature Model

**Growth Model of *L. monocytogenes***

Growth Model of total flora

Competition Model MPD

Concentration *L. monocytogenes* (t)

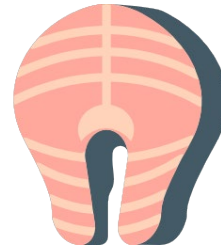
Concentration *L. monocytogenes* at consumption

# Distribution of CCs in cold smoked salmon (Møller-Nielsen et al. 2017, Painset et al. 2019)

- 294 strains collected in EFSA Baseline survey

CCs	Number of isolates	Total	Virulence group
CC1	1	37 (12.6%)	Hyper
CC2	4		
CC6	10		
CC7	8		
CC87	6		
CC101	7		
CC451	1		
CC3	9	105 (35.7%)	Medium
CC5	1		
CC8	49		
CC14	5		
CC20	4		
CC21	1		
CC59	6		
CC177	1		
CC155	24		
CC403	4		
new CC	1		
CC9	37	152 (51.7%)	Hypo
CC121	93		
CC19	3		
CC31	5		
CC193	4		
CC204	8		
ST124	2		
<b>Total</b>	<b>294</b>		

# And now, let's run the QMRA models !



Prevalence  
10.4%

**Specific**

**Generic**

(%)	Slow	Fast	Total
Hypervirulent	0.4	12.2	<b>12.6</b>
Medium virulence	1.1	34.6	<b>35.7</b>
Hypovirulent	1.6	50.1	<b>51.8</b>
Total	<b>3.0</b>	<b>97.0</b>	100

$T_{\min}$  : 1 empirical distribution  
of 166 values

**$r$ -values** : 1 empirical  
distribution of 26 values

$T_{\min}$  : 2 distributions = slow, fast

**$r$ -values** : 3 distributions = hyper-,  
medium, hypo-virulent



# Output of the QMRA models

## Specific

Nb of cases (%)	Slow	Fast	Total
Hypervirulent	9	547	<b>556 (97%)</b>
Medium virulence	0.3	17	<b>17 (3%)</b>
Hypovirulent	0.0	0.1	<b>0.1 (0.02%)</b>
Total	<b>9.5 (1.7%)</b>	<b>564.2 (98.3%)</b>	<b>574</b>

≠

## Exposure

(%)	Slow	Fast	Total
Hypervirulent	0.4	12.2	<b>12.6</b>
Medium virulence	1.1	34.6	<b>35.7</b>
Hypovirulent	1.6	50.1	<b>51.8</b>
Total	<b>3.0</b>	<b>97.0</b>	<b>100</b>

## Generic

**978 cases >> 574 cases**

(≠ population structure)



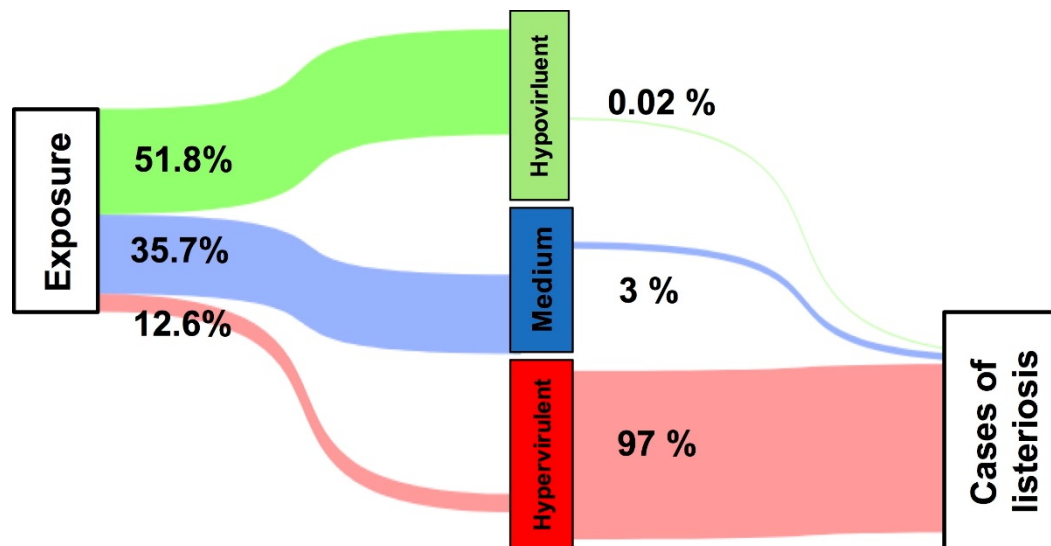
## Exposure

Hypervirulent	<b>19% (&gt; 13%)</b>
Medium virulence	<b>58%</b>
Hypovirulent	<b>23% (&lt; 52%)</b>



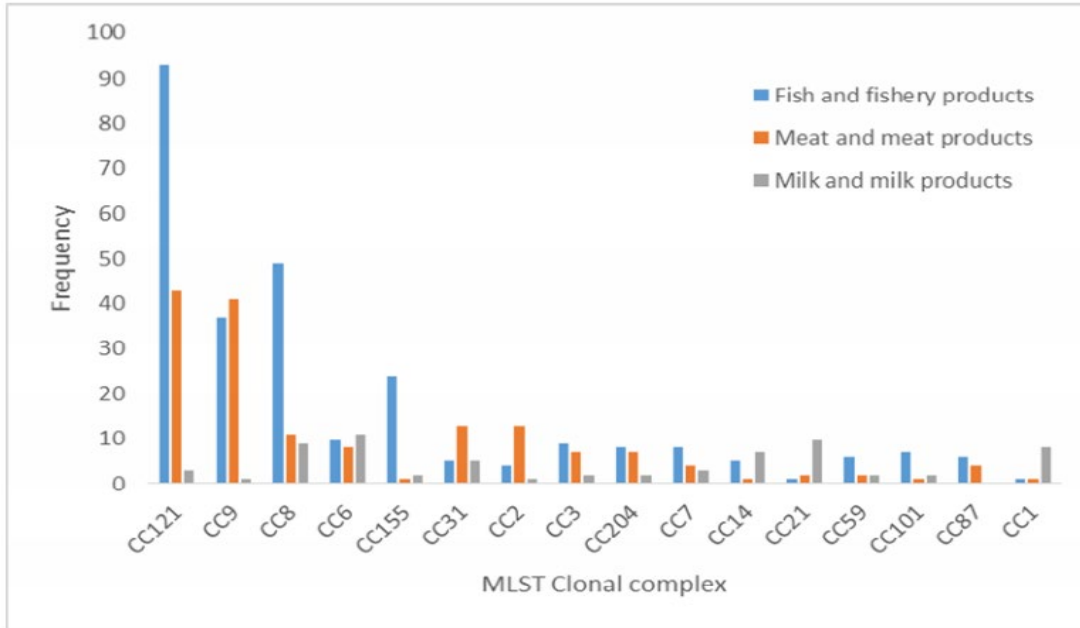
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# Conclusions



- Proof of concept (integration of WGS in risk assessment)
- Strong hypothesis:  $r$ -values ↔ CCs ↔ clinical frequency
- Important issue: consider the population structure of strains
- Near future → Biomarkers  
→ Accessible data
- Adapting or refinement of intervention strategies (hypervirulent strains ?)

# Perspectives



(Nielsen et al. 2017)

- What about other RTE food products? Not enough genomes to be confident on diversity
- EJP OH LISTADAPT: collection of strains from main RTE food categories



Thank you for your attention !

Many thanks to  
Lena Fritsch (the PhD student)  
Pr. Jean-Christophe Augustin

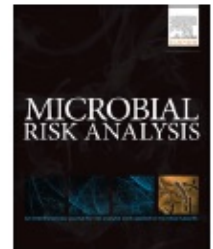


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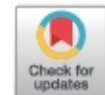
Microbial Risk Analysis

journal homepage: [www.elsevier.com/locate/mran](http://www.elsevier.com/locate/mran)



Full length article

Next generation quantitative microbiological risk assessment: Refinement of the cold smoked salmon-related listeriosis risk model by integrating genomic data



Lena Fritsch<sup>a</sup>, Laurent Guillier<sup>a</sup>, Jean-Christophe Augustin<sup>a,b,\*</sup>