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The increase of *Listeria monocytogenes* lineage type I in South African food factories

Prof Pieter Gouws

Director: Centre for Food Safety, Stellenbosch University

Food Safety

Safety first

We are all consumers

We all want safe nutritious food

We can prevent foodborne illness

We can promote food safety

We all have a role to play

Food safety is in our hands

Food safety is everyone's businesses

Everyone is responsible for food safety and health

- **Food Scientist and Nutritionists**
  - Programs that focus on healthy and safe food
    - Make safe food and easy choice
- **Food businesses**
  - Promote a food safety culture
    - Engage employees, suppliers and stakeholders to grow the food safety culture
- **School and tertiary institutions**
  - Support food safety education
    - Include food safety education as part of professional development
- **Consumers**
  - Being informed and promote food safety
    - Consumers have the power to drive change

Key Messages

The safe production of food requires an understanding of how microorganisms contaminate food, and how they may be controlled

- There is no food security without food safety.
- Foods and food factories are complex ecosystems.
- The food environment is composed of
  - Intrinsic factors (pH, water activity, nutrients)
  - Extrinsic factors (Temperature, other bacteria, cleaning chemicals)
- *Listeria* – specific growth conditions
  - Gram +, ubiquitous in the environment, everywhere,
  - But also, intracellular pathogen in humans and animals

Whole Genome Sequencing

WGS – have revolutionized the understanding and control of *Listeria* – precision food safety

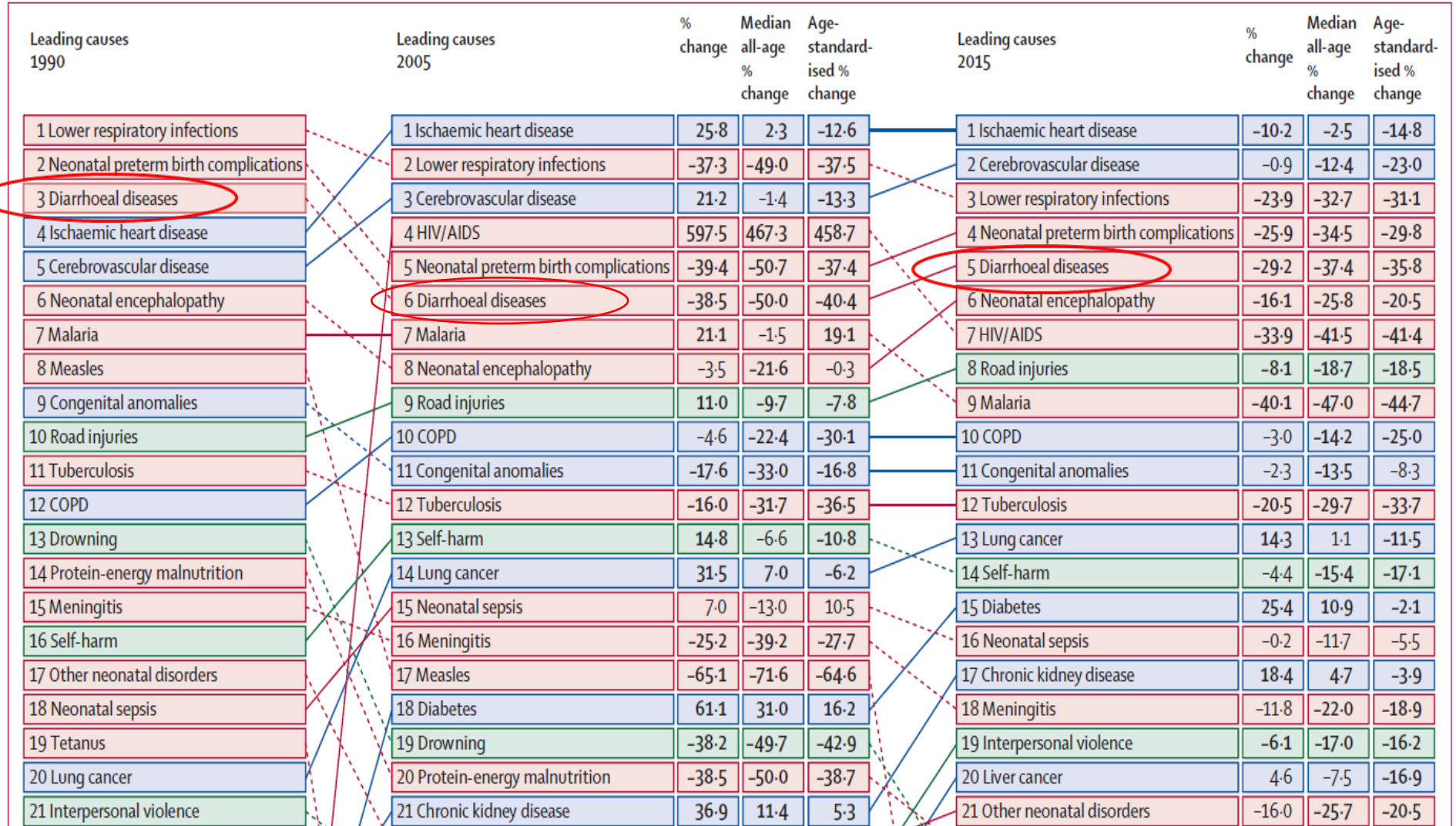
- Tracking and tracing food borne disease outbreaks.
- Managing public health outbreaks.
- Track pathogens in factory environments and tailor interventions.
- Resistant genes
- Precision food safety tool.

Food safety and discissions on food safety must be based on science

- Food scientists, nutritionists, food chemists, food microbiologists, veterinarians, medical doctors and toxicologists, to name a few, advise what food production, processing, handling and preparation practices are needed to make food safe.
- When safe practices are employed across the food chain, food becomes safe.
- Incompetent consultants and advisors
  - Decisions not based on sound scientific principles

Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015

- *Lancet* 2016; 388: 1459–544



Leading causes 1990	Leading causes 2005	% change	Median all-age % change	Age-standardised % change	Leading causes 2015	% change	Median all-age % change	Age-standardised % change
1 Lower respiratory infections	1 Ischaemic heart disease	25·8	2·3	-12·6	1 Ischaemic heart disease	-10·2	-2·5	-14·8
2 Neonatal preterm birth complications	2 Lower respiratory infections	-37·3	-49·0	-37·5	2 Cerebrovascular disease	-0·9	-12·4	-23·0
3 Diarrhoeal diseases	3 Cerebrovascular disease	21·2	-1·4	-13·3	3 Lower respiratory infections	-23·9	-32·7	-31·1
4 Ischaemic heart disease	4 HIV/AIDS	597·5	467·3	458·7	4 Neonatal preterm birth complications	-25·9	-34·5	-29·8
5 Cerebrovascular disease	5 Neonatal preterm birth complications	-39·4	-50·7	-37·4	5 Diarrhoeal diseases	-29·2	-37·4	-35·8
6 Neonatal encephalopathy	6 Diarrhoeal diseases	-38·5	-50·0	-40·4	6 Neonatal encephalopathy	-16·1	-25·8	-20·5
7 Malaria	7 Malaria	21·1	-1·5	19·1	7 HIV/AIDS	-33·9	-41·5	-41·4
8 Measles	8 Neonatal encephalopathy	-3·5	-21·6	-0·3	8 Road injuries	-8·1	-18·7	-18·5
9 Congenital anomalies	9 Road injuries	11·0	-9·7	-7·8	9 Malaria	-40·1	-47·0	-44·7
10 Road injuries	10 COPD	-4·6	-22·4	-30·1	10 COPD	-3·0	-14·2	-25·0
11 Tuberculosis	11 Congenital anomalies	-17·6	-33·0	-16·8	11 Congenital anomalies	-2·3	-13·5	-8·3
12 COPD	12 Tuberculosis	-16·0	-31·7	-36·5	12 Tuberculosis	-20·5	-29·7	-33·7
13 Drowning	13 Self-harm	14·8	-6·6	-10·8	13 Lung cancer	14·3	1·1	-11·5
14 Protein-energy malnutrition	14 Lung cancer	31·5	7·0	-6·2	14 Self-harm	-4·4	-15·4	-17·1
15 Meningitis	15 Neonatal sepsis	7·0	-13·0	10·5	15 Diabetes	25·4	10·9	-2·1
16 Self-harm	16 Meningitis	-25·2	-39·2	-27·7	16 Neonatal sepsis	-0·2	-11·7	-5·5
17 Other neonatal disorders	17 Measles	-65·1	-71·6	-64·6	17 Chronic kidney disease	18·4	4·7	-3·9
18 Neonatal sepsis	18 Diabetes	61·1	31·0	16·2	18 Meningitis	-11·8	-22·0	-18·9
19 Tetanus	19 Drowning	-38·2	-49·7	-42·9	19 Interpersonal violence	-6·1	-17·0	-16·2
20 Lung cancer	20 Protein-energy malnutrition	-38·5	-50·0	-38·7	20 Liver cancer	4·6	-7·5	-16·9
21 Interpersonal violence	21 Chronic kidney disease	36·9	11·4	5·3	21 Other neonatal disorders	-16·0	-25·7	-20·5

Burden of food borne illness

Campylobacter, Salmonella, Listeria

- Leading cause in humans are *Campylobacter*, but
- *Listeria* and *Salmonella* are causing more deaths
- ECDC / EFSA (2021) 1% of reported illness due to *Listeria monocytogenes*, but is far the leading cause of death
  - 55% of reported deaths in 2020 (of foodborne illness) due to *Listeria monocytogenes*
  - Fatality rate 18 – 22%
- Rates of *Listeria* infection has increased in Europe since 2020
  - Aging population
  - Availability and consumption of RTE foods
  - Emerging virulence potential of *Listeria* strains, peaks in summer months

When food is not safe ……..

- When food is not safe and nutritious,
  - the economy suffer

Emerging Trends (2019 - 2022)

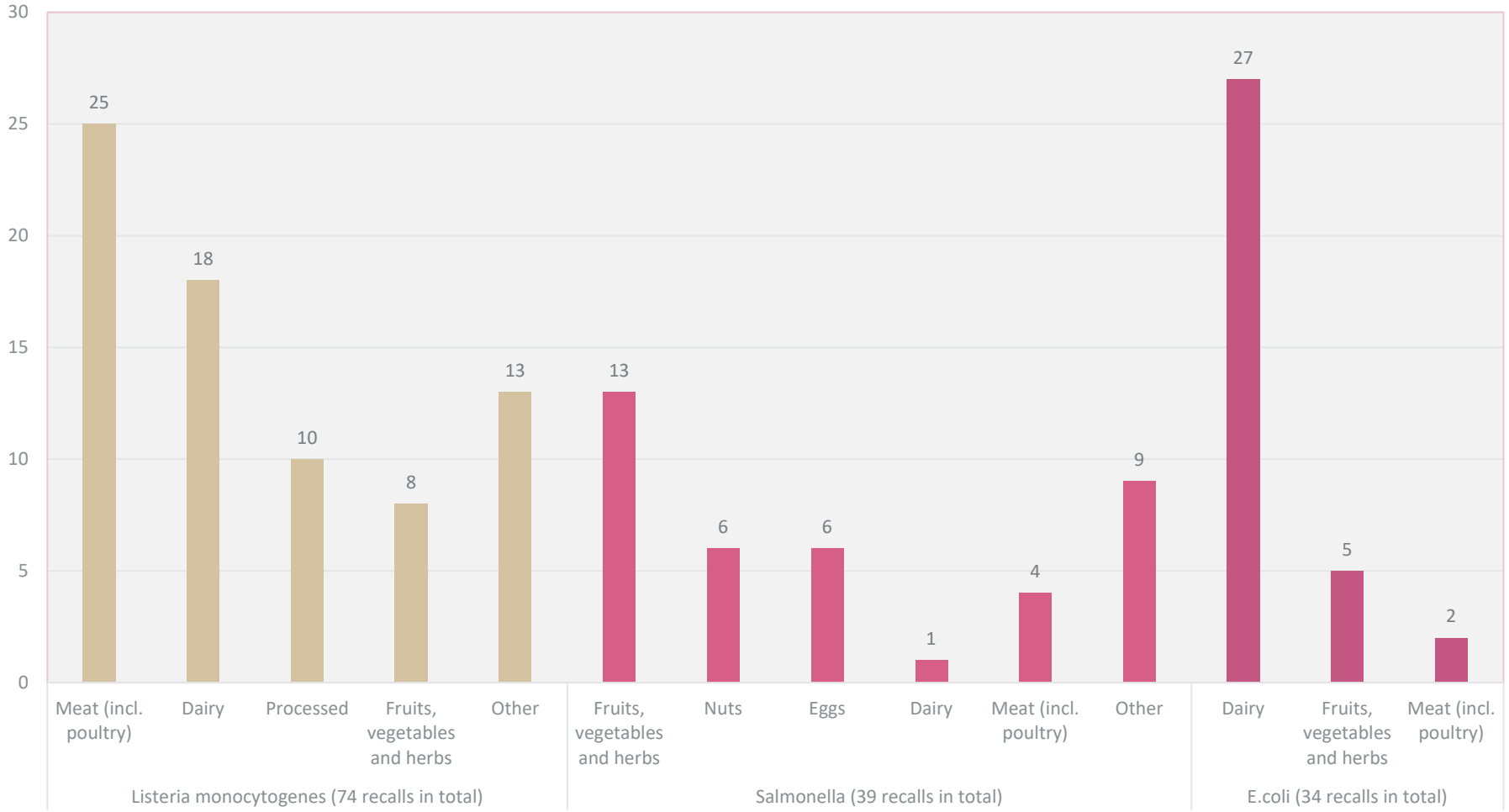


Photo illustration

CDC reports that outbreak traced to enoki mushrooms has ended; concerns continue about this imported commodity

By Coral Beach on April 8, 2023

Federal officials say an outbreak of Listeria infections traced to enoki mushrooms has ended.

The Centers for Disease Control and Prevention reports that the outbreak sickened five people in four states from coast to coast: California, Nevada, Michigan and New Jersey. No one died, but all five of the patients required hospitalization.

Labels of recalled ground cinnamon

Ground cinnamon suspected as 30 sick in Spain

By News Desk on April 16, 2023

At least 30 people are part of an outbreak linked to ground cinnamon from Vietnam.

People who fell sick in Spain with Clostridium perfringens infections reported symptoms including vomiting and diarrhea.

The Spanish Agency for Food Safety and Nutrition (AESAN) said it had been informed by officials in Madrid of Clostridium perfringens in a brand of ground cinnamon.

Fresh Express recalls salad kit products because of a positive Listeria test

By News Desk on April 7, 2023

Fresh Express Inc. is recalling three varieties of branded and private-label salad kit products produced at the company's Morrow, GA, facility because of a possible health risk from Listeria monocytogenes.

Chicken suspected in multi-country Salmonella outbreak

By Joe Whitworth on March 31, 2023

An outbreak of Salmonella has affected more than 200 people in Europe, the United Kingdom, and the United States.

The Salmonella Virchow outbreak started in June 2017 and most cases have been linked to local restaurants serving kebab meat. At least one person in the United States had gotten sick. A female patient from California had a travel history to Paris in 2019 and needed hospital treatment.

Food safety is an essential public health issue

- The potential threat to public health from foodborne diseases continues to increase with expanding urbanisation and the global distribution of food
- Global demand for food continues to increase
- Prepared consumer foods are one of the fastest growing sectors
- Consequences of a failed food safety policy are costly
- Paradigm shift to risk-based methods for analysis.
- *Science based food controls are essential to produce food products*

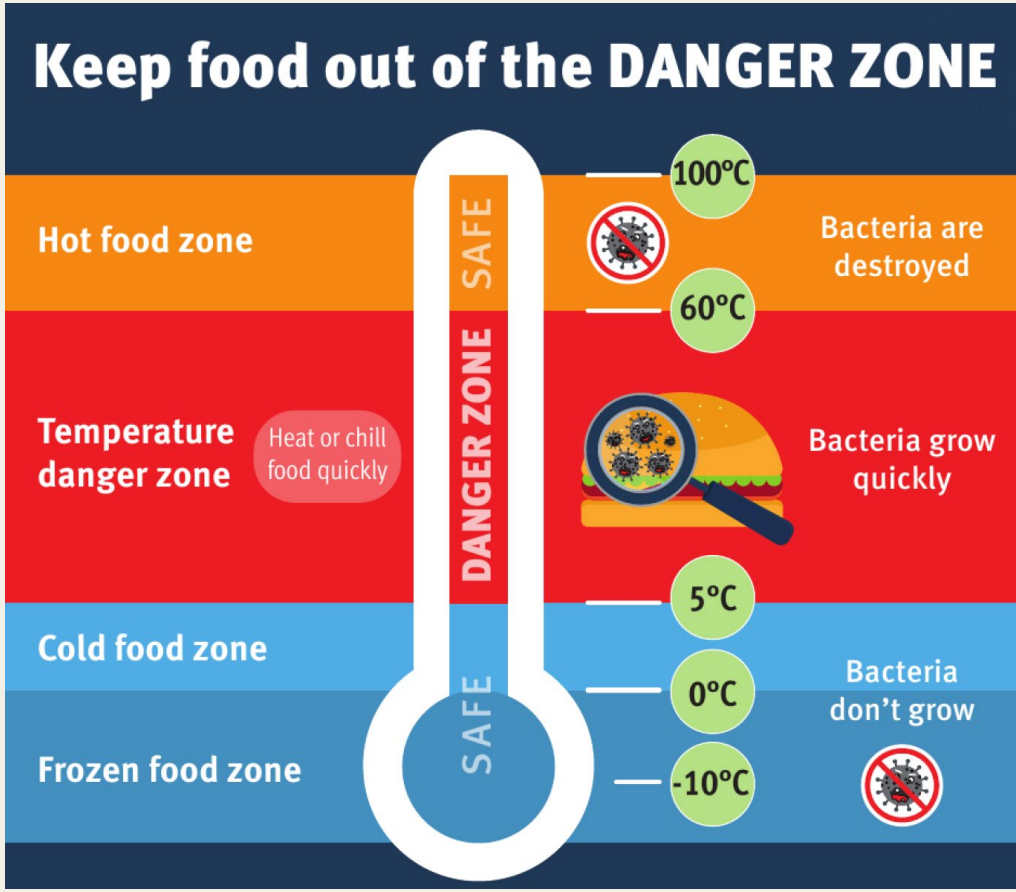
One health aproach

- Human health and animal health are interdependent
- At the same time, both depend on the environment

Control of pathogenic organisms

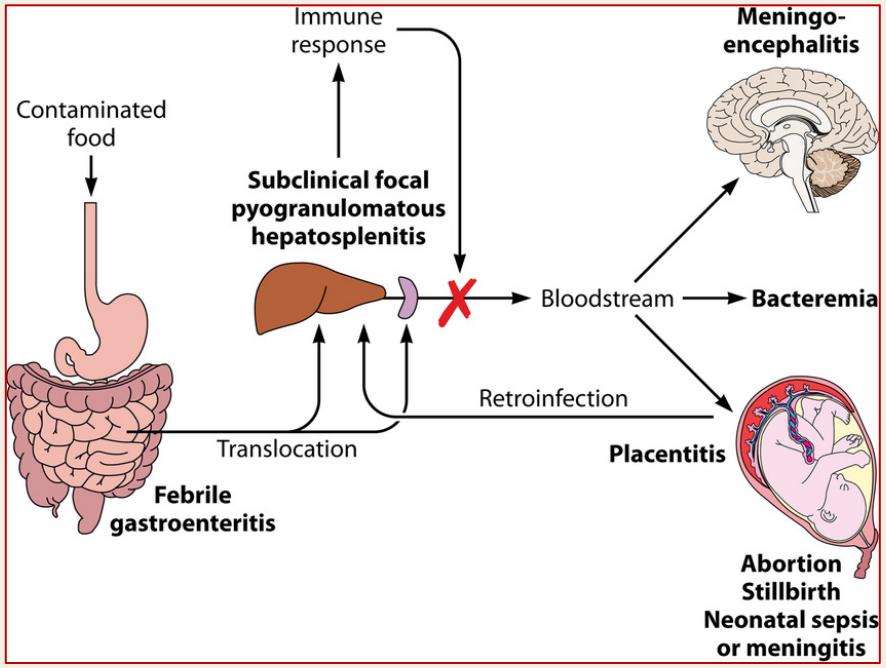
Understand the nature of the problem organism

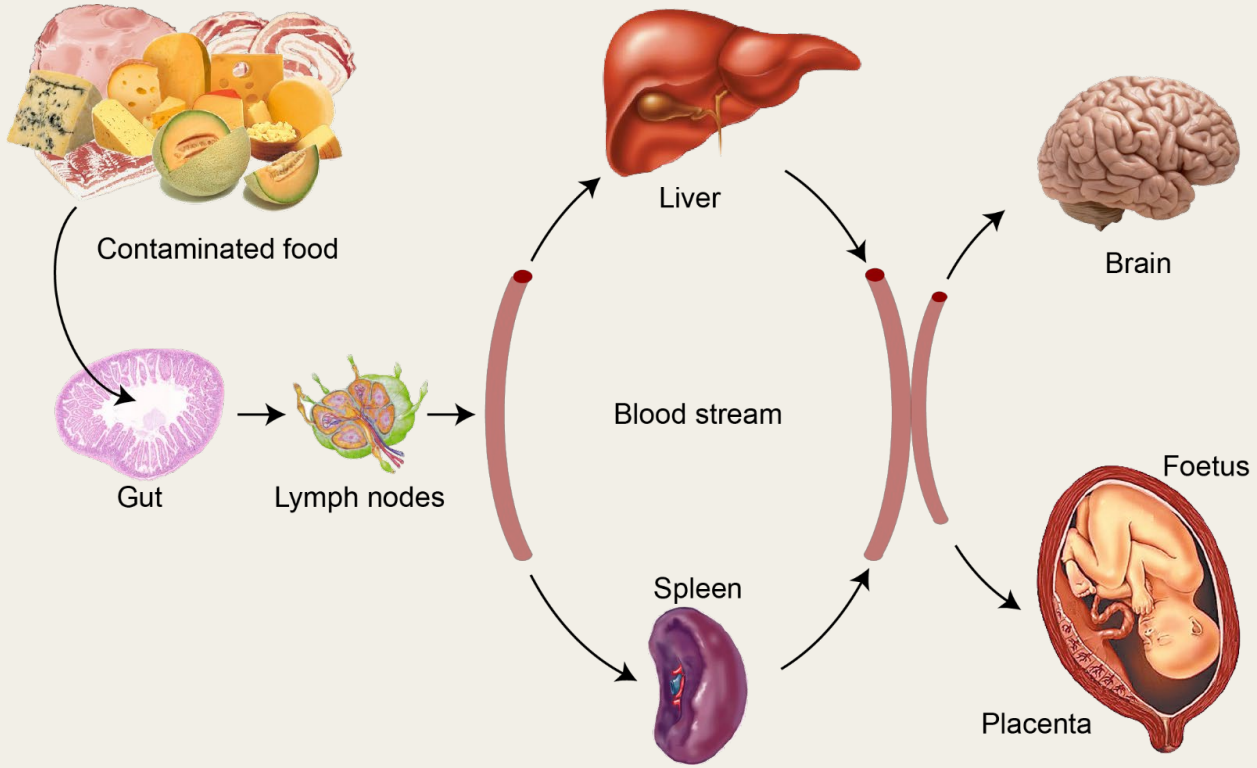
- Prevent contamination
  - (Keep them out)
- Destroy foodborne disease agents
  - (Kill them)
- Prevent multiplication of foodborne disease agents
  - (Control them)



Listeriosis

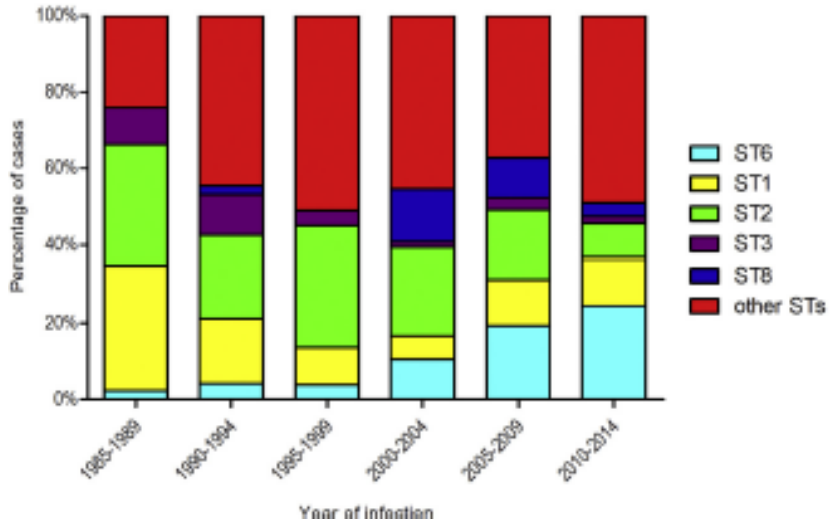
Listeria monocytogenes





Meningitis cases in the Netherlands from 1985 - 2014

- Kremer *et al.*, 2017



Novel listerial plasmid and an efflux transporter, ***emrC***, to be associated with the emergence of meningitis caused by *L. monocytogenes* **ST6** in the Netherlands, **possibly through decreased susceptibility to disinfecting agents**.

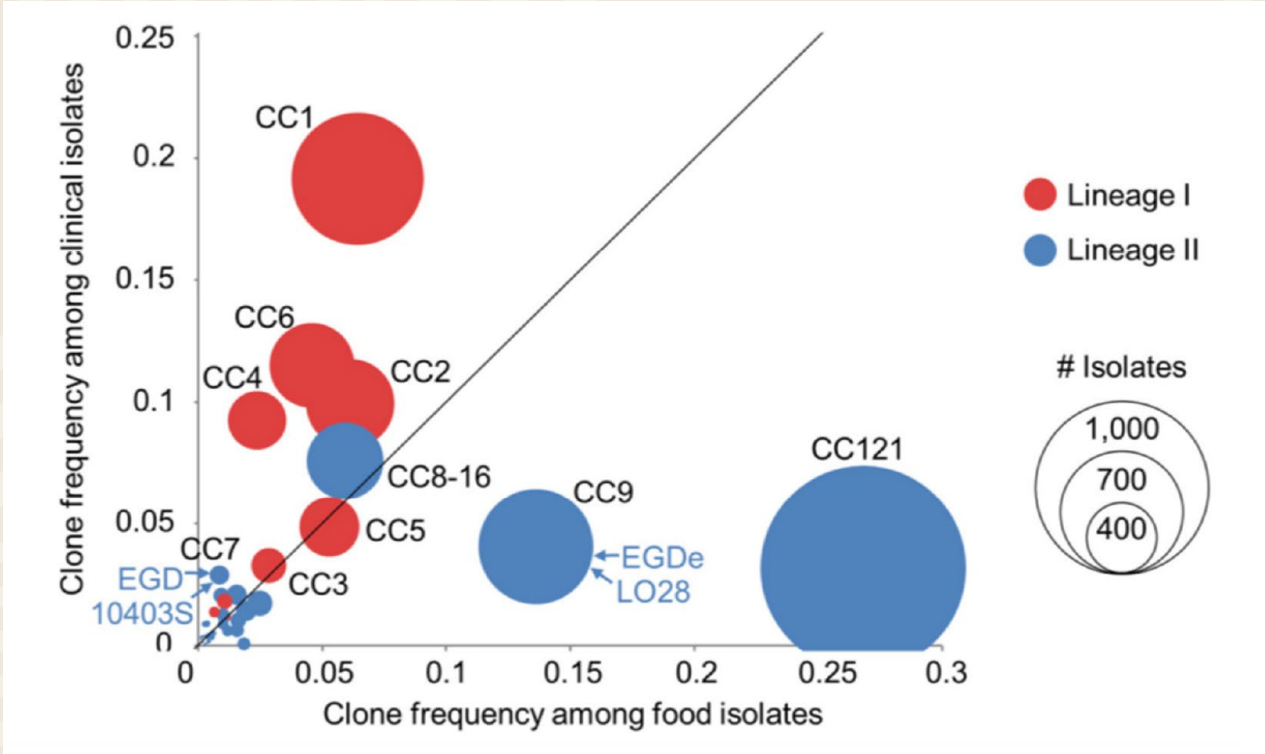
The *emrC* gene encodes an efflux protein that pumps quaternary ammonium compounds out of the cell **and increases the capacity to form a biofilm.**

Benzalkonium chloride is extensively used in the food-processing industry as a disinfectant and sterilization agent thus select for resistance mechanisms.

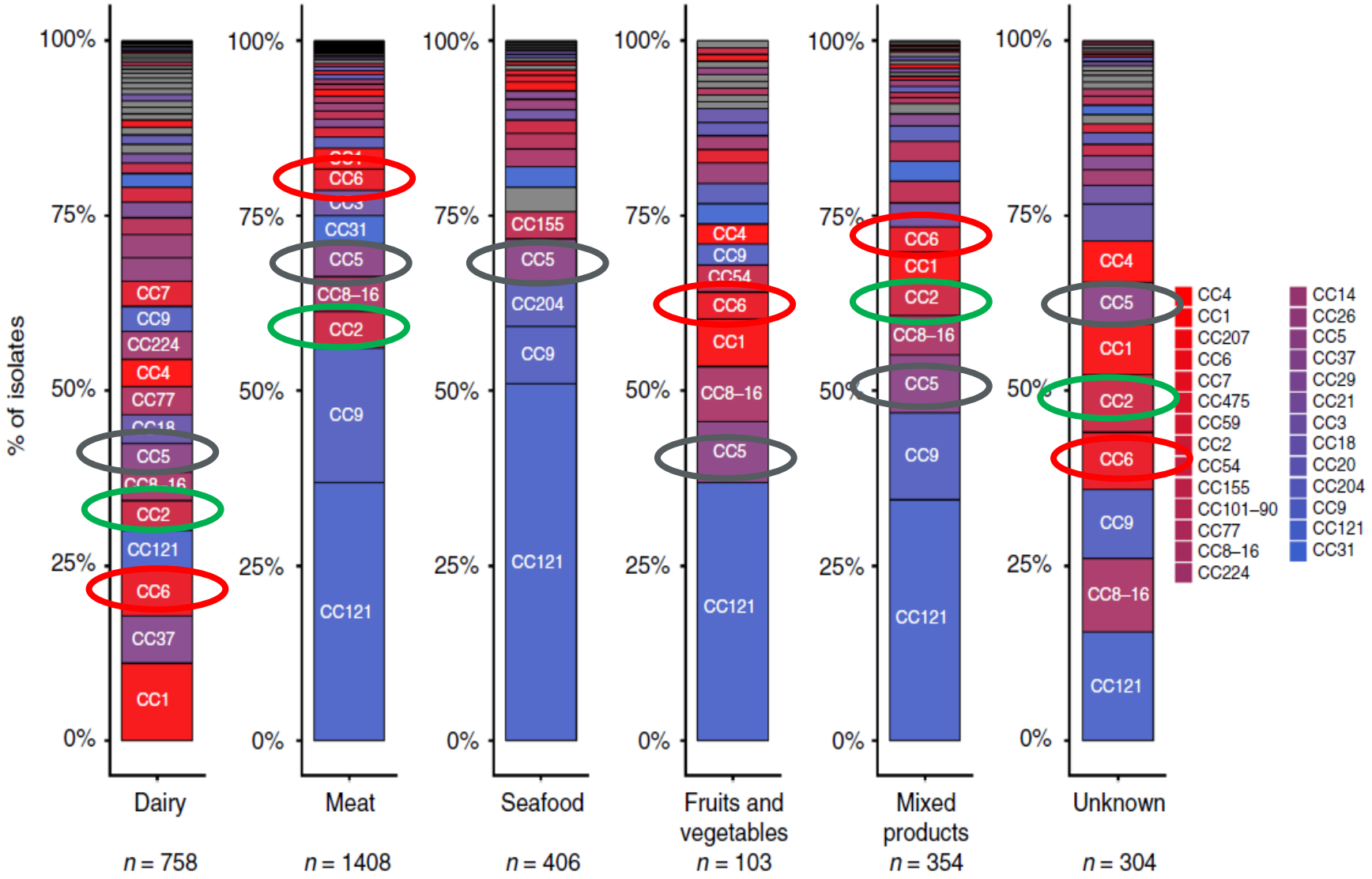
Listeria monocytogenes virulence

Serotypes and clonal complexes

- Hypervirulent
  - Associated with illness
  - the genetic characteristics associated with hypervirulence are found to be absent in those clones that are more associated with foods
- Hypovirulent
  - more associated with foods and with less capacity to cause disease.

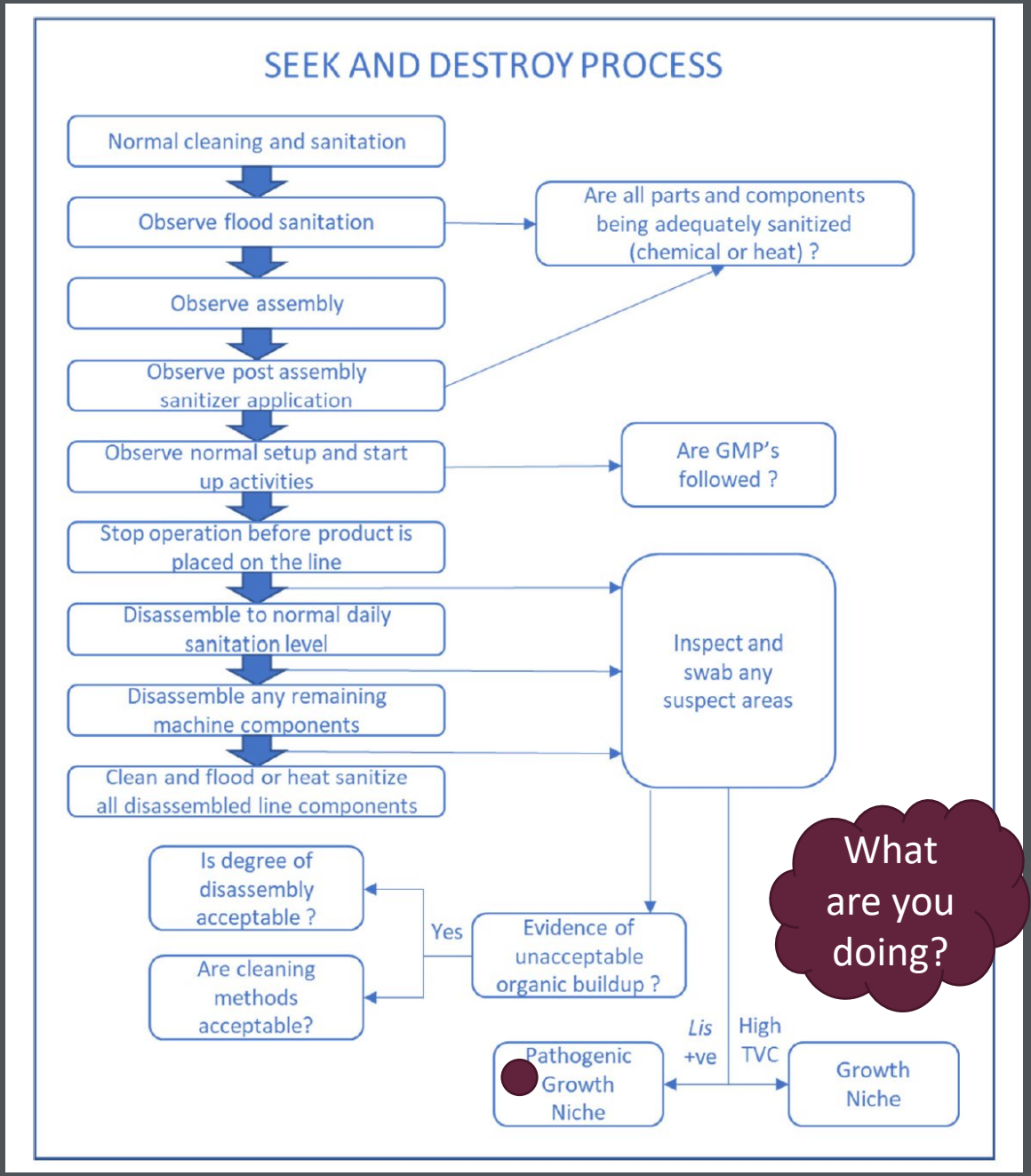


CC / ST 2, 5, 6 proportions in different food categories



EU FAO/WHO and US food legislation

- Regulation (EC) No. 2073/2005 specifies the criteria and testing requirements for certain foodstuffs, including that RTE foods that do not support the growth of *L. monocytogenes* do not exceed contamination levels <100cfu/g, and that RTE foods that do support the growth of *L. monocytogenes* do not exceed this contamination level during the product's shelf-life
- Accepted levels of *Listeria* contamination in RTE foods is important, as in the EU there is a risk-based acceptance of its presence in RTE food at levels that will not cause illness in the general population, consistent with Codex principles and the risk assessment of the WHO
- This risk-based approach of the EU and FAO/WHO is contrary to US food legislation which classifies any level of *L. monocytogenes* as an adulterant in RTE foods



Addressing *Listeria* contamination in the food processing environment requires a step-wise problem solving approach

It is difficult if not impossible to eliminate *Listeria* from the food processing environment because of its ubiquitous nature and particular survival characteristics

Listeria is particularly well adapted to grow in the environmental conditions that are present in food processing facilities and particularly within the chilled foods sector

Table 1.5. Growth and survival limits of *L. monocytogenes* {FSAI, 2011a}

Parameter	Growth Range	Optimal Growth	Can Survive (but no growth)[a]
Temperature (°C)	-1.5 to 45	30 to 37	-18
pH	4.2 to 9.5	7	3.3 to 4.2
Water Activity (a_w)	0.90 to >0.99	0.97	<0.90
Salt Concentration (%)	<0.5 to 12	n/a	≥20

[a] *Survival period will vary depending on nature of food and other factors*

Listeria typically enters food processing plants via raw food ingredients

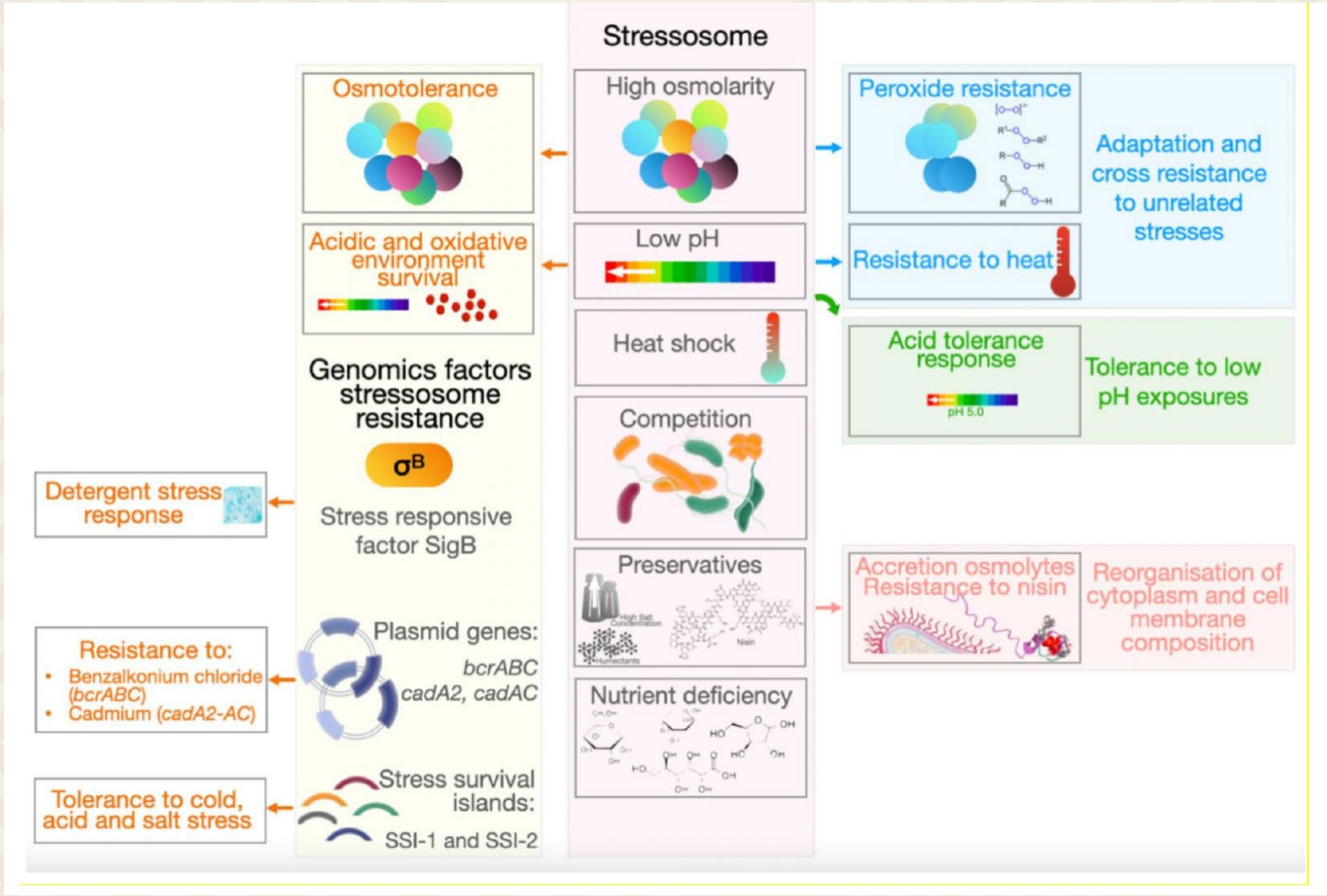
Study on newly opened food processing plant

- in the drains of the raw processing areas during production in the first month of operations
- after 4 months in raw drains after the cleaning cycle, including a strain that went on to be isolated repeatedly in the raw environment and also on occasion in the cooked area
- after 6 months in the cooked environment, with several strains isolated during repeat visits from that point onwards
- personnel, equipment, trolleys could be identified as a source of contamination of the strains isolated inside the plant

Processing plant environment

- Most common source of prepared food contamination by *Listeria ?*
- Persistent strains
- Growth niches
  - Allow organism to evade cleaning and disinfection
  - Cracks in floors, unpolished welds, biofilms
- Ineffective sanitisers and disinfectants
  - Persistent strains
  - QAC resistance mechanisms
  - Biofilms

Yet not all strains are equal.



Lineage I and II (historical data)

- At the population level, lineage II isolates were more frequently recovered from foods and food processing environments than lineage I, leading to speculation that lineage II isolates may have a greater adaptation to survive and grow in food-related environments and an increased ability to persist.
- Most studies suggest a higher prevalence of lineage I isolates among human listeriosis cases
- Lineages III and IV are rare and isolates of these groups show the highest prevalence among animal isolates
- At the clone level, the hypervirulent clones CC1, CC2, CC4 and CC6 predominate in clinical samples and are of lineage I, serogroup IVb, while the hypovirulent clones CC9 and CC121 predominate in foods and are of lineage II, and of serogroups IIc and IIa respectively.

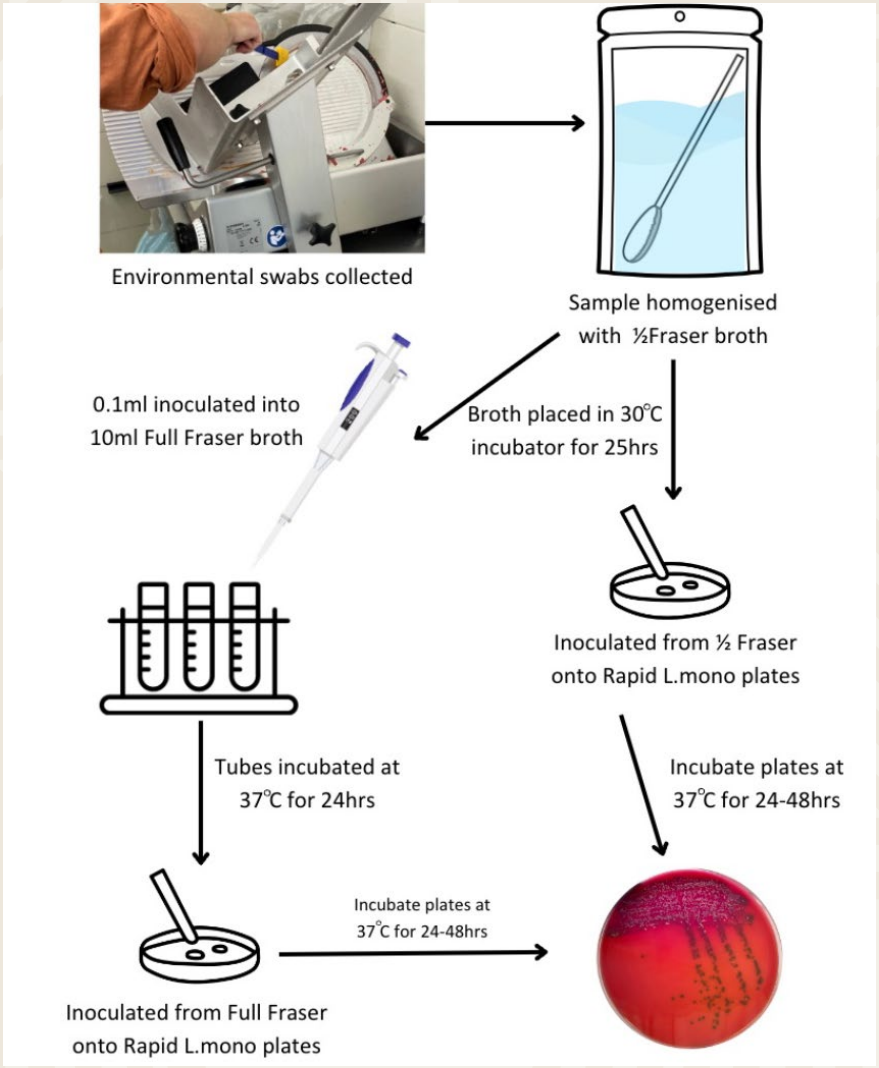
Listeria monocytogenes

- Lineage I = Hypervirulent
- Genes conferring to
  - Greater capacity to cause disease

- Lineage II = Hypovirulent
- Genes conferring to
  - Disinfection tolerance
  - Environmental stresses, such as biocides, cold temperature, high slat, etc

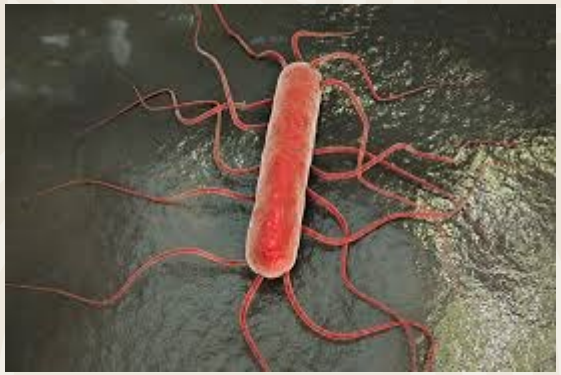
Delicatessens

- Environmental swabs were collected from delicatessens in South Africa. Swabs (4 cm2) were taken from different points within each facility, including hand contact points, scullery areas, meat slicers, food cooking areas and drains. Each swab collected was tested *for Listeria monocytogenes* (ISO 11290-1/A1:2005Results confirmed with VITEK®2 Compact Automated System.
- *L. monocytogenes* was detected in 100% of the drains, 75% of the scullery areas and 50% of the food preparation areas throughout the sampling period. The *Hly*A gene was used to confirm the *L. monocytogenes* results through DNA extraction and Polymerase Chain Reaction (PCR) testing.
- Resistant to QAC's



Food processing environments

- 42 isolates in this study were assigned to lineage type I
- Serotypes and sequence types belonging to lineage type I have a higher virulence potential than those belonging to lineage type II
- These results indicate that strains with a higher virulence potential have become dominant within several food processing environments
- Multiple genes tested confer resistance towards QAC-sanitisers



Survivability of lineage types

- The following factors may also be contributing towards survivability
  - Acquisition of resistance gene(s)
  - Factory processing or cleaning factors (sanitiser selection) may be favouring lineage I
  - Lineage I may be constantly reintroduced due to a common raw material
  - Are we sitting on a time bomb?
  - Start to work in interdisciplinary teams to solve complex problems

- **Lineage I = Hypervirulent**
- Genes conferring to
  - Greater capacity to cause disease

- **Lineage II = Hypovirulent**
- Genes conferring to
  - Disinfection tolerance
  - Environmental stresses, such as biocides, cold temperature, high slat, etc.

Summary

- *Listeria* are still a huge issue in South Africa
- *L. monocytogenes* typically enter food processing facilities with contaminated raw food ingredients, and if they become established in a niche in the processing environment they may cross-contaminate foods being processed and consequently cause foodborne illness and outbreaks.
- The design and management of food processing environments is therefore crucial to prevent contamination, as well as staff and consumables involved in raw and cooked processing, hygienic design of the built environment and processing equipment, effective sanitation, personnel training, microbiological surveillance of the processing environment, and effective corrective action protocols if *L. monocytogenes* is detected.

Summary

- Population epidemiology studies of *L. monocytogenes* have revealed that not all lineage types are equal in terms of causing disease, with ST1, ST2, ST4 and ST6 classified as hypervirulent clones and associated with a greater proportion of disease and of severe infection.
- Lineage type I is becoming dominant in food processing plants tested.
- Bacterial genomes are dynamic and can mutate within the timeframe of an outbreak or a contamination event investigation or processing plant, and an appreciation of the background mechanisms and potential rate of change is important to the appropriate interpretation of data in identifying most likely to be from a common source.
- Controlling foodborne pathogens, and *L. monocytogenes* in particular in food processing facilities is of critical strategic importance for a food business.

We speculate

- Biofilm formation in the presence of disinfectant may represent an important attribute among certain resistant and persistent strains of *L. monocytogenes*.
- Acquisition of resistance gene(s) to QAC in Lineage I increases the capacity to form biofilms.
- Therefore, Lineage I will become the persistent strain of *Listeria monocytgenes.*

- *Alternative cleaning protocols:*
- *?*
- *?*
- Incompetent consultants and advisors
  - Decisions not based on sound scientific principles

Partnerships in food safety today will reap future rewards

- Safe food production improves economic opportunities by enabling market access and productivity. However, unsafe or contaminated food leads to trade rejections, economic losses and food loss and waste.
- Centre for Food Safety and industry partnerships.
  - Database of WGS data
    - Better decision making
    - Control pathogens
    - Risk based
    - Future food safety experts
- UCD-CFS industry model

Work together

Only when we work together, can we achieve safer food for better health

If food is not safe, it is not food

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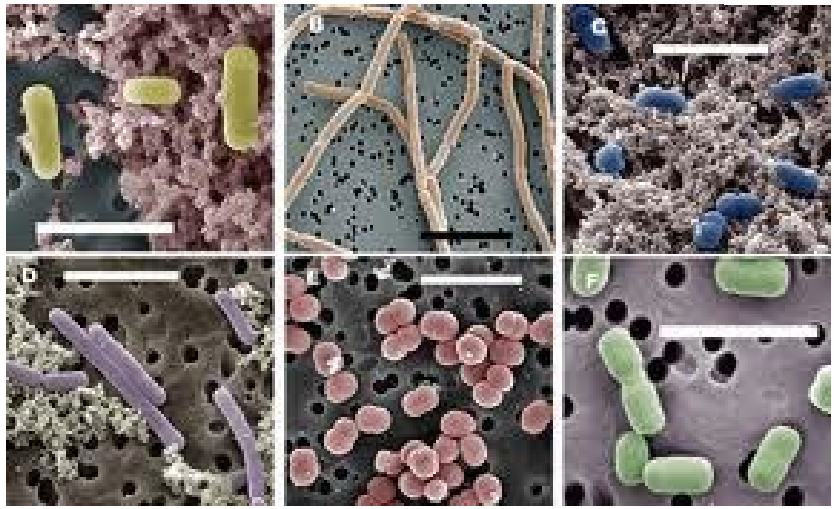
Louis Pasteur



“Messieurs, c’est les microbes qui auront le dernier mot”
(Gentlemen, it is the microbes that will have the last word)

thank you | enkosi | dankie

The tragic coronavirus pandemic of 2020-2022 opened the world's eyes anew to the urgent need for a better understanding of microorganisms, whether viruses or bacteria, in order to develop best practices for reducing the risk of dangerous infections. Ideally, every household should have sufficient knowledge of how viruses and other kinds of microorganisms can damage human and animal health. Now, with exquisite timing, Prof Pieter Gouws at the Centre for Food Safety (CFS), in the Department of Food Science at Stellenbosch University, and food scientist Dr Michaela van den Honert, have collaborated on a scientific household guide for "living with little monsters", introducing the reader to an array of potentially harmful microorganisms. Nor have the authors neglected the bacteria which play a positive role, for example, in the human gut. They have gathered the latest scientific evidence for an extensive set of descriptions of specific microbes to watch out for and how best to minimise the risk of being infected by them. By so doing, they can empower ordinary consumers, along with their families, to live healthier, less risky, daily lives.

"There's no point for scientists to have all this information about 'germs' if they don't share it to make the world a better place."

Prof Steven Forsythe

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Living with little Monsters

Dr M van den Honert and Prof P Gouws with MJ Lee

Living with little Monsters

An Illustrated Household Guide to Managing the Hidden World of Microbes

Dr Michaela van den Honert
and Prof Pieter Gouws with Michael J Lee

Flat size: 349.8mm × 244.1mm | Finished width: 169.9mm | Spine: 10mm

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