

Pharma & Medical Applications of Microbial Biodiversity
 Université PARIS-SACLAY

Salmonella and Salmonellosis

Maria PARDOS DE LA GANDARA
 Deputy Director
 National Reference Centre for *E. coli*, *Shigella* & *Salmonella*
 (Enteric Bacterial Pathogens Unit)
 Institut Pasteur
 7/02/2024

Maria Pardos de la Gandara

- 2003 MD: Universidad de Zaragoza (Saragossa, Spain)
- 2008 Specialist in Microbiology and Parasitology (Saragossa, Spain)
- 2010 PhD: Universidad de Zaragoza (Saragossa, Spain)
 - o 18 months at the CNR-ESS, Institut Pasteur (Paris, France)
- 2010 Post-Doc: Centre National de la Recherche Scientifique (Gif-sur-Yvette, France)
 - o 6 months at the Bossi Lab, Center for Molecular Genomics
- 2011 Post-Doc: The Rockefeller University (New York, US)
 - o 7 years at the Tomasz Lab, Laboratory of Microbiology and Infectious Diseases
- 2018 Deputy Director: CNR-ESS
 - o 6 years at the Institut Pasteur (Paris, France)

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EPIDEMIOLOGICAL SURVEILLANCE: NRC-ESS

Duties of the National Reference Centre for *E. coli*, *Shigella* & *Salmonella*

1. **Microbiological expertise**
 - Identification
 - Development of new typing methods (MLST + *rfb* + *fljB* + *rfbB*)
2. **Contribute to the epidemiological surveillance**
 - Follow evolutive and seasonal trends for the different serotypes
 - Early detection of emerging epidemiological phenomena
 - Use state-of-the-art sub-typing methods (CRISPOL, cgMLST) for the investigation of outbreaks
3. **Surveillance of the resistance to antibiotics**
 - Genomic (Resistome)
 - Phenotypic (Antibiograms: MIC, DD)

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EPIDEMIOLOGICAL SURVEILLANCE: NRC-ESS

<https://www.pasteur.fr/fr/sante-publique/CNR/ies-cnr/escherichia-coli-shigella-salmonella/rapports-d-activite>

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TAXONOMY: Who's Salmonella ?

Gram negative rod, facultative anaerobe, mobile (flagella)

Family:	Enterobacteriaceae	
Genus:	Salmonella	
Species:	<i>S. enterica</i>	<i>S. bongori</i>
Subspecies:	<ul style="list-style-type: none"> <i>S. enterica enterica</i> (I) <i>S. enterica salamae</i> (II) <i>S. enterica arizonae</i> (IIIa) <i>S. enterica diarizonae</i> (IIIb) <i>S. enterica houtenae</i> (IV) <i>S. enterica indica</i> (VI) <i>S. enterica</i> (VII)* <i>S. enterica</i> (VIII)* 	
Serotype:	Typhi, Dublin, Kentucky, Mbandaka, Arechavaleta, ... (> 2 500)	

* Cacciola A, et al. Microbiol Genomics, 2019

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HABITAT: SOURCE OF INFECTION

Ubiquitous:

- Intestinal carriage in vertebrates:
 - o *S. e. enterica*: hot-blooded animals (mammals, birds)
 - o Other subspecies: cold-blooded animals (reptiles)
- Pathogenic for different animals:
 - o Strictly humans: Typhi, Paratyphi A, B, C
 - o Strictly other animals: Abortusovis, Gallinarum, Abortusequi, ...
 - o Both: certain niche predilection
 - Poultry: Enteritidis, Hadar, Virchow, Newport, Senftenberg, ...
 - Swine: Derby, Typhimurium, ...
 - Bovine: Dublin, Panama, Montevideo, ...

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Habitat: source of infection

Source of infection: minor serotypes (nontyphoidal *Salmonella*, NTS)

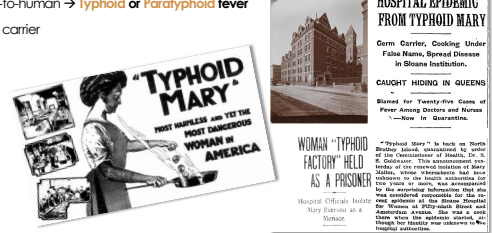
- Zoonanthroposis → **Gastroenteritis**
 - **Food poisoning**
 - Contact with animals (pets, farms)
 - Indirectly through contamination of water and surfaces (→ vegetables)



Habitat: source of infection

Source of infection: major serotypes (typhoidal *Salmonella*, TS)

- Human-to-human → **Typhoid or Paratyphoid fever**
- Healthy carrier



Dissemination of *Salmonella* in the kitchen



Dissemination of *Salmonella* in the kitchen



Dissemination of *Salmonella* in the kitchen



Dissemination of *Salmonella* in the kitchen



Dissemination of *Salmonella* in the kitchen



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Dissemination of *Salmonella* in the kitchen



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Dissemination of *Salmonella* in the kitchen



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Dissemination of *Salmonella* in the kitchen



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Dissemination of *Salmonella* in the kitchen



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Dissemination of *Salmonella* in the kitchen




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What to do?

At the individual level:

- Wash **WELL** hands and cooking tools
- Use separated surfaces to prepare meat and vegetables
- Cook well all types of meat
- Avoid non-pasteurized/UHT milk




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What to do?

At the institutional level:

- Identify the pathogen
- Treat the patient
- Epidemiological Surveillance measures




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What to do?

At the institutional level:

- Identify the pathogen



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IDENTIFICATION

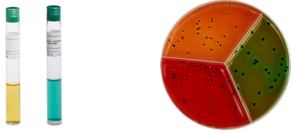
- Identification of genus, species and subspecies:
 - **Phenotypic: Biochemistry**
 - Proteomic: MALDI-TOF
 - Genetic: quantitative PCR
- Serotyping:
 - Phenotypic: Serotyping by plate agglutination
 - Genotypic: Serotyping by sequencing: MLST + *fljC* + *fljB*
→ **Whole Genome Sequencing (WGS)**

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CULTURE

1. Search for *Salmonella enterica*

- Enriched media: **Selenite**, Müller-Kauffmann
- Selective media: **SSA**, Hektoen, **XLT4**




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Phenotypic identification

2. Genus and species: *Salmonella enterica*

- Biochemical (metabolic) **general** characteristics:

Lactose -	LDC +	H ₂ S +	Gas/Glucose +	Indole -
Urease -	ONPG -	GDC +	ADH +	TDA -
Gelatinase -	VP -	DNAse -	Adonitol -	Glycerol -
Galacturonate -	Simmons Citrate +			



mdpib.com.jv
 bioquip.com
 Soliman, S., et al. (2014). 2012
 CNR-ESS

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Phenotypic identification

3. Subspecies:

- Biochemical (metabolic) **specific** characteristics:

	I	II	IIIa	IIIb	IV	VI	(old V)
	<i>S. e. enterica</i>	<i>S. e. solanoe</i>	<i>S. e. oranoe</i>	<i>S. e. diarizonae</i>	<i>S. e. houtense</i>	<i>S. e. indica</i>	<i>S. bongori</i>
Duclal	Dul	+	+	—	—	—	d
β-Galactosidase	ONPG	—	—	+	+	—	d
Maltolase	Mol	—	+	+	+	—	—
Gelatinase (charcoal)	Gel	—	+	+	+	+	+
Sorbitol	Sorb	+	+	+	+	—	+
Growth on KCN	KCN	—	—	—	+	—	+
D-Tartrate	dTar	+	—	—	—	—	+
Galacturonate	Galact	—	+	—	+	+	+
β-Glucuronidase	gncH	+	+	—	+	+	+
β-Glucuronidase	β-Glu	d	d	—	+	—	d
Mucate	Muc	+	+	+	—	—	+
Sialine	Sial	—	—	—	+	—	+
Lactose	Lac	—	—	+	—	d	—

White & Kaufmann-Le Minor Scheme (2011)

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Proteomic identification

MALDI-TOF: "Matrix Assisted Laser Desorption Ionization – Time of Flight"

- Lysis of proteins and identification of peptide fragments: "Peptide Mass Fingerprint" (PMF)

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Proteomic identification

2. Genus, species, subspecies: *Salmonella enterica enterica*

- 5 to 10 peaks: species
- *Salmonella* spp. would need over > 300 peaks for all serotypes
- Newest generations manage to identify some serotypes

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GENETIC IDENTIFICATION

qPCR-based syndromic Kits:

- No culture needed (analysis directly from clinical sample)
- Simultaneous search for all suspected microorganisms:
 - Virus
 - Bacteria
 - Parasites
- For the main infectious syndromes:
 - Respiratory
 - Gastrointestinal
 - Meningoencephalitis
 - Sepsis

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Genetic identification

→ Genus: *Salmonella* spp.

- qPCR: quantification of copies for each target
- Identification of genetic targets: *spaO* (BDMax™), *flrA* (Roche LightMix™), *invA*
- For *Salmonella* spp. only the **genus** can be detected
- No bacterial isolation: no antibiogram, no epidemiological surveillance (CNR)

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IDENTIFICATION

- Identification of genus, species and subspecies:
 - Phenotypic: Biochemistry
 - Proteomic: MALDI-TOF
 - Genetic: quantitative PCR
- Serotyping:
 - Phenotypic: Serotyping by plate agglutination
 - White-Kauffmann-Le Minor Scheme (W-KLM)
 - Genotypic: Serotyping by sequencing: MLST + *fljC* + *fliB*
 - Whole Genome Sequencing (WGS)

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SEROTYPING BY AGGLUTINATION

White-Kauffmann-Le Minor Scheme
Gold Standard technique for 90 years:

- Agglutination on plate with polyclonal antibodies
- **19 polyvalent + 151 monovalent**

White-Kauffmann-Le Minor Scheme, 2007
https://www.scribd.com/document/100000000/White-Kauffmann-Le-Minor-Scheme-2007

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Somatic antigen « O »

Antigen:

- Hexoses, in the Lipopolysaccharide chain (LPS)
- ~20 genes (*rfb* region)
- Agglutination with **7 polyvalent sera + 65 monovalent sera**

Sarantis PV, et al. Frontiers in Cellular Microbiology 2019

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Flagellar antigen(s) « H »

Antigen:

- Polymers of flagellin protein
 - Diphasic (H1, H2)
 - eventually mono / triphasic
- Genes *fljC* (50 alleles) and *fljB* (~30 alleles)
- Agglutination with **12 polyvalent sera + 85 monovalent sera**
- **Phase inversion** on Sven-Gard agarose

Hoodson JA, et al. J Mol Commun 2017

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Capsular antigen « Vi »

Antigen:

- "Virulence" (invasive)
- Polysaccharide:
 - **Typhi +++**
 - **Paratyphi C**
 - **Dublin**

de Jong HC, et al. BMC Microbiol 2015

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Serotyping by agglutination: WHO-CC

White-Kauffmann-Le Minor Scheme

- **World Health Organisation Collaborating Centre**
 - Institut Pasteur, Paris, France
 - Center for Diseases Control and Prevention, Atlanta, États Unis
 - Institut für Hygiene und Umwelt, Hamburg, Allemagne
- All serotypes of *Salmonella* (> 2.500)

White-Kauffmann-Le Minor Scheme, 2007

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Serotyping by agglutination

White-Kauffmann-Le Minor Scheme (1926 – 1965 → 2007):

- The evolution of serotype naming:
 - First → syndrome: **typhi, typhimurium, Choleraesuis**
 - Then → family name of person identifying for the first time: **Virchow**
 - Now → geographic (first identification): **Dublin, Kentucky, Lutetia**
- The serotype is determined by the combination of the antigenic formula:

Serotype	Ag O	Ag H1	Ag H2	Autres
Enteritidis	9,12	g,m	-	-
Typhimurium	1,4,[5],12	i	1,2	-
Typhi	9,12	d	-	Vi

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AGGLUTINATION → WGS

Adapting the White-Kauffmann-Le Minor Scheme *in silico*

● Agglutination
● Agglutination & PCR (7g-MLST)
● Whole Genome Sequencing

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Serotyping by WGS

Adapting the White-Kauffmann-Le Minor Scheme *in silico*

- **Region rfb :**
 - Genomic region encoding for the Somatic Antigen « O »
 - Complex, eventually not specific enough
- **MLST :**
 - Seven housekeeping genes: *aroC, dnaN, hemD, hsd, purE, sucA, thrA*
 - EnteroBase database (enterobase.warwick.ac.uk)
- **fliC + fliB :**
 - Genes encoding for the flagellar phases H1 & H2

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Serotyping by WGS

Adapting the White-Kauffmann-Le Minor Scheme *in silico*

- The serotype is inferred from: MLST + « O » group (rfb region) + flagellar phases (fliC + fliB) :

Sérotype	MLST	Ag O	Ag H1	Ag H2	Autres
Enteritidis	11, 183	O:9 (D1)	g,m	-	-
Typhi	1, 2	O:9 (D1)	d	-	Vi
Koessen	48	O:2 (A)	l,v	1,5	-
Miami	48	O:9 (D1)	a	1,5	-
Panama	48	O:9 (D1)	l,v	1,5	-

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What to do?

At the institutional level:

- Treat the patient

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TREATMENT

- Gastroenteritis
 - ✓ Symptomatic support
 - ✓ Hydration
- Typhoid Fever and Complications
 - ± 3rd Generation Cephalosporins (3GC)
 - ± Fluoroquinolones
 - ± Azithromycin

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MECHANISM OF ACTION OF ANTIBIOTICS

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Mechanism of action: beta-lactams

Beta-lactams show affinity for the **transpeptidase** (« Penicillin-Binding-Protein ») (**PBP**)

- The binding between **BL** + PBP blocks the peptidoglycan cross-linking
 - and by doing so, the synthesis or reparation of a stable wall

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Mechanism of action: quinolones

Quinolones show affinity for the **gyrase (GyrA)** et la **topoisomérase (parC)** subunits

- The binding between **Q** + **G/T** stops DNA synthesis or repair:
 - Quinolones stabilise the topoisomerase-DNA cleavage complex in which there is a double-strand break.
 - If the cleavage complex is not resolved, replication and transcription cannot happen, which causes **slow bacterial cell death**.
 - If the topoisomerase is removed, the double-strand break is free, but if left unrepaired, it leads to the fragmentation of the chromosome, which causes **rapid bacterial cell death**.

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Mechanism of action: macrolides

Azithromycin shows affinity for the **50S ribosomal subunit**

- The binding between **AZI** + 50S stops protein synthesis at the ribosome

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Salmonella spp.

Salmonella spp. is naturally **SUSCEPTIBLE** to all antibiotics

Antibiotique	≤ 4 S
Acide nalidixique	≤ 4 S
Ampicilline	≤ 1 S
Azithromycine	8 S
Cefotaxime	≤ 0,25 S
Ceftazidime	≤ 0,5 S
Chloramphenicol	≤ 8 S
Ciprofloxacine	0,03 S
Colistine	2 S
Gentamicine	≤ 0,5 S
Méropénème	≤ 0,03 S
Sulfaméthoxazole	32 S
Tétracycline	≤ 2 S
Tigécycline	≤ 0,25 S
Triméthoprime	≤ 0,25 S

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Salmonella spp.

Salmonella spp. can become resistant to antibiotics

Antibiotique	≤ 128 S
Acide nalidixique	≤ 128 S
Ampicilline	≤ 64 S
Azithromycine	≤ 64 S
Cefotaxime	≤ 4 S
Ceftazidime	≤ 32 S
Chloramphenicol	≤ 128 S
Ciprofloxacine	≤ 64 S
Colistine	≤ 1 S
Gentamicine	≤ 1 S
Méropénème	≤ 0,5 S
Penicilline	0,06 S
Méropénème	0,06 S
Sulfaméthoxazole	≤ 128 S
Tétracycline	≤ 64 S
Tigécycline	≤ 1 S
Triméthoprime	≤ 2 S

Quinolones, Azithromycin, Beta-lactams (c3c), Quinolones

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MECHANISMS OF RESISTANCE TO ATB

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Resistance to antibiotics: *Salmonella*

Natural resistance:

- Innate, stable, shared by all bacteria belonging to the same species:
 - Vertical transmission to the offspring
 - "Wildtype" phenotype: the most susceptible observed among strains of one species

Salmonella spp.

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Resistance to antibiotics: *Salmonella*

Acquired resistance:

- Due to a **chromosomal mutation** on the sequence of a gene or its regulatory system:
 - Vertical transmission to the offspring
- By **horizontal transfer** between bacteria
 - Possible transfer between different species
 - Transformation : DNA fragments free in the environment
 - Transduction : Bacteriophages → Prophages
 - Conjugation : Plasmids

```

VLDGDDHRAAYKXIAIVVGDVDTKXTHPRDIAAGDVRRAQDFFL
VLDGDDHRAAYKXIAIVVGDVDTKXTHPRDIAAGDVRRAQDFFL
VLDGDDHRAAYKXIAIVVGDVDTKXTHPRDIAAGDVRRAQDFFL
VLDGDDHRAAYKXIAIVVGDVDTKXTHPRDIAAGDVRRAQDFFL
                    
```

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RESISTANCES IN SALMONELLA

- Resistance to beta-lactams
- Resistance to quinolones
- Resistance to azithromycin

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Resistance to beta-lactams

Acquired resistance:

- bla*TEM-1, *bla*CTX-M-9, *bla*CMY-2, *bla*OXA-48

Antibiotics	Wildtype	Penicillinase	ESBL	Cephamycinase	Carbapenemase
Amoxicillin (Aminop.)	S	R	R	R	R
3GC	S	S	R	R	R
Cephamycins	S	S	S	R	R
Carbapenems	S	S	S	S	R

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Resistance to quinolones

Intrinsic resistance:

- Point mutations on chromosomal 'quinolone-resistance determining regions' (QRDR):
 - *gyrA*, *gyrB*, *parC*, *parE*
 - Low to high level resistance

Acquired resistance:

- Genes obtained by plasmid conjugation:
 - *qnr*, *qepA*, *acc6'*-*lb-cr*, *oqxAB*
 - Low-level resistance

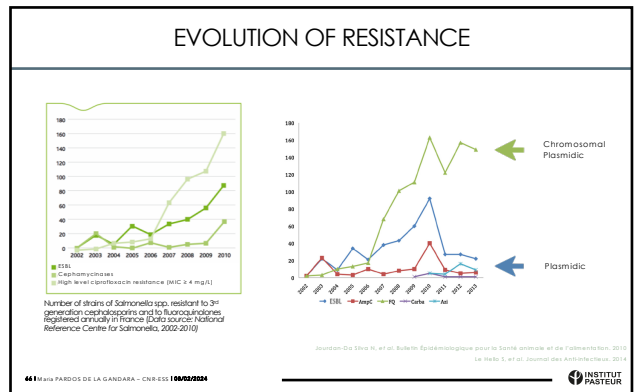
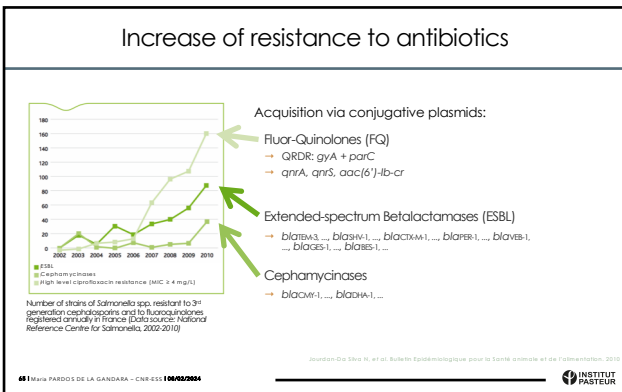
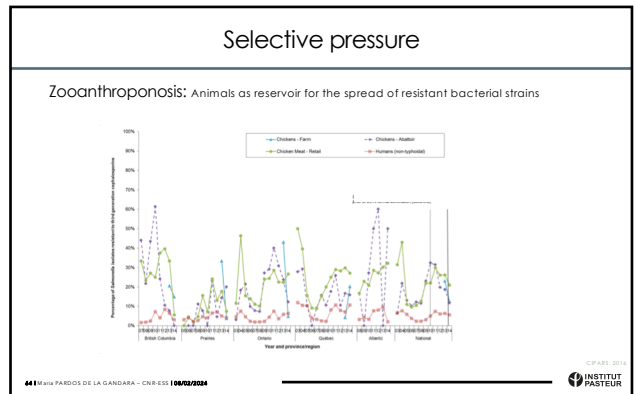
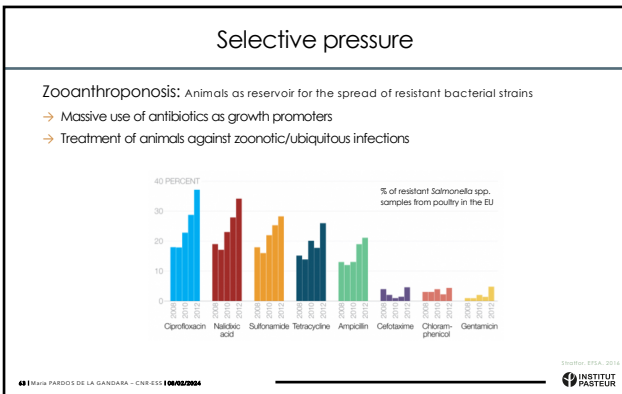
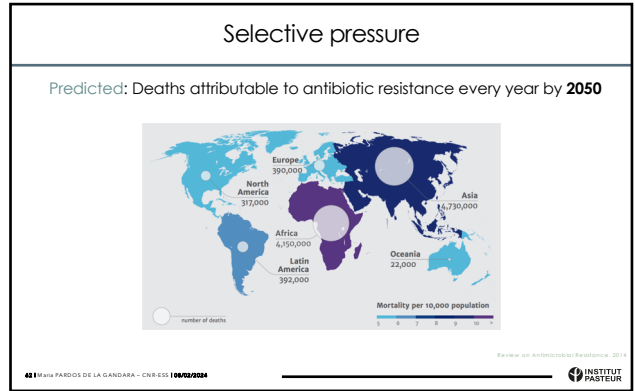
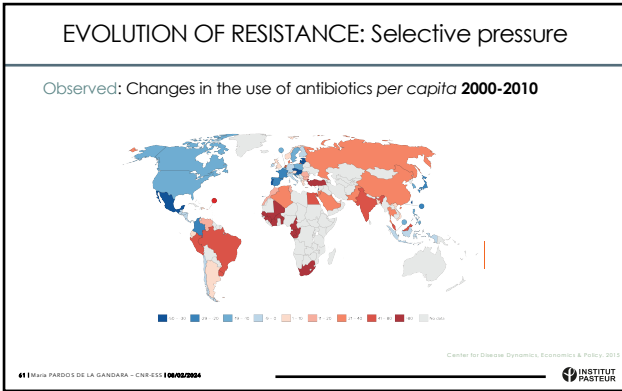
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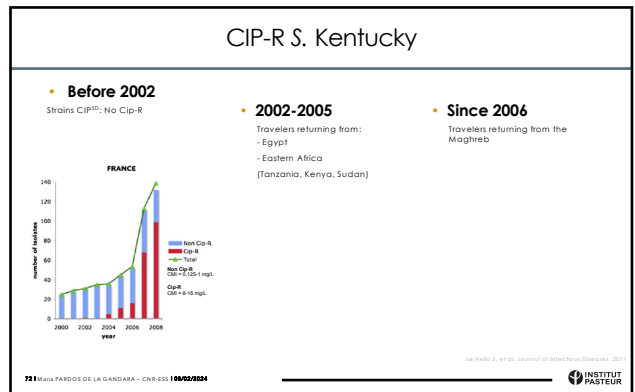
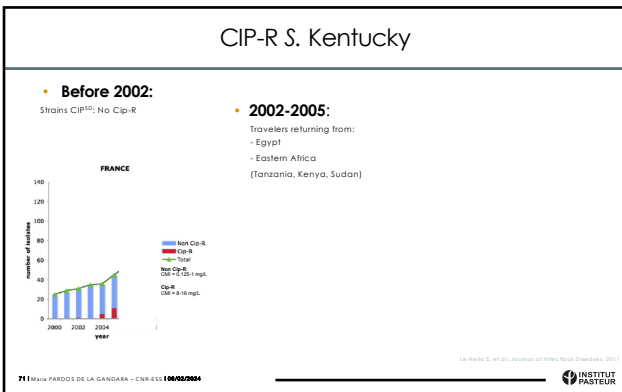
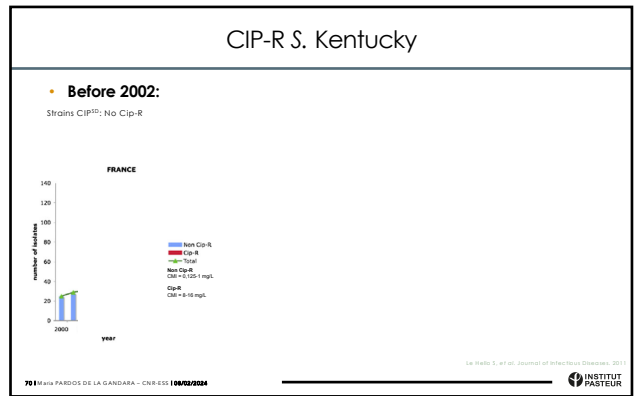
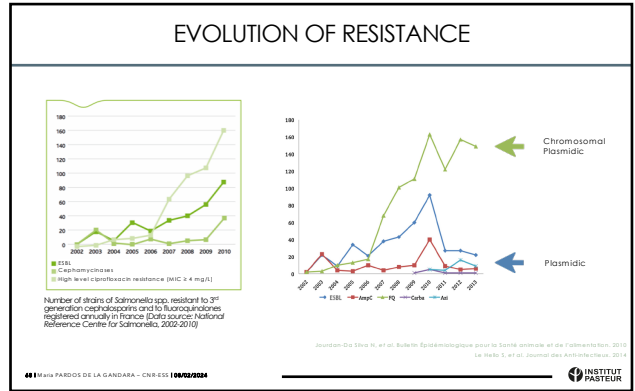
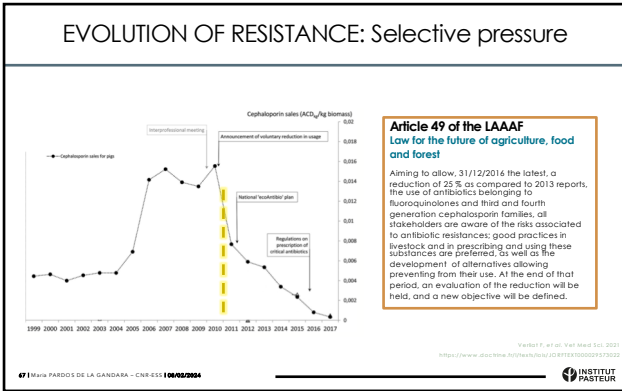
Resistance to azithromycin

Acquired resistance:

- mph(A)* gene: macrolide 2'-phosphotransferase

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CIP-R S. Kentucky

- Before 2002**
Strains CIP^{RS}; No CIP-R
- 2002-2005**
Travelers returning from:
 - Egypt
 - Eastern Africa (Tanzania, Kenya, Sudan)
- Since 2006**
Travelers returning from the Maghreb

Wahl FA, et al. Emerging Infectious Diseases. 2008
de Toledo S, et al. Journal of Infectious Diseases. 2011

Asp87As Asp87Tyr Asp87Gly

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CIP-R S. Kentucky

Multidrug-resistant strains (BL-AM-TE-CIP)

- Origin in Egypt:
- Intensive aquaculture in tanks introduced in Egypt in the 1950s
 - Egypt produces 80% of farm-raised fish in Africa
 - Integrative aquaculture: fish fed with:
 - Poultry residues
 - Fish residues
 - Mixed rice-fish culture

Sapich A, et al. Environmental International. 2008
El-Sayed AFM, et al. FAD. 2007
Wayne Clagson R & Datta J. Food Safety. 2011

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CIP-R S. Kentucky

Since 2013 in poultry in France

Epidemiological investigation of highly ciprofloxacin resistant *Salmonella Kentucky* detected for the first time in French avian production

Investigation épidémiologique du premier foyer lié à *Salmonella Kentucky* hautement résistante aux fluoroquinolones détecté en élevage avicole en France

Gudon F, et al. Bulletin Epidémiologique pour la Santé Animale et de l'Élevage Vétérinaire. 2017

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What to do?

At the institutional level:

- Epidemiological Surveillance measures

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EPIDEMIOLOGICAL SURVEILLANCE

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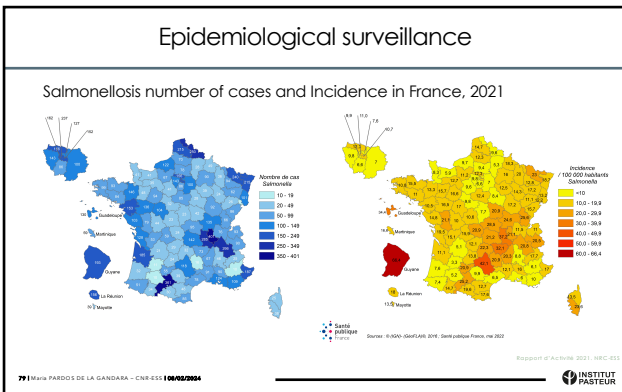
Epidemiological surveillance

Pyramid of the foodborne infection surveillance in France:

- Clinical outcomes in red
- Sources of information in blue

Van Cauwen D, et al. Bulletin Epidémiologique Hebdomadaire. 2018

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Epidemiological surveillance: Compulsory Declaration

Salmonellosis issue of a Compulsory Declaration:

- Typhoid and Paratyphoid Fever
- Collective food-poisoning (CFP, TIAC)

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Epidemiological surveillance: Compulsory Declaration

Typhoid and Paratyphoid Fever

- Human to human contamination
- Healthy carrier (~ Biofilm)
 - Gallbladder
 - Kidneys
 - Anatomical abnormalities
 - Foreign bodies, prosthesis

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Epidemiological surveillance: Compulsory Declaration

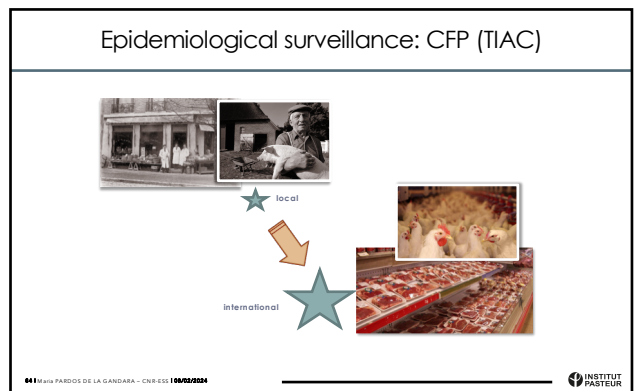
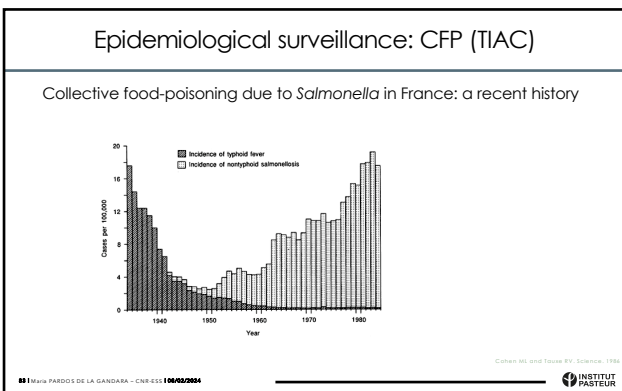
Collective food-poisoning (CFP)

[rToxi-infection alimentaire collective, TIAC]

- Identification of two or more cases having similar symptoms, generally gastroenteritis, for which one common food source can be incriminated
- Spur to launch investigations:
 - Potential consequences for Health 🏥, Economy 💰, Society 🏘️
 - Identify the sources (food, products at risk)
 - Stop the transmission
 - Guide the prevention measures and evaluate their impact

→ Compulsory declaration in France since 1987

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Epidemiological surveillance: Compulsory Declaration

Collective food-poisoning (CFP, TIAC)
Salmonella as a zoonosis:

- Poultry: Enteritidis, Hadar, Virchow, Newport, Senftenberg, ...
- Swine: Derby, ...
- Cattle: Dublin, Panama, Montevideo, ...

Also contamination of **water** and cross-contamination of **surfaces, vegetables**

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Epidemiological surveillance: CFP (TIAC)

Sankey diagram of the distribution of the top five *Salmonella* serotypes causing human salmonellosis in the EU, by food-animal source, 2020:

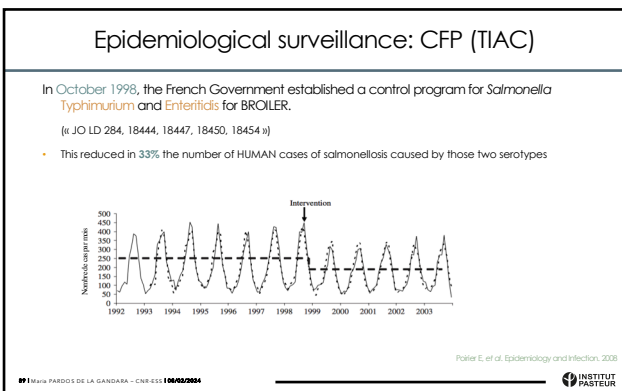
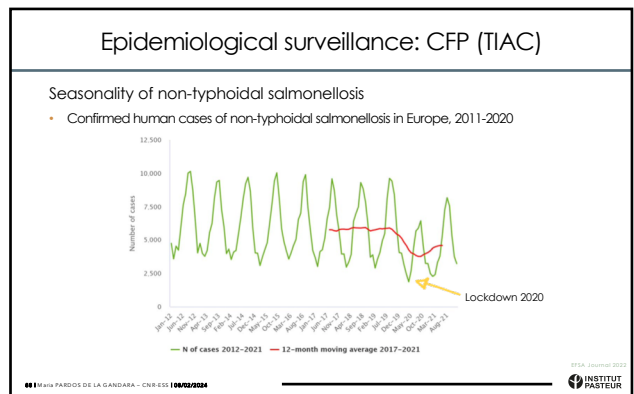
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Epidemiological surveillance: CFP (TIAC)

Yearly distribution of serotypes in humans in France

Rank	2017***	2018*	2019*	2020*	2021
1	Enteritidis (2 109)	Enteritidis (2 486)	Enteritidis (2 212)	Enteritidis (1 880)	Enteritidis (3 199)
2	1,4,[5],12i:- (2 101)	1,4,[5],12i:- (2 251)	1,4,[5],12i:- (1 818)	1,4,[5],12i:- (1 820)	1,4,[5],12i:- (2 086)
3	Typhimurium (1 919)	Typhimurium (1 933)	Typhimurium (1 083)	Typhimurium (793)	Typhimurium (1 044)
4	Infantis (212)	Newport (296)	Infantis (296)	Napoli (169)	Infantis (256)
5	Typhi (159)	Infantis (286)	Typhi (214)	Infantis (162)	Chester (156)
6	Newport (154)	Kentucky (177)	Newport (186)	Newport (144)	Napoli (147)
7	Kentucky (136)	Napoli (165)	Napoli (181)	Derby (132)	Derby (126)
8	Derby (130)	Typhi (159)	Derby (138)	Panama (106)	Newport (101)
9	Chester (86)	Chester (157)	Panama (133)	Dublin (81)	Coelin (95)
10	Panama (100)	Derby (135)	Stanley (126)	Typhi (84)	Montevideo (93)

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- ### MICROBIOLOGICAL SURVEILLANCE: methods
- **Phenotypic:**
 - Immunoassays → Serotyping
 - **Molecular:**
 - Enzymatic restriction → Pulsed-Field Electrophoresis on Gel (PFGE)
 - PCR (amplification and sequencing) → Multi-Locus Variant Analysis (MLVA) → CRISPOL-typing (CRISPR) → Multi-Locus Sequence Typing (MLST)
 - **Genomics:**
 - Whole Genome Sequencing → Core genome MLST (cgMLST), Hierarchical Clustering of cgMLST (HierCC) → SNP trees
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MICROBIOLOGICAL SURVEILLANCE

- Phenotypic:**
 - Immunoassays → Antigen-Antibody reaction (Serotyping)



Group O-4 (B)

Presentation of factor O:27 was modified. See page 8.

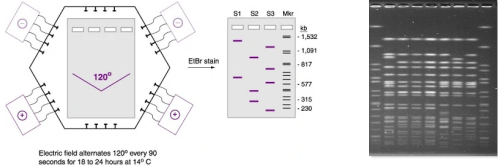
Type	Somatic (O) antigens		Flagellar (H) antigens		
	Phase 1	Phase 2	Other		
H	4,12	6A ₁₂			
Banana	1,4,12,12	6B	1,5,6		
Melon	4,12,12	6B	1,6,6A		
Typylomonium	1,4,12,12	1	1,2		
Lemon	1,4,12,12	1	1,5		
Alpaga	4,12	1	1,6		
Peach	4,12	1	6A,6		



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MICROBIOLOGICAL SURVEILLANCE

- Molecular:**
 - Enzymatic restriction → Pulsed-Field Electrophoresis on Gel (PFGE)



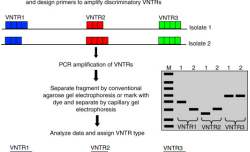
Electric field alternates 120° every 90 seconds for 18 to 24 hours at 14°C

http://www.pfge.com/wh/wh/Pulsed_Field_Elec...

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MICROBIOLOGICAL SURVEILLANCE

- Molecular:**
 - PCR (amplification and sequencing) → Multi-Locus Variant Analysis (MLVA)



Van Steffen, A. & S. K. Highgate, Eds. Encyclopedia of Microbial Sciences. Academic Press, 2012

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MICROBIOLOGICAL SURVEILLANCE

- Molecular:**
 - PCR (amplification and sequencing) → CRISPR-typing (CRISPR)

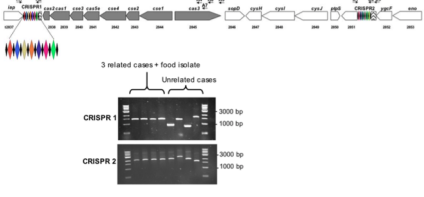
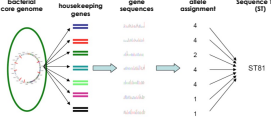


Figure 1.41.01. FoodNet, 2012

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MICROBIOLOGICAL SURVEILLANCE

- Molecular:**
 - PCR (amplification and sequencing) → Multi-Locus Sequence Typing (MLST)



ST	aroC	aroN	hemD	hsd	purE	sucA	thrA
11	0	2	3	7	6	6	11
11	0	2	3	7	6	6	11
11	0	2	3	7	6	6	11
24	00	09	12	9	5	5	2
24	00	09	12	9	5	5	2
24	00	09	12	9	5	5	2
24	00	09	12	9	5	5	2
24	00	09	12	9	5	5	2
24	00	09	12	9	5	5	2

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2711011/>

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MICROBIOLOGICAL SURVEILLANCE

- Genomics:**
 - Whole Genome Sequencing (WGS)
 - Core genome MLST (cgMLST)
 - Hierarchical Clustering of cgMLST (HierCC)
 - Single nucleotide polymorphism (SNP)

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MICROBIOLOGICAL SURVEILLANCE

Microbiological surveillance by Genomics

- Pan-genome:**
All genes present in at least one strain of the species
- Core genome:**
Those genes present in all strains of the species
- Accessory Genome:**
Genes present in some but not in all strains of the species

Shobkolli S.J. et al. Trends in Parasitology, 2021

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Microbiological surveillance: cgMLST

Microbiological surveillance by Genomics

- cgMLST : core genome MLST
- Allelic sequences for 3 002 genes

Madden M.C. et al. Nature Reviews Microbiology, 2013
Adapted from Zhou Z. et al. BMC Genomics, 2020

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Microbiological surveillance

Microbiological surveillance by Genomics

- cgMLST on 3002 genes :

Adapted from Zhou Z. et al. BMC Genomics, 2020
https://doi.org/10.1186/s12854-020-0363-6

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Microbiological surveillance

Microbiological surveillance by Genomics

- Hierarchical Clustering of cgMLST (HierCC) on 3002 genes :

→ HC2850: ≤ 2850 alleles different between genomes (≥ 152 identical) ≈ **subspecies**

Adapted from Zhou Z. et al. BMC Genomics, 2020
Zhou Z. et al. BMC Genomics, 2020

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Microbiological surveillance

Microbiological surveillance by Genomics

- Hierarchical Clustering of cgMLST (HierCC) on 3002 genes :

→ HC2850: ≤ 2850 alleles different between genomes (≥ 152 identical) ≈ **subspecies**

→ HC2000: ≤ 2000 alleles different between genomes (≥ 1 002 identical) ≈ **superlineage (~ serotype)**

Adapted from Zhou Z. et al. BMC Genomics, 2020
Zhou Z. et al. BMC Genomics, 2020

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Microbiological surveillance

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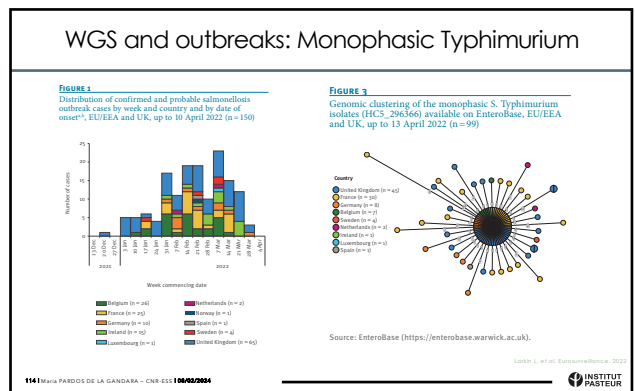
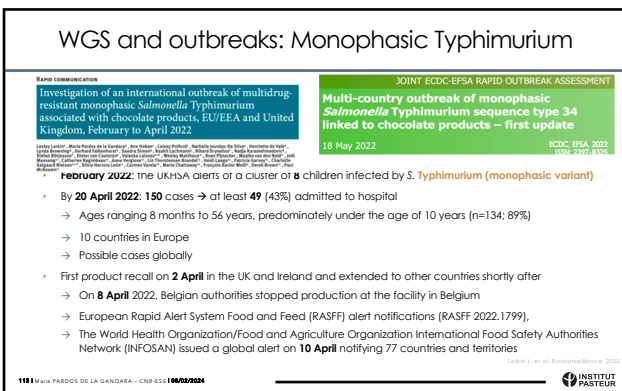
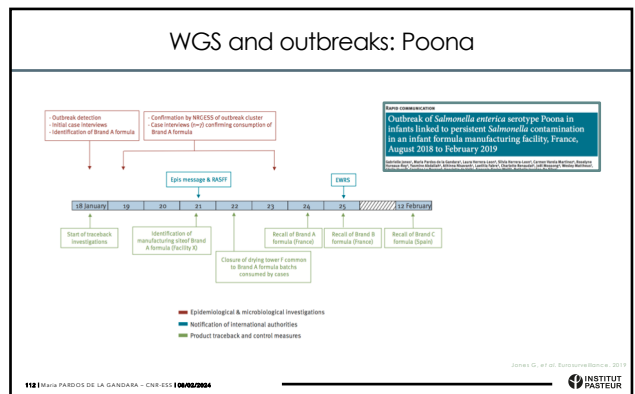
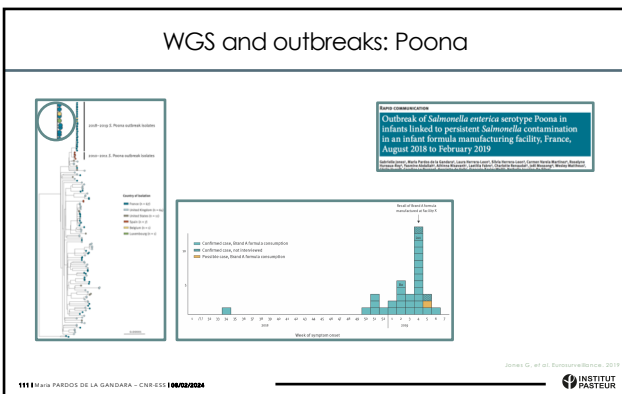
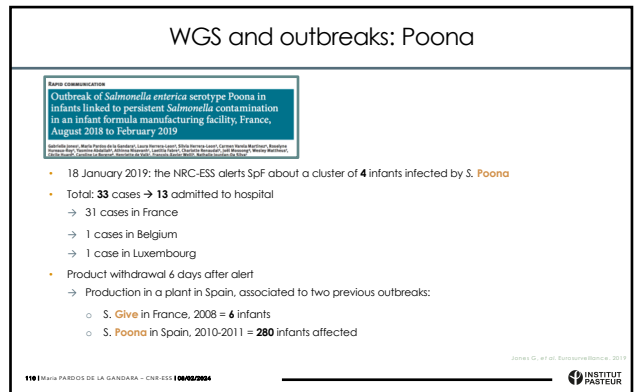
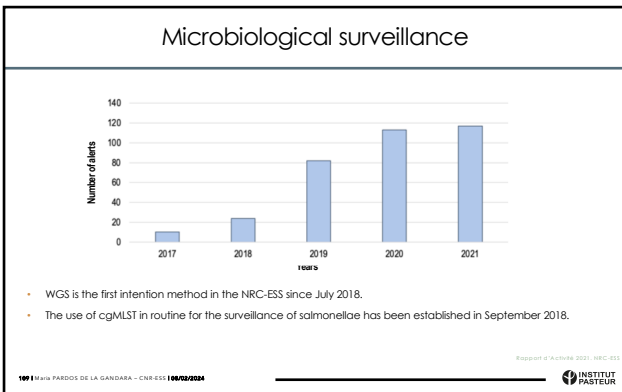
→ HC2000: ≤ 2000 alleles different between genomes (≥ 1 002 identical) ≈ **superlineage (~ serotype)**

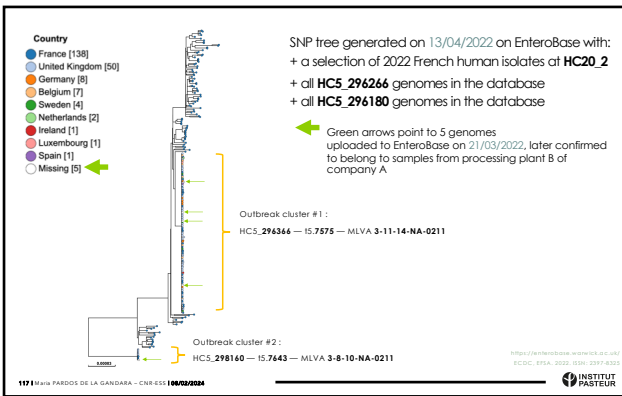
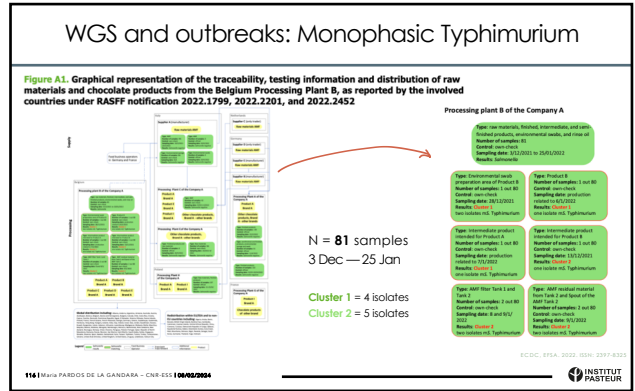
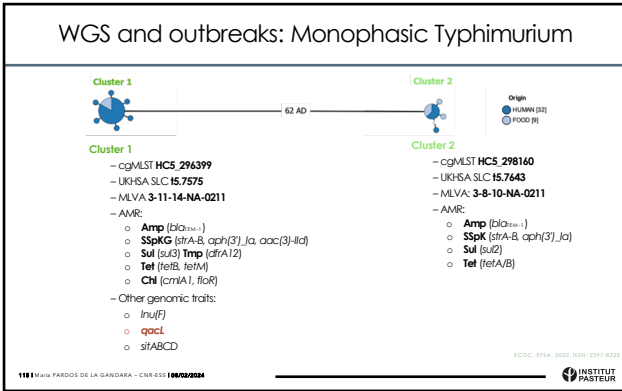
→ ...

→ HCS: ≤ 5 alleles different between genomes (≥ 2 997 identical) ≈ **outbreak**

Adapted from Zhou Z. et al. BMC Genomics, 2020
Zhou Z. et al. BMC Genomics, 2020

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THANK YOU FOR YOUR ATTENTION !

CNRS ESCHERICHIA COLI, SHIGELLA, SALMONELLA

m.pardo@pasteur.fr

ECCDC, EFSA, 2020, 2021, 2022/2024

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