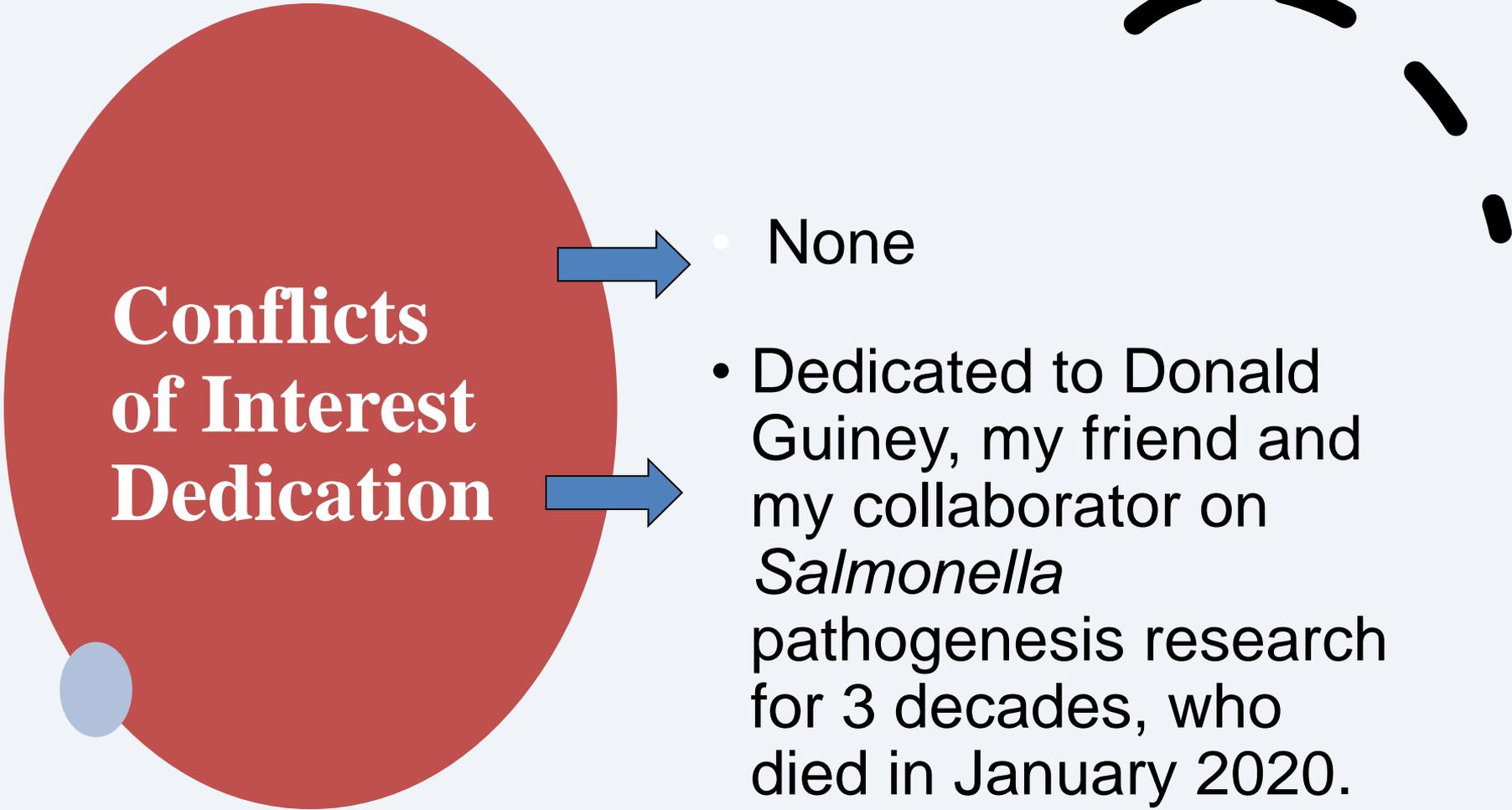


Invasive Non- typhoidal *Salmonella enterica* Infections

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Conflicts of Interest Dedication

- None
- Dedicated to Donald Guiney, my friend and my collaborator on *Salmonella* pathogenesis research for 3 decades, who died in January 2020.

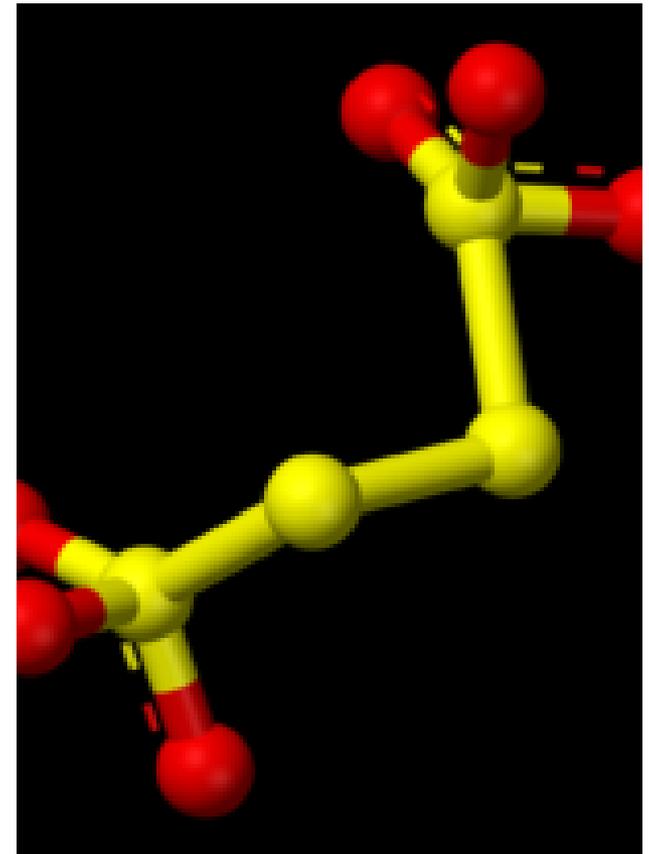
Leland S. Rickman, M.D.

- Lee was an important member of the ID Division at UCSD. He was born in Cleveland and earned a bachelor's degree in chemical engineering and a medical degree from the University of Michigan, did an internship and residency at UCSD and an ID fellowship at the Naval Hospital in San Diego. Five years later in 1990 I hired him to work in our HIV research. However, he wanted a more clinical position, so I cobbled together salary from the Hospital, and he was launched on a wonderful career in hospital epidemiology and clinical teaching. His enthusiasm for physical diagnosis was legendary, and he would leave a patient a run of to find a resident or fellow with a “you got to see this.” He was the bulwark of our clinical program at UH until his sudden and unexplained death, soon after arriving in Africa in June 2003.

The Genus *Salmonella*

- Gram negative bacilli in the Family *Enterobacteriales*.
- Motile (via peritrichous flagella)
- Non-lactose fermenting
- Produce H₂S from cysteine
- Grow in the presence of tetrathionate

used in vitro stool cultures, but tetrathionate is made in the inflamed gut and the *Salmonella* can use it as an electron acceptor, a huge advantage over the competing microbiota in the gut.



Salmonella Terminology

- *Salmonella* species used to be named based on **two polymorphic antigens**:
 - **O** (lipopolysaccharide)
 - **H** (flagellum); most *Salmonella* can alternately express either of two antigenically distinct flagella
- Genetic analysis concluded that there are only 2 species of *Salmonella*: *enterica* (further divided into 6 subspecies) and *bangori*.

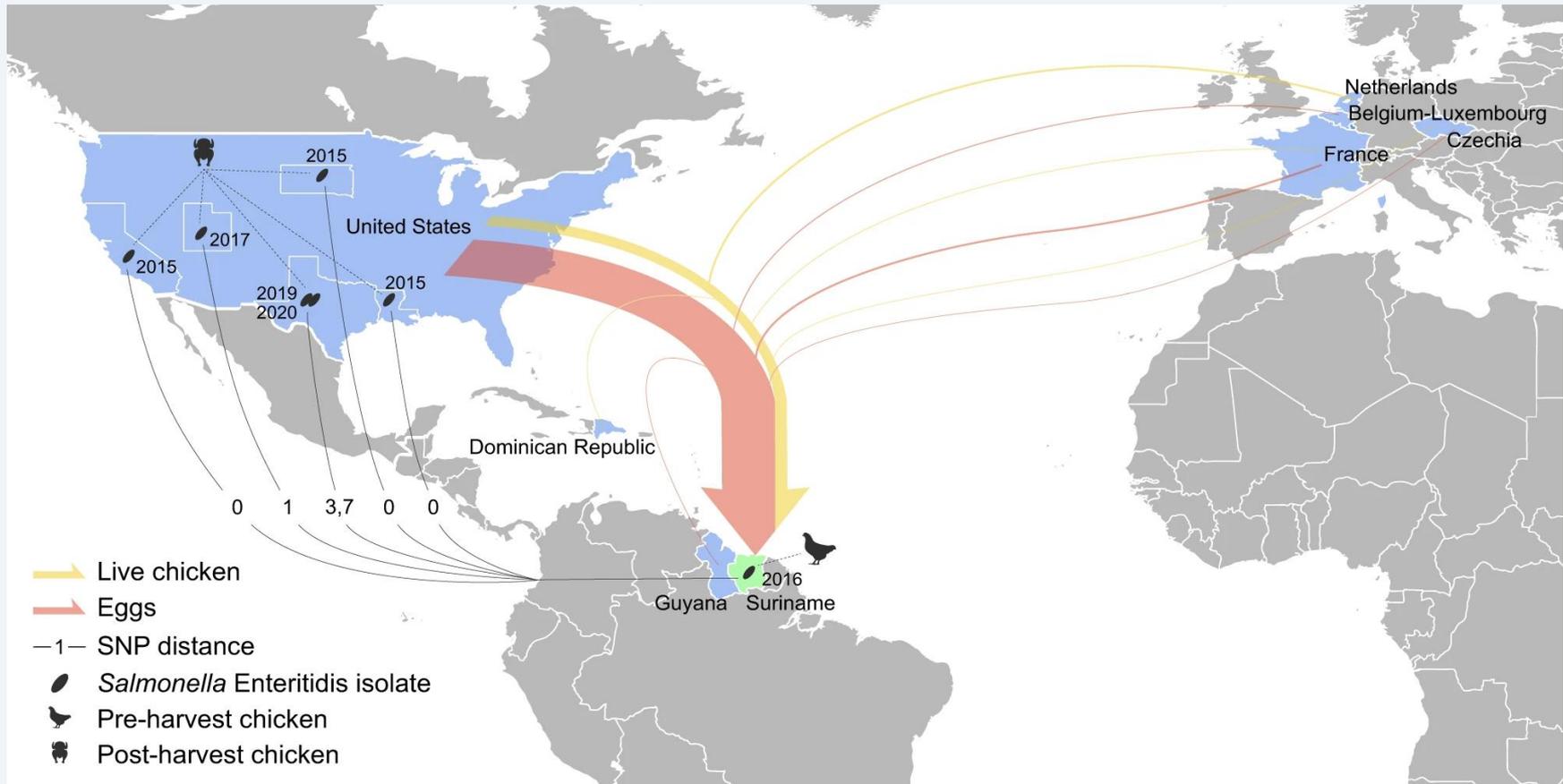
Salmonella Taxonomy

- **NAMES** **NUMBERS**
- *S. enterica* 2,557
 - *S. enterica* subsp. *enterica* 1,531
 - *S. enterica* subsp. *salamae* 505
 - *S. enterica* subsp. *arizonae* 99
 - *S. enterica* subsp. *diarizonae* 336
 - *S. enterica* subsp. *Houtenae* 73
 - *S. enterica* subsp. *Indica* 13
- *S. bangori* 22
- However, some species names such as *S. typhi* and *S. choleraesuis* have epidemiological and clinical importance so they were not abandoned but relegated to serovar status, e.g., *S. enterica enterica* serovar Typhi. For consistency, this convention was adapted for all serovars.

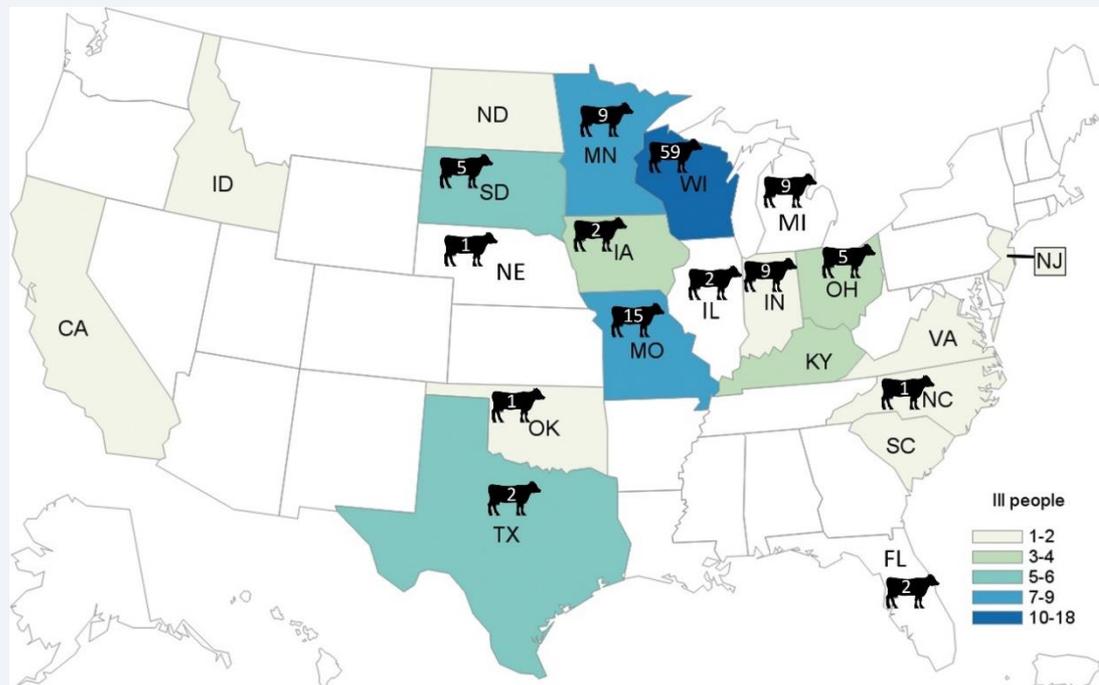
NTS Epidemiology; The Poster Child for One Health

- There are an estimated 93 million NTS infections, with 155,000 fatalities every year.
- Spread is fecal oral from contaminated food or water.
- Attack rates are highest in sub-Saharan Africa, including 75–388 cases per 100,000 children and 2,000–7,500 cases per 100,000 HIV-infected adults.
- 95% of NTS infections are gastroenteritis, but 5% present as bacteremia and systemic infections; these are called invasive nontyphoidal salmonellosis (iNTS).

Global Spread of *S. Enteritidis* Linked to Distribution of Chickens and Eggs from the USA.



Outbreak of *S. Heidelberg* Infections in Cattle and People



- Nichols M. et al Outbreak of Multidrug-Resistant *Salmonella* Heidelberg Infections Linked to Dairy Calf Exposure, United States, 2015-2018. Foodborne Pathog Dis. 2022 Jan 6. doi: 10.1089/fpd.2021.0077. PMID: 34989634

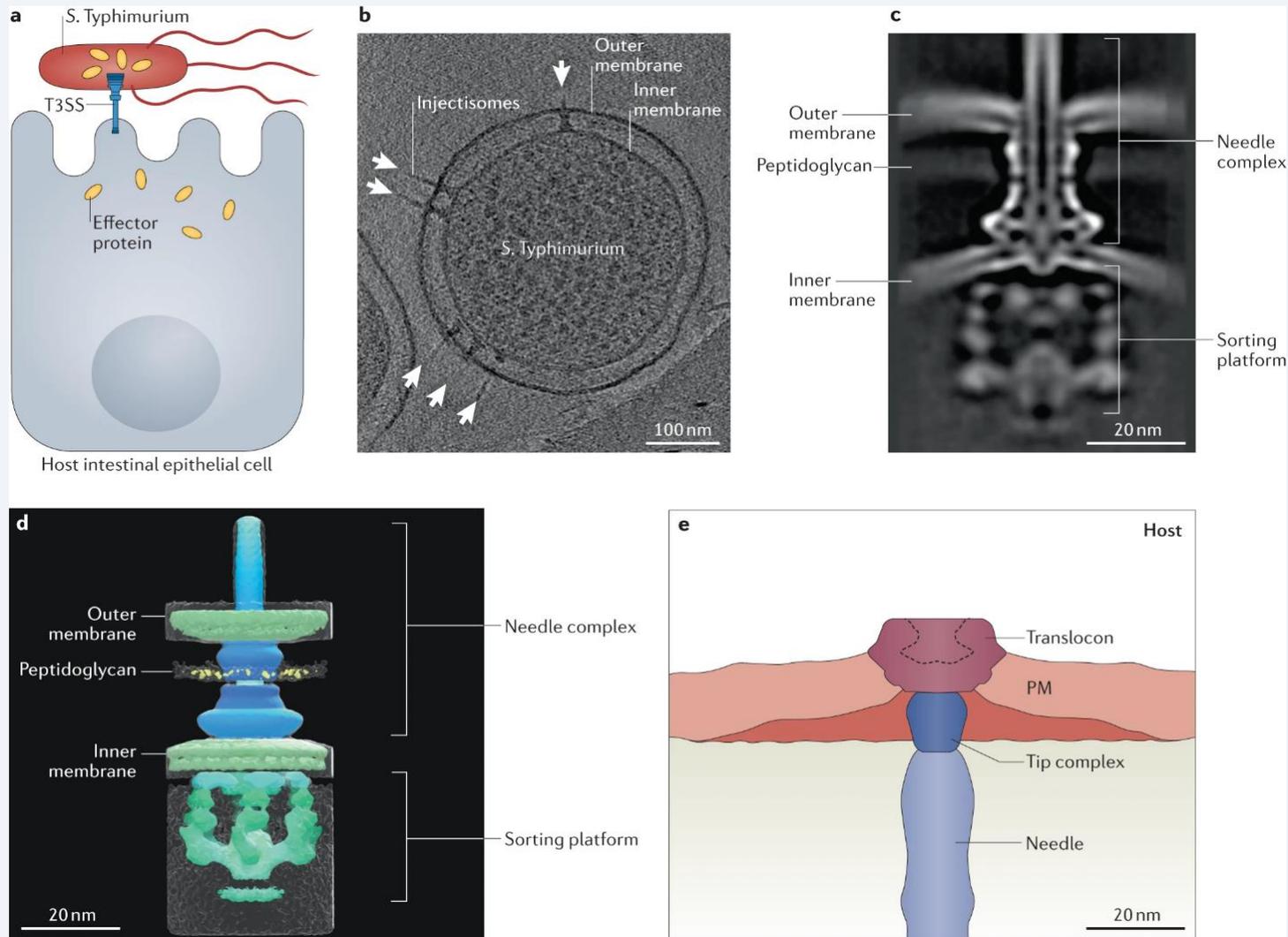
Syndrome	Serovars	Epidemiology	Predisposing host factors
Gastroenteritis	Many	Foodborne, animal exposure	Achlorhydria, prior antibiotics
Enteric (typhoid) fever	Typhi‡, Paratyphi A, (B and C less commonly)	Via fecal contamination of food or water by carriers or acutely ill people.	HLA-B*27:05
Invasive (bacteremia and extra-intestinal infections)	Choleraesuis Dublin Enteritidis Typhimurium Bovismorbificans Arizona Gallinarum	Foodborne (Raw milk, raw cheese, undercooked eggs, or undercooked pork). Animal exposure Folk remedies from rattle snakes	Malnutrition Hemolytic anemias T cell immunosuppression Malignancy Mutations or acquired autoimmunity that compromises IL-12/IFN γ activity Schistosomiasis Chronic granulomatous disease

Pathogenesis

What makes some *Salmonella enterica* serovars more pathogenic than *E. coli* ?

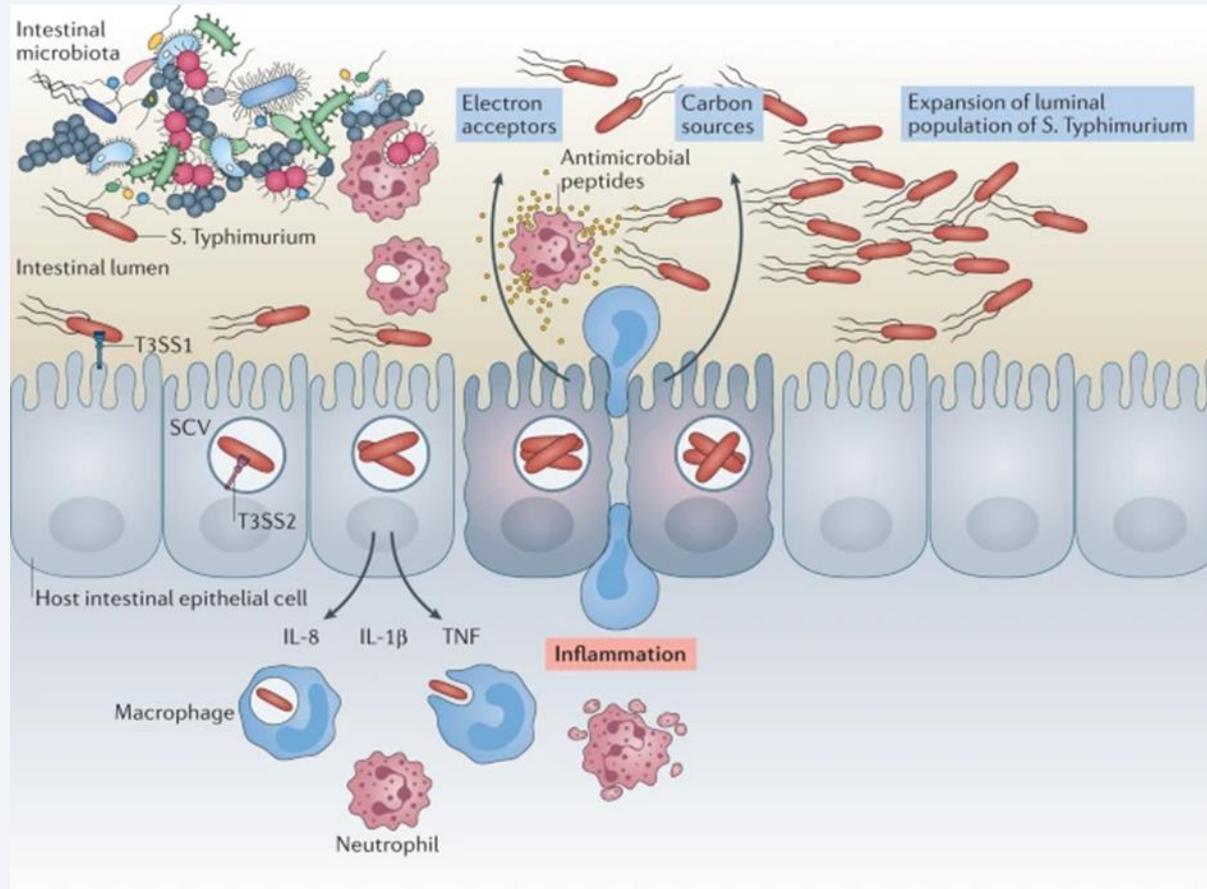
- Transposon inserts into the chromosome that encode virulence genes: Salmonella Pathogenicity Islands or spi.
 - spi1 and spi 2 both encode type 3 secretion systems, but the triggers for their and the regulation of their expression are different.
-

T3SS in Salmonella



Galán, J.E. *Salmonella* Typhimurium and inflammation: a pathogen-centric affair. *Nat Rev Microbiol* **19**, 716–725 (2021). <https://doi.org/10.1038>

Salmonella's Interactions with the Intestine



Galán, J.E. *Salmonella Typhimurium* and inflammation: a pathogen-centric affair. *Nat Rev Microbiol* **19**, 716–725 (2021). <https://doi.org/10.1038>

Salmonella Virulence plasmids (spv)

- Large non-transmissible plasmids that are present in only a small number of serovars
- Each serovar has a unique plasmid
- All spv plasmids contain a highly conserved 5 gene operon that encodes 4 secreted protein.
- All host-adapted NTS carry a spv EXCEPT Typhi and Paratyphi
 - Choleraesuis
 - Dublin
 - Abortus equi
 - Abortus ovis
 - Gallinarum
 - Pullorum
- Also carried by some but not all strains of *S. Typhimurium* and *S. Enteritidis*.
- 4 spv proteins are secreted by the spi2 apparatus when bacteria are intracellular.
- Those proteins interfere with different aspects of macrophage function.

Invasive *S. Typhimurium* Infections are More Likely to Have spv Genes Than are Fecal Isolates.

Source	Year	Number	# spv positive (%)
Feces			
	1983	29	10 (34)
	1990	14	4 (14)
	1991	36	19 (53)
	Total	79	33 (42)
Blood*			
	1988	12	8 (67)
	1990	30	24 (80)
	Total	42	32 (76)

chi-square
p-value is
.000301

* Includes 2 each from abscesses and urine, and 1 each from ascites fluid and ear exudate.

Fierer J, Krause M, Tauxe R, Guiney D. Salmonella typhimurium bacteremia: association with the virulence plasmid. J Infect Dis. 1992 Sep;166(3):639-42.

Invasive *S. Typhimurium* Infections are More Likely to Have *spv* Genes Than are Fecal Isolates.

spv	Blood culture	Stool culture
+	12	17
-	0	12

P <0.02

Heithoff DM, Shimp WR, Lau PW, Badie G, Enioutina EY, Daynes RA, Byrne BA, House JK, Mahan MJ. Human *Salmonella* clinical isolates distinct from those of animal origin. *Appl Environ Microbiol.* 2008 Mar;74(6):1757-66

Syndrome	Serovars	Epidemiology	Predisposing host factors
Gastroenteritis	Many	Foodborne, animal exposure	Achlorhydria, prior antibiotics
Enteric (typhoid) fever	Typhi, Paratyphi A, (B and C less commonly)	Fecal contamination of food or water by carriers or acutely ill people.	HLA Class II genes
Invasive (bacteremia and extra-intestinal infections)	Choleraesuis Dublin Enteritidis Typhimurium Bovismorbificans Arizona Gallinarum	Foodborne (Raw milk, raw cheese, undercooked eggs, or undercooked pork). Animal exposure Folk remedies from rattle snakes	Malnutrition Hemolytic anemias Malignancy T cell immunosuppression Mutations or acquired auto-immunity that compromises IL-12/IFN γ activity Schistosomiasis Chronic granulomatous disease

NTS infections in Cancer Patients at M.D. Anderson Hospital

Clinical presentation	No. (%)			GE= gastroenteritis
	Episodes	Severe sepsis or septic shock	Death within 30 days	
Acute GE	22 (25)	8 (36)	0 (0)	
Acute GE with bacteremia	12 (13)	6 (50)	1 (8)	
Primary bacteremia	27 (31)	10 (37)	2 (7)	
Focal infection	27 (31)	5 (19)	4 (15)	
Total	88 (100)	29 (33)	7 (8)	

Mori N, et al. Clinical presentation and outcomes of non-typhoidal Salmonella infections in patients with cancer. BMC Infect Dis. 2021 Sep 29;21(1):1021. doi: 10.1186/s12879-021-06710-7. PMID: 34587893; PMCID: PMC8482602.

Leukemia and Lymphoma are Highest Risk Malignancies for iNTS Infections

Table 1.—Type of Underlying Condition in 95 Patients With Salmonellosis

Neoplastic Disease	Total	Children	Adults	Adults >65
Leukemia	21	11	10	...
Acute lymphatic	6	4	2	...
Acute myelocytic	6	2	4	...
Acute stem cell	5	5
Chronic myelocytic	2	...	2	...
Leukolymphosarcoma	2	...	2	...
Lymphoma	25	...	21	4
Hodgkin's disease	9	...	7	2
Lymphosarcoma	7	...	7	...
Reticulum cell sarcoma	8	...	6	2
Mycosis fungoides	1	...	1	...
Gastrointestinal	9	...	3	6
Gynecologic	5	...	5	...
Breast	5	...	4	1
Genitourinary	5	...	3	2
Respiratory	5	...	3	2
Head and neck	2	...	1	1
Miscellaneous	9			
Embryonal cell carcinoma	1	1
Sarcoma	3	1	2	...
Malignant melanoma	1	1
Myoblastoma	2	1	1	...
Wilm's tumor	2	2
Nonneoplastic disease	9	1	7	1
Total	95	17	60	18

Wolfe M, et al. Salmonellosis in Patients With Neoplastic Disease; A Review of 100 Episodes at Memorial Cancer Center Over a 13-Year Period. *Arch Intern Med.* 1971;128(4):546-554. doi:10.1001

Complications of iNTS Bacteremia

- Endovascular infections – consider this diagnosis whenever there is high grade bacteremia, e.g., 4/4 bottles +, especially in the absence of diarrhea..
 - *Endocarditis* - rare but very destructive with high rate of abscess formation and valve destruction and ~ 40% mortality.
 - Surgery is often indicated.

Complications of iNTS Bacteremia

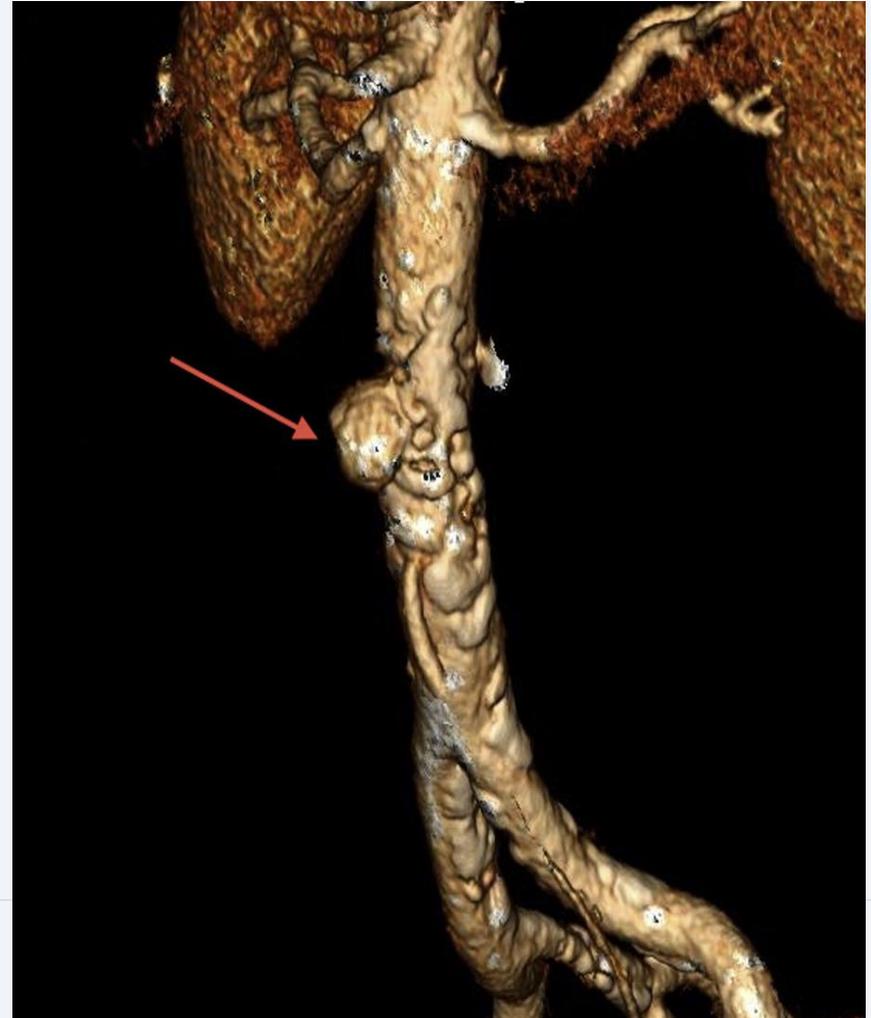
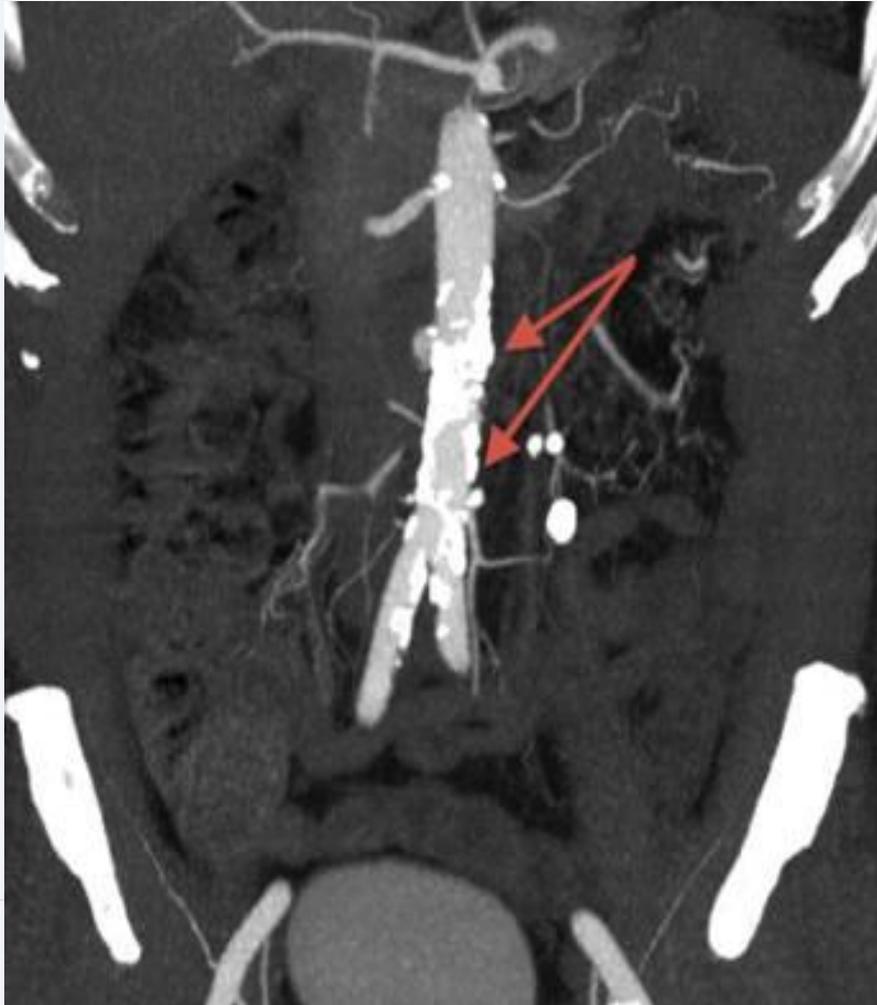
- Endovascular infections
- *Aortitis* - infections of pre-existing atherosclerotic. Most commonly occur in infra-renal aorta.
 - Consider the diagnosis in everyone >65 y.o. or known to have a prior atherosclerotic aneurysm.
 - Use imaging to look for it in anyone with Salmonella thoracic vertebral osteo
- Treatment is antibiotics and surgery. Surgery may be open or TAVR, especially if there is bleeding. If foreign body is inserted may need lifetime antibiotic suppression

CT Scans of Four Aortic Aneurysms



D1

Recreation of an Infra-Renal Mycotic Aneurysm of Atherosclerotic Aorta



Complications of iNTS Bacteremia

- **Osteomyelitis** is always hematogenous. In adults it involves axial skeleton. In kids, it targets the epiphyses of long bones or bone infarcts in patients with sickle cell disease. Acute osteo usually responds to antibiotics. Can result in chronic osteo that can be dormant for decades.
 - **Septic arthritis** - usually in old prosthetic joints. Requires joint replacement and antibiotics.
-

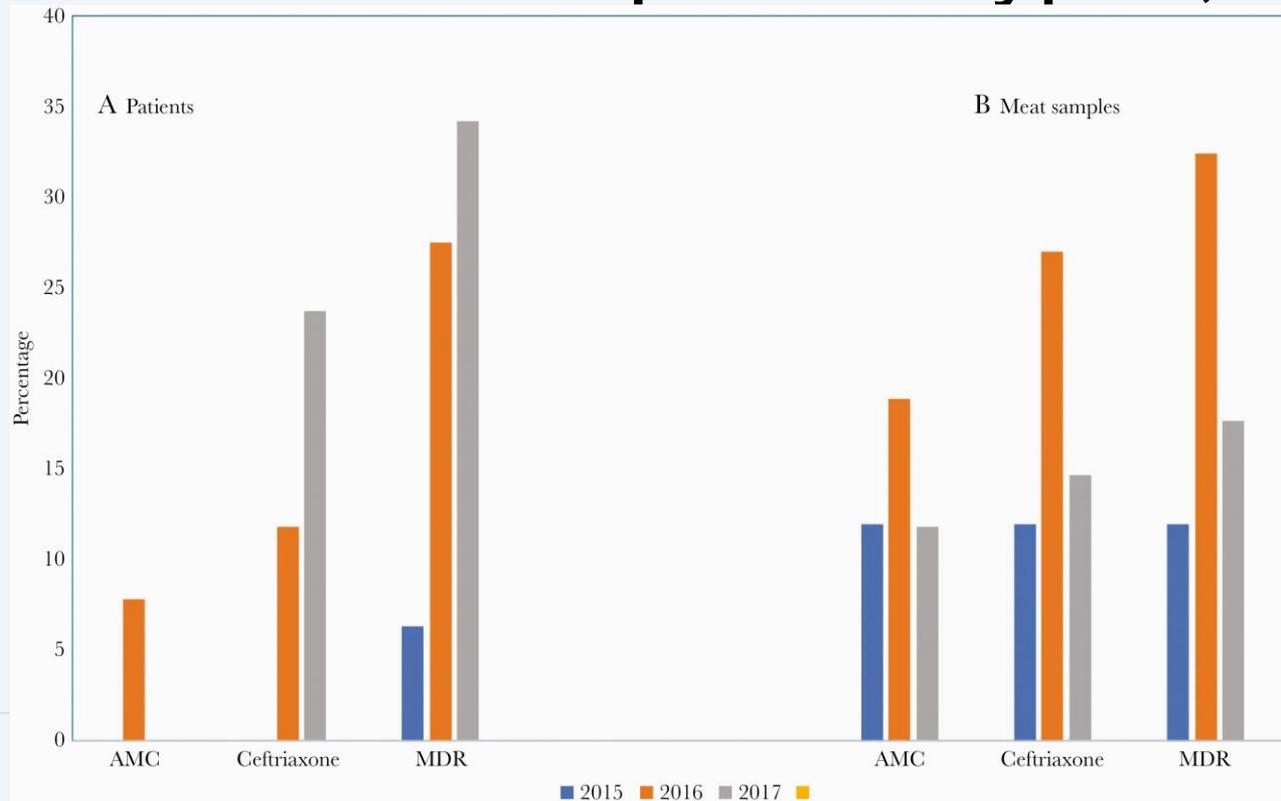
Complications of iNTS Bacteremia

- **Meningitis.** Common in infants and children in sub-Saharan Africa. High mortality. Rare in adults, but the most likely cause of enteric GNR infection if not iatrogenic or Strongyloides-related.
- **Focal abscesses.** *Salmonella* target macrophages so more often in liver or spleen. Blood cultures may be negative.
- **Urinary tract.** can be ascending in women and in catheterized men, but otherwise hematogenous.

Treatment of NTS

- Only antibiotics that can enter macrophages are effective.
 - Aminoglycosides and polymyxins are not effective.
- Uncomplicated gastroenteritis does not need to be treated. Salmonella colitis may be an exception.
- Drug resistance of Salmonella is an increasing problem everywhere.

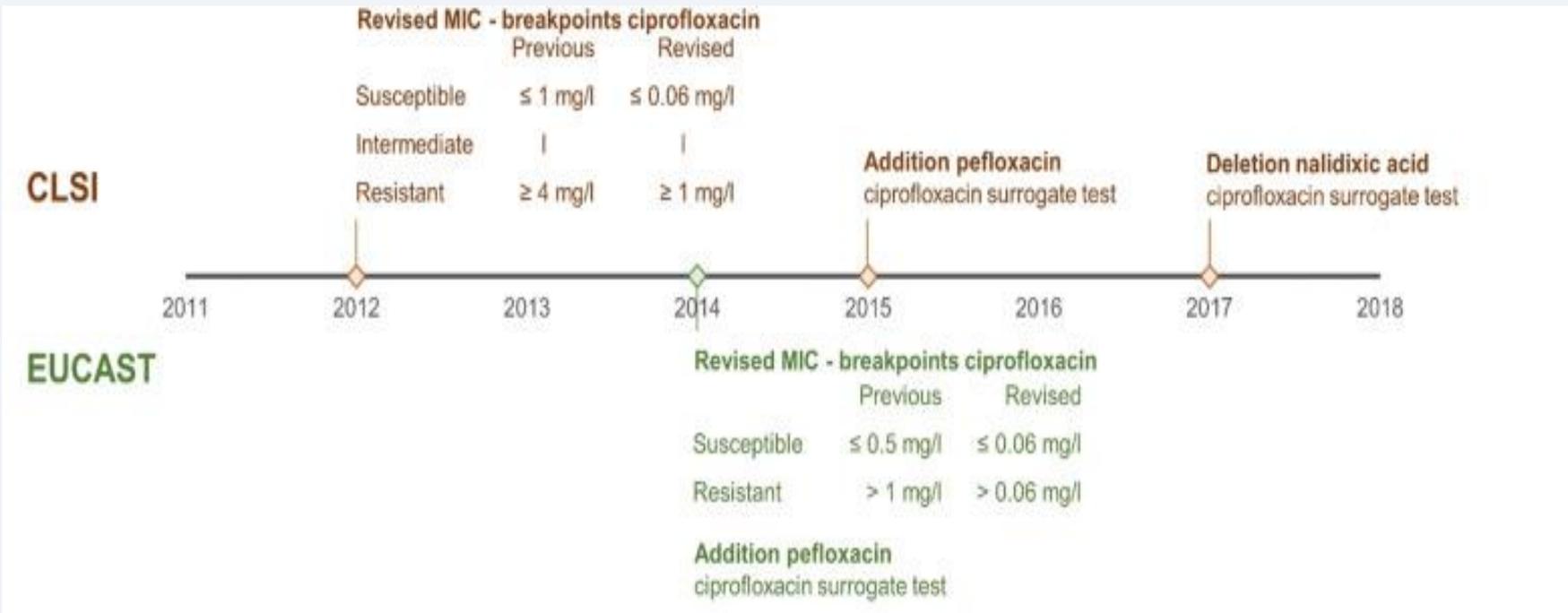
Increasing Antimicrobial Resistance Over Time in NTS isolates from People and Meat in Pennsylvania With the Same Sequence Types, PA.



Potentially Useful Antibiotics to Treat iNTS

- ampicillin/amoxicillin
- amoxicillin + clavulanic acid
- Tmp/smx
- chloramphenicol
- third generation cephalosporins (ceftriaxone)
- fluoroquinolones (ciprofloxacin)

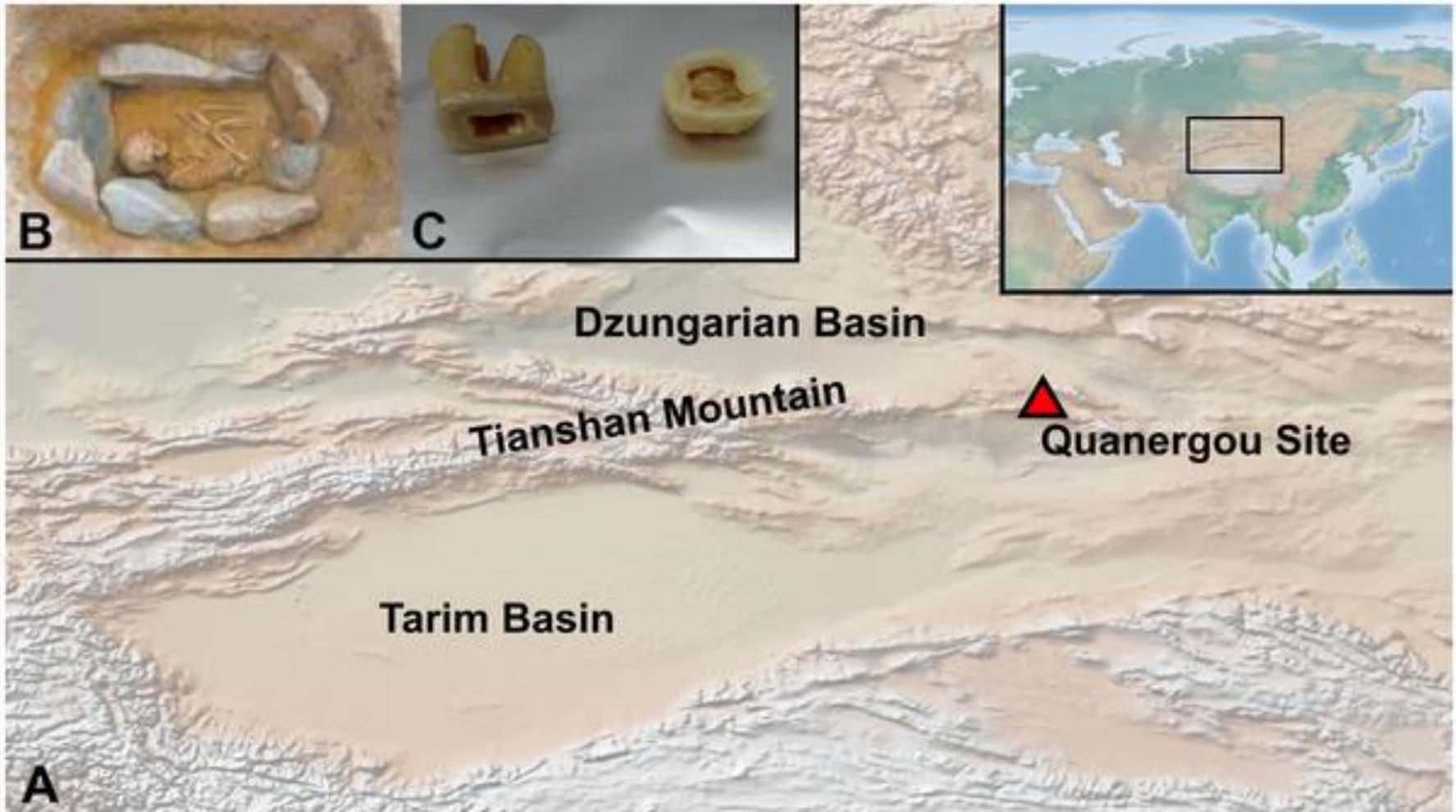
Surreptitious Cipro Resistance of Salmonella



Summary

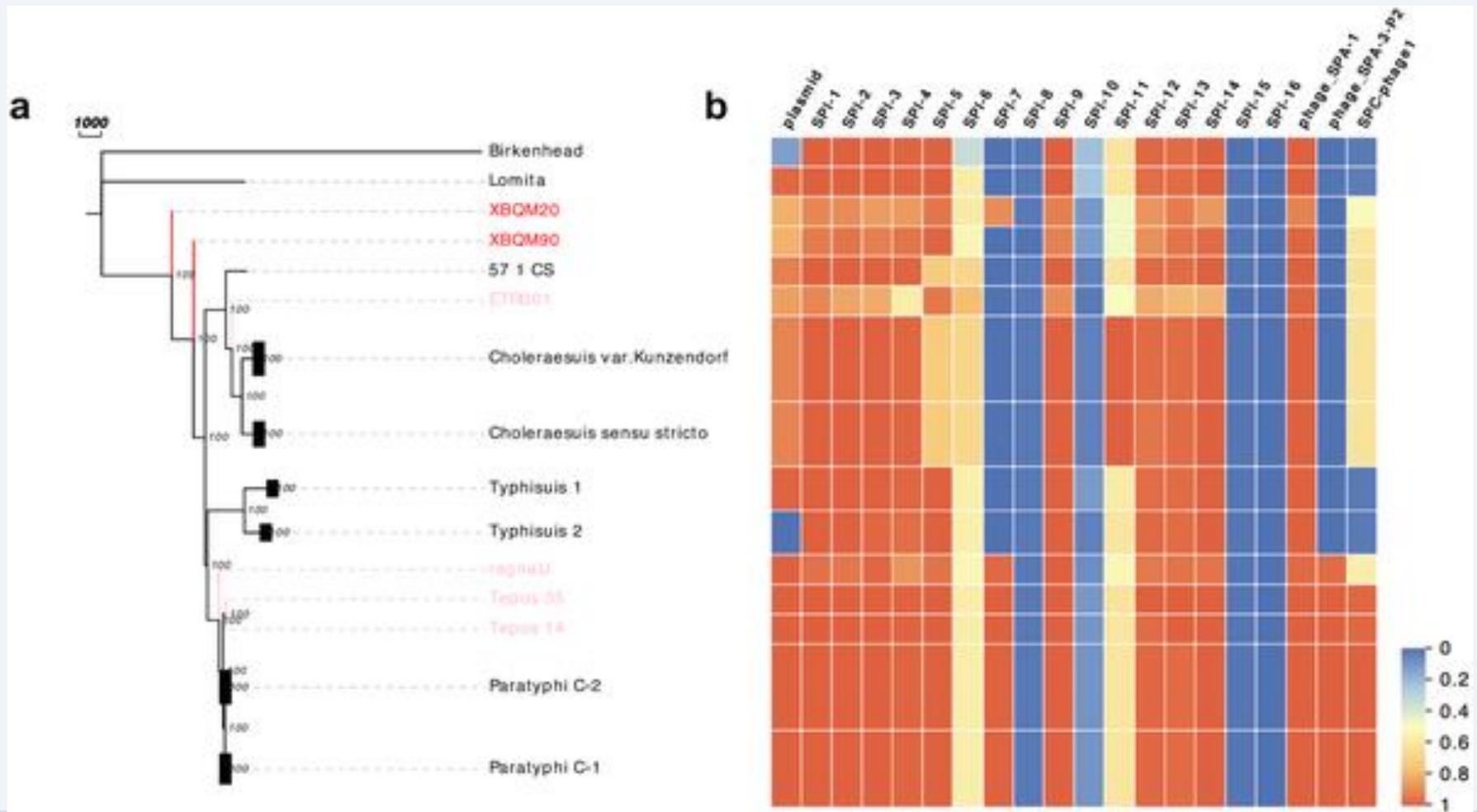
- iNTS are uncommon but potentially lethal.
 - iNTS is characterized by bacteremia or focal extra-intestinal infection in absence of diarrhea.
 - Risk factors for iNTS infections are both a function of the pathogens' genes (*spv* plasmid) and the susceptibility of the host.
 - Antimicrobial therapy is required, but resistance to our best drugs is increasing. This is largely due to the use of antibiotics in animal feed.
-

Isolation of *S. enterica* DNA from Teeth of Bronze Age Skeletons in Xinjiang China (3,000-1,200 BCE)



Wu X, Ning C, Key FM, Andrades Valtueña A, Lankapalli AK, et al. (2021) A 3,000-year-old, basal *S. enterica* lineage from Bronze Age Xinjiang suggests spread along the Proto-Silk Road. PLOS Pathogens 17(9): e1009886.

Phylogenetic tree of the Para C lineage and the gain and loss of virulence factors.

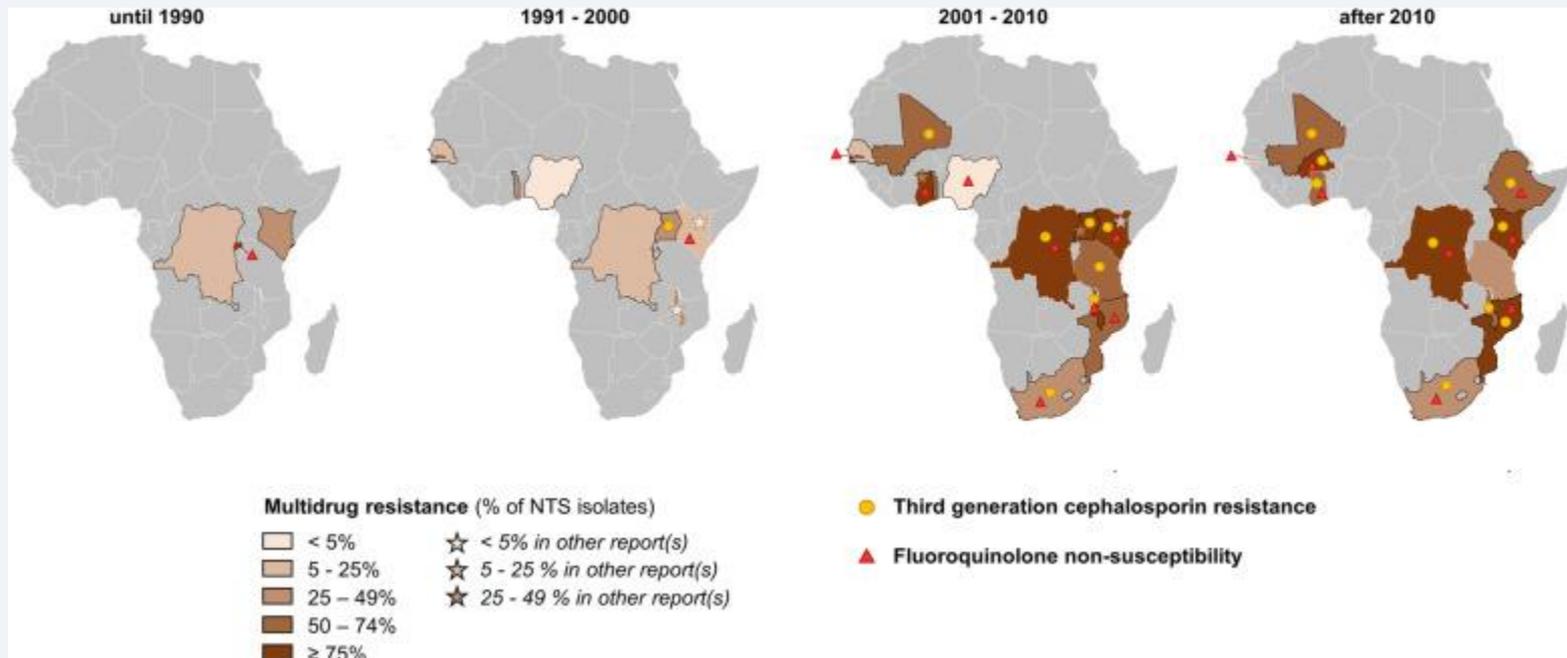


Wu X, Ning C, Key FM, Andrades Valtueña A, Lankapalli AK, et al. (2021) A 3,000-year-old, basal *S. enterica* lineage from Bronze Age Xinjiang suggests spread along the Proto-Silk Road. *PLOS Pathogens* 17(9): e1009886.

<https://doi.org/10.1371/journal.ppat.1009886>

<https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1009886>

Spread of MDR NTS in Sub-Saharan Africa



Tack B et al. DR Congo: Emergence of O5-negative Salmonella Typhimurium and extensive drug resistance. PLoS Negl Trop Dis. 2020 Apr 2;14(4):e0008121.

Non-fecal *S. Typhimurium* Isolates From Humans and Animals are More Likely to be *spv* +

Host	Source	Plasmid +	Plasmid -
Animal	Fecal	15	0
	Other	5	0
Human	Fecal	19	10
	Blood	12	0

Heithoff DM et al. Human Salmonella clinical isolates distinct from those of animal origin. Appl Environ Microbiol. 2008:1757-66. doi: 10.1128/AEM.02740-07.