Invasive Non-typhoidal *Salmonella enterica* Infections

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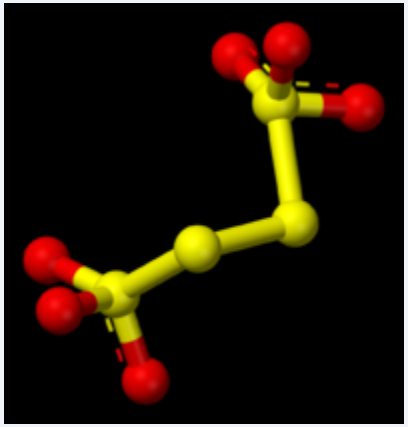
Conflicts of Interest Dedication

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

The Genus *Salmonella*

- Gram negative bacilli in the Family Enterobacterales.
- Motile (via flagella)
- Non-lactose fermenting
- Produce H_2S
- Grow in the presence of tetrathionate.



Salmonella Terminology

- Formerly, species were designated on the basis of two polymorphic antigens and now the same methods are used to identify serovars:
  - O or lipopolysaccharide
  - H or flagella; most *Salmonella* can alternately express two antigenically distinct flagella
- Genetic analysis concluded that there are only 2 species of *Salmonella*.
- Because names like *S. typhi* and *S. choleraesuis* have epidemiological and clinical importance they were not abandoned but relegated to serovar status, e.g., *S. enterica enterica* serovar Typhi. For consistency, adapted for all serovars.

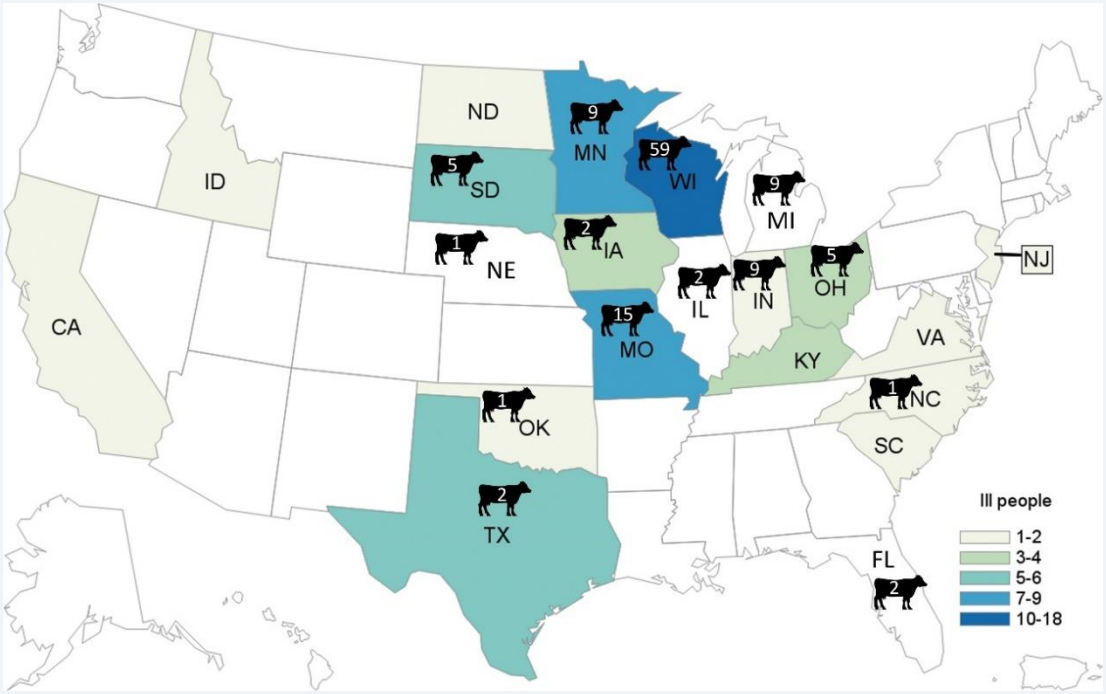
Salmonella Taxonomy

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

NTS Epidemiology

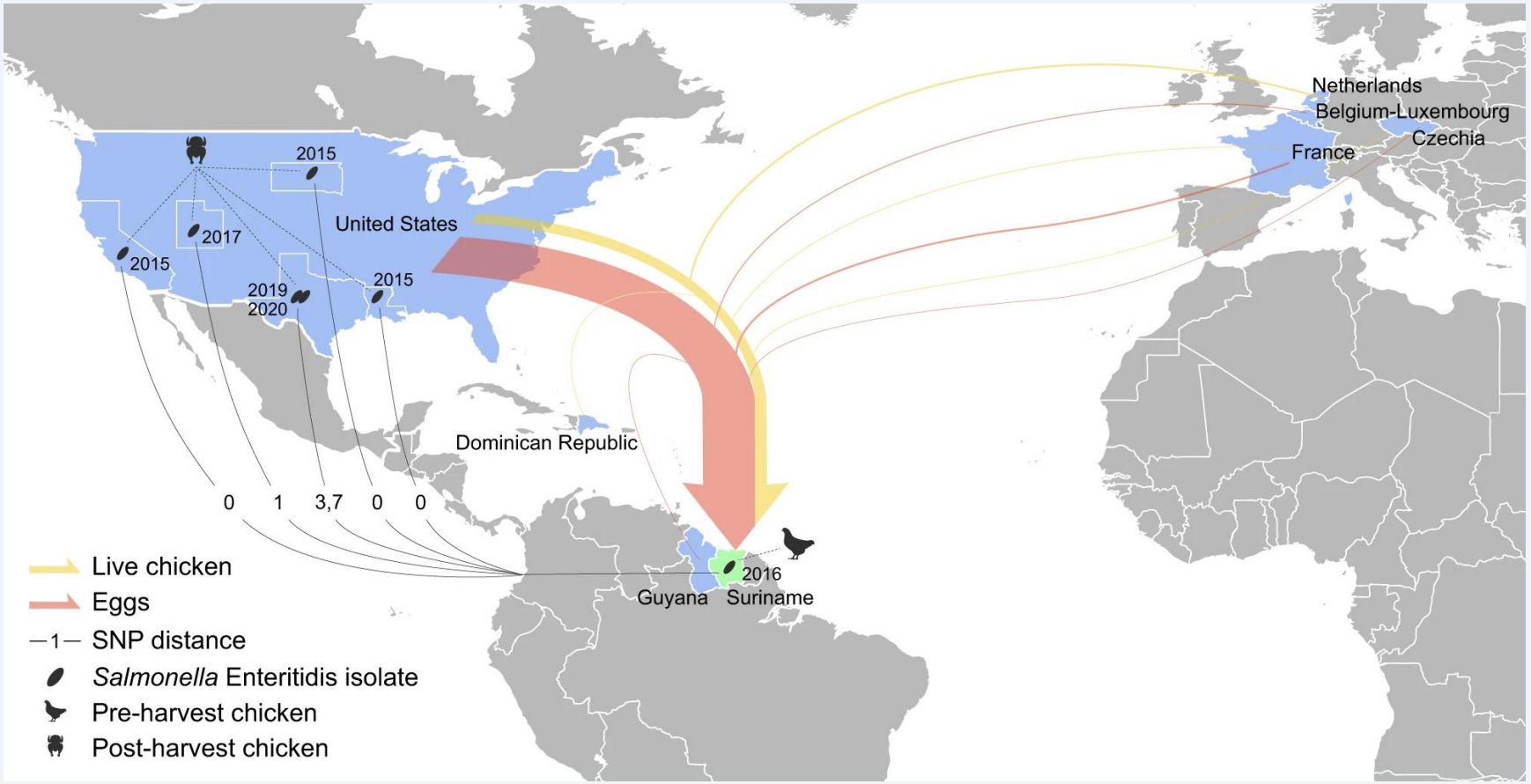
- There are an estimated 93 million NTS infections, with 155,000 fatalities every year.
- Spread is fecal oral from contaminated food or water
- Attack rates are highest in sub-Saharan Africa, including 75–388 cases per 100,000 children and 2,000–7,500 cases per 100,000 HIV-infected adults.
- In 95%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

Outbreak of S. Heidleberg 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

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



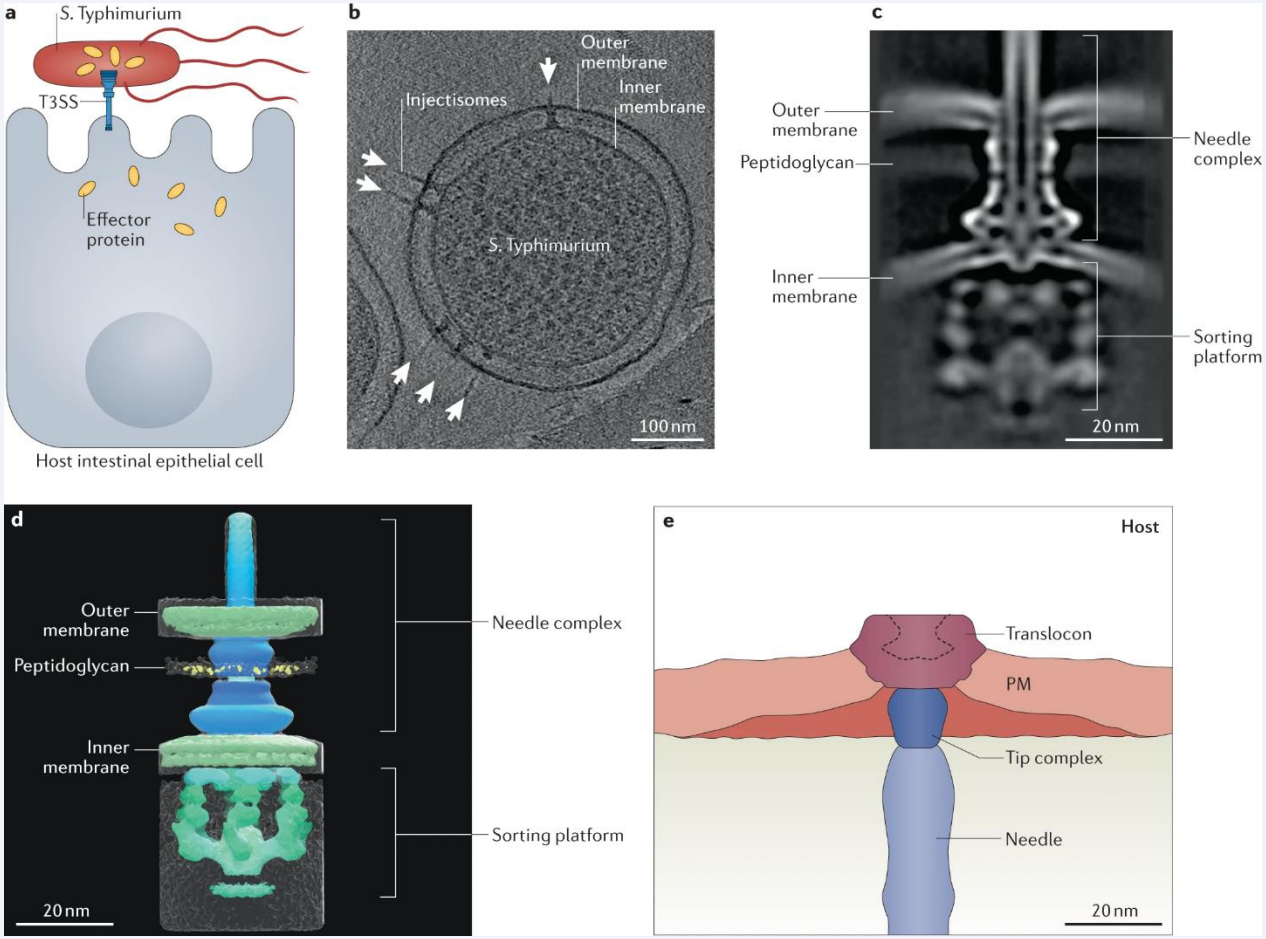
Syndrome	Serovars	Epidemiology	Predisposing host factors
Gastroenteritis	Many	Foodborne, animal exposure	Achlorhydria, prior antibiotics
Enteric (typhoid) fever	Typhi‡, Paratyphi A, (B and C less commonly)	Via fecal contamination of food or water by carriers or acutely ill people.	HLA Class II
Invasive (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 auto-immunity that compromises IL-12/IFNγ activity Schistosomiasis Chronic granulomatous disease

Pathogenesis

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

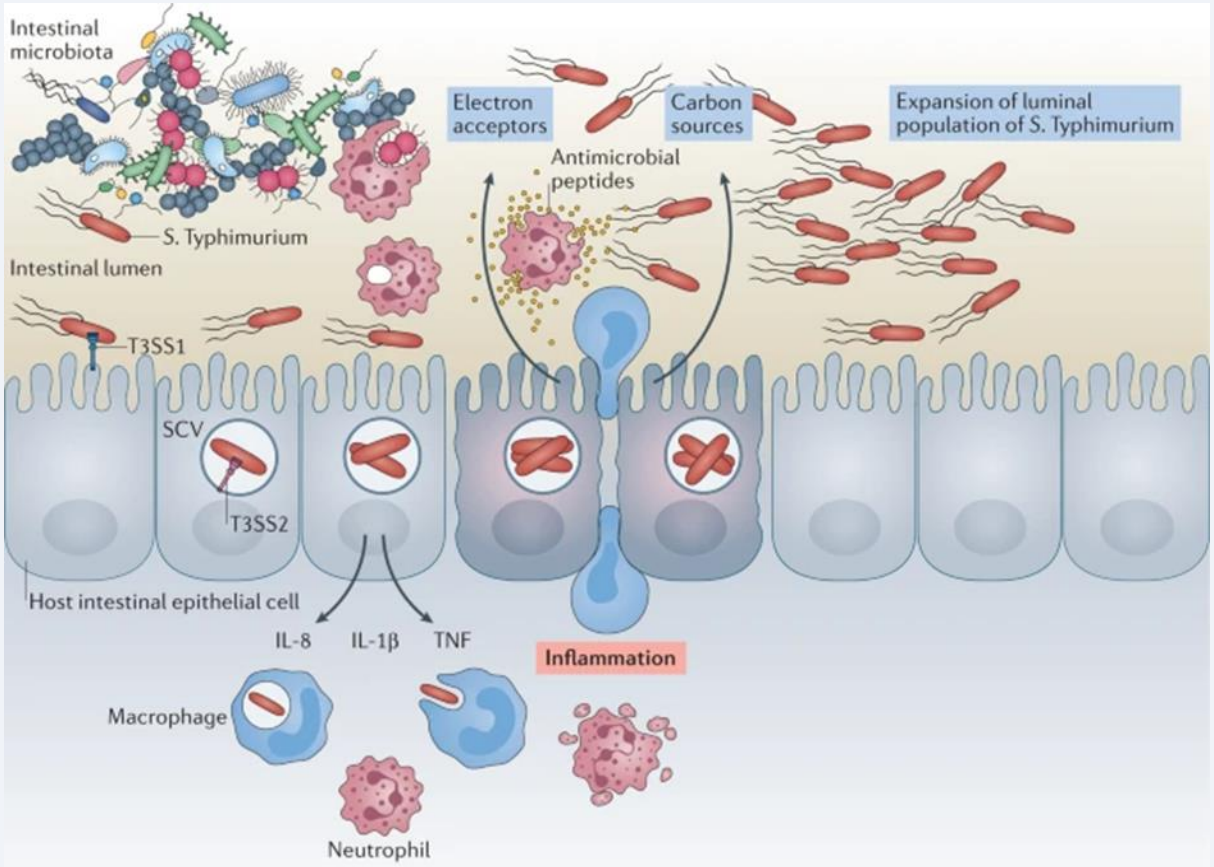
- Transposon inserts in the chromosome that encode virulence genes, but their expression is tightly regulated: Salmonella Pathogenicity Islands or spi.
- spi1 and spi 2 both encode type 3 secretion systems, but the triggers for their expression are different.

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



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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*. Typhimuium 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.

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.

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 I 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.

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

Clinical presentation	No. (%) 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)

GE= gastroenteritis

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

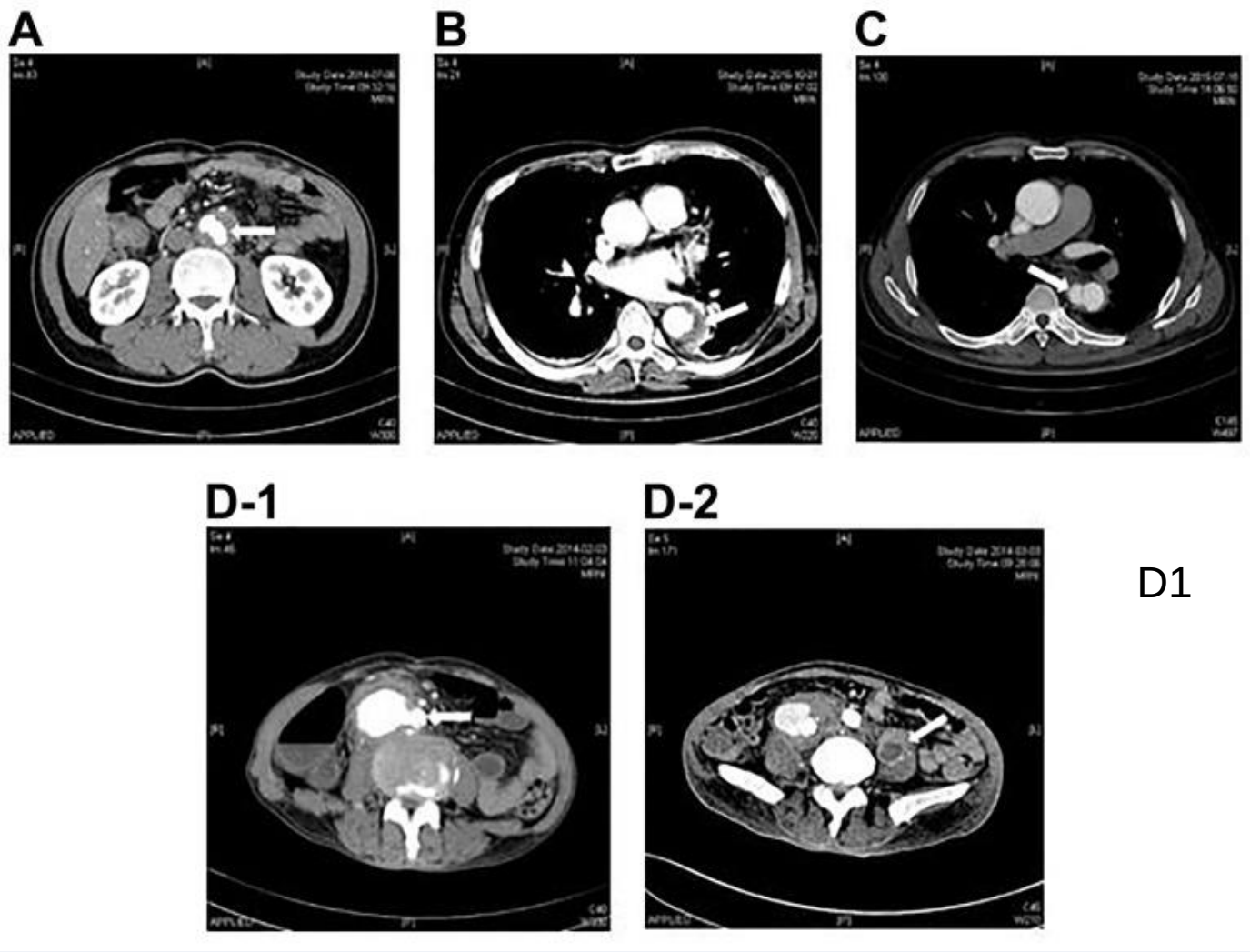
Neoplastic Disease	Total	Children	Adults	Adults >65
Leukemia	21	11	10	...
Acute lymphatic	6	4	2	...
Acute myelocytic	6	2	4	...
Acute stem cell	5	5	...	...
Chronic myelocytic	2	...	2	...
Leukolymphosarcoma	2	...	2	...
Lymphoma	25	...	21	4
Hodgkin's disease	9	...	7	2
Lymphosarcoma	7	...	7	...
Reticulum 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.
  - *Endocarditis* - rare but very destructive with high rate of abscess formation and valve destruction and ~ 40% mortality
  - *Aortitis* - infections of pre-existing atherosclerotic aneurysms or contiguous spread from adjacent infected vertebrae. Most commonly occur in infra-renal aorta.
    - Consider the diagnosis in everyone >65 y.o. or known to have a prior aneurysm.
    - Use imaging to look for it in anyone with Salmonella thoracic vertebral osteo

CT Scans of Four Aortic Aneurysms



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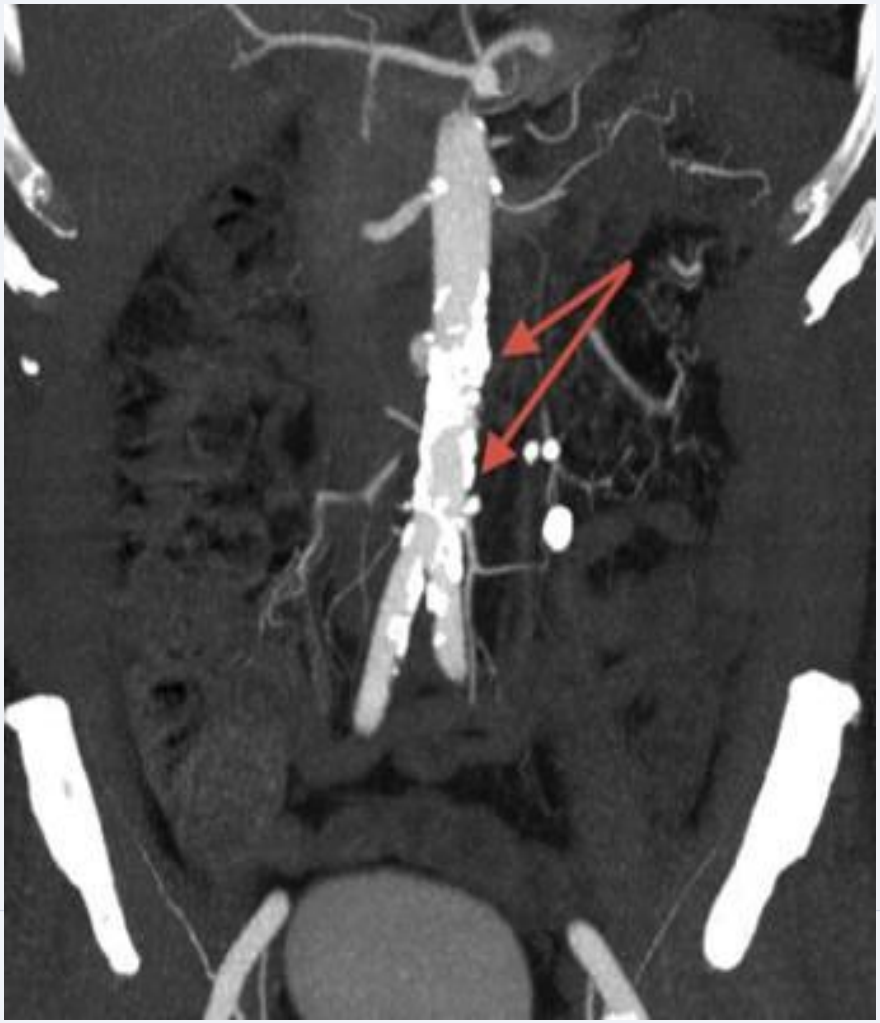
Recreation of an Infra-Renal Mycotic Aneurysm of Atherosclerotic Aorta



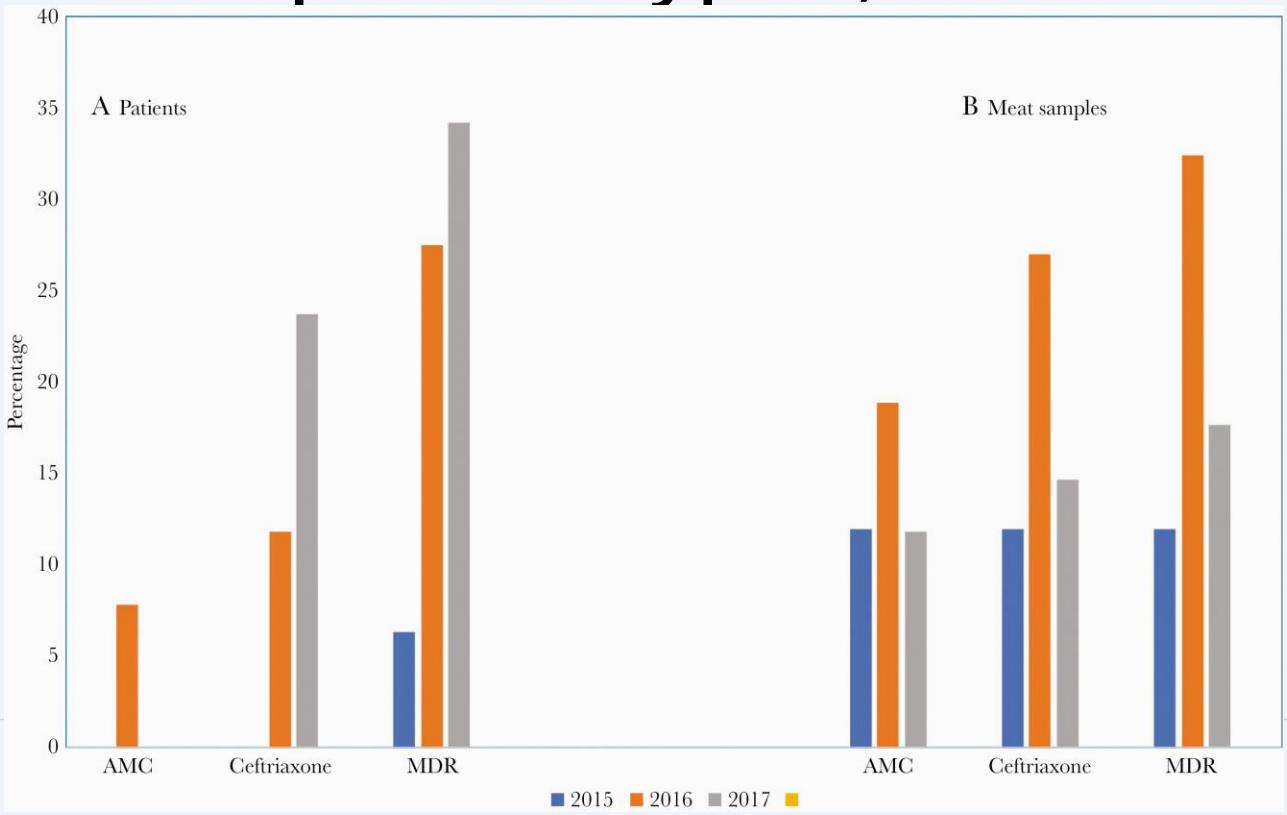
Complications of iNTS Bacteremia

- Osteomyelitis is always hematogenous. In adults it involves axial skeleton. In kids the epiphyses of long bones. In patients with sickle cell disease can also infect bone infarcts. 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.

Antimicrobial Resistance in NTS isolates from Stool Cultures and Meat in Pennsylvania With the Same Sequence Types, PA.

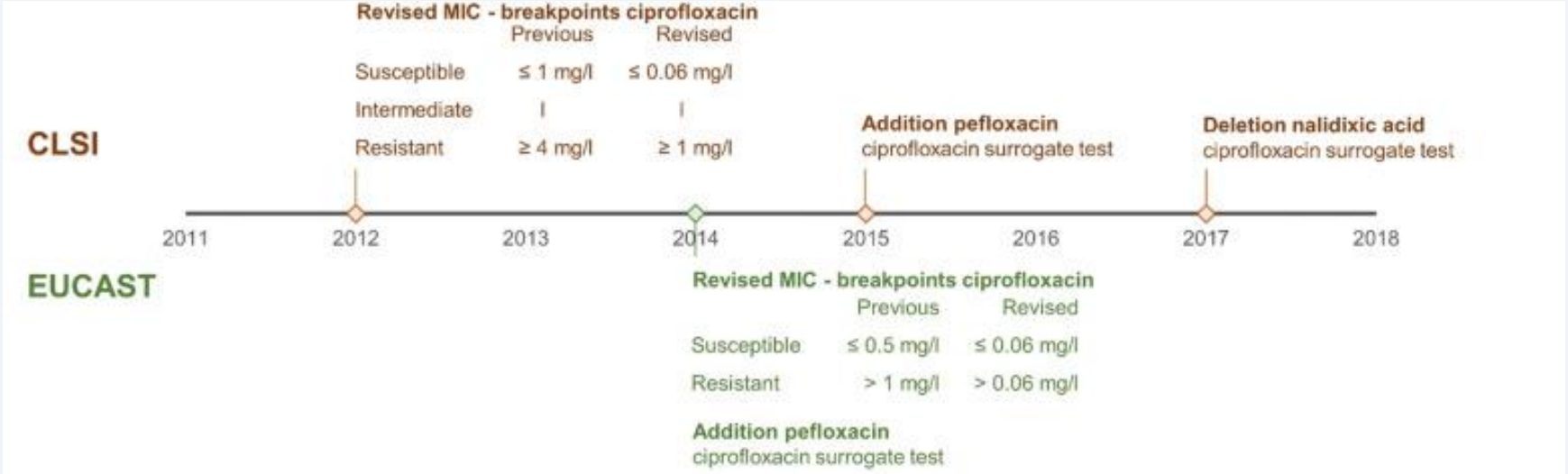


M'ikanatha MM et al. *Open Forum Infect Dis*, Volume 8, Issue 8, August 2021,

Potentially Useful Antibiotics to Treat iNTS

- ampicillin/amoxicillin, Augmentin
- co-trimoxazole
- chloramphenicol
- third generation cephalosporins (ceftriaxone)
- fluroquinolones (Cipro)

Timeline of Changes in Cipro MIC Interpretation

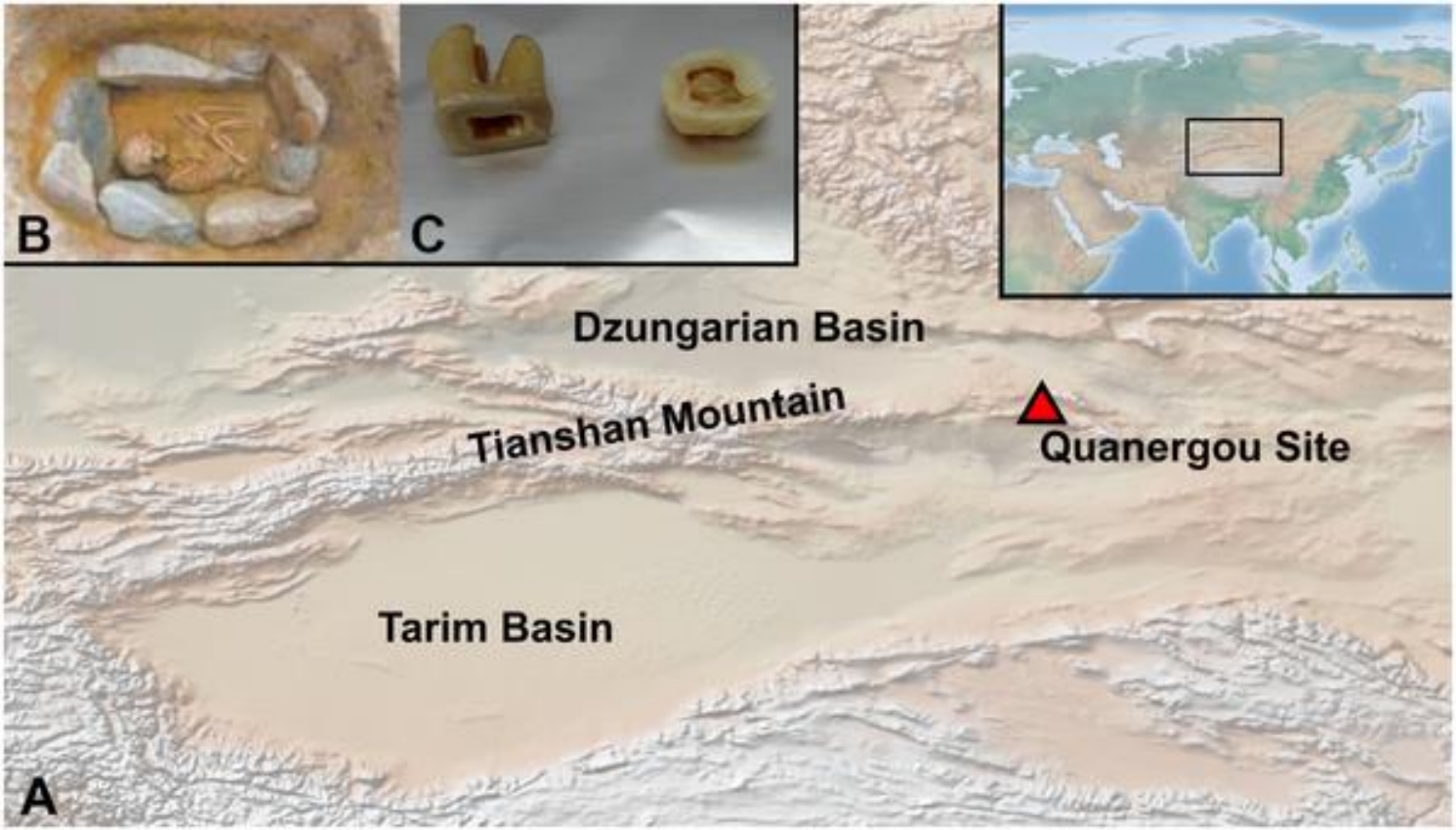


Antibiotic class and agent	AWaRe classification	Definitions
Ampicillin, amoxicillin TMP – SMX Chloramphenicol	Access Access Access	**Multidrug resistance (MDR):** co-resistance to ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol
Third generation cephalosporins: Ceftriaxone Cefotaxime	 Watch Watch	**Third generation cephalosporin resistance (C3G-resistance):** resistance to any third generation cephalosporin or presence of ESBL Extended spectrum beta lactamase producers (ESBL): testing no longer recommended since revised cephalosporin breakpoints in 2010
Fluoroquinolones: Ciprofloxacin	 Watch	**Fluoroquinolone non-susceptibility (FQNS):** presence of FQR, DCS, resistance to nalidixic acid or resistance to pefloxacin Ciprofloxacin: revised breakpoints since 2010: Fluoroquinolone resistance (FQR): MIC ≥1 mg/l Decreased ciprofloxacin resistance (DCS): MIC between >0.06 mg/l and <1 mg/l Nalidixic acid or pefloxacin resistance (disk diffusion) predict DCS and FQR
Azithromycin	Watch	No interpretative criteria yet

Summary

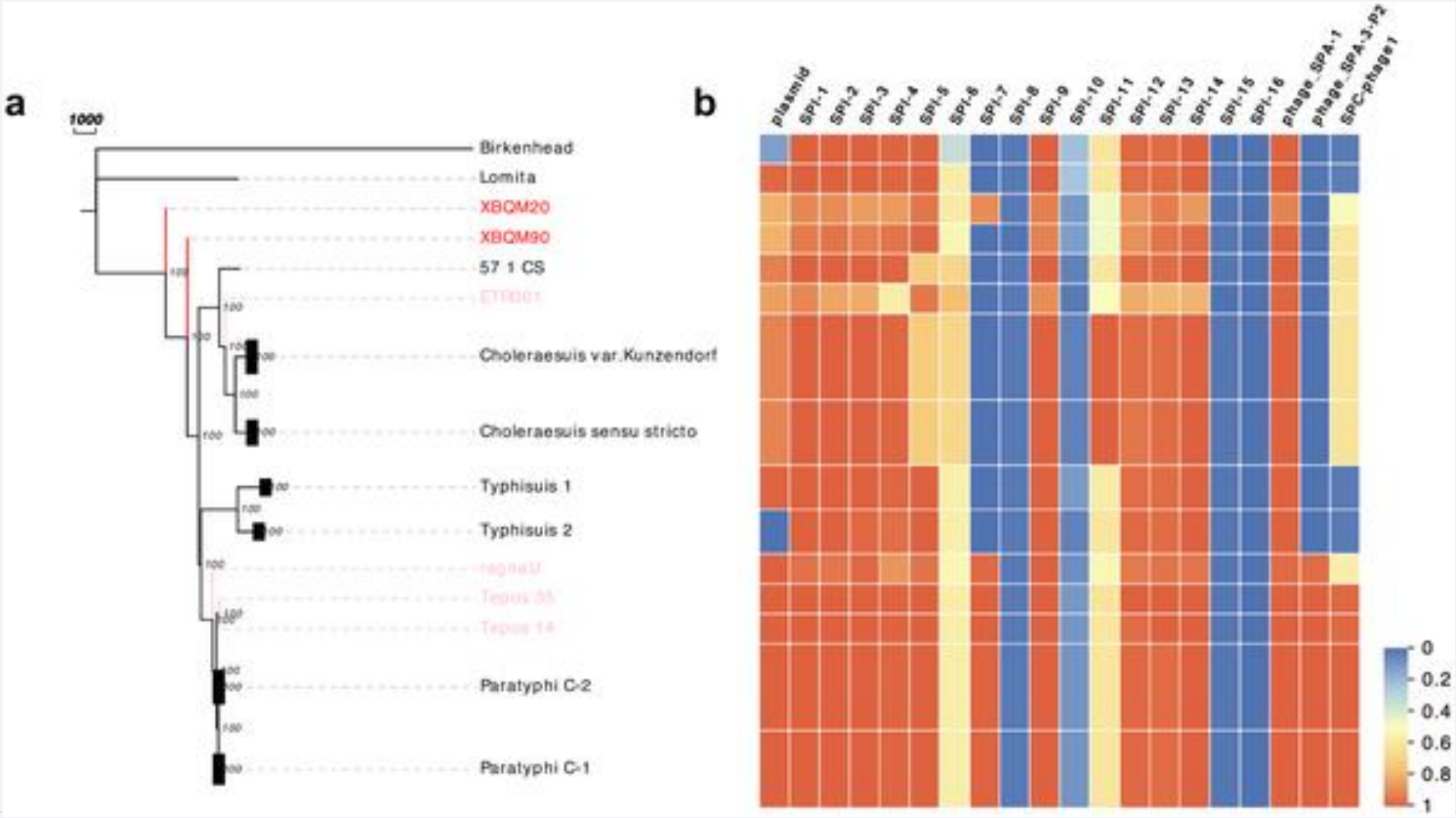
- iNTS are uncommon but potentially lethal.
- iNTS is characterized by bacteremia or focal extra-intestinal infection in absence of diarrhea.
- Antimicrobial therapy is required but resistance to our best drugs is increasing. This is a function of agricultural use of antibiotic in feed.
- Risk factors for iNTS infections are both a function of the *spv* plasmid in the infecting serovar and the susceptibility of the host.

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.



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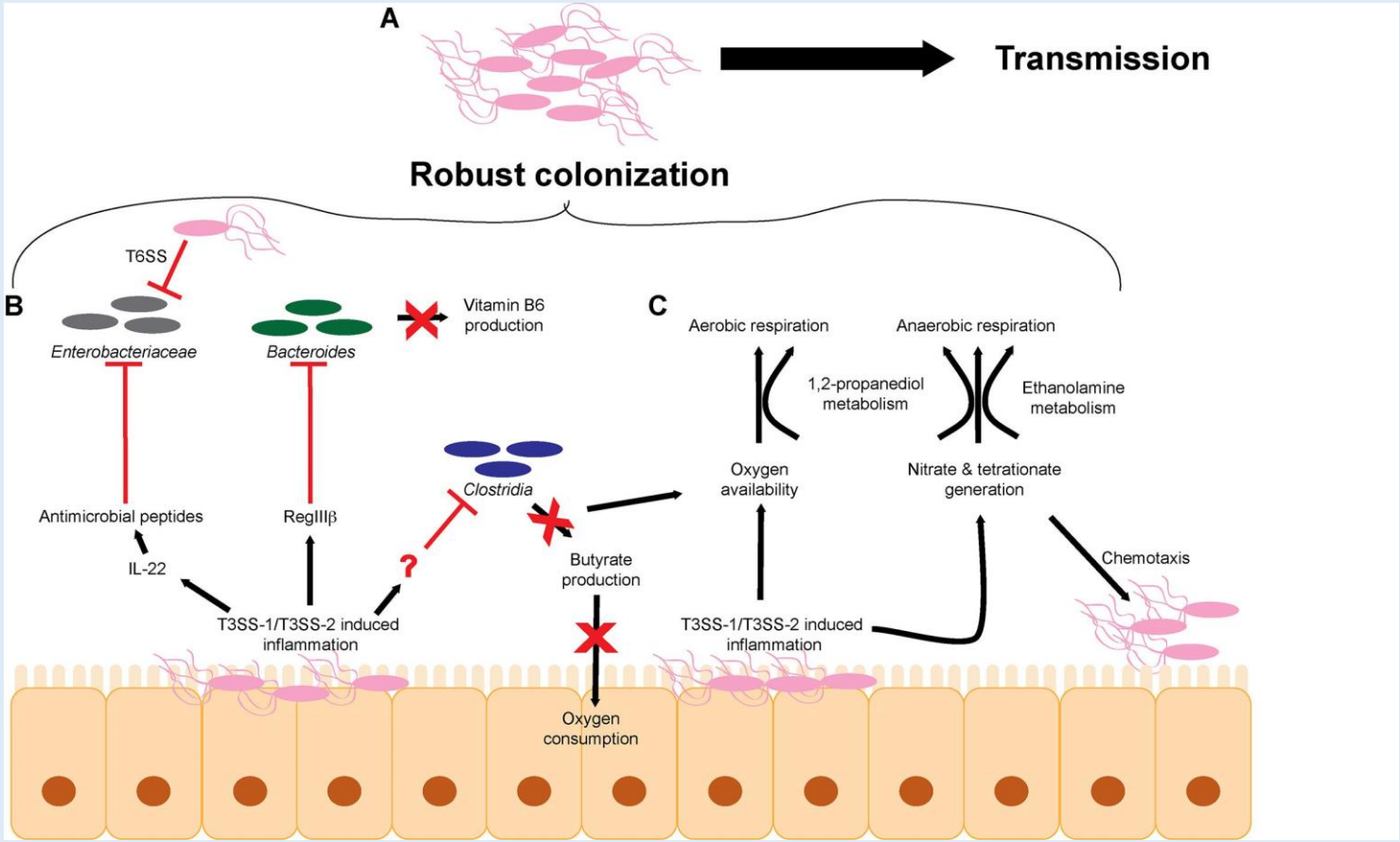
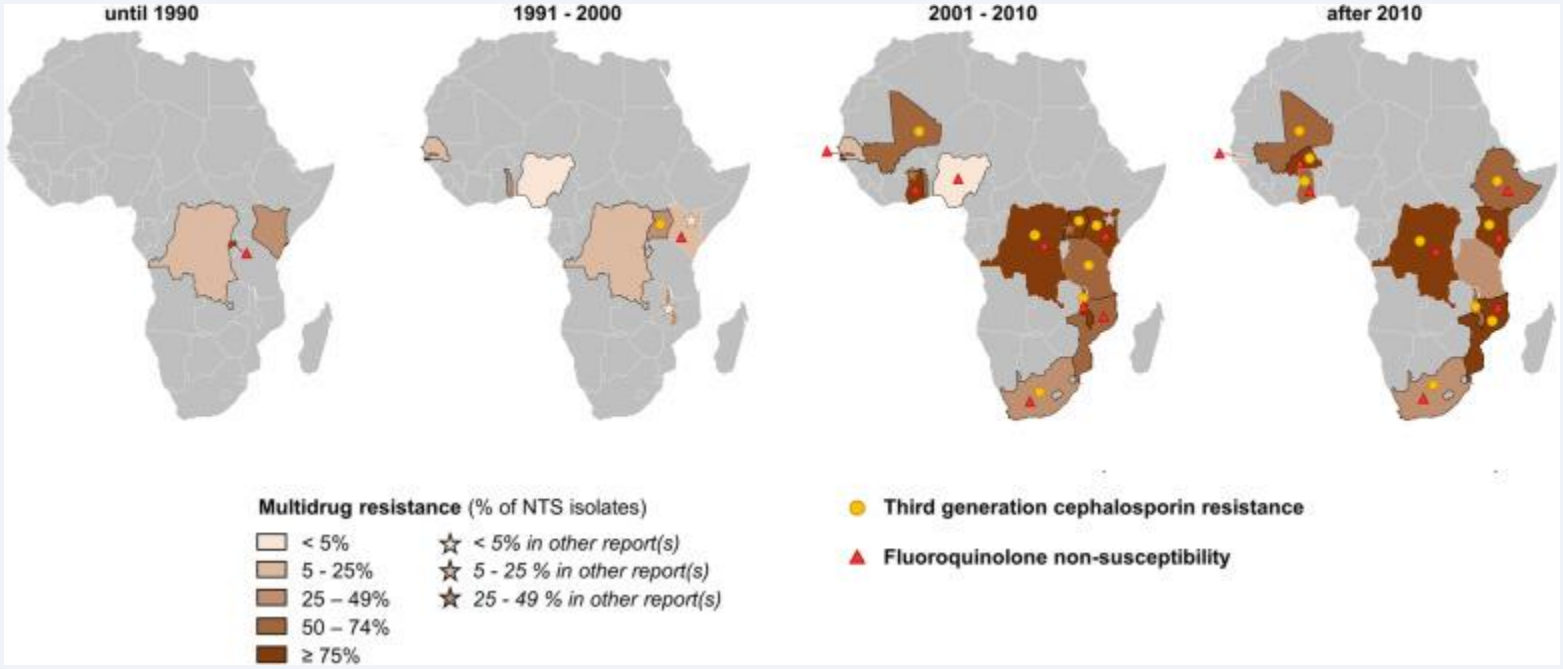
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1009886

How *S. enterica* Typhimurium Out-competes the Normal Gut Flora



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.