

A case-control investigation into the household distribution of invasive *Salmonellae* in Blantyre, Malawi

Malawi-Liverpool-Wellcome Trust
Clinical Research Programme

Melita Gordon
Reenesh Prakash

Reservoirs of iNTS - what is known already?



Uncertain functional host adaptation of ST313 and other iNTS serovars and clades

Kariuki et al, Kenya, J Med Micro 2006

127 index invasive disease iNTS

32/467 (family contacts carried Salmonella, 65% matched index by PFGE)

4 environmental isolates also similar, but very few animal isolates

Dione et al, Gambia PLOS NTD 2011

GEMS 14 NTS cases (8 diarrhoea, 6 healthy controls)

210 household animals (chicken, sheep goat)

21 animal NTS isolates (10%), MLST and serotyping

no sequence type overlap between human enteric and animal isolates

Nyirenda et al unpublished, Malawi 2014-15

60 healthy children aged 6-18 months sampled every month

~30% healthy children carried culture positive NTS short-term (higher by molecular)

Of these, **50% were Salmonella Typhimurium ST313**

International consensus meeting on Invasive Salmonella Disease



November 2014, MLW, Malawi



BILL & MELINDA
GATES *foundation*

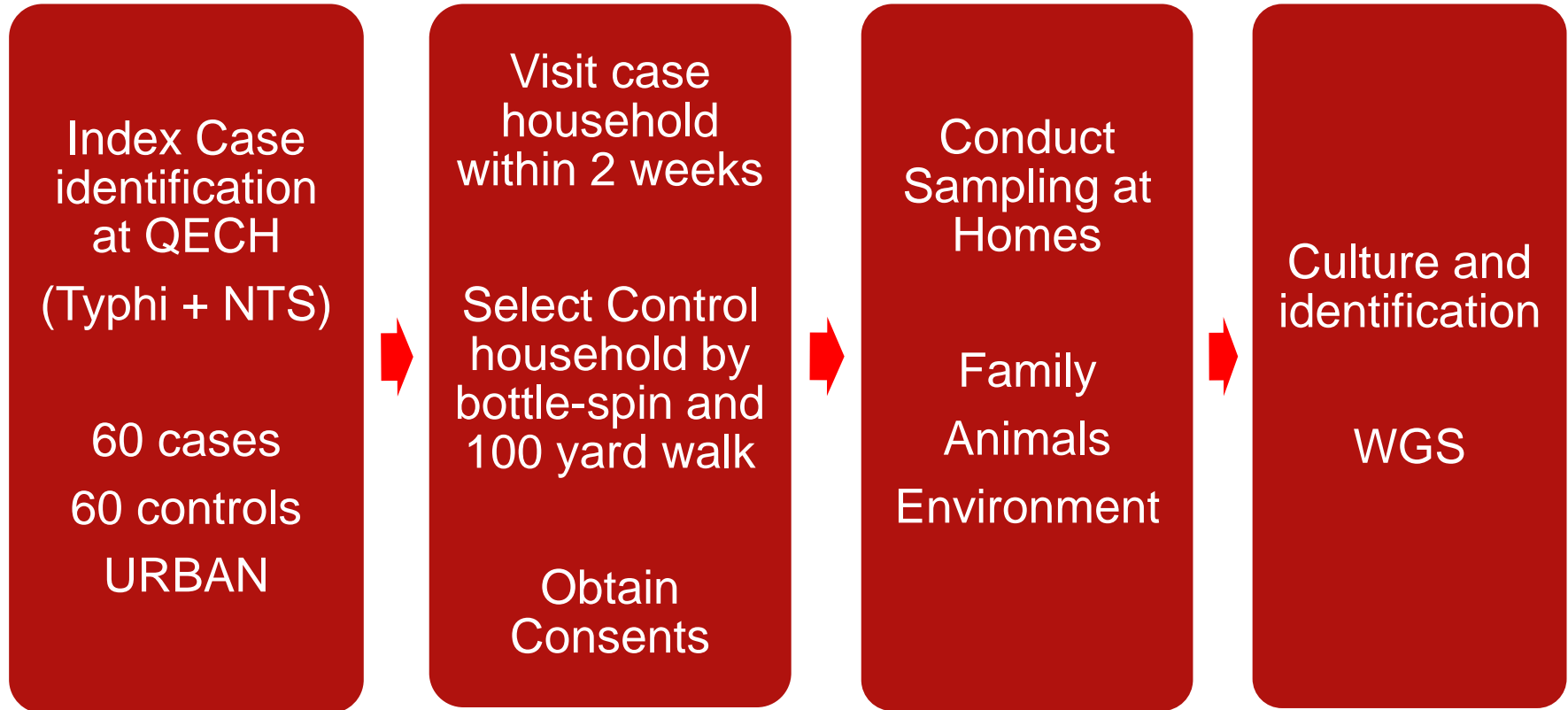
Case control study of reservoirs of invasive *Salmonella* disease



Objectives

- Identify household shedding and household environmental reservoirs of invasive *Salmonellae* within Blantyre
- Compare invasive to human, animal and environmental strains using whole genome sequencing
- information to inform control strategies for *Salmonella* infections in Africa

Recruitment and sampling



Household (HH)	iNTS Household		S.Typhi Household	
	Case n=27	Control n=27	Case n=33	Control n=33
Median (IQR) or %				
HH members	5 (5-7)	4 (3-6)	6 (4-7)	5 (4-6)
No. of rooms	4 (3-5)	3 (2-4)	3 (3-4)	4 (3-4)
HH keeps animals %	32	12	26	27
Has bathroom %	16	4	6	10
Walls unbaked mud %	4	20	13	23
Walls baked mud %	12	24	23	7
Walls plastered %	84	56	65	70
Tin roof %	100	96	97	97

Socioeconomic and sanitation	iNTS Household		S.Typhi Household	
	Case n=25	Control n=25	Case n=31	Control n=31
Socioeconomic:				
Electricity supply %	12	8	0	3
HOH can read & write %	100	80	90	90
Sanitation:				
Pit latrine no slab %	40	48	38	37
Pit latrine with slab %	52	48	58	60
Flush toilet %	8	4	3	3
No. sharing toilet	9 (7-18)	9 (5-13)	12 (8-16)	9 (6-14)
Soap Available %	56	48	61	73

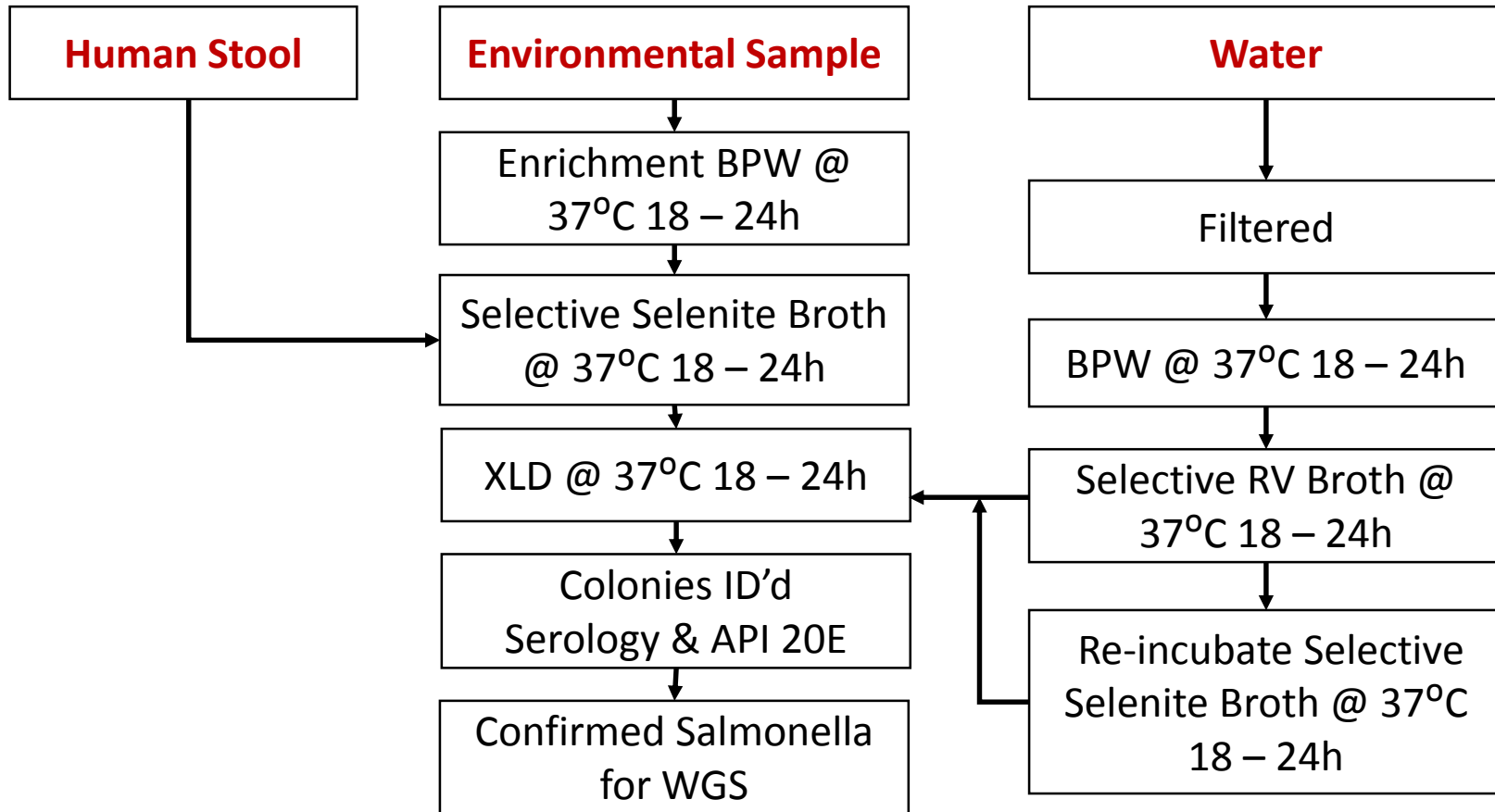
Drinking water	iNTS Household		S.Typhi Household	
	Case n=25	Control n=25	Case n=31	Control n=31
Piped into house %	8	0	3	1
Piped into plot %	28	24	19	17
Public tap or standpipe %	48	68	65	60
Public bore hole %	16	8	6	10
Other %	0	0	6	10
Water Treatment used %	16	16	16	20

Sampling





- **Stool** (Human, adults and children)
- **Animal stool/rectal swabs** (chickens, pigs, cows, goats, sheep, cats, dogs, gecko)
- **Sterile boot sox** - perimeter, latrine, rubbish, bedroom, cooking areas
- **Water** (Stored, puddles, vessels)
- **Food** (left over)





Total samples 1510	Case Total Samples	No. of Salmonella Isolated	% Positive	Control Total Samples	No. of Salmonella Isolated	% Positive
Human Stool	273	11	4	282	4	4
Animal Rectal Swab	17	0	0	9	1	0
Animal Stool	32	2	6	34	1	6
Bootsocks	305	6	2	297	24	8
Food	67	0	0	52	0	0
Water	108	0	0	98	0	0
Total	802	19	2.4	708	30	4.2



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S Typhi : households and controls (34 + 34)



	Case Total Samples	No. of Salmonella Isolated	% Positive	Control Total Samples	No. of Salmonella Isolated	% Positive
Human Stool	157	2	1.3	121	4	3.3
Animal Rectal Swab	8	0	0	6	1	16.7
Animal Stool	14	2	14	17	1	6
Boots socks	168	5	3	166	17	10
Total	442	9	2	394	23	5.8

iNTS : households and controls (26 + 26)



	Case Total Samples	No. of Salmonella Isolated	% Positive	Control Total Samples	No. of Salmonella Isolated	% Positive
Human Stool	116	9	8	97	0	0
Animal Rectal Swab	9	0	0	31	0	0
Animal Stool	18	0	0	7	0	0
Bootsocks	137	1	1	131	7	5
Food	26	0	0	24	0	0
Water	54	0	0	42	0	0
Total	360	10	2.8	314	7	2.2

Summary of isolation rates



- ❖ Multiple serovars isolated across the physical and living household environment

3% overall isolation by culture

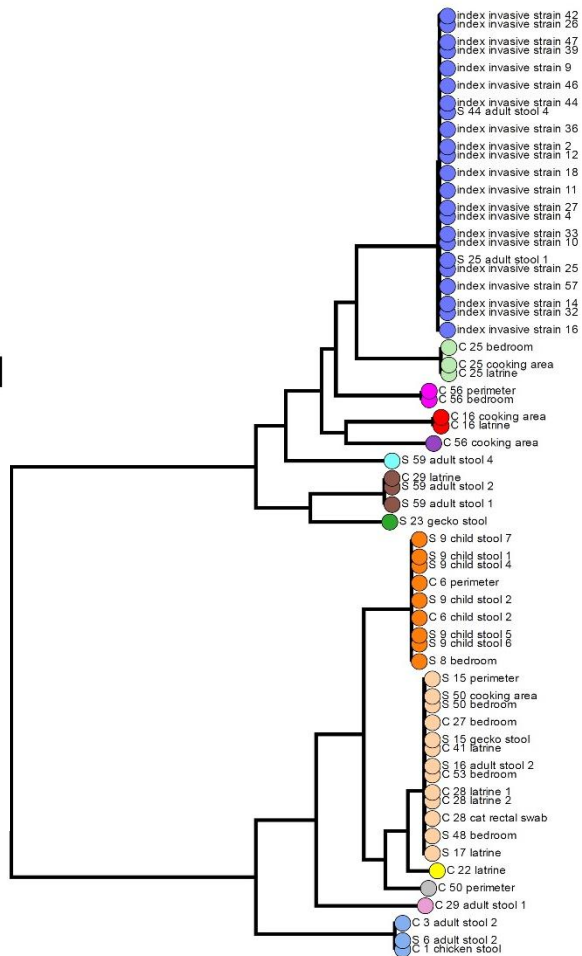
Isolation 4% human, 6% animals, 5% soil

- ❖ No isolates from food or water – sampling / methodological



Predicted serovar

- Aberdeen
- Agoueve|Cubana
- Amager
- Djama
- Gaminara
- Hadar
- Havana|II 1,13,22:g,t:[1,5]
- II 40:b:-
- II 42:r:-|IIIb 42:r:-:[z50]
- Mgulani
- Montevideo
- Ogbete|II 43:z:1,5
- Senftenberg
- Typhimurium



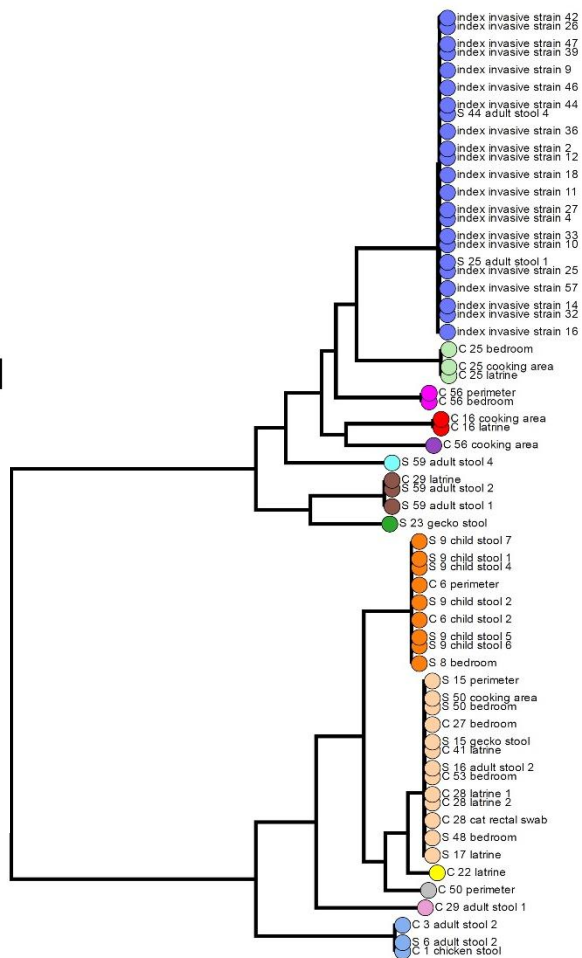
Illumina sequencing
Enterobase phylogenetic trees

(S. Typhi blood stream
isolates not included)



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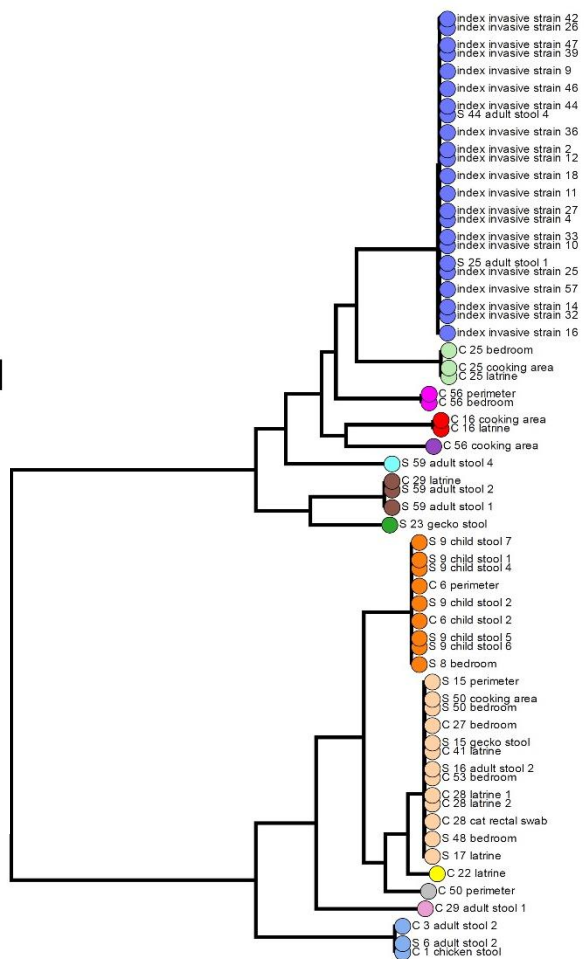


**Invasive NTS cases
S. Typhimurium ST313**



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**Invasive NTS cases
S. Typhimurium ST313**

Bootsox house areas (controls)

Family members stool

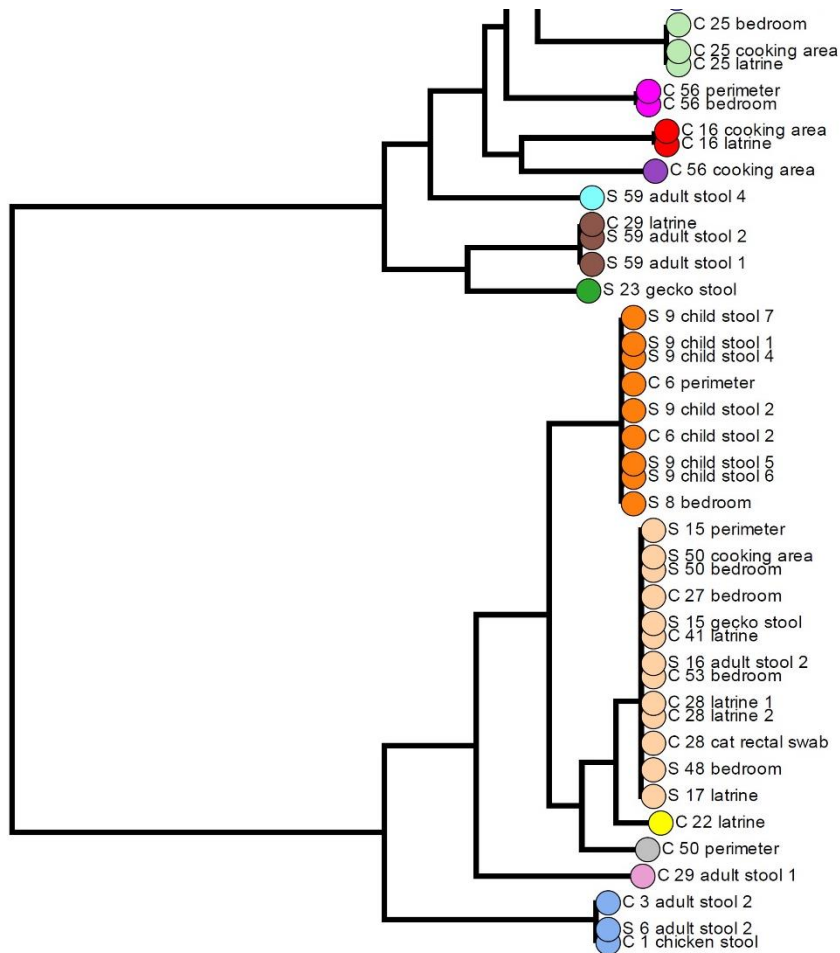
Bootsox house areas (cases & controls)

Family members stool



Predicted serovar

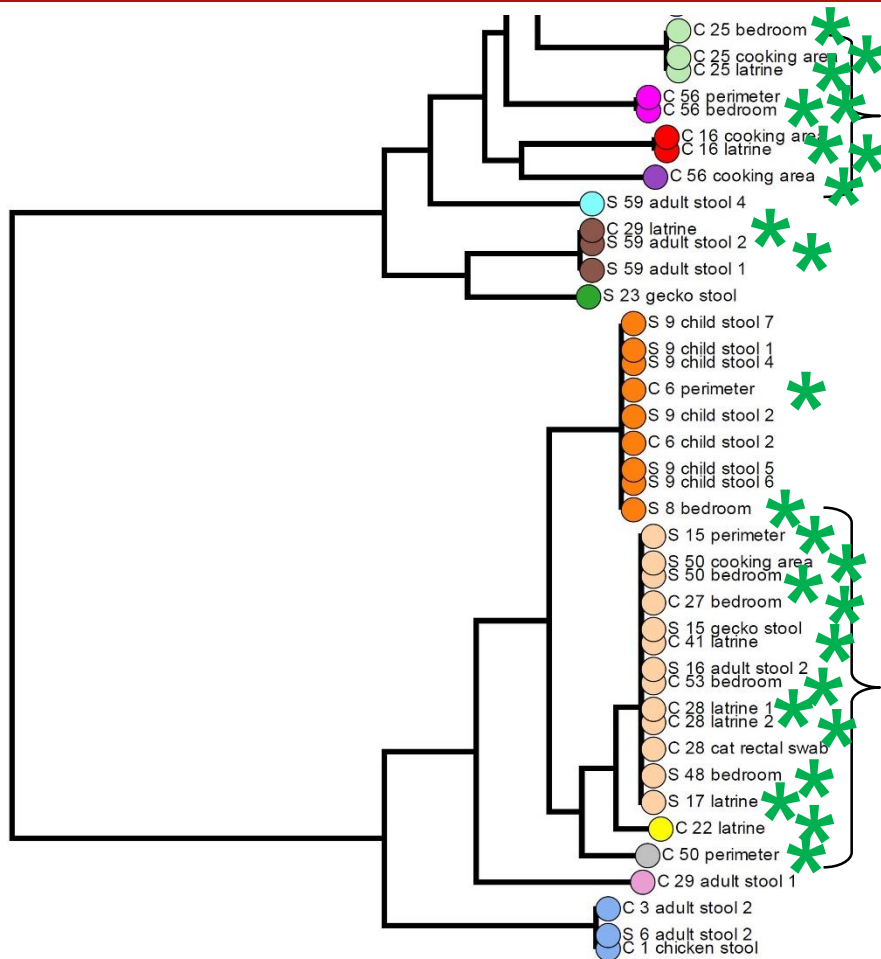
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* Boot sox samples



Bootsox house areas (controls)

latrine, cooking area, bedroom, perimeter, rubbish tip

Bootsox house areas (cases & controls)

latrine, cooking area, bedroom, perimeter, rubbish tip

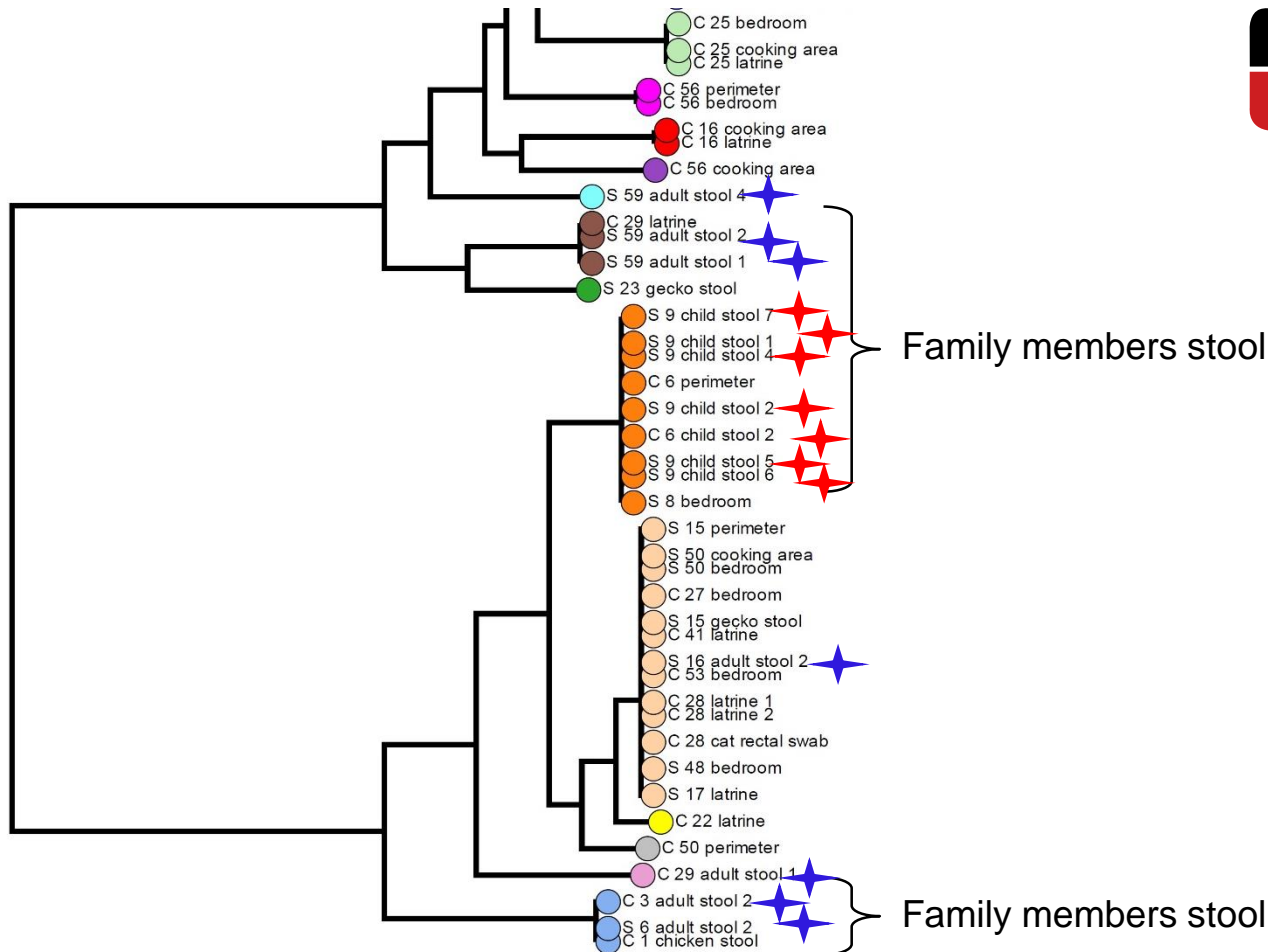




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- Adult stool
- Child stool

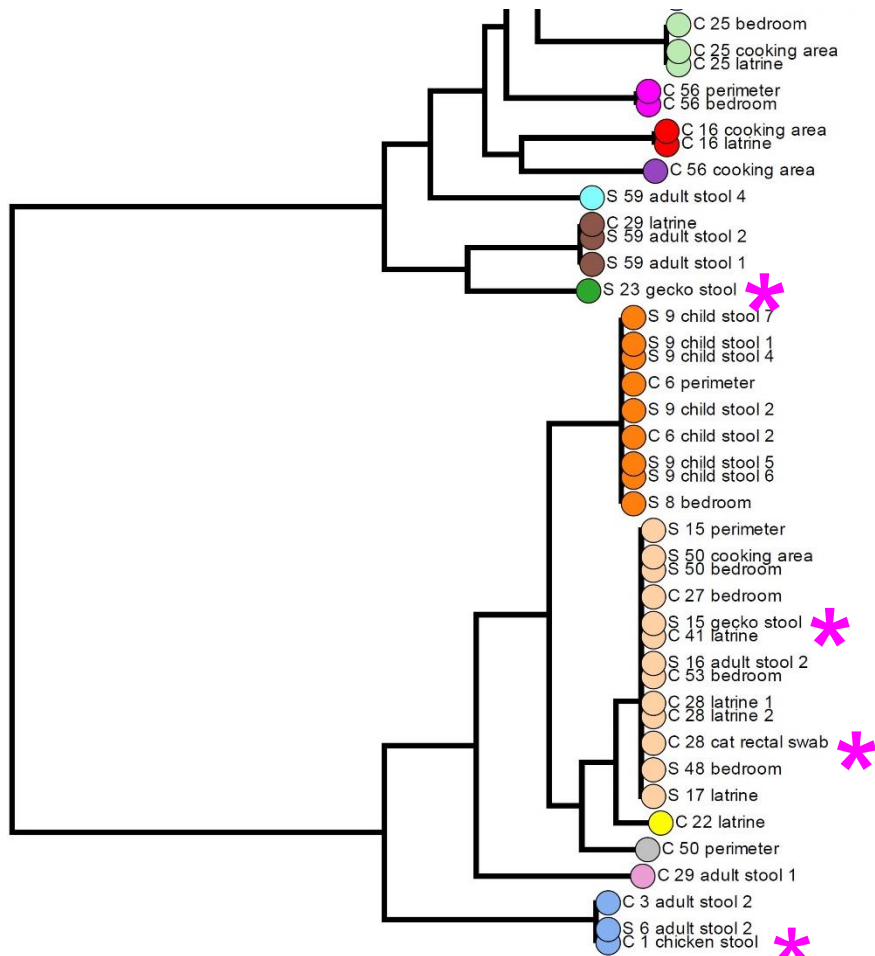




Predicted serovar

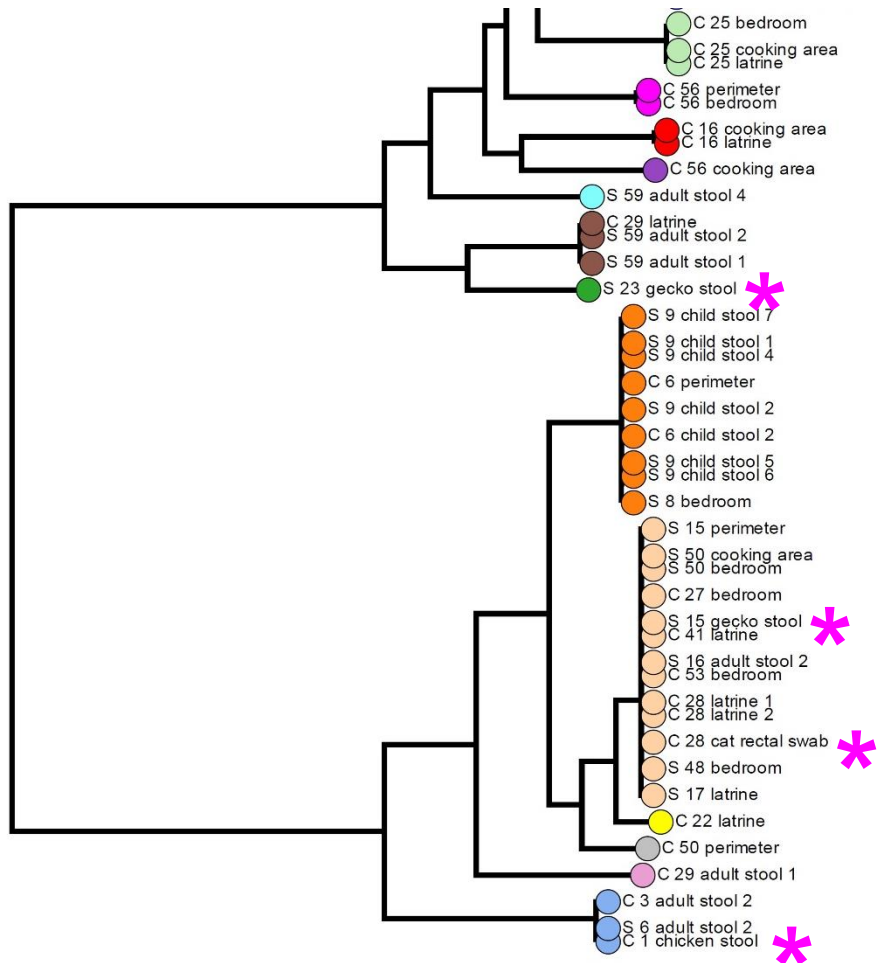
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* Animal isolate



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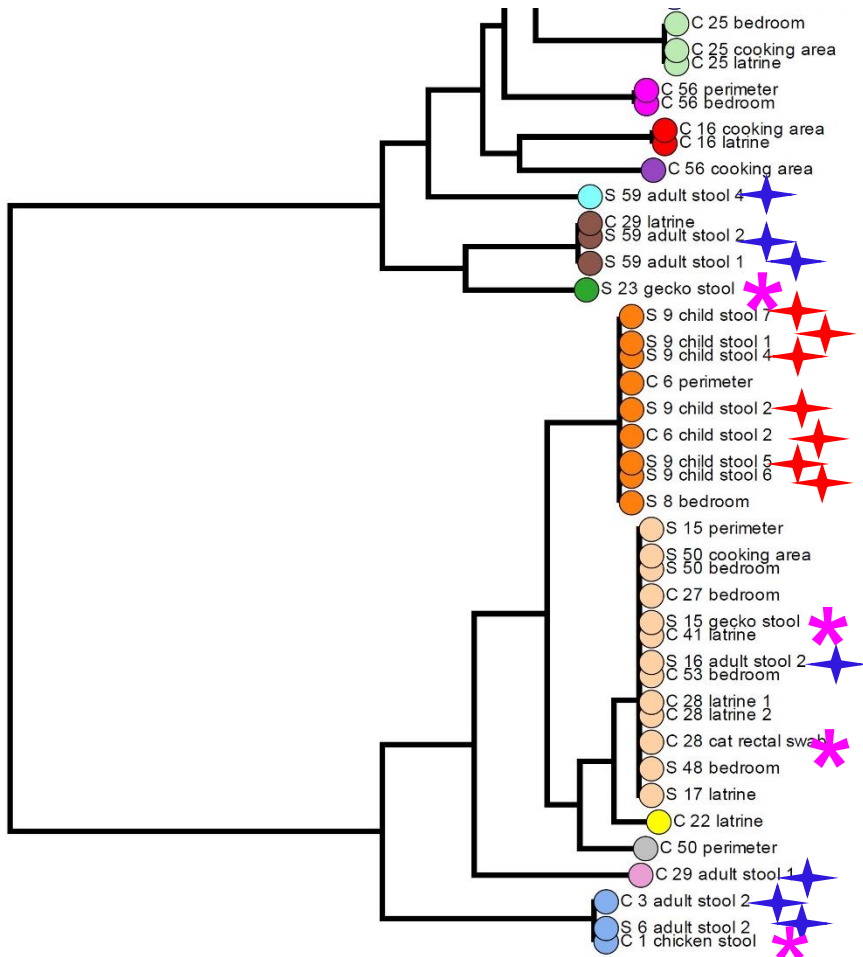


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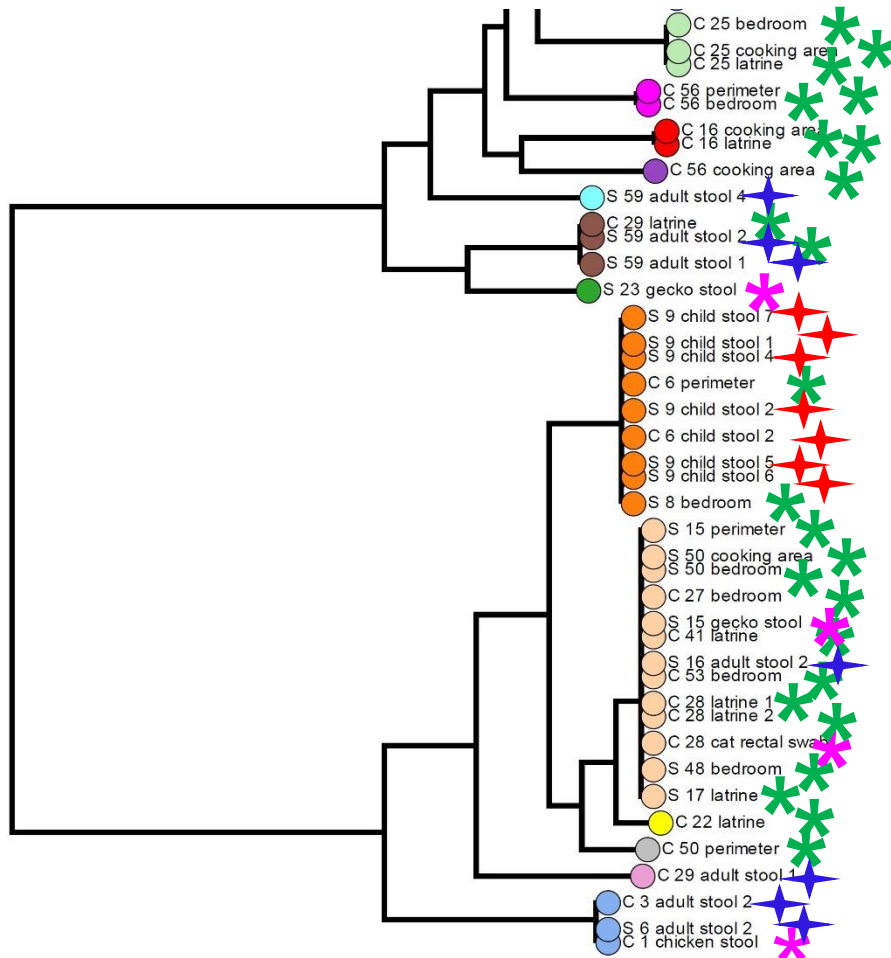


Mixing of human and animal carried serovars

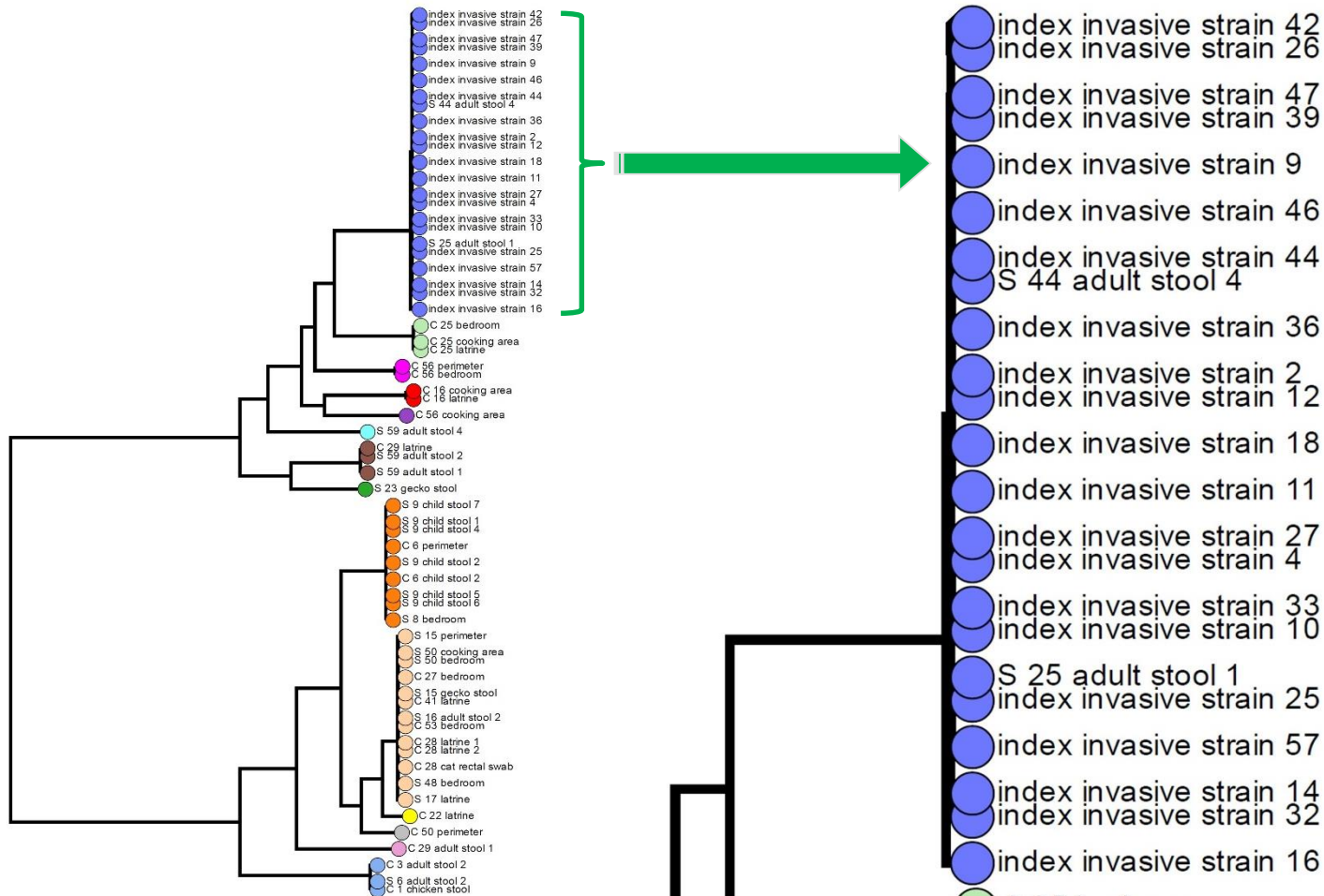
- Adult stool
- Child stool
- Animal isolate

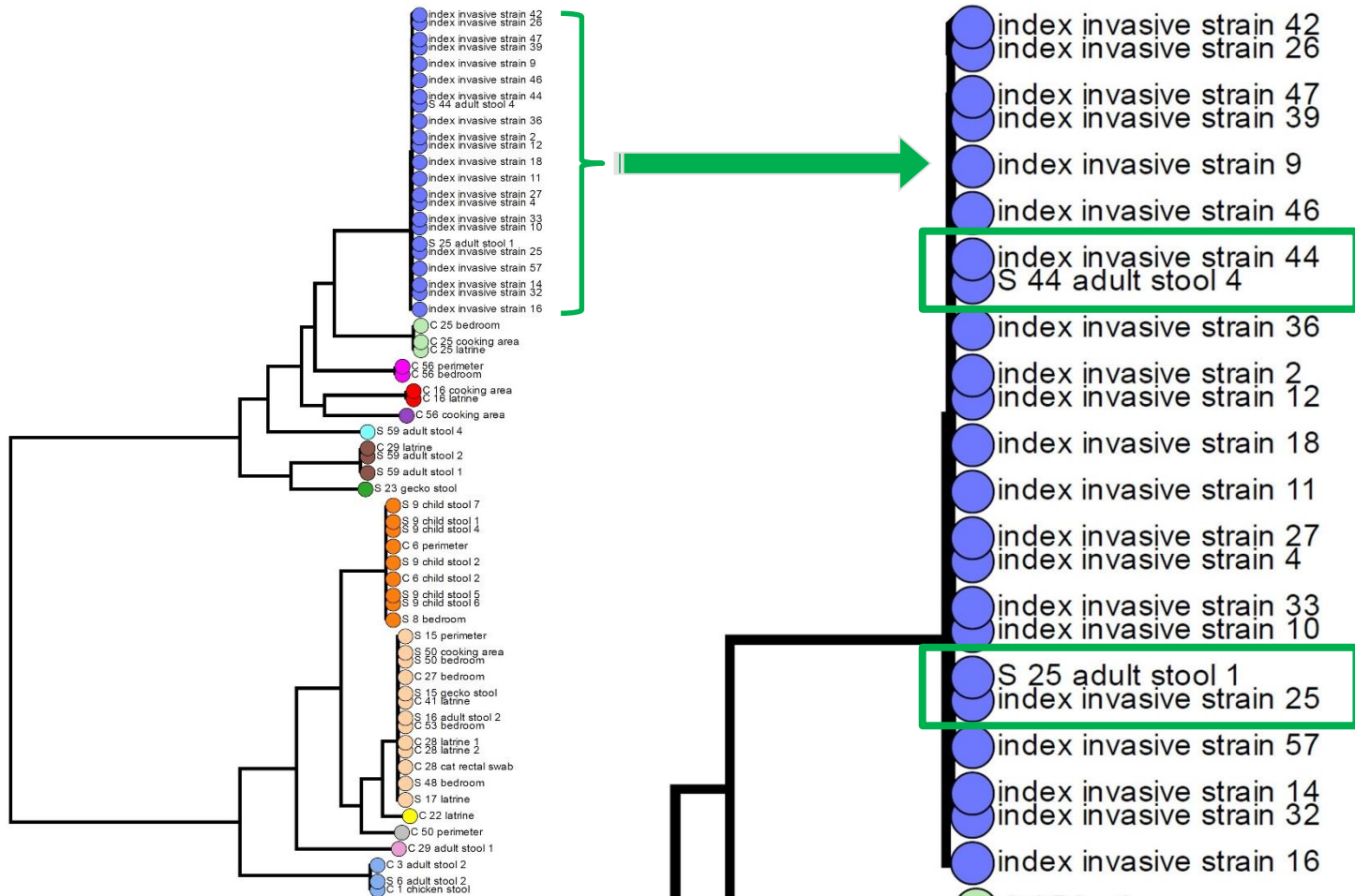
Predicted serovar

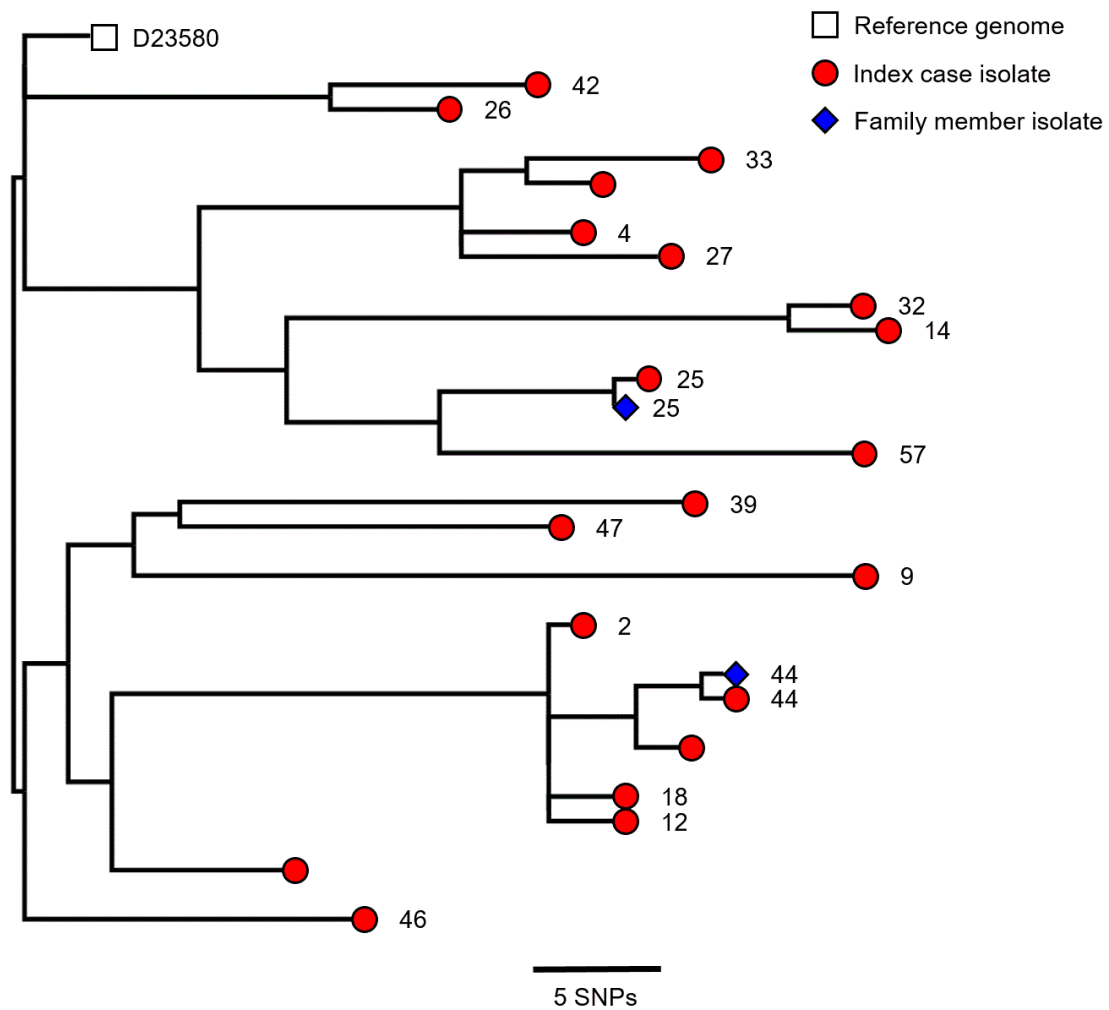
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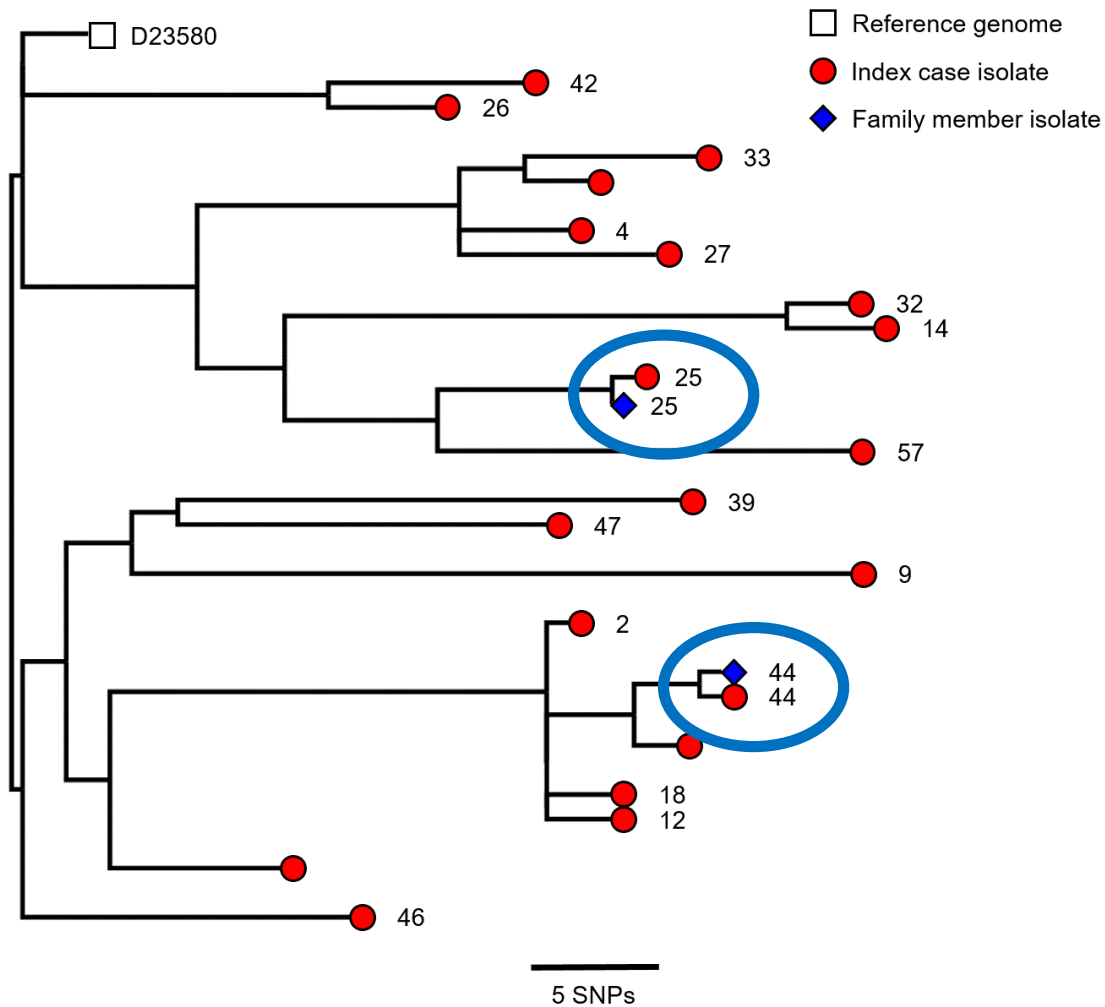


- Adult stool
- Child stool
- Animal isolate
- Boot sox isolates









Summary & conclusions



- ❖ iNTS and Typhoid case-control study – human, animal and environmental, urban slum setting
- ❖ Multiple serovars isolated across the physical and living household environment
 - 3% overall isolation by culture
 - Isolation rate 4% human, 6% animals, 5% soil
 - No isolates from food or water – sampling and methodological issues
- ❖ Clear overlap between asymptomatic human and animal serovars
- ❖ A phylogenetic ST313 match found only with family members (1 adult, one child)
- ❖ **Supports (but does not prove) mainly human reservoir or iNTS**
- ❖ **Supports (but does not prove) human to human transmission of iNTS**

Reenesh Prakash





Thank you

MALAWI

Reenesh Prakash
Leonard Koolman
Franziska Olgemoeller
Rose Nyirenda
Brigitte Denis

LIVERPOOL

Chisomo Msefula
Nick Feasey
Paul Wigley
Jay Hinton
Sian Jones

Chisomo Msefula
Robert Heyderman

BILL & MELINDA
GATES *foundation*

