

Pan genome clustering reveals the role of prophage elements in the evolution and adaptation of *Salmonella* Enteritidis

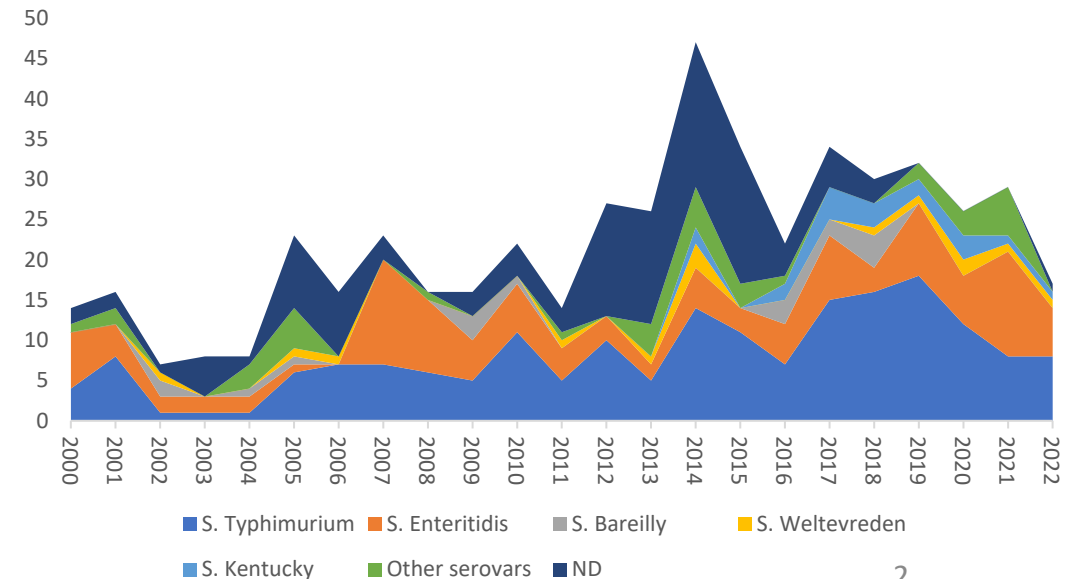


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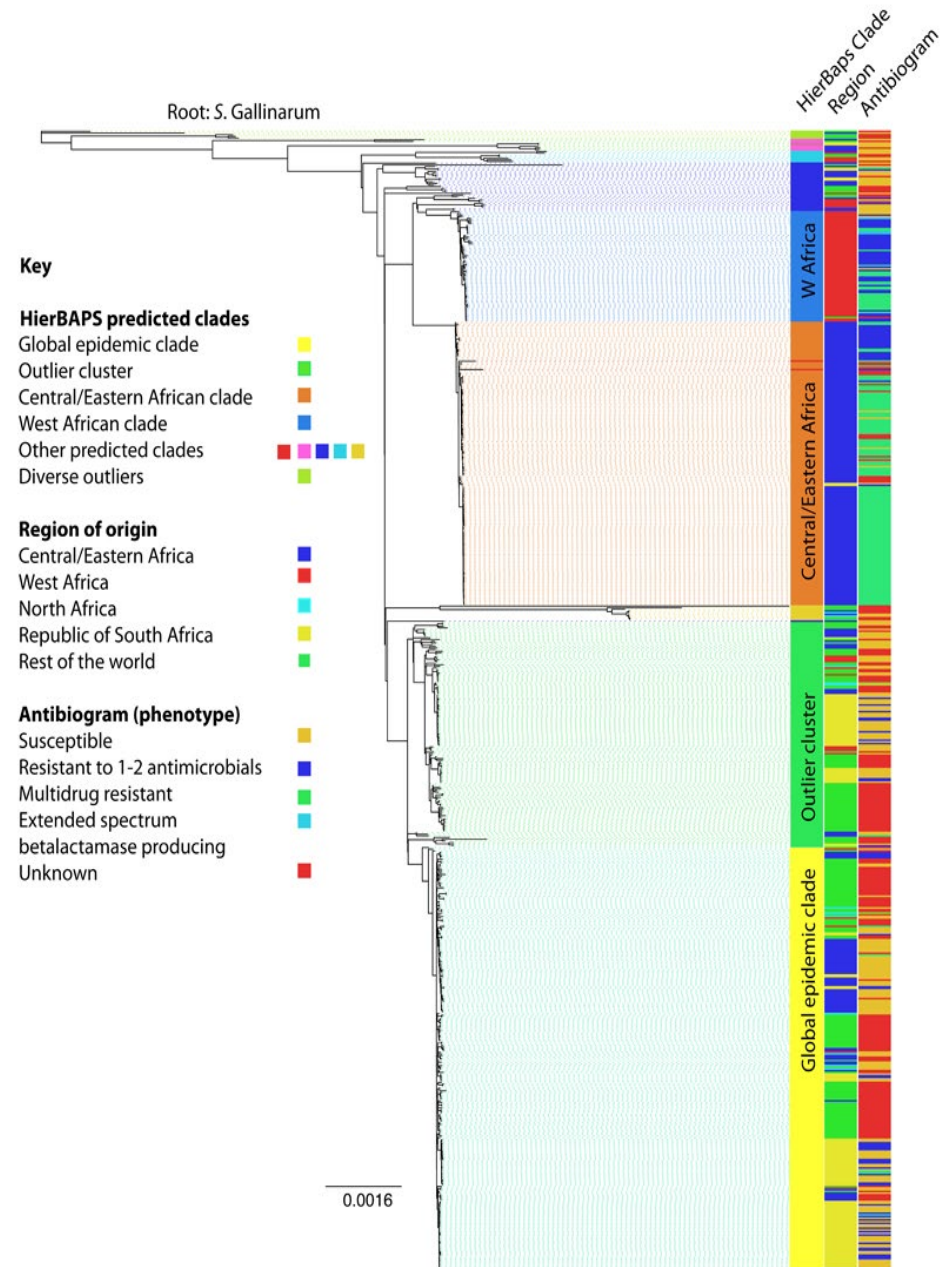
Background

- ❖ S. Enteritidis is known to cause iNTS diseases in sub-Saharan African countries
- ❖ As of 2017, 79% of the 535,000 cases and 85% of the 77,500 deaths have been reported in sub-Saharan Africa (Stanaway et al., 2019)
- ❖ In South Asian countries, the prevalence of iNTS has been largely underestimated
- ❖ At our center, S. Enteritidis is the 2nd most common iNTS (30%) after S. Typhimurium (49%)
- ❖ The genomic insights, human adaptation and clinical features of this serovar are currently not known

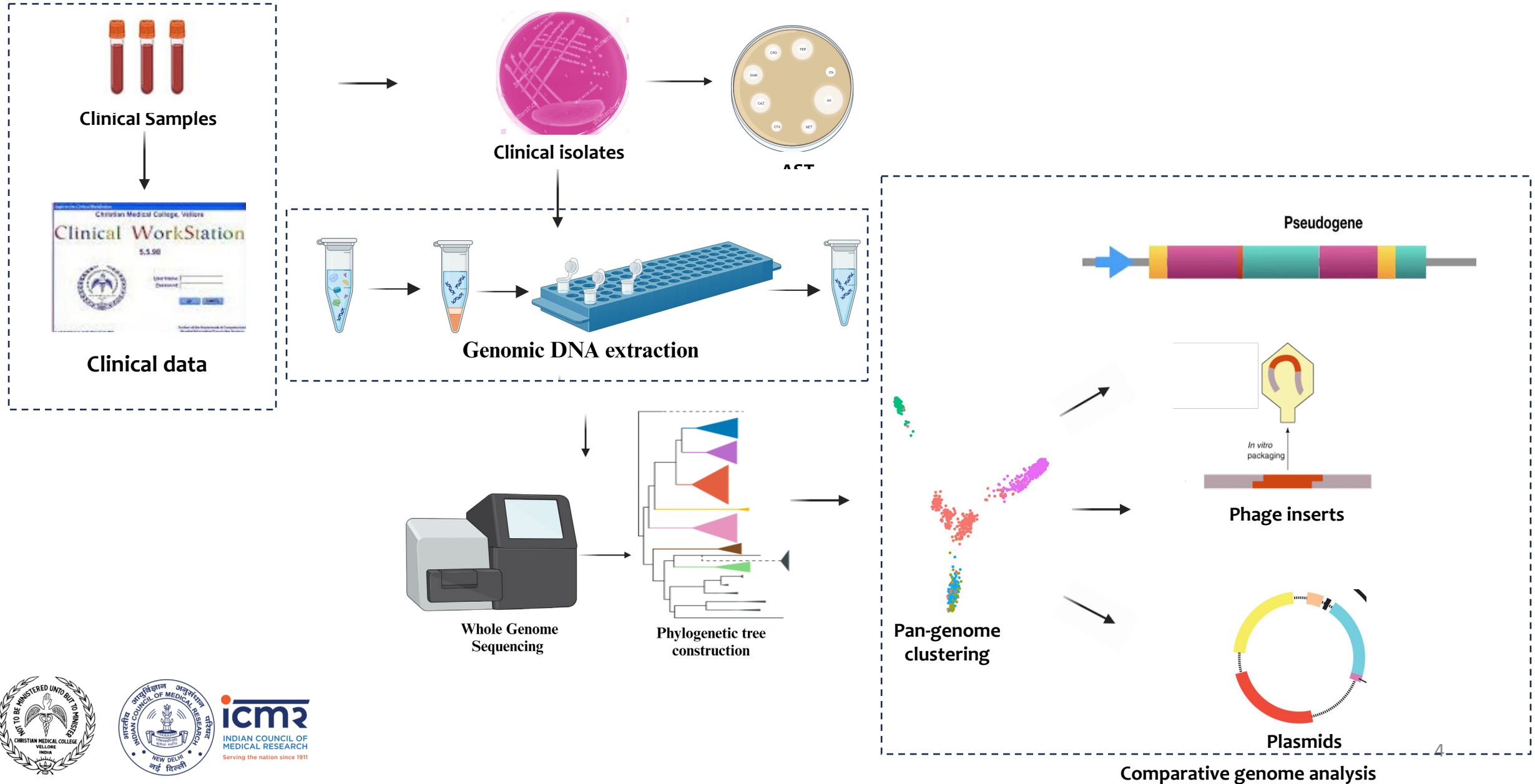


Genomic insights : What we know so far!

- ✓ The dominant clone **ST11** carried diverse strains of *S. Enteritidis*
- ✓ Seven-gene MLST classification not robust
- ✓ Population Structure
 - ❖ East African clade
 - ❖ West African clade
 - ❖ Global Outlier/Atlantic
 - ❖ Global Epidemic
- ✓ AMR burden mostly on both the African clades
- ✓ Characteristic phages in different clades
- ✓ Variants of pSEN plasmids



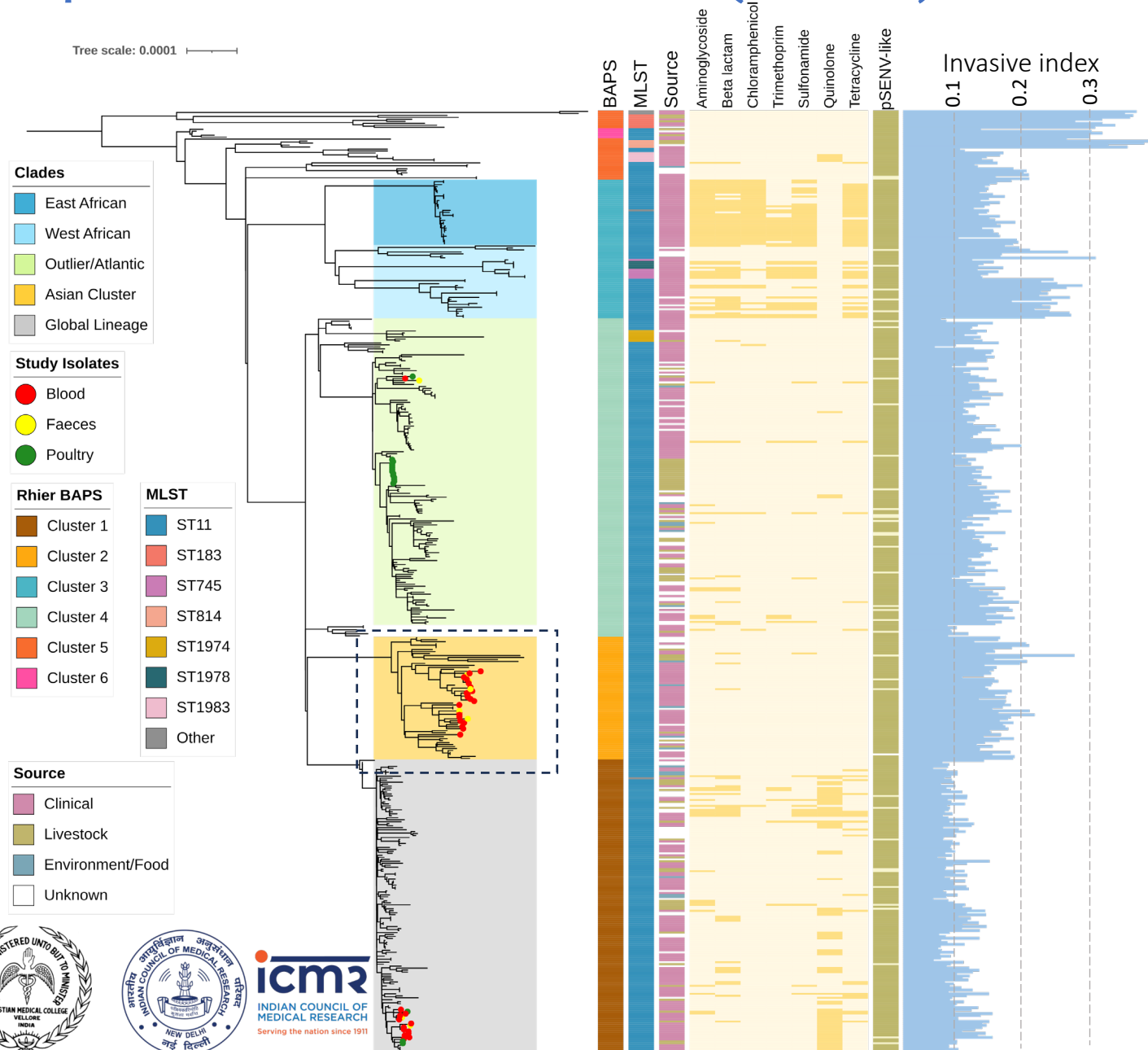
Methods



Clinical data

Variable	Overall n (%)/median (IQR) (n = 101)	Immunosuppressive conditions n (%)/median (IQR)		p-value	OR	95% CI
		Yes (n=58)	No (n=43)			
Sex (Male)	66 (65.34)	36 (62.1)	30 (69.76)			
Age	34.5 (9 – 54.5)	36 (25 – 50)	24.5 (0 – 55.75)			
Pediatric (<16 years)	28 (27.72)	9 (15.52)	19 (44.18)			
Duration of fever	6 (3 – 10)	7 (4 - 11)	5 (1 – 8)			
Co-infection	18 (17.82)	11 (18.96)	7 (16.28)	0.797	1.2	0.18 ± 0.0741
Antibiotic combination therapy	66 (65.34)	38 (65.52)	28 (65.12)	1	1.02	0.65 ± 0.0936
Abdominal pain	27 (26.73)	16 (27.58)	11 (25.58)	1	1.11	0.27 ± 0.0858
Ascites	12 (11.88)	9 (15.51)	2 (4.65)	0.066	4.27	0.12 ± 0.0644
Cough	31 (30.69)	18 (31.03)	13 (30.23)	1	1.04	0.31 ± 0.0897
Diarrhoea	36 (35.64)	19 (32.76)	17 (39.53)	0.532	0.75	0.36 ± 0.0936
Fever	76 (75.25)	46 (79.31)	30 (69.77)	0.351	1.66	0.75 ± 0.0839
Pneumonia	22 (21.78)	14 (24.14)	8 (18.6)	0.627	1.39	0.22 ± 0.08
Septic Shock	20 (19.8)	16 (27.59)	4 (9.3)	0.025	3.71	0.2 ± 0.078
Unhealthy/ Anaemia	60 (59.41)	38 (65.52)	22 (51.16)	0.158	1.81	0.59 ± 0.0956
Recovery	84 (83.17)	47 (81.03)	36 (83.72)			
Death	11 (10.89)	8 (13.79)	3 (6.98)	0.345	2.13	0.11 ± 0.0605

Population structure of SE (n=476)



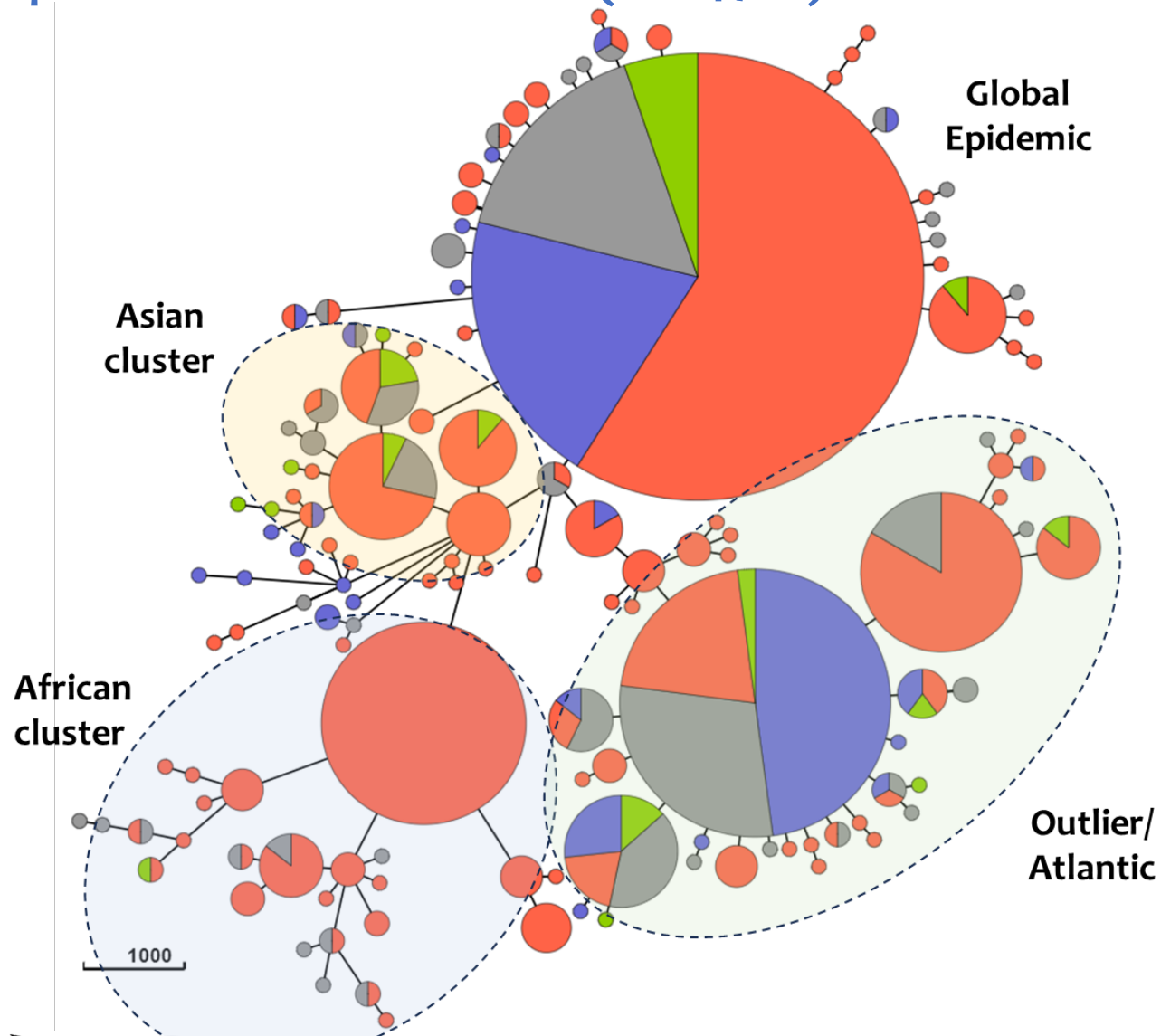
Phenotypic characterization

- ✓ Isolates confirmed by serotyping & biochemical
- ✓ Susceptible to all tested antibiotics except FQ

Genomic characterization

- ✓ Study isolates (n=65); Clinical – 48, Poultry – 17
- ✓ Isolates belong to ST11 or SLVs
- ✓ New phylogroup predominantly of invasive isolates from India (Asian cluster)
- ✓ **Phylogroup distribution - Clinical**
 - ✓ Asian cluster (61.9%)
 - ✓ Global epidemic (33.3%)
- ✓ **Phylogroup distribution – Poultry**
 - ✓ Global Outlier (82.3%)

Population structure (n=476)

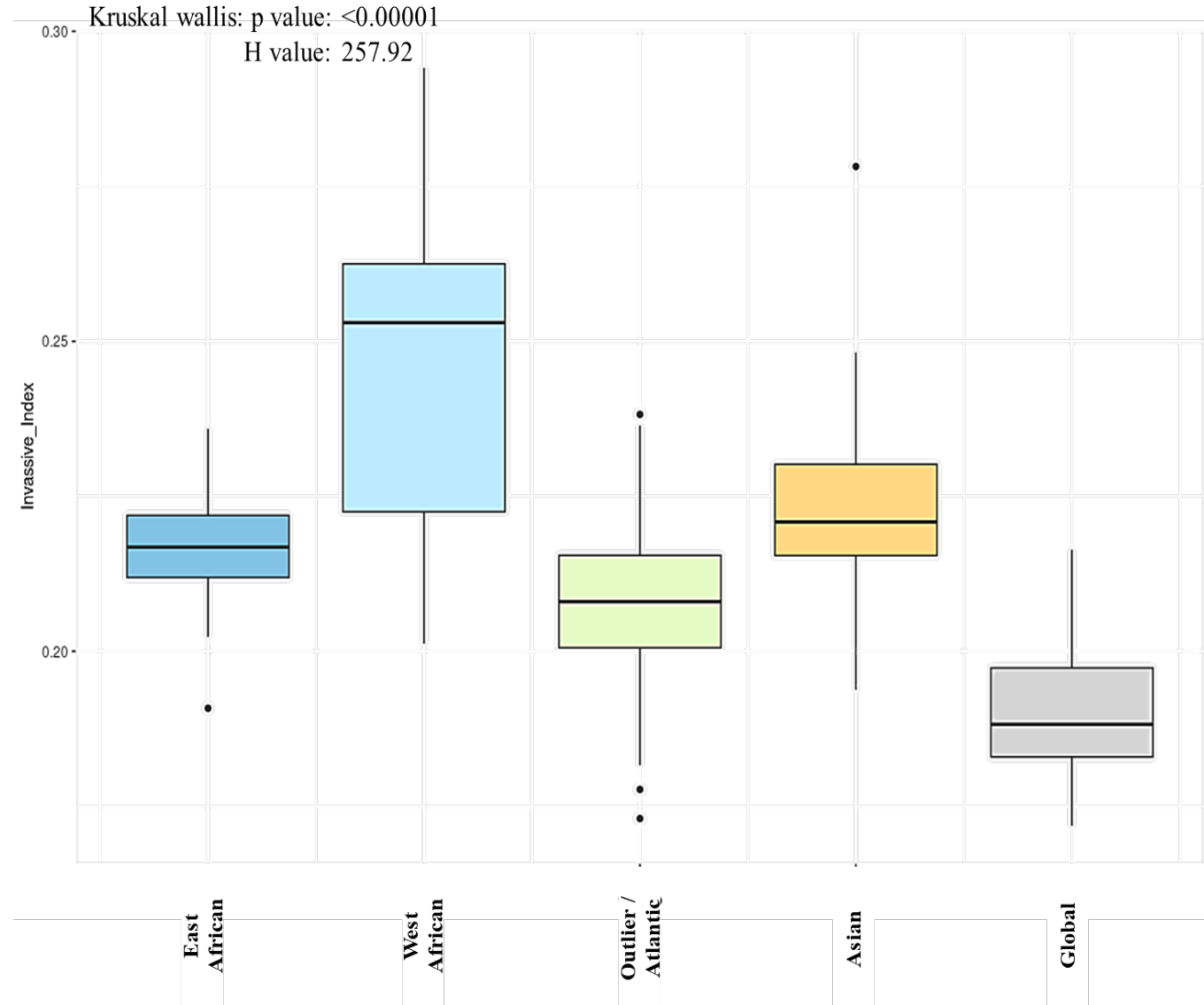


- ✓ African cluster predominantly of clinical isolates
- ✓ Global epidemic and Outlier cluster consist of clinical, Environmental and livestock isolates
- ✓ Asian cluster was diverse but majorly composed of clinical isolates

Invasiveness Index & HDCS

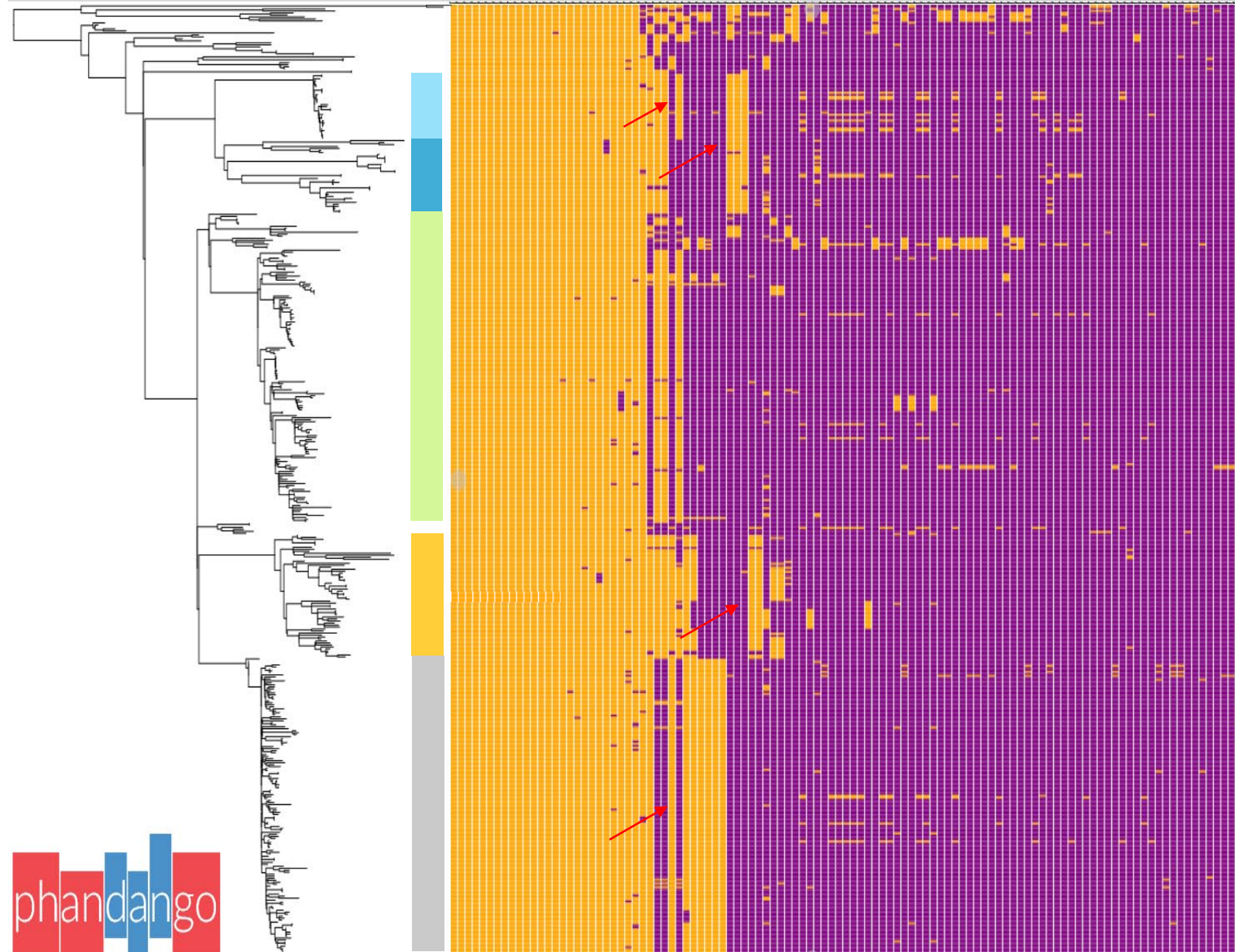
- ✓ S. Enteritidis phylogroups remain to be gastrointestinal (DBS <0.5)
- ✓ Invasiveness index varied among the phylogroups
 - ✓ West African (median = 0.253, SD = 0.25)
 - ✓ Global epidemic (median = 0.188, SD = 0.01)
 - ✓ Asian clade (median 0.221, SD= 0.013)
- ✓ High genome degradation in African and Asian

Phylogroup	No. of Characteristic HDCS
East African	13
West African	9
Asian	8

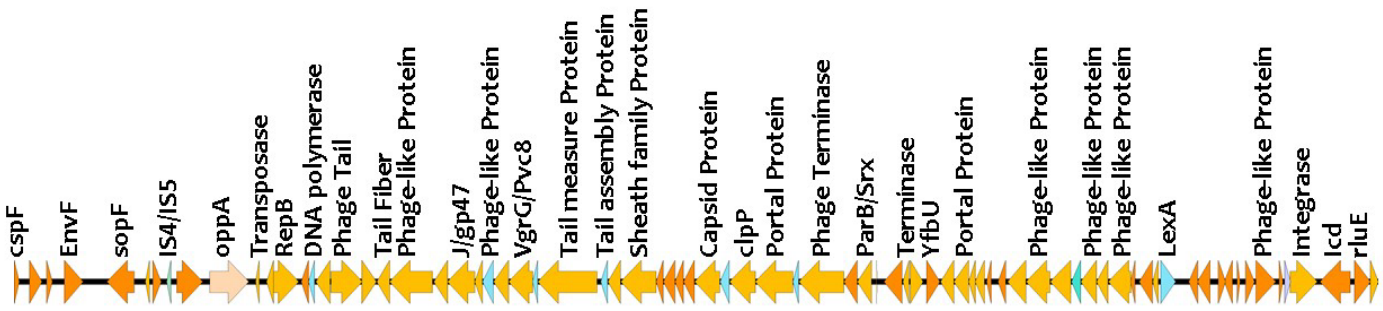
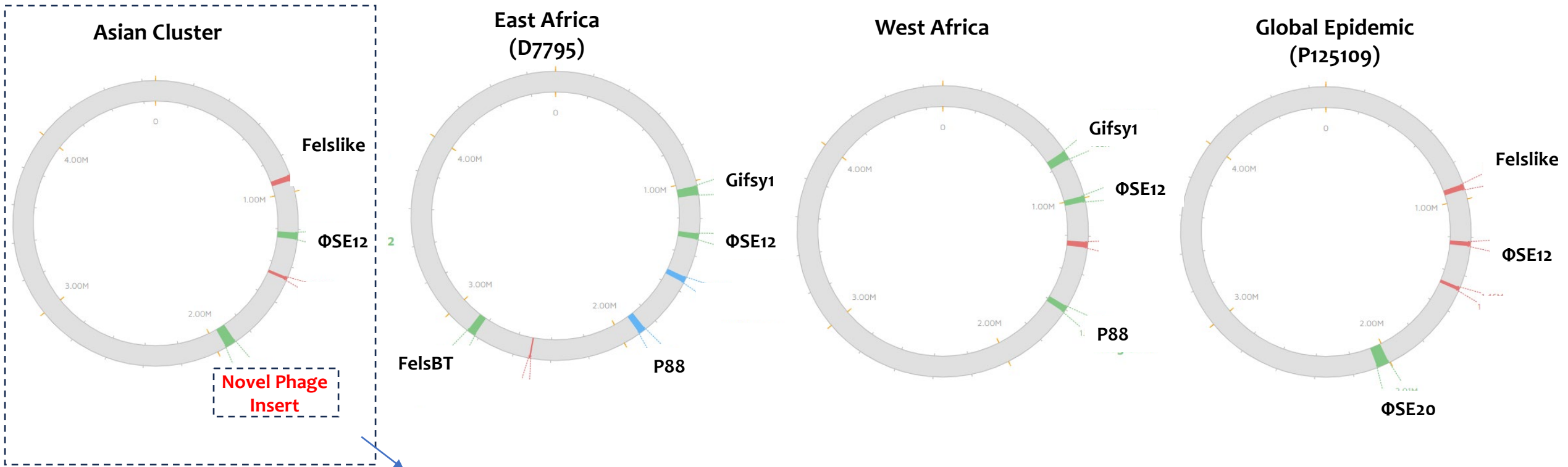


Pangenome Clustering

- ✓ Evidence of gene flux associated with the phylogroups
- ✓ Lineage specific core genes were separated using Twilight
- ✓ Insertions were characterized as prophages by Phaster



Phage inserts



Summary

- Severely **immunosuppressed** individuals are at a higher risk of iNTS
- Majority of *S. Enteritidis* causing invasive infection from India belonged to an emerging lineage that we propose to refer to as the **Asian cluster**
- Genotypic differences observed in isolates within the **Asian cluster** compared to strains found in **livestock** (Global Outlier)
- Isolates belonging to the Asian cluster exhibited the **second highest invasiveness index**, following the West African clade
- Asian cluster was characterized a **high level of HDCS** and a **distinctive prophage repertoire**



Acknowledgements



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Thank you...!

