

# Role of Bacteriophage Defense Systems in the Spread of Drug-Resistant *Salmonella* Typhi

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Scientist

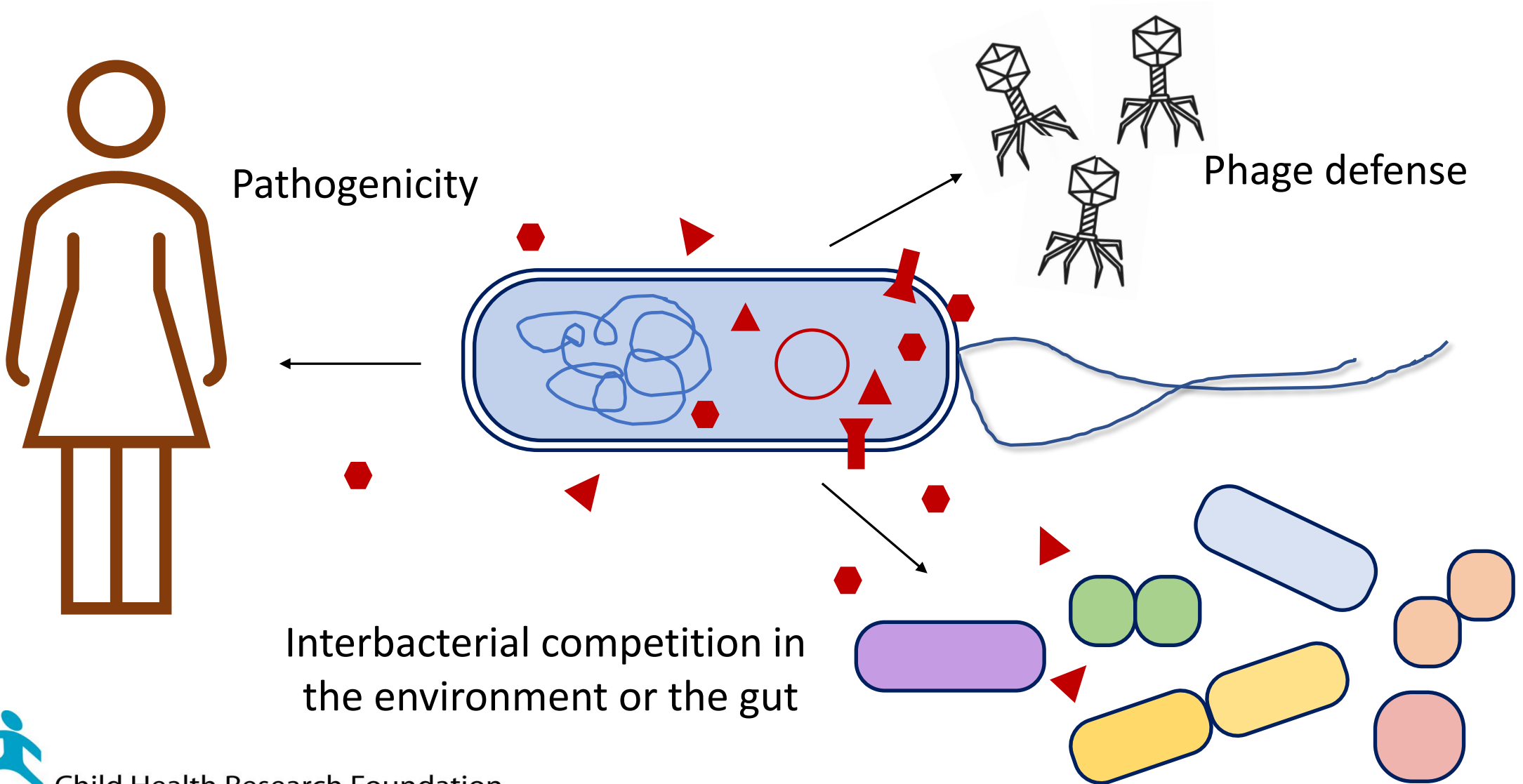
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# Interactions of *Salmonella* Typhi in the environment



# Phages and *Salmonella* Typhi: a short history

- Phages against *Salmonella* Typhi were reported in the 1930's
- 1950s, "Phage Typing" system was developed
- 2010: *Pickard et al* sequenced 7 Typhi phages
  - Use Vi as a receptor
  - Belonged to five different families

*Bull. Org. mond. Santé* } 1955, 13, 109-170  
*Bull. Wld Hlth Org.* }

## WORLD SURVEY OF TYPHOID AND PARATYPHOID-B PHAGE TYPES

A. FELIX, D.Sc., F.R.S.

*Lister Institute of Preventive Medicine, London*  
*Joint Chairman and Secretary, International Committee for Enteric Phage Typing*

Manuscript received in March 1955

### SYNOPSIS

The results achieved through international co-operation in the field of enteric phage typing and the methods by which these results were obtained are reviewed. The data are derived mainly from reports submitted by the author to the International Committee for Enteric Phage Typing (ICEPT) on the occasion of its meetings during the Fifth and Sixth International Congresses for Microbiology. Phage typing of *S. typhi* and *S. paratyphi B* are examined, and the progress achieved since the inception of the ICEPT is discussed. The necessity of calculating frequency distribution of types by foci is emphasized, and the author gives a summary of the results of surveys of the geographical distribution of typhoid and paratyphoid-B phage types.



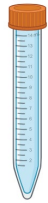
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# Isolating phages against *Salmonella* Typhi



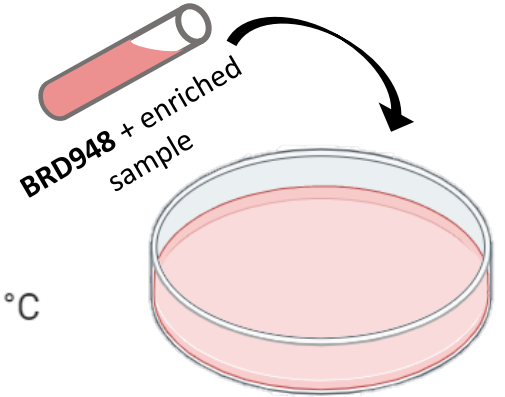
Collect environmental water  
(Sewage, lakes, rivers, ponds, stagnant water)



Filtration to remove bacteria  
and large particles



Incubation with **BRD948**  
for phage enrichment



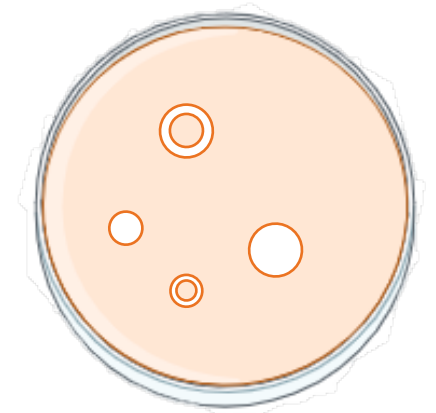
Lawn co-Culture over  
agar plate



Jason Andrews



Senjuti Saha



Phage plaques

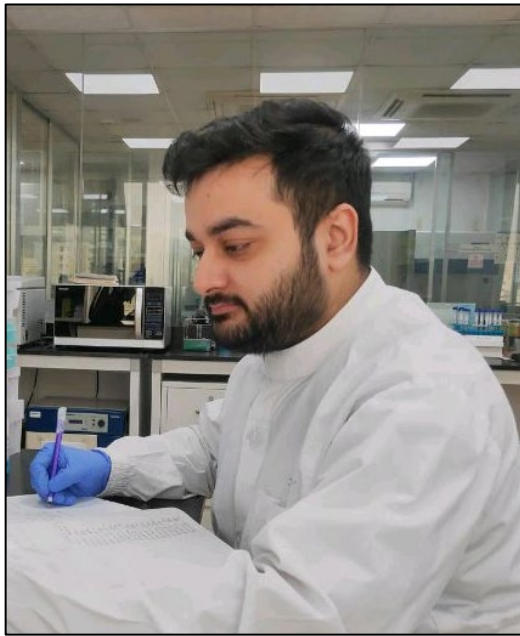
# Isolation of phages

	Dhaka			Mirzapur			Chittagong			Total		
	N	Total Collection	Phages	N	Total Collection	Phages	N	Total Collection	Phages	N	Total Collection	Phages
<b>Total</b>	<b>67</b>	<b>212</b>	<b>83</b>	<b>4</b>	<b>316</b>	<b>5</b>	<b>23</b>	<b>275</b>	<b>25</b>	<b>94</b>	<b>803</b>	<b>113</b>

# Posters and oral talk

**Development of low-cost environmental surveillance method for effective typhoid fever**

Shuborno Islam, Oral talk



**Exploring Diversity and Environmental Dynamics of *Salmonella* Typhi and its Bacteriophages**

Rathindranath Kabiraj, Abstract: 48



**Mapping typhoid fever in Bangladesh using environmental surveillance**

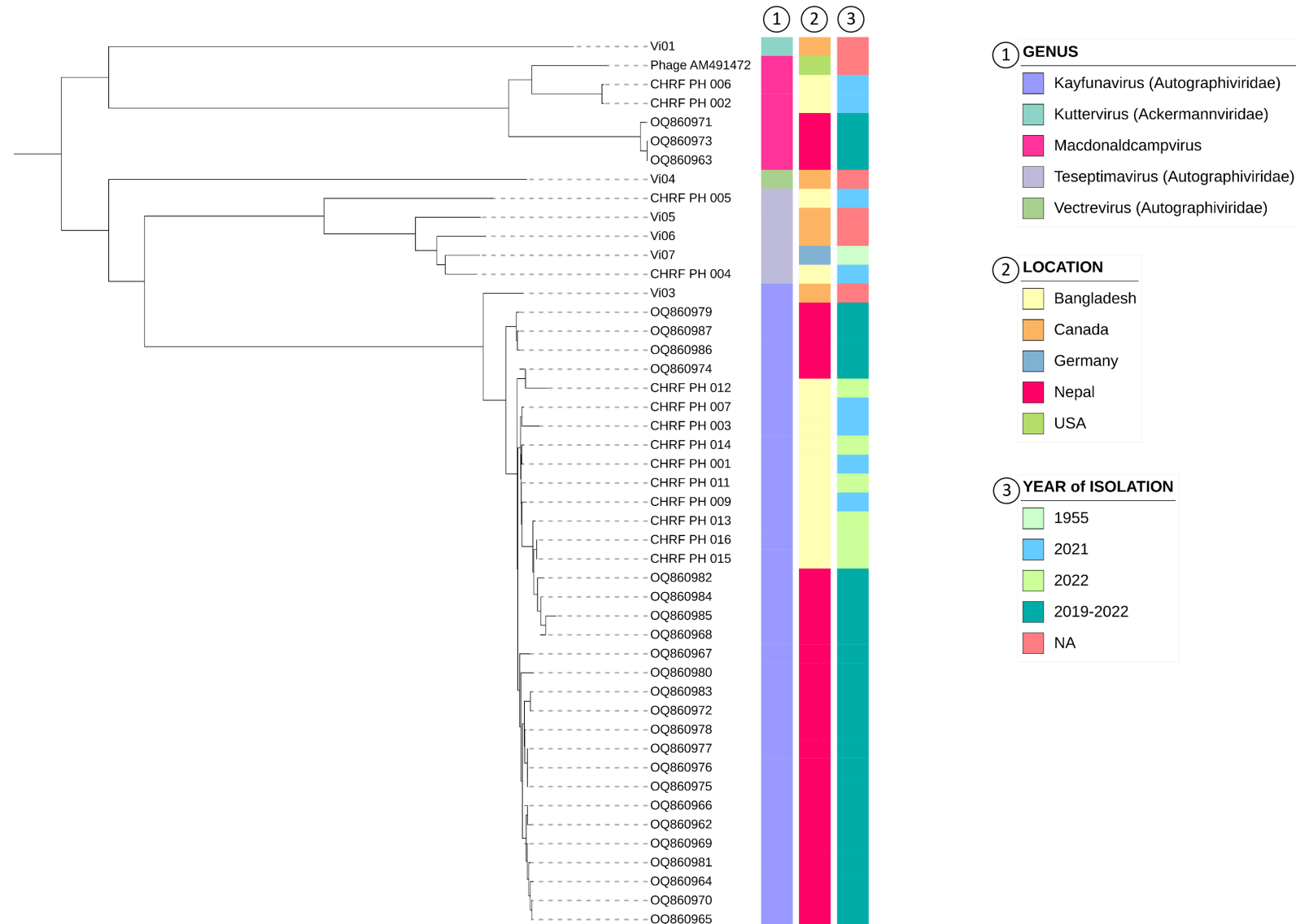
Al Amin, Abstract: 07





# What is diversity of phages in *Salmonella* Typhi?

- Sequenced 14 phages from Bangladesh
- Compared this with 26 phages from Nepal and 6 phages from Pickard et al
- 3/5 families identified by Pickard circulating in Nepal and Bangladesh



Are there differences between different genotypes in their phage susceptibility?



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# Identification of an active BREX system in 4.3.1.3.Bdq

**AMR**

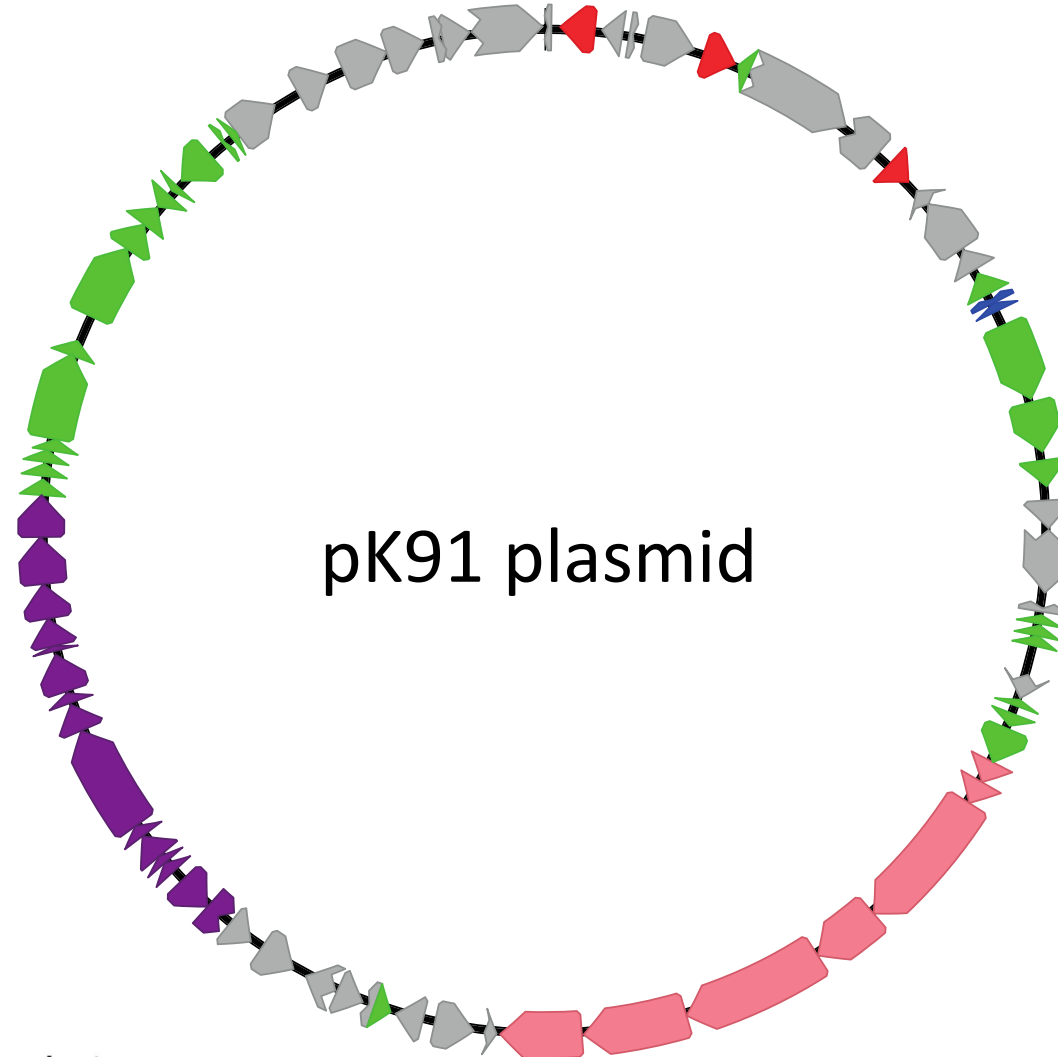
Transposase/replication

**T4SS**

**BREX system**

**Primarily unknown**

1. **blaTEM-1 (ampicillin)**
2. **qnr (quinolone)**



# Identification of an active BREX system in 4.3.1.3.Bdq

**AMR**

Transposase/replication

**T4SS**

**BREX system**

**Primarily unknown**

pK91 plasmid

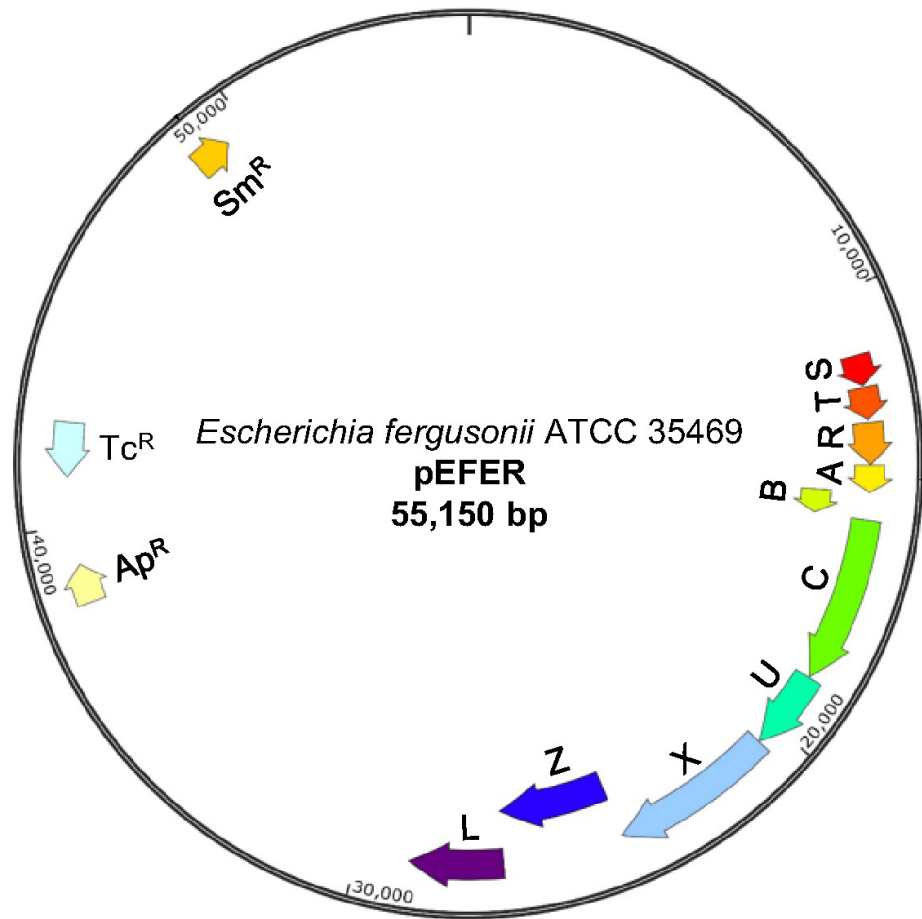
## Type IV Secretion System:

- Conjugative system
- DNA release/uptake system
- Effector system

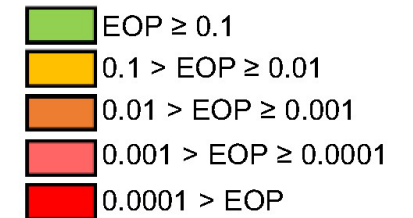
## BREX system:

- Discovered in 2015 (Sorek Lab)
- Phage defense system
- Found in bacteria and archaea
- Not described in typhoidal *Salmonella* yet

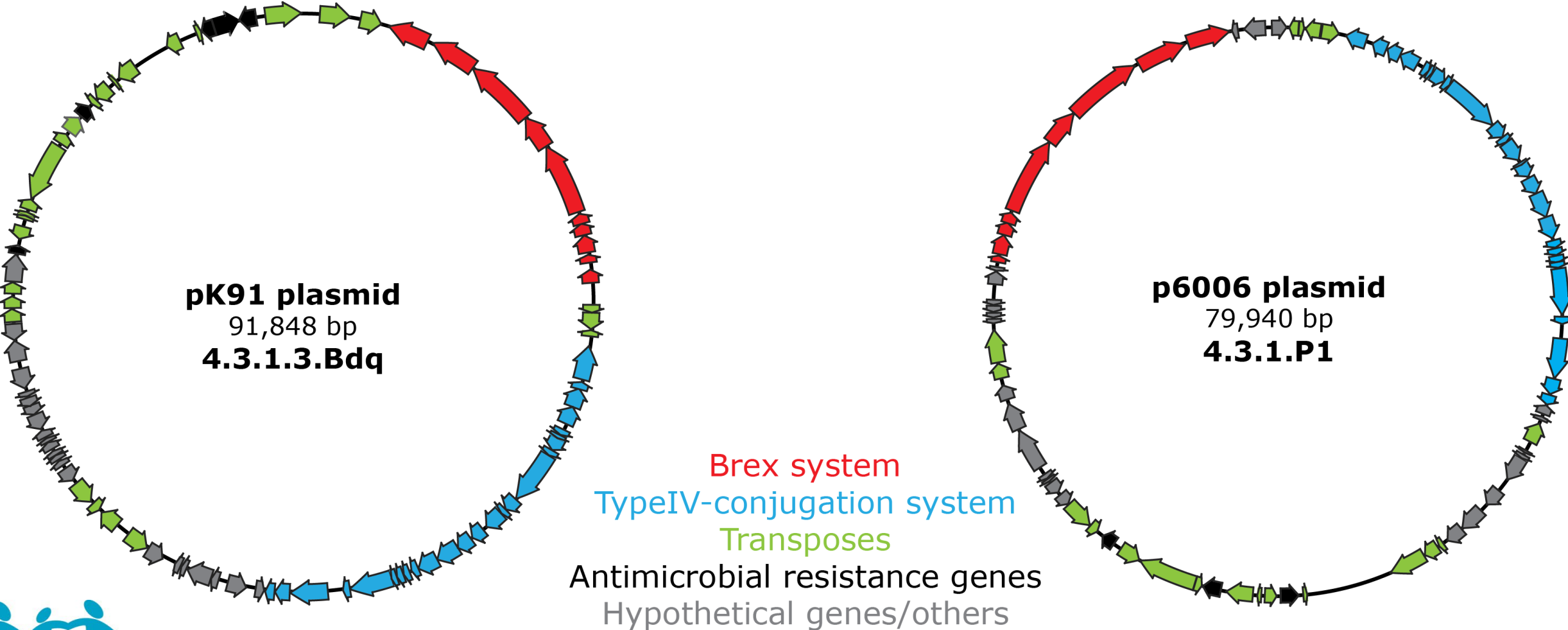
# *Salmonella* Typhi BREX system is related to active BREX system in *Escherichia fergusonii*



$\Phi$	pEFER EOP $\pm$ SD	pEFER- <i>brxA</i> ::Tn5 EOP $\pm$ SD
AL25	1.03 $\pm$ 0.23	0.90 $\pm$ 0.22
Alma	0.73 $\pm$ 0.18	0.97 $\pm$ 0.03
Bam	5.75 $\times 10^{-4}$ $\pm$ 5.19 $\times 10^{-4}$	1.01 $\times 10^{-3}$ $\pm$ 7.24 $\times 10^{-4}$
Baz	0.73 $\pm$ 0.58	1.40 $\pm$ 1.17
BB1	0.48 $\pm$ 0.21	1.32 $\pm$ 0.7
BGP	4.00 $\times 10^{-2}$ $\pm$ 5.40 $\times 10^{-2}$	0.14 $\pm$ 0.13
BHP	1.21 $\pm$ 0.42	1.29 $\pm$ 0.50
CP	<1.36 $\times 10^{-8}$ $\pm$ 4.72 $\times 10^{-9}$	0.17 $\pm$ 0.17
CS16	5.95 $\times 10^{-8}$ $\pm$ 7.35 $\times 10^{-8}$	2.73 $\times 10^{-3}$ $\pm$ 9.56 $\times 10^{-4}$
EH2	<3.44 $\times 10^{-7}$ $\pm$ 1.06 $\times 10^{-7}$	1.94 $\pm$ 1.36
EL	<3.31 $\times 10^{-8}$ $\pm$ 1.81 $\times 10^{-8}$	0.18 $\pm$ 6.06
Geo	<2.19 $\times 10^{-8}$ $\pm$ 8.30 $\times 10^{-9}$	4.96 $\times 10^{-2}$ $\pm$ 3.95 $\times 10^{-3}$
Jura	1.07 $\pm$ 0.45	1.22 $\pm$ 0.63
Mak	2.43 $\times 10^{-3}$ $\pm$ 3.17 $\times 10^{-4}$	3.73 $\times 10^{-3}$ $\pm$ 2.37 $\times 10^{-3}$
Mav	<2.37 $\times 10^{-8}$ $\pm$ 1.71 $\times 10^{-8}$	3.43 $\times 10^{-7}$ $\pm$ 3.97 $\times 10^{-7}$
NP	<4.11 $\times 10^{-8}$ $\pm$ 8.09 $\times 10^{-9}$	0.17 $\pm$ 8.37 $\times 10^{-2}$
NR1	<3.26 $\times 10^{-8}$ $\pm$ 1.73 $\times 10^{-8}$	0.13 $\pm$ 8.76 $\times 10^{-2}$
PATM	<4.17 $\times 10^{-8}$ $\pm$ 8.07 $\times 10^{-9}$	1.06 $\pm$ 0.16
Pau	<2.86 $\times 10^{-8}$ $\pm$ 1.69 $\times 10^{-8}$	1.19 $\pm$ 0.76
QOTSP	4.92 $\times 10^{-7}$ $\pm$ 8.18 $\times 10^{-7}$	0.10 $\pm$ 7.52 $\times 10^{-2}$
SAP	<7.95 $\times 10^{-8}$ $\pm$ 2.44 $\times 10^{-8}$	1.74 $\pm$ 0.13
Sipho	2.04 $\times 10^{-3}$ $\pm$ 2.09 $\times 10^{-3}$	3.12 $\pm$ 2.47
Solly	4.21 $\times 10^{-7}$ $\pm$ 2.37 $\times 10^{-7}$	0.11 $\pm$ 9.91 $\times 10^{-2}$
Some	<1.40 $\times 10^{-7}$ $\pm$ 3.32 $\times 10^{-8}$	1.97 $\pm$ 1.62



# BREX system is also found on the XDR strain of Salmonella Typhi



# Summary:

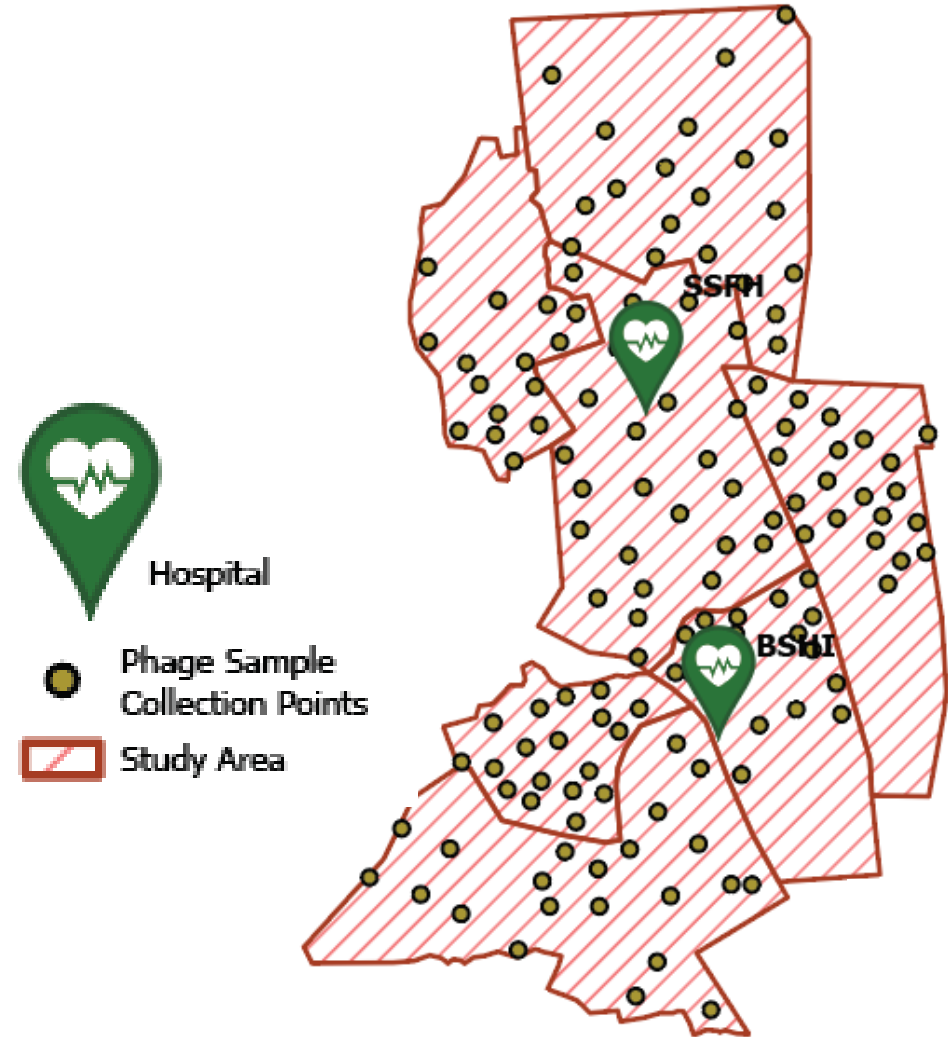
- Diverse set of Typhi phages were isolates from regions endemic for typhoid fever
- There is an active interaction between the phages and Typhi collected from the same geographical region
- Certain drug-resistant strains such as XDR (4.3.1.P1) and 4.3.1.3.Bdq lineages contain “active” phage-defense systems on the plasmids also carrying antimicrobial resistance genes

**Phage defense systems may contribute to the fitness of Typhi, thereby affecting which lineages spread in a geographical location**



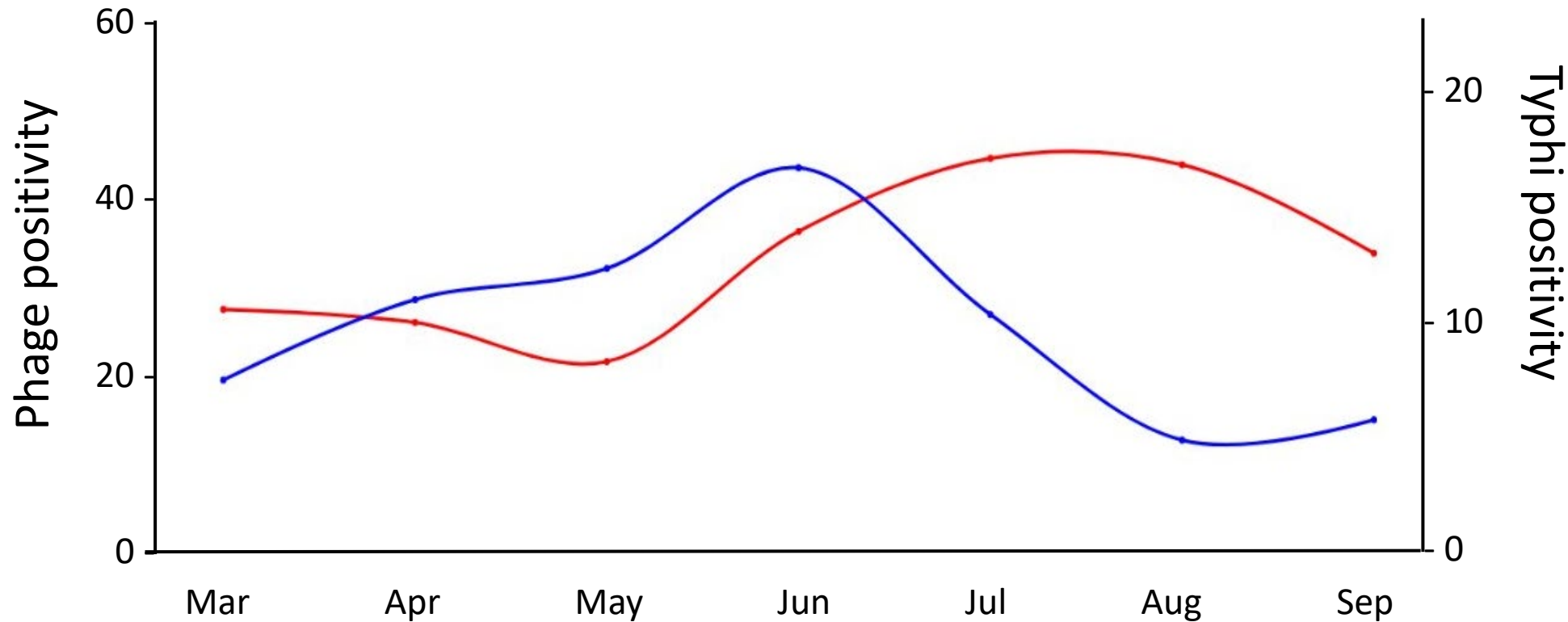
# How do Typhi phages affect *Salmonella* Typhi?

- Catchment area comprising of 7 thanas around two large pediatric hospitals in Dhaka
- We have been collecting Typhi strains from this catchment area since 2016
- Selected 140 locations to sample phages per month
- Identify correlation between Typhi/phage abundance





# Temporal relationship between Phage positivity and Typhi positivity in an endemic setting





# Thank You!!



**Jason Andrews, Stanford**

**Kesia D. Silva, Stanford**

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