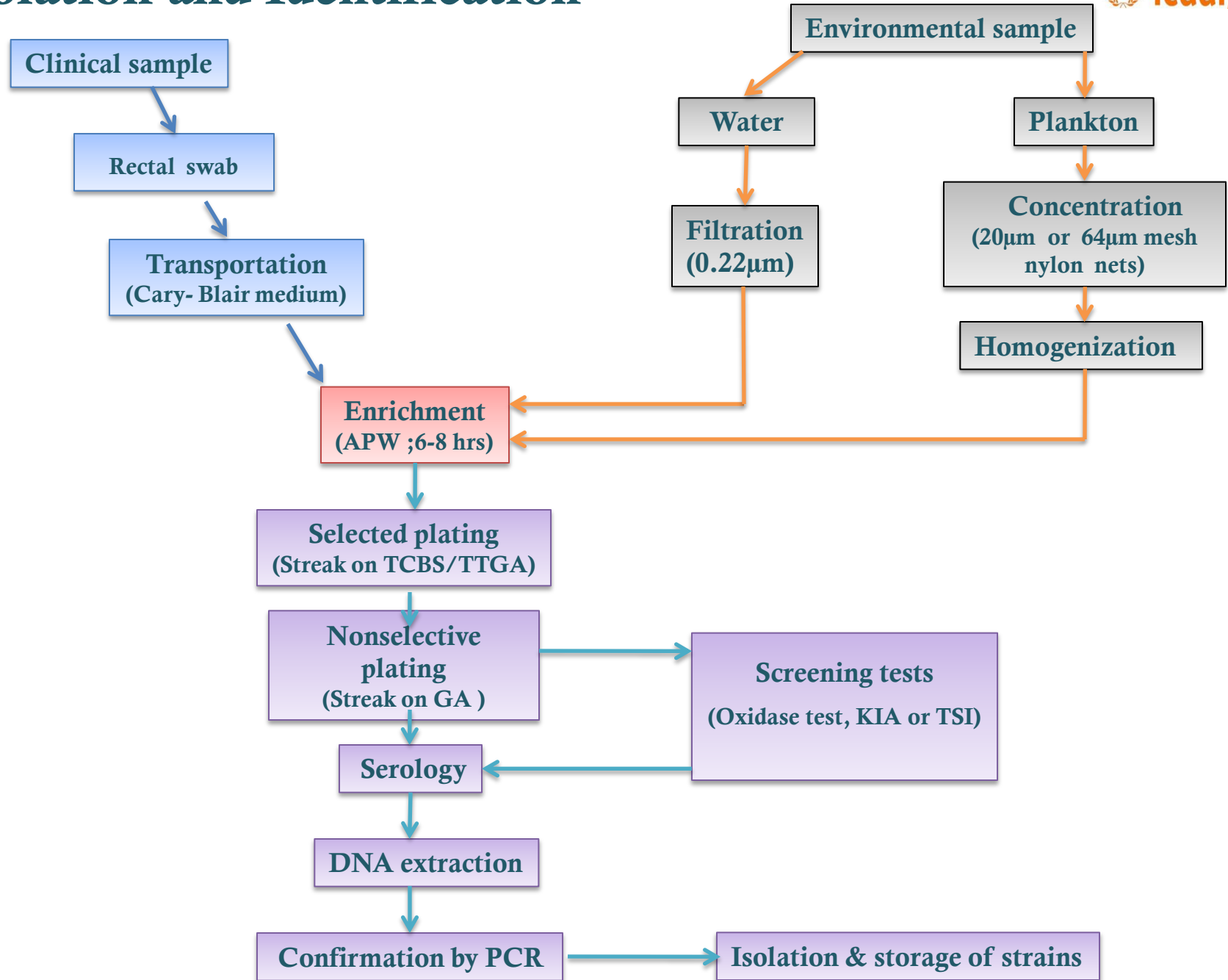


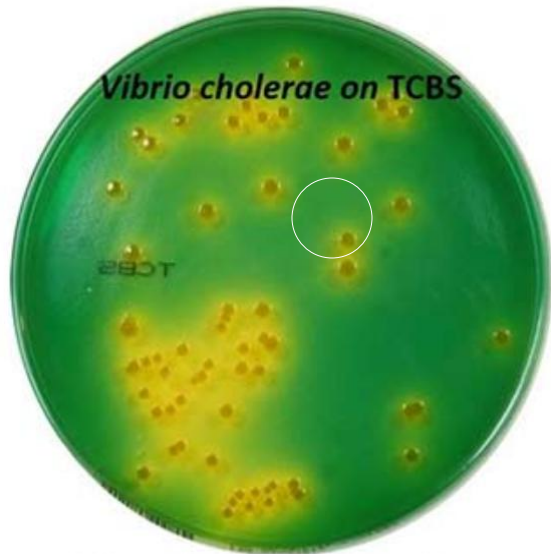
Cholera detection and *Vibrio cholerae* isolation and characterization : icddr,b perspective

Munirul Alam, PhD, FBAS, FAAM
Senior Scientist & Head, Molecular Ecology
and Metagenomics Laboratory, icddr,b
(International Centre for Diarrheal Disease
Research, Bangladesh)
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Isolation and Identification



Culturing *Vibrio cholerae* from suspected cholera stool samples



Rapid
Dipstick test
+Ve



Streak
enriched
stool on
TCBS & TTGA
plates



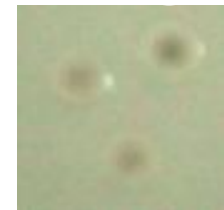
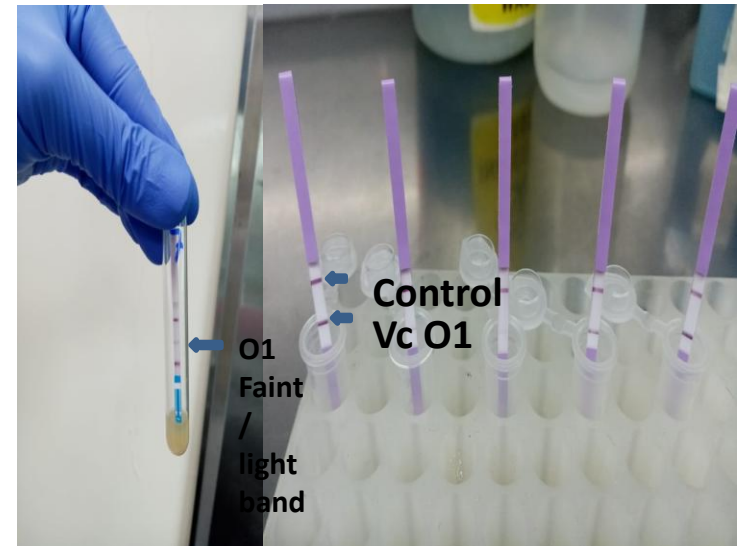
Subculture
on GA plates



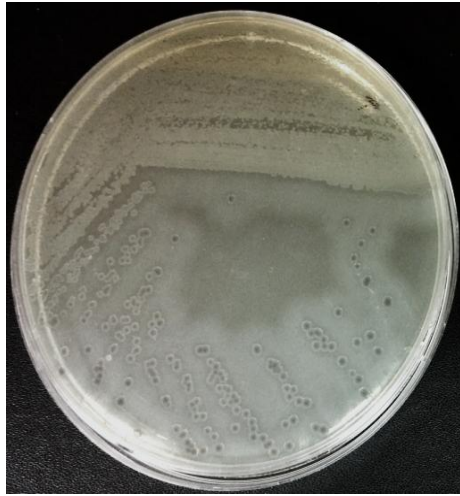
Serology
confirmatio
n for O1 &
O139



Strain Stock
in
LB+glycerol
for -80C



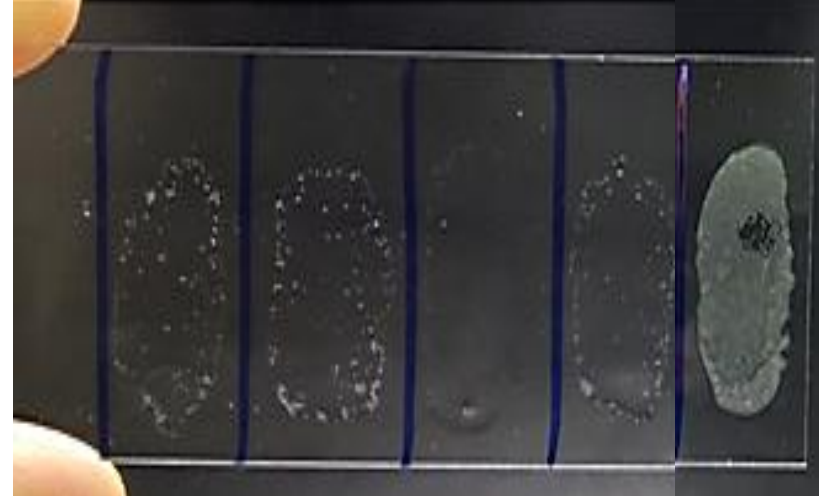
Vibrio cholerae isolation and confirmation by culture and serology



TTGA (selective)



GA (non-selective)



Serological tests: O1/O139, Inaba/Ogawa

Short-term and long-term storage of *V. cholerae* strains

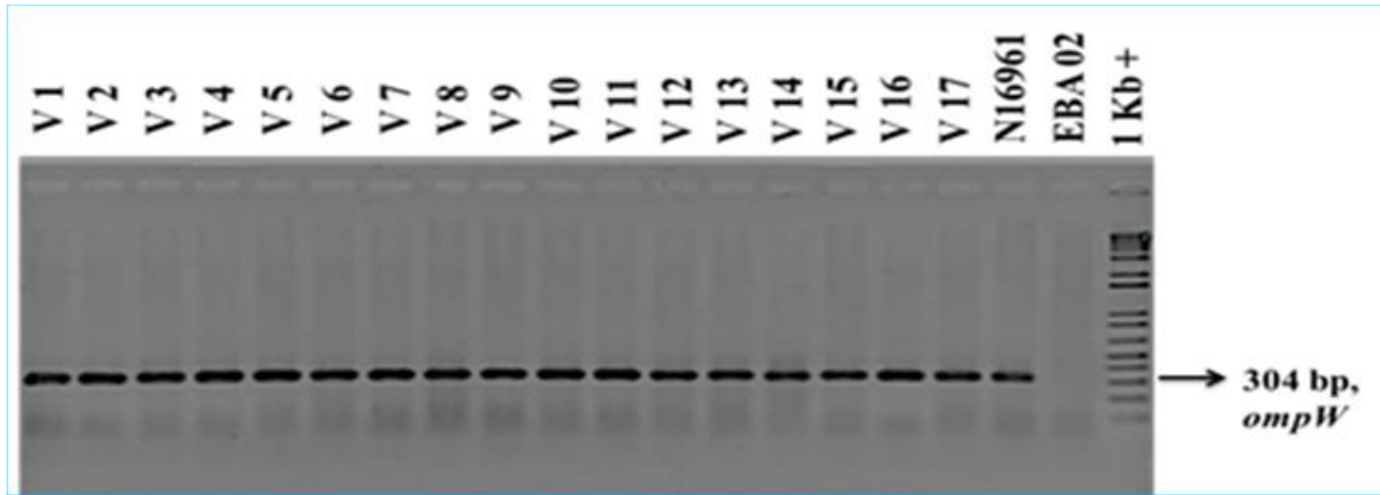


T1N1 soft agar and store at RT



Cryopreserved in LB + 30% glycerol at -80°C

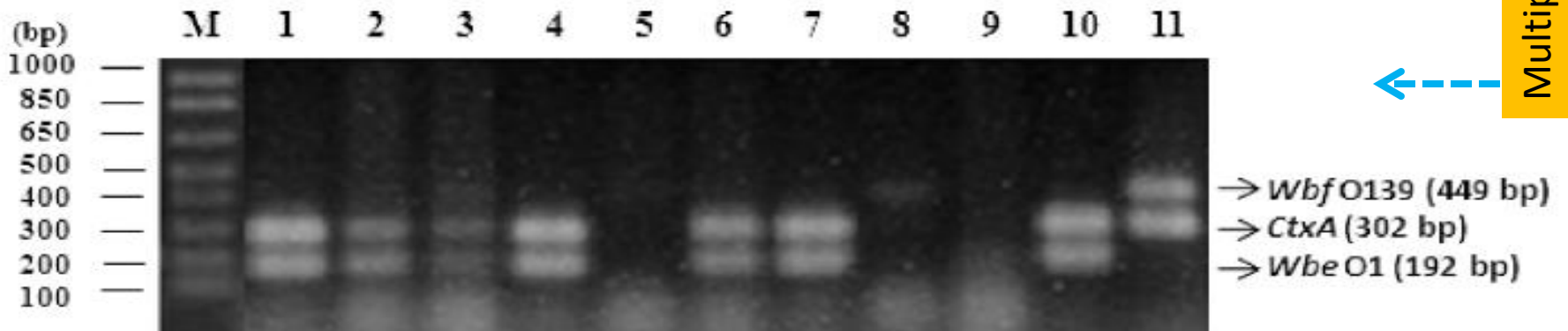
Molecular detection of toxigenic *Vibrio cholerae* O1 and O139



Simplex PCR



Multiplex PCR



Biotypes classical & El Tor: Emergence of altered El Tor in South-east Asia

Table 1 - Type of *ctxB* encoded in El Tor *V. cholerae* isolates from 1991 to 2006.

Year	<i>ctxB</i> type*		Region‡ (number of isolates)
	El Tor	classical	
1991	6		Southeast Asia (5), South Asia (1)
1992	5		Southeast Asia (3), Latin America (2)
1993	2	3	Southeast Asia (5)
1994	1	2	Southeast Asia (2†), East Asia (1)
1995		10	Southeast Asia (10)
1996		1	Southeast Asia (1)
1997		14	Southeast Asia (11), East Asia (3)
1998		2	Southeast Asia (1), East Asia (1)
1999		4	Southeast Asia (1), South Asia (3)
2000		1	Africa (1)
2001		2	Southeast Asia (2)
2002		1	Southeast Asia (1)
2003		0	
2004		8	Southeast Asia (3), South Asia (5)
2005		3	Southeast Asia (2), South Asia (1)
2006		2	Southeast Asia (1), South Asia (1)
	14	53	

*: *ctxB* typing was performed by MAMA-PCR on 203rd nucleotide of *ctxB* gene.

†: One harbored El Tor *ctxB* and the other harbored classical *ctxB*.

‡: Africa; Madagascar. East Asia; China, Korea, North Korea. South Asia; India. Southeast Asia; Indonesia, Philippine, Singapore, Thailand. Latin America; Bolivia, Peru.

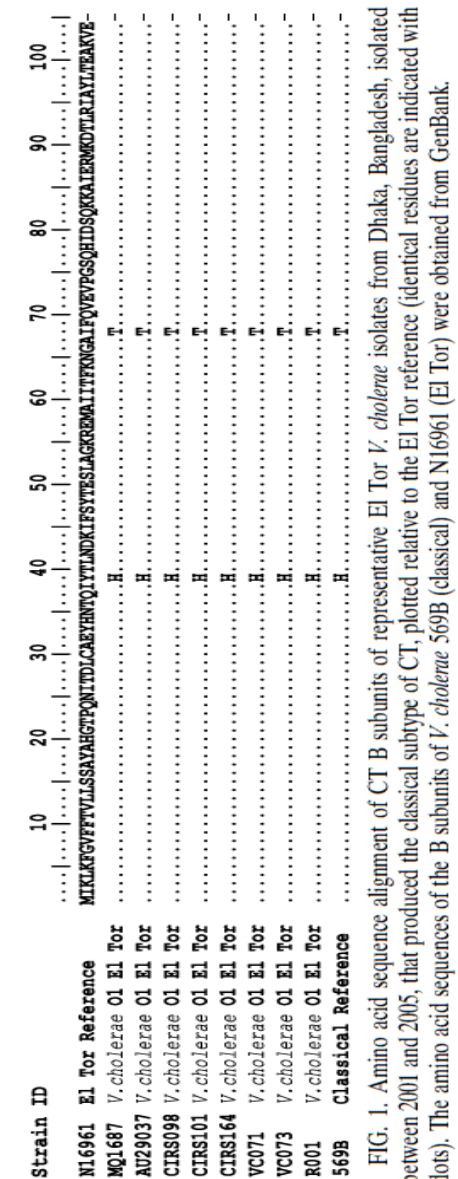
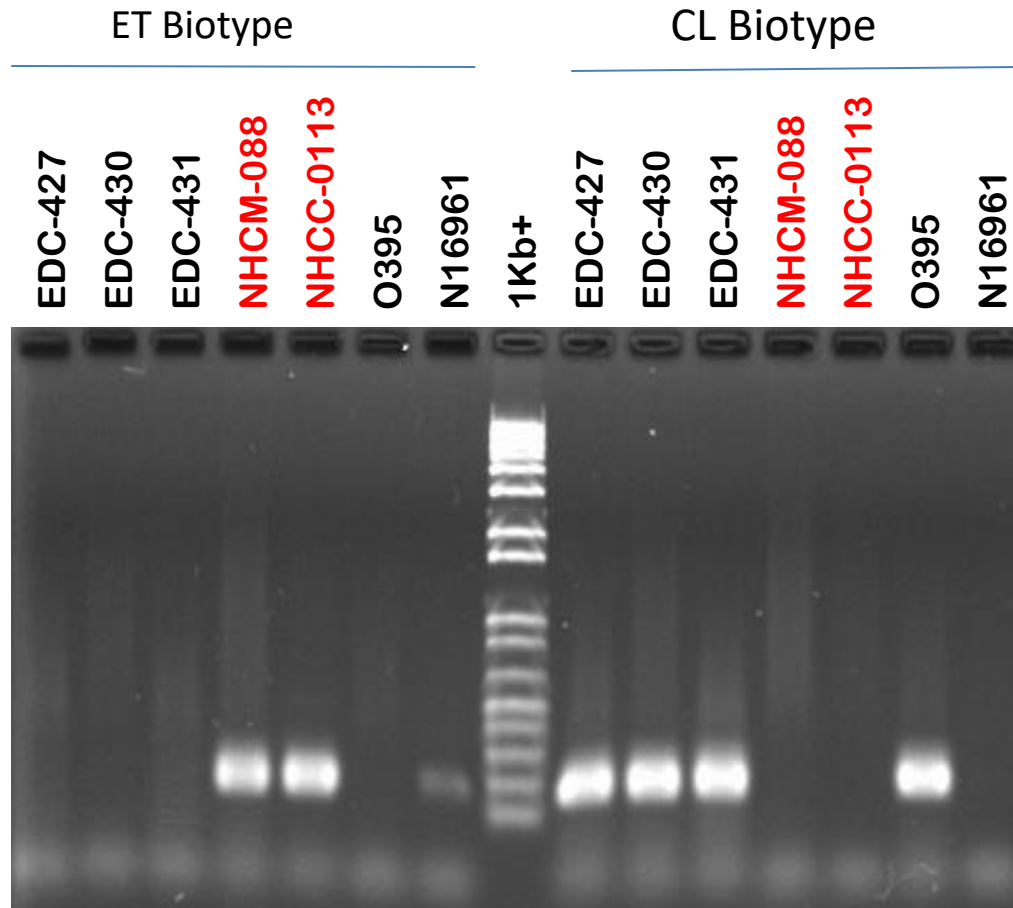


FIG. 1. Amino acid sequence alignment of CT B subunits of representative El Tor *V. cholerae* isolates from Dhaka, Bangladesh, isolated between 2001 and 2005, that produced the classical subtype of CT, plotted relative to the El Tor reference (identical residues are indicated with dots). The amino acid sequences of the B subunits of *V. cholerae* 569B (classical) and N16961 (El Tor) were obtained from GenBank.

Nair et al., 2006

Biotype determination by simplex MAMA PCR

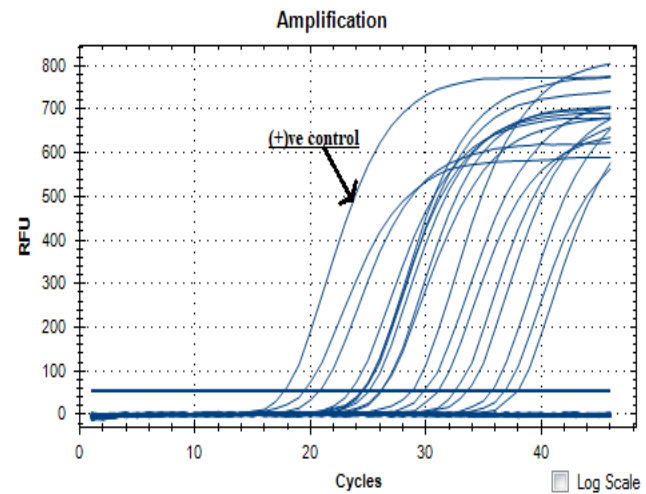
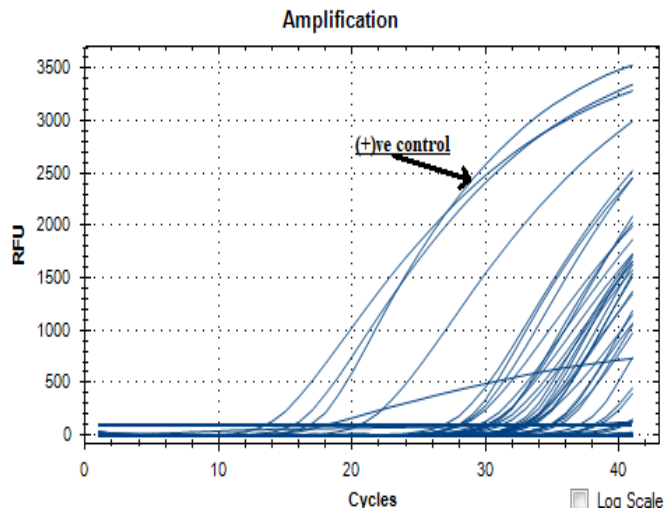


qPCR detection of *Vibrio cholerae*

Target gene	Primer - probe	Sequence
Vibrio Cholerae O1	<i>Rfb/wbeF</i>	CCAGATTGTAAAGCAGGATGGA
	<i>Rfb/wbeR</i>	GGTCATCTGAAGTACAAC
	VCO1	Fam – CCCGGAGTTTGTAAAGCCCACTACCGGG - Dabcyl
Vibrio Cholerae O139 rbf	<i>wbfF</i>	CATACCAACGCCCTTATCCATT
	<i>wbfR</i>	GCATGACTGGCATCCCAAAT
	VCO139	Cy5 – CGGGTGAGAAAAGACAGCAATAACACCCG - Dabcyl
<i>viuB</i>	<i>viuB-qpcr-F</i>	TCGGTATTGTCTAACGGTAT
	<i>viuB-qpcr-R</i>	CGATTCGTGAGGGTGATA
	<i>viuB</i>	FAM - TCATTTGGC/ZEN/CAGAGCATAAACCGGT - 3IABkFQW

qPCR detection of *Vibrio cholerae*

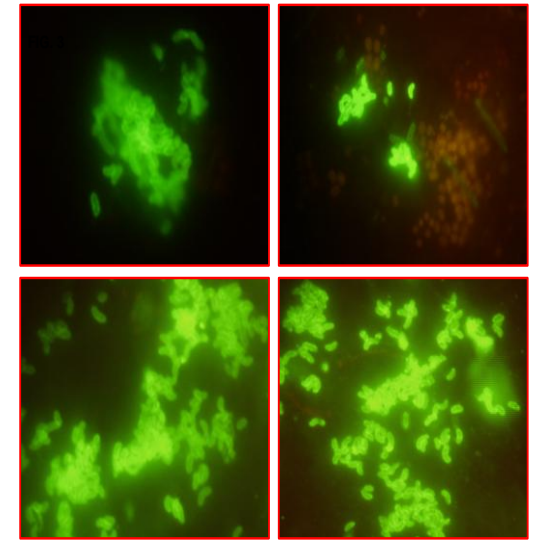
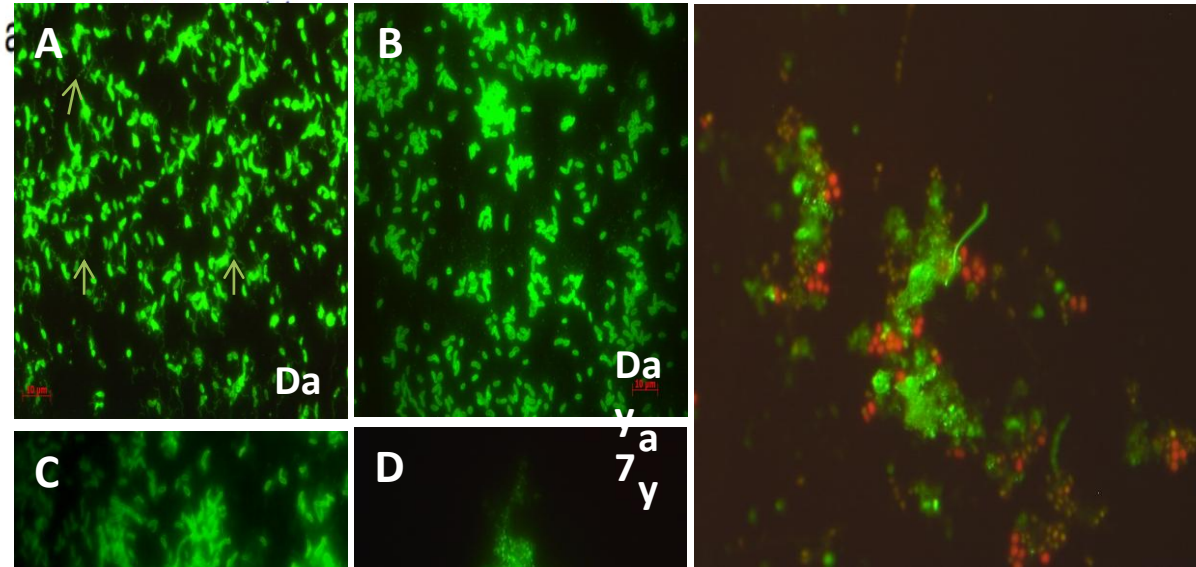
Amplification plot



***Vibrio cholerae* confirmation from qPCR amplification of *viuB* and *rfbO1* genes**

Biofilms in water, its role and impact in human disease transmission

Anwar Huq¹, Chris A Whitehouse^{2,*}, Christopher J Grim^{1,4}, Munirul Alam³



Viable but nonculturable *Vibrio cholerae* O1 in biofilms in the aquatic environment and their role in cholera transmission

Munirul Alam*, Marzia Sultana*, G. Balakrish Nair*, A. K. Siddique*, Nur A. Hasan*, R. Bradley Sack†, David A. Sack*, K. U. Ahmed*, A. Sadique*, H. Watanabe‡, Christopher J. Grim§, A. Huq§, and Rita R. Colwell¶^{§¶}

*International Center for Diarrhoeal Disease Research, Bangladesh, Dhaka-1000, Bangladesh; †Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205; ‡Department of Bacteriology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan; §Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202; and ¶Institute for Advanced Computer Studies, University of Maryland, College Park, MD 20742



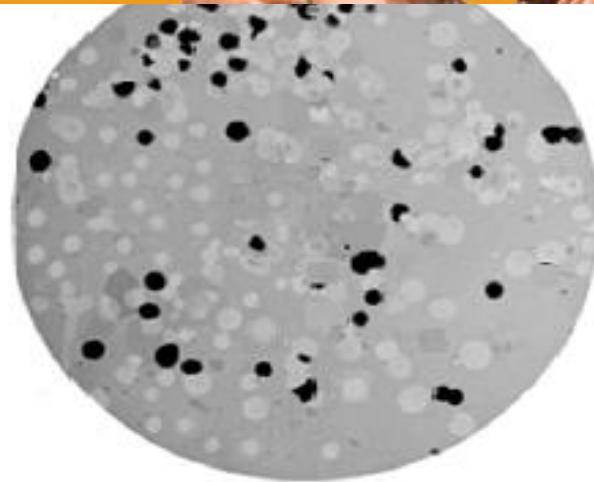
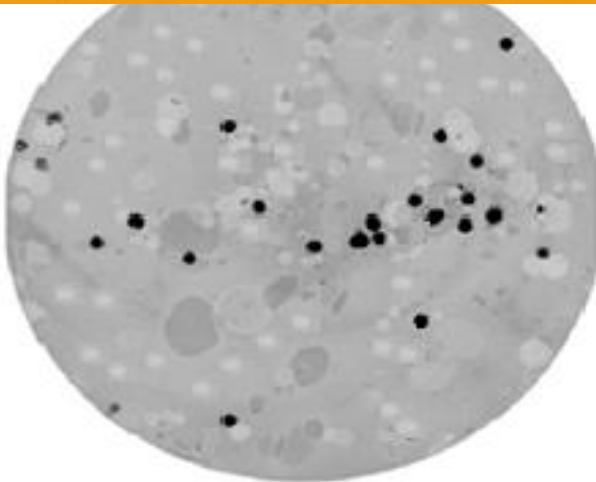
Biofilms Comprise a Component of the Annual Cycle of *Vibrio cholerae* in the Bay of Bengal Estuary

Marzia Sultana,^a Suraia Nusrin,^a Nur A. Hasan,^{a,b,d} Abdus Sadique,^a Kabir U. Ahmed,^a Atiqul Islam,^a Anwar Hossain,^c Ira Longini,^d Azhar Nizam,^b Anwar Huq,^e Abul K. Siddique,^a David A. Sack,^f Richard B. Sack,^f Rita R. Colwell,^{e,f,g} Munirul Alam^a

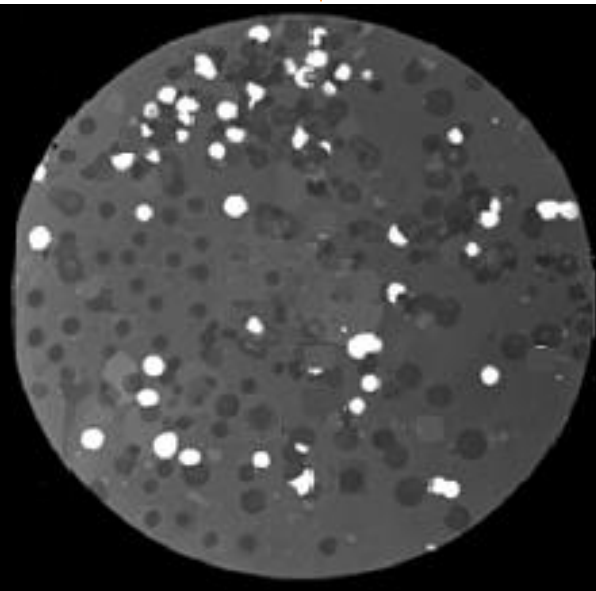
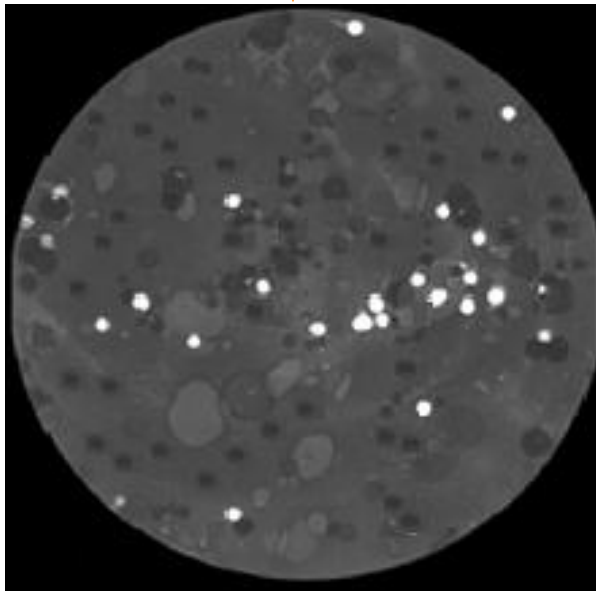


icddr,b

KNOWLEDGE FOR GLOBAL LIFESAVING SOLUTIONS



V. cholerae culture on NSA aided by colony blot hybrid.



Direct
Enrichment
Colony blot
PCR
DFA
....

Environmental Genome Project of *Vibrio cholerae* is ongoing at icddr,b (W- DFID: 2019 – 2022) jointly with Sanger (UK) & PGIMER (India)

icddr,b,
Dhaka,
Bangladesh
Munirul Alam (PI)
Dinesh Mondal (Co-PI)

Archived *V. cholerae*
(n=5000)

&

Prospectively isolated
V. cholerae from
aquatic environment
& monitor...

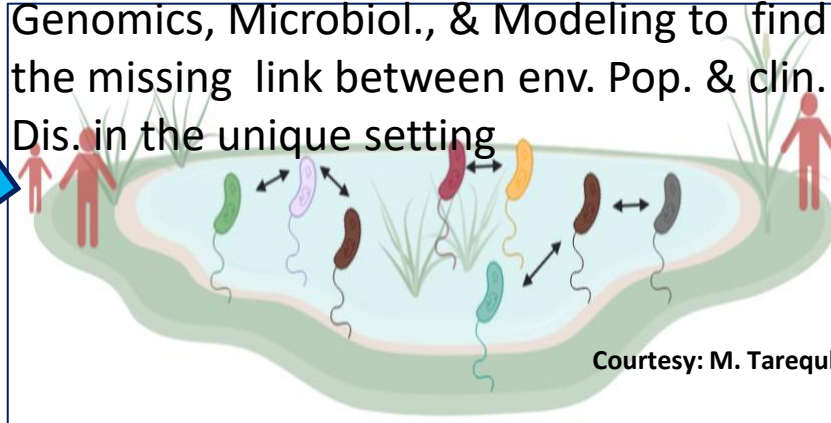
Sanger Institute,
Cambridge,
United Kingdom
Nicholas Thomson
(Co-PI)

PGIMER,
Chandigarh,
India
Neelam Taneja
(Co-PI)

Prospectively
isolated *V.*
cholerae from
aquatic
environment

On target, progressing.....

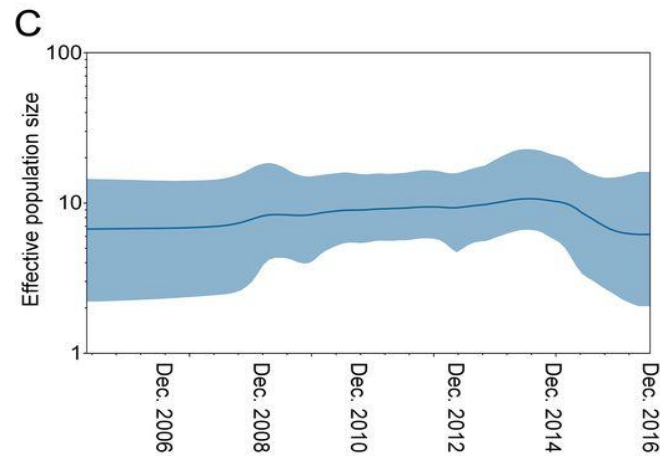
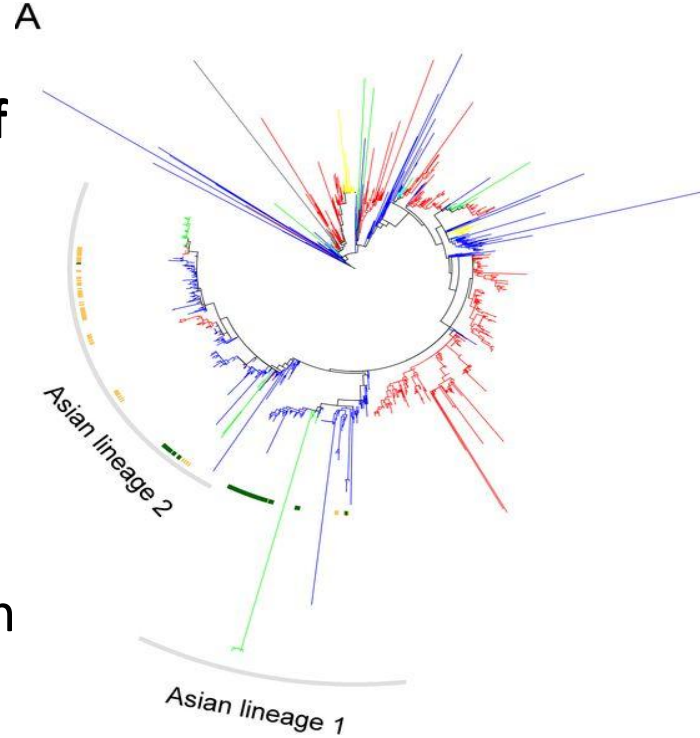
Genomics, Microbiol., & Modeling to find
the missing link between env. Pop. & clin.
Dis. in the unique setting



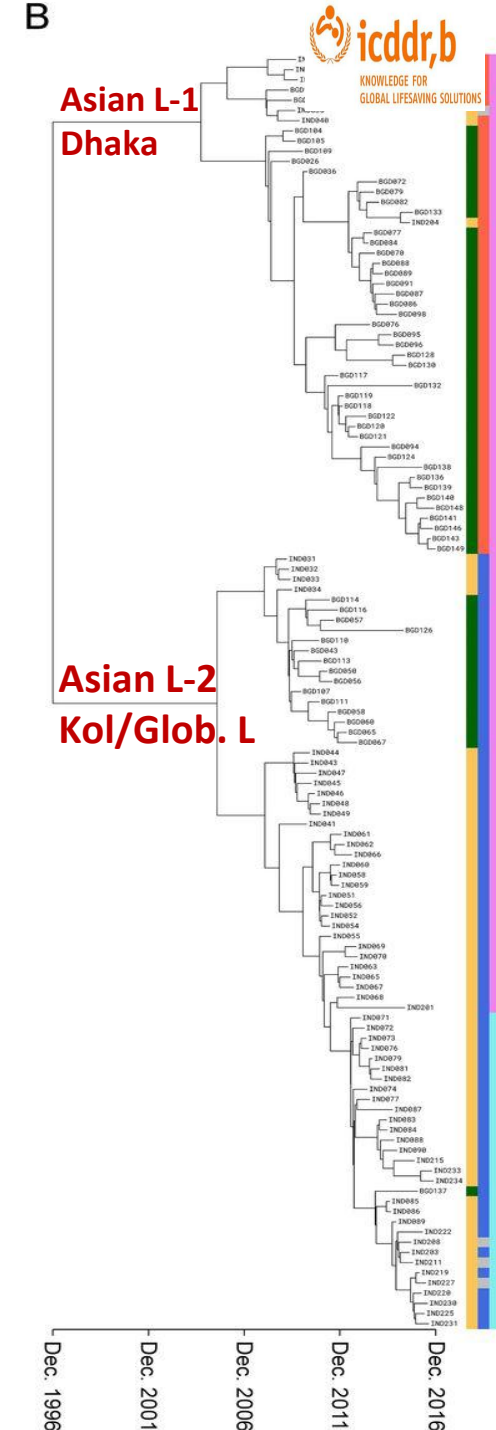
Whole-Genome Analysis of Clinical *Vibrio cholerae* O1 in India and Bangladesh

Revealed two Asian lineages:

- Lineage 1: predominant in Bangladesh, and found in other countries of Asia
- Lineage 2: dominant in India, and found in Syria and Haiti



Morita & Alam *et al.*, 2020 *mBio*



Genomic attributes of *Vibrio cholerae* O1 responsible for 2022 massive cholera outbreak in Bangladesh

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Accepted: 9 February 2023

Published online: 01 March 2023

Check for updates

Md Mamun Monir¹, Mohammad Tarequl Islam¹, Razib Mazumder², Dinesh Mondal², Kazi Sumaita Nahar¹, Marzia Sultana¹, Masatomo Morita³, Makoto Ohnishi³, Anwar Huq⁴, Haruo Watanabe³, Firdausi Qadri¹, Mustafizur Rahman¹, Nicholas Thomson^{5,6}, Kimberley Seed⁷, Rita R. Colwell^{4,8}, Tahmeed Ahmed⁹ & Munirul Alam¹✉

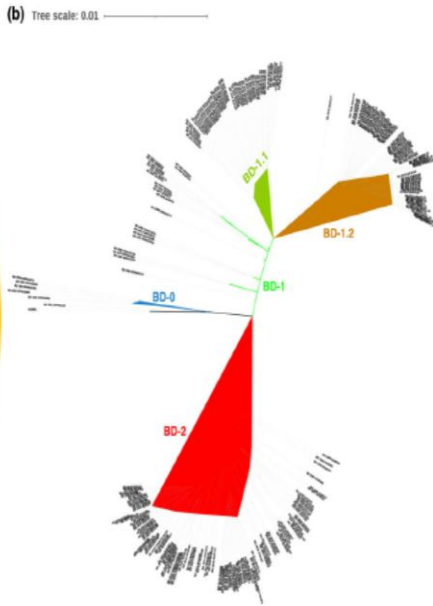
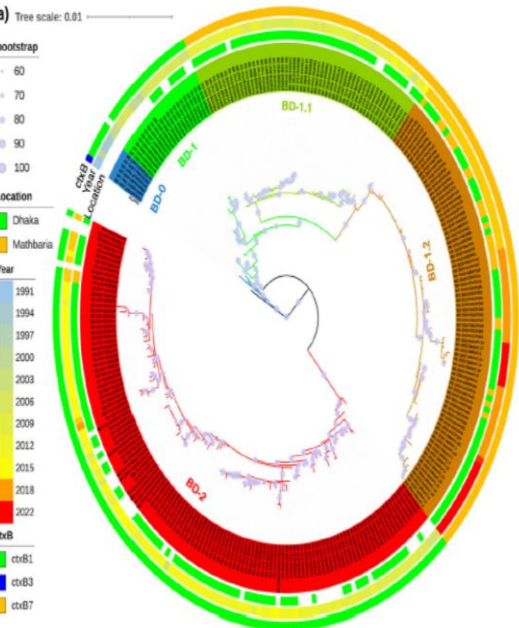


Fig. 2 | Phylogenetic relatedness of *V. cholerae* O1 DT104 strains isolated during the 2022 cholera outbreak in Dhaka, Bangladesh. **a** Maximum likelihood phylogenetic tree of representative strains of the clade, where BD-1, BD-1.1, and BD-1.2 clustered with

RESEARCH Open Access



Vibrio cholerae O1 associated with recent endemic cholera shows temporal changes in serotype, genotype, and drug-resistance patterns in Bangladesh

Fatema Tuz Jubyda^{1,2†}, Kazi Sumaita Nahar^{1†}, Indrajeet Barman^{1†}, Fatema-Tuz Johura¹, Mohammad Tarequl Islam¹, Marzia Sultana¹, Wali Ullah¹, Jarin Tasnim¹, Sahitya Ranjan Biswas¹, Md Mamun Monir¹, Christine Marie George³, Andrew Camilli⁴, Niyaz Ahmed^{1,5}, Allen G. Ross^{1,6}, John D. Clemens¹ and Munirul Alam^{1*}

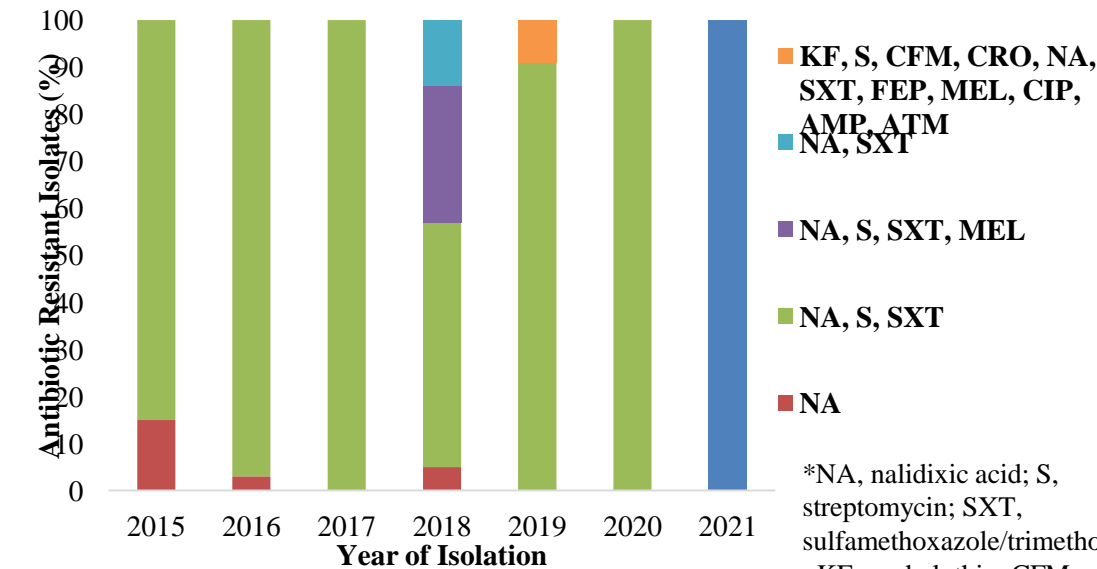


Fig 3: Antibiotic resistance profile of *V. cholerae* O1 strains isolated from Dhaka, 2015-2021

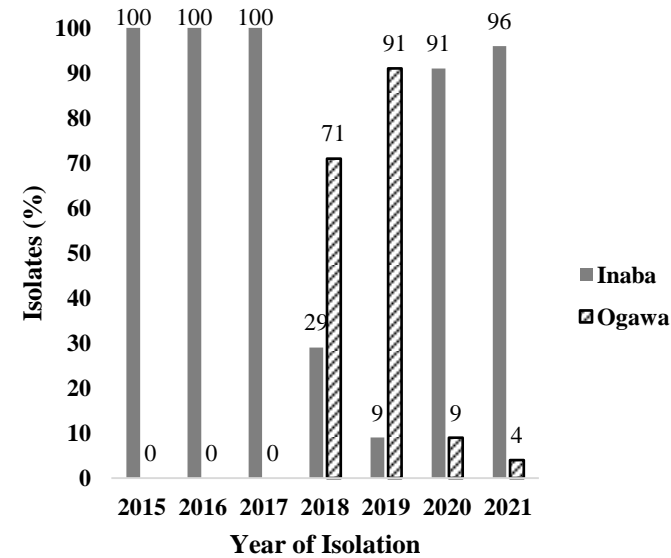


Figure 1: Temporal distribution of serotypes among *Vibrio cholerae* O1 strains isolated from Dhaka, 2015-2021

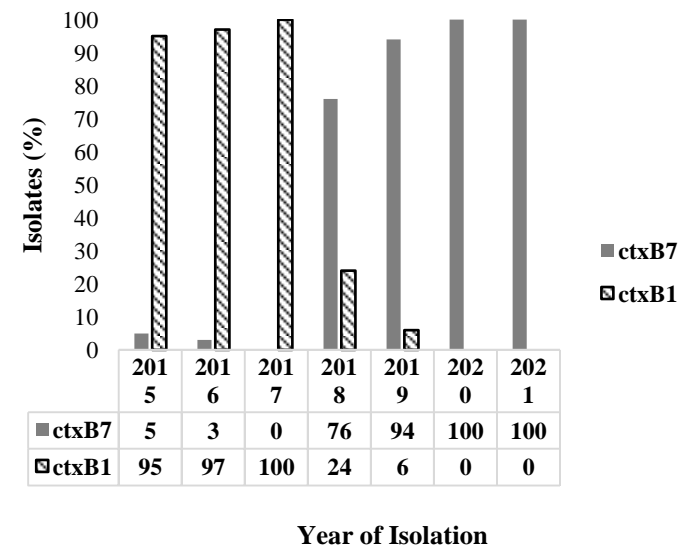
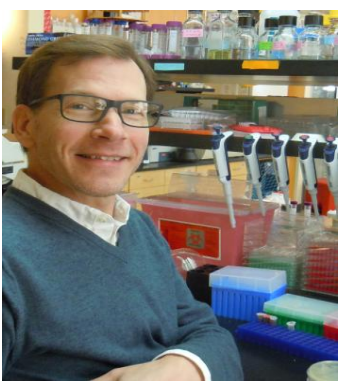


Figure 2: Temporal distribution of *ctxB* alleles among *Vibrio cholerae* O1 strains isolated from Dhaka, 2015-2021

*NA, nalidixic acid; S, streptomycin; SXT, sulfamethoxazole/trimethoprim; KF, cephalothin; CFM, cefixime; CRO, ceftriaxone; FEP, cefepime; MEL, levofloxacin; CIP, ciprofloxacin; AMP, ampicillin; ATM, aztreonam.



Prof. Andrew Camilli,
Tufts



Dr. Mimi Yen PhagePro



Dr. Kim Seed, Univ.
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Prof. Yan
Boucher
Natl. Univ.
Singapore



Dr. Tania Dottorini
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THANK YOU



Canada



National Institutes of Health
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GATES foundation