AMERICAN UNIVERSITY OF BEIRUT
FACULTY OF MEDICINE

WHO CC for Reference & Research on Bacterial Pathogens

CHOLERA GENOMIC SURVEILLANCE IN LEBANON

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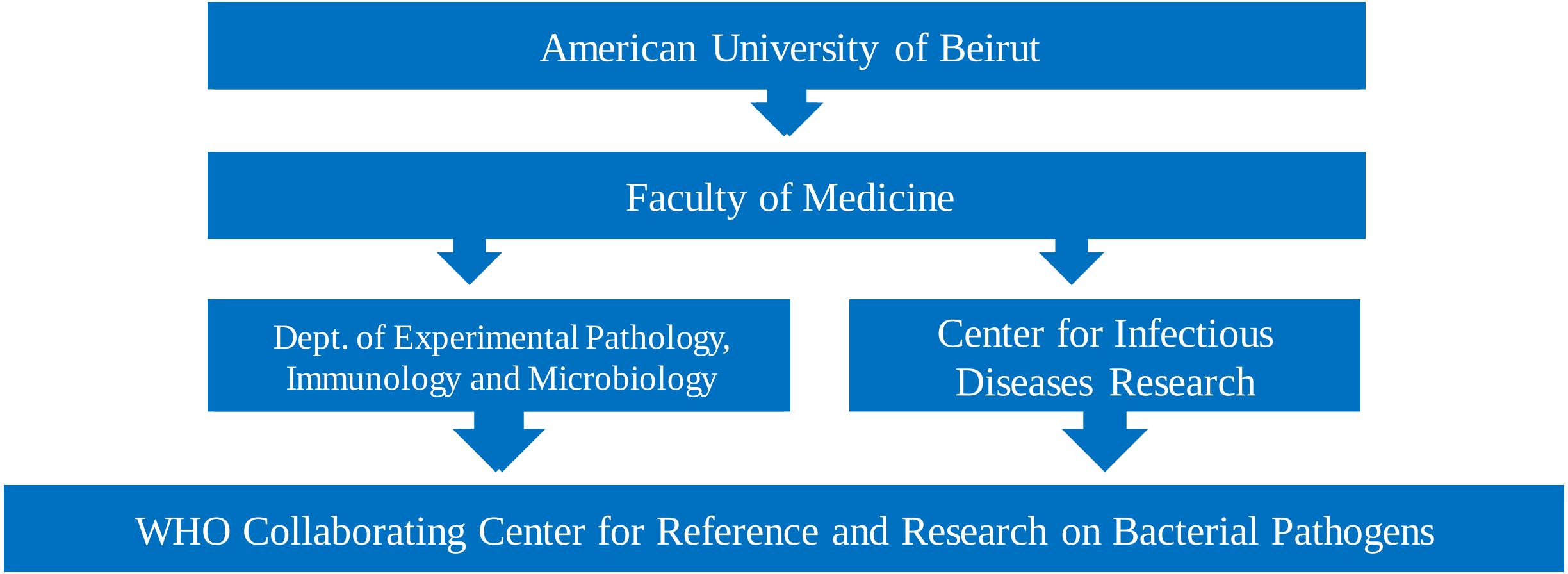
Co-Director Center for Infectious Diseases Research (CIDR)

Organizational Structure



Objectives of Laboratory Testing for Cholera

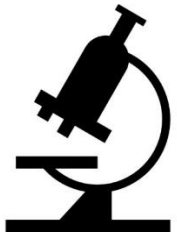
- Timely, accurate, and reliable laboratory results are critical for detecting cases and confirm outbreaks of cholera that may spread rapidly if not contained.

- The objectives of the laboratory diagnosis of cholera include:
  - Confirming alerts and declaring outbreaks
  - Monitoring antibiotic susceptibility
  - Characterizing the circulating strains
  - Identifying changes in the virulence
  - Supporting epidemiologic investigations
  - Declaring the end of an outbreak.

- At least one laboratory in the country should be operational and capable of isolating and identifying *Vibrio cholerae* by culture -or PCR if available- and performing antibiotic susceptibility testing in the country.

- The designated reference laboratory should ensure provision of transport media and reagents, training of technicians and monitoring the quality of examinations.

Laboratory Tests for Cholera

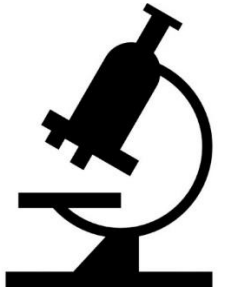


- **Traditional Phenotypic Detection Methods of *Vibrio cholerae***
  - **RDTs & Culture**

- **Traditional Molecular Detection Methods of *Vibrio cholerae***
  - **Nested Multiplex PCR Panels**
- **Biotyping**
- **Antimicrobial Susceptibility Testing**

- **Traditional & Advanced Genotyping Methods**
  - **Pulsed-field Gel Electrophoresis (PFGE)**
  - **Whole Genome Sequencing (WGS)**

Traditional Detection Methods



Example: *Vibrio cholerae*

- **Rapid diagnostic kits for *V. cholera* identification are available**

CRYSTAL® VC RAPID DIAGNOSTIC TEST (RDT)

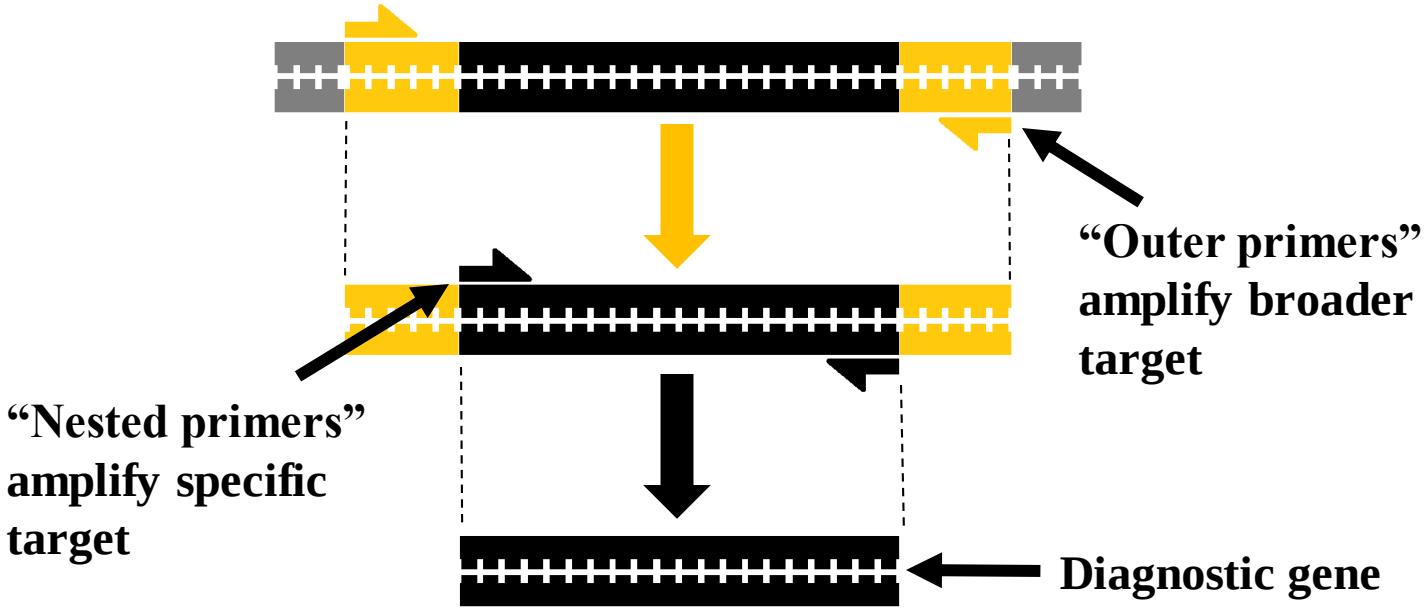
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

- **Utilizes nitrocellulose strips coated with anti-O1 and anti-O139 antibodies**
- **Provides a result within 15-30 minutes directly from patient stool sample**
- **Can be performed on site in the field during an outbreak**
- **Identifies toxigenic *V. cholerae* serogroups O1 and O139**

Traditional Molecular Techniques

Multiplex Nested PCR:

- Genomic DNA of infecting pathogens can be extracted directly from the patient sample
- Detection of diagnostic genes using Simplex Nested PCR can identify the infecting pathogen



- Applying Multiplex Nested PCR would identify several pathogens in a single PCR run
- This highly sensitive identification process requires less than 24hrs

CHOLERA SURVEILLANCE IN LEBANON

Culture on TCBS

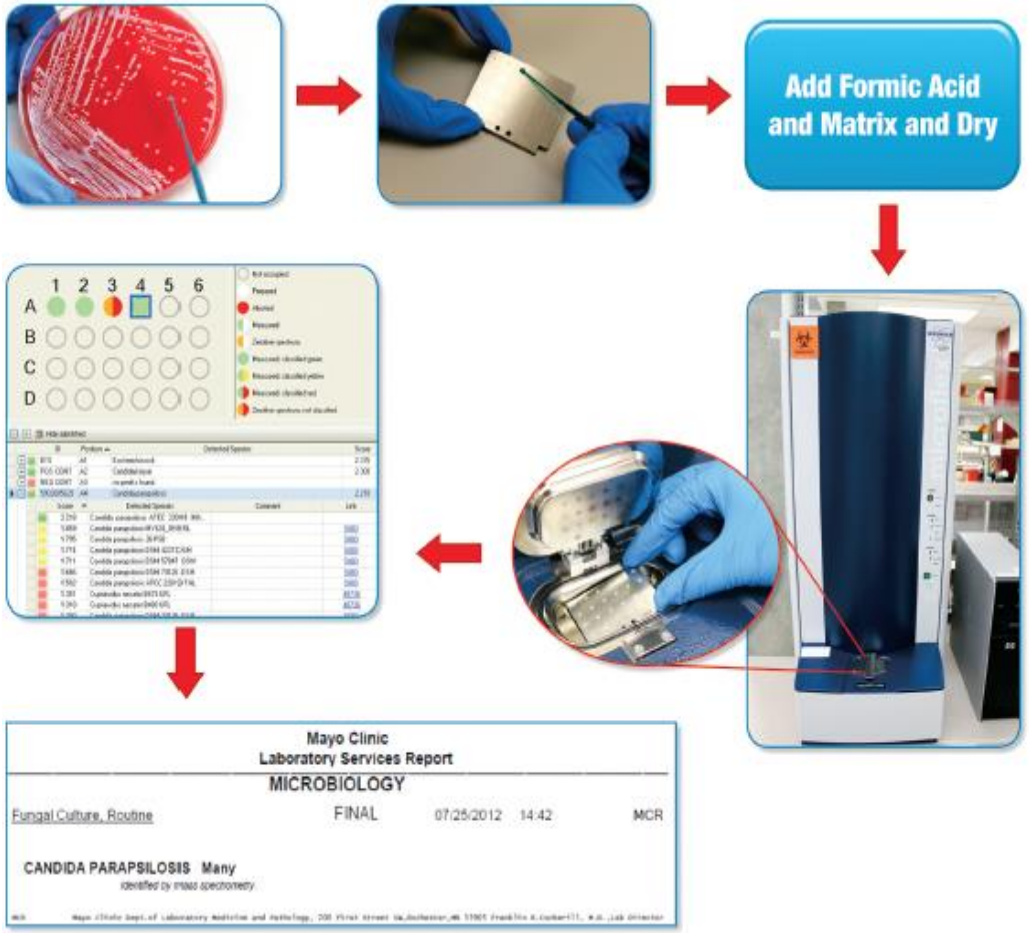
Example: *Vibrio cholerae*

- **Traditional isolation of *V. cholerae* from fecal specimens involves several steps**

Culture in AUB Bacteriology and Molecular Microbiology Lab EPIM WHO CC

Biotyping using MALDI-TOF

- Using a plastic or wooden stick, loop, or pipette tip, a colony is picked from a culture plate to a spot on a MALDI-TOF MS target plate.
- One or many isolates may be tested at a time.
- In this example, cells are treated with formic acid on the target plate.
- The spot is overlain with 1–2 μL of matrix and dried.
- The plate is placed in the ionization chamber of the mass spectrometer.
- A mass spectrum is generated and compared against a database of mass spectra by the software, resulting in identification of the Organism.



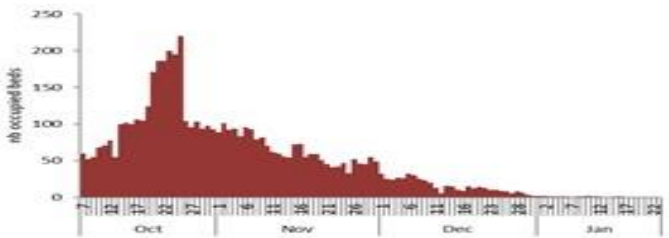
REPUBLIC OF LEBANON
MINISTRY OF PUBLIC HEALTH
EPIDEMIOLOGICAL SURVEILLANCE PROGRAM

Cholera Surveillance Report
24 Jan 2023

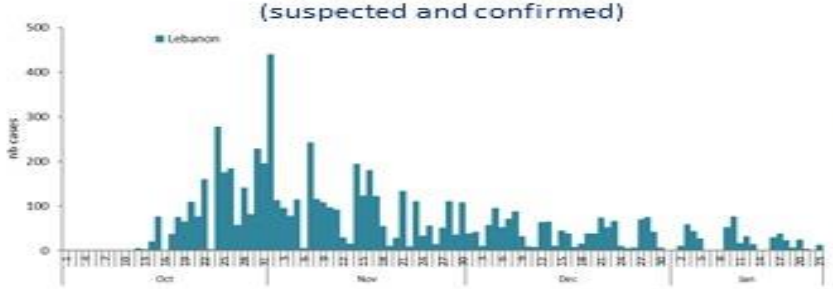
Call Centers
1760 (Red Cross)
1787 (MOPH)

All cases (suspected and confirmed)		Confirmed Cases		Deaths (confirmed)	
New (past 24 h)	Cumulative	New (past 24 h)	Cumulative	New	Cumulative
13	6268	0	671	0	23

Occupied hospital beds
(suspected and confirmed)

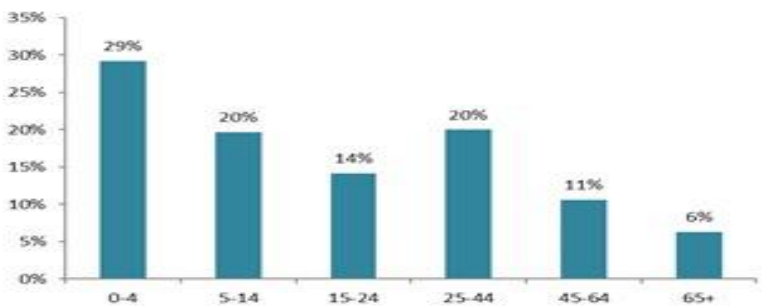


By date of report
(suspected and confirmed)

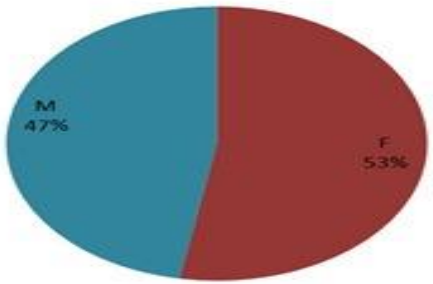


By locality (confirmed cases)

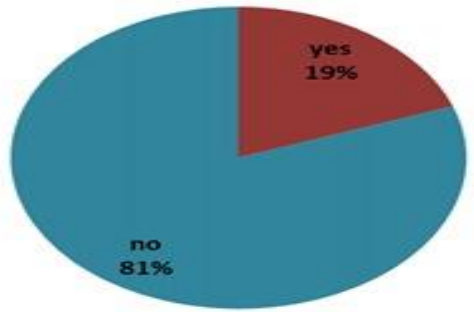
By age group
(suspected and confirmed cases)



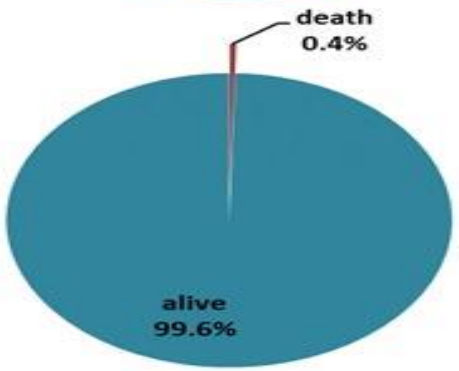
By Sex
(suspected and confirmed)



By Hospital Admission
(suspected and confirmed)



By Outcome
(suspected and confirmed)



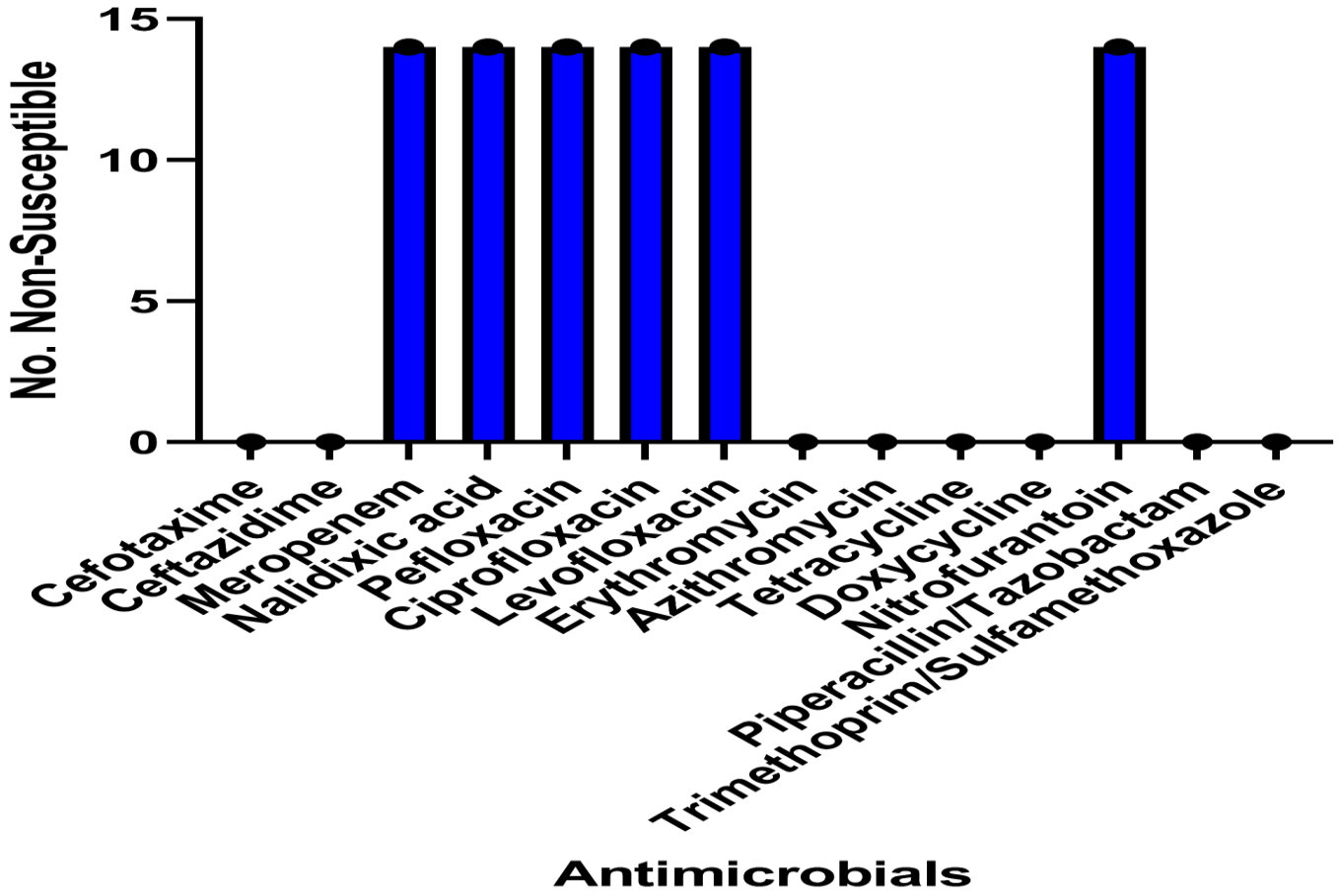
Antimicrobial Susceptibility Testing

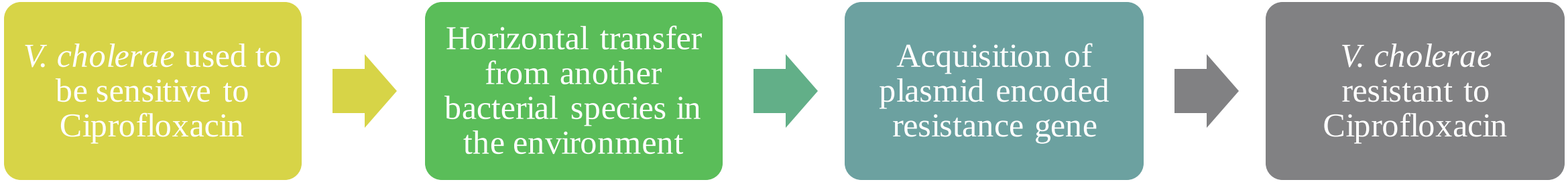
- We have so far completed the Antimicrobial Susceptibility testing using **disc diffusion (DD)** assay that was validated with **broth microdilution (BMD)** assay on 68/300 pure cultures showing *Vibrio cholerae* isolates.
- Both susceptibility tests were repeated 3 times and they were reproducible and results matched. Phenotypic tests results matched also genotypic results.

Antimicrobial Susceptibility Testing

Sample ID		AST													
	Cefotaxime	Ceftazidime	Piperacillin/tazobactam	Meropenem	Nalidixic acid	Pefloxacin	Ciprofloxacin	Levofloxacin	Erythromycin	Azithromycin	prim-sulfamet	Tetracycline	Doxycycline	O129	Nitrofurantoin
S1	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S2	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S3	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S4	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S5	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S6	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S7	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S8	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S9	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S10	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S11	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S12	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S13	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R
S14	S	S	S	R	R	R	I	I	S	S	S	S	S	R	R

Antimicrobial Susceptibility Testing





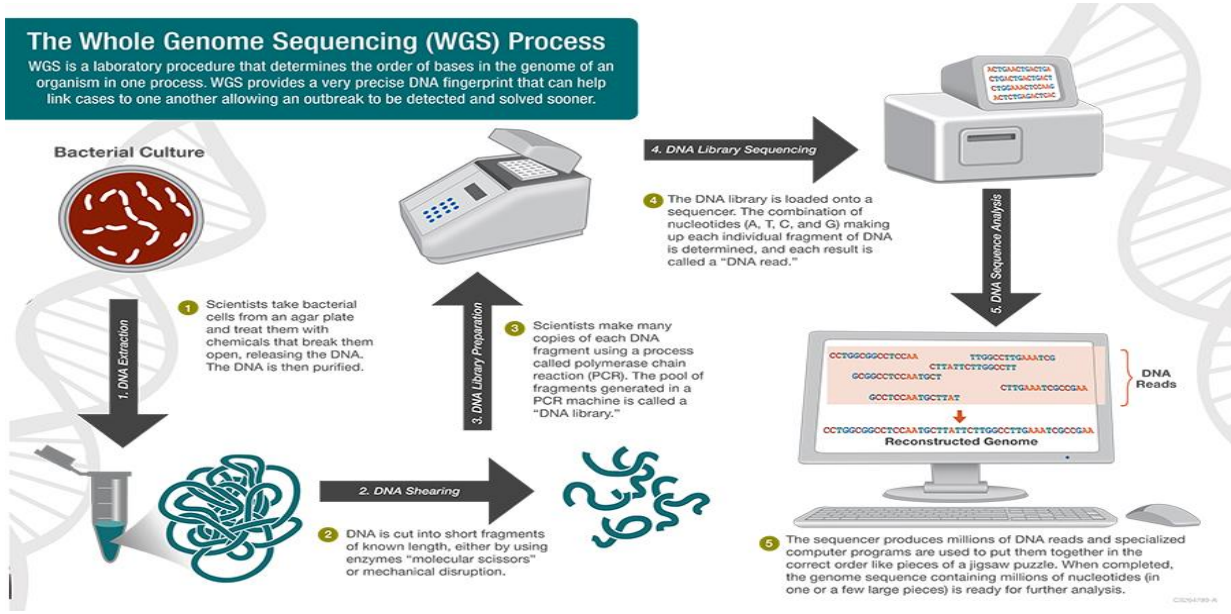
- **Azithromycin** would be for now the main recommendation if available.
- If *V. cholerae* proved to be sensitive to doxycycline it would be helpful (as it can be treated with a single dose). Doxycycline testing is another option.

GENOMIC SURVEILLANCE IN LEBANON

Whole Genome Sequencing (WGS)

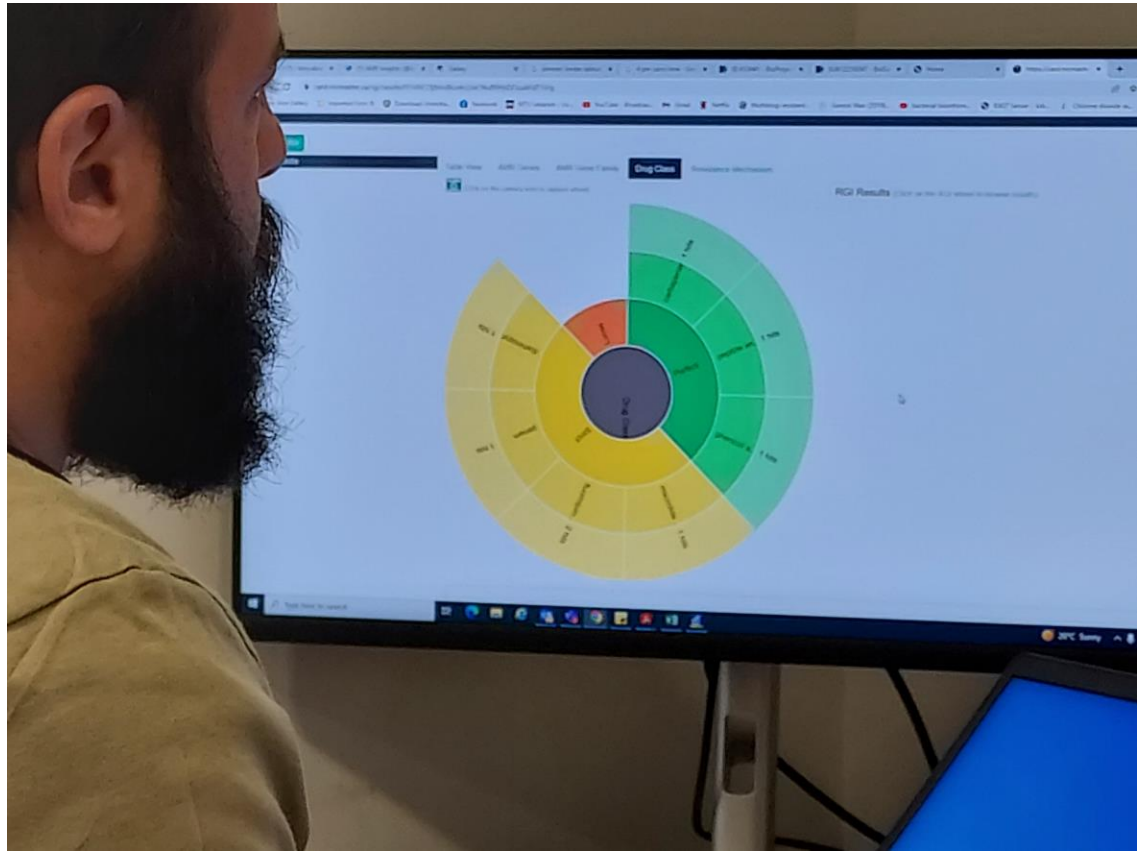
- Capable of the concurrent detection and full characterization of *Vibrio spp*.
- Relies on genomic DNA extracted from bacterial isolate
- Identifies serogroups, biotypes, virulence determinants, antimicrobial resistance genes, and the presence of plasmid and/or bacteriophages in a single run
- Provides the highest resolution genomic typing method that can compare samples to source as well as construct phylogenic trees

- The future of laboratory-based surveillance of microbes implicated in disease outbreaks



https://www.cdc.gov/pulsenet/pathogens/wgs.html

NGS in AUB Bacteriology and Molecular Microbiology Lab EPIM WHO CC

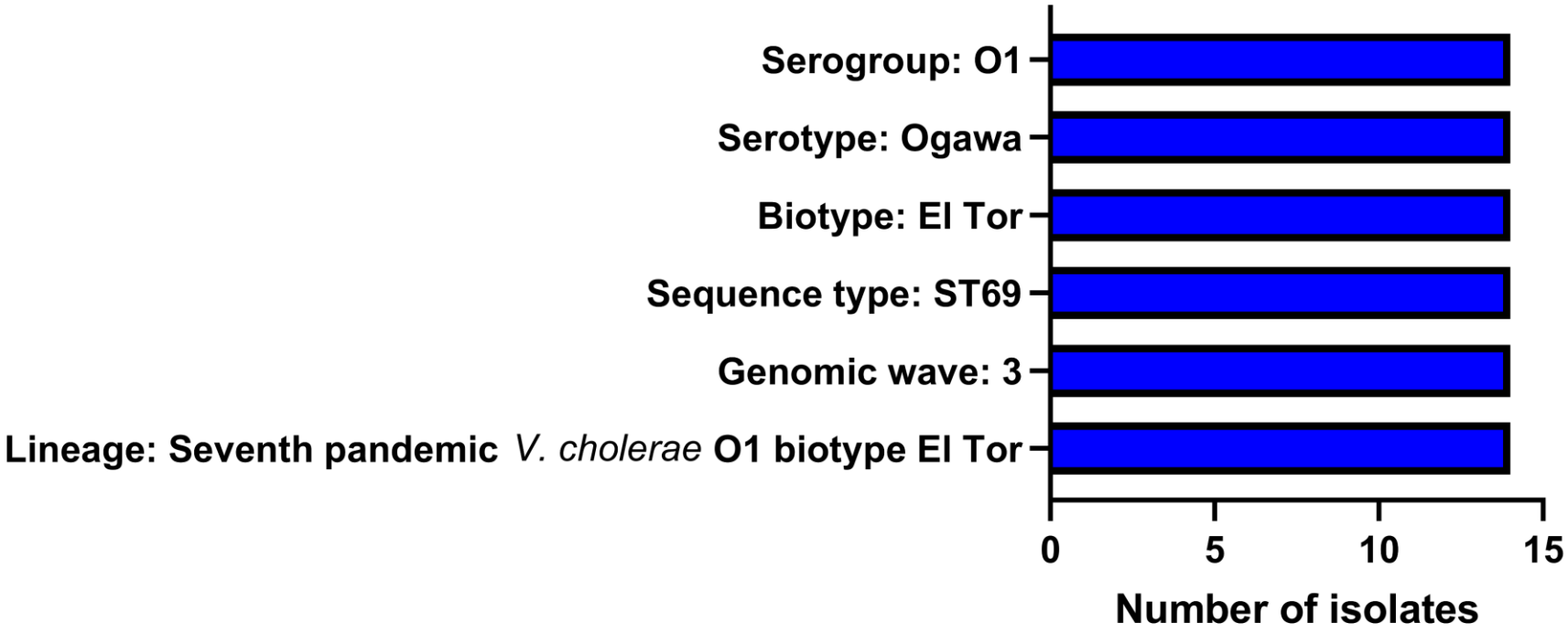


Strain Typing Using NGS

14 representative of 68 samples sequenced

Sample ID	WGS									
	Serogroup	Serotype	Biotype	Sequence Type	Genomic Wav	lineage	wbeT	rfbO1	tcpA	ctxB
S1	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S2	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S3	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S4	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S5	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S6	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S7	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S8	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S9	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S10	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S11	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S12	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S13	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7
S14	O1	Ogawa	El Tor	ST69	3	Seventh pandemic *V. cholerae* O1 biotype El Tor	wt Eltor	wt	A266G	ctxB7

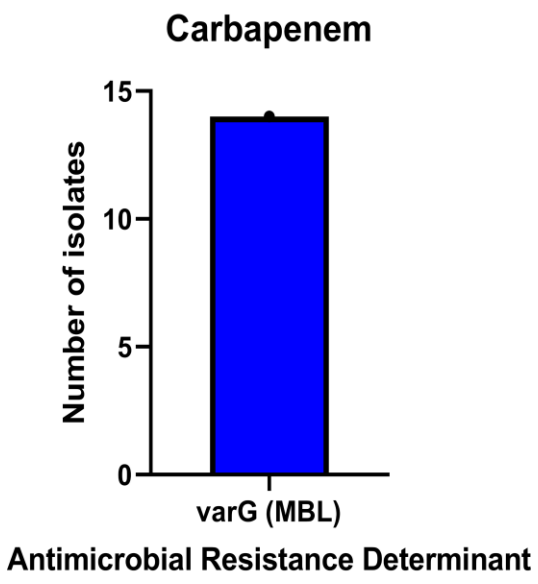
Strain Typing Using NGS

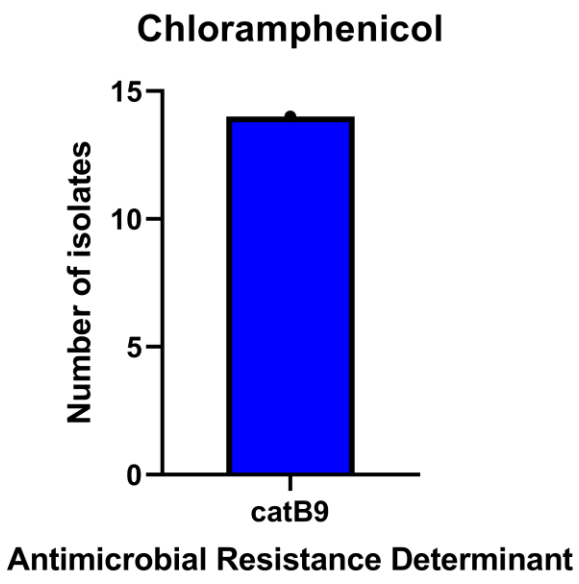


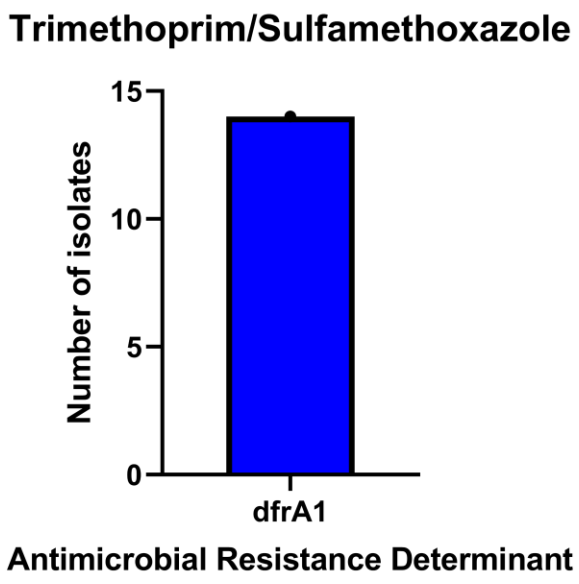
Antimicrobial Resistance Determinants (Genes)

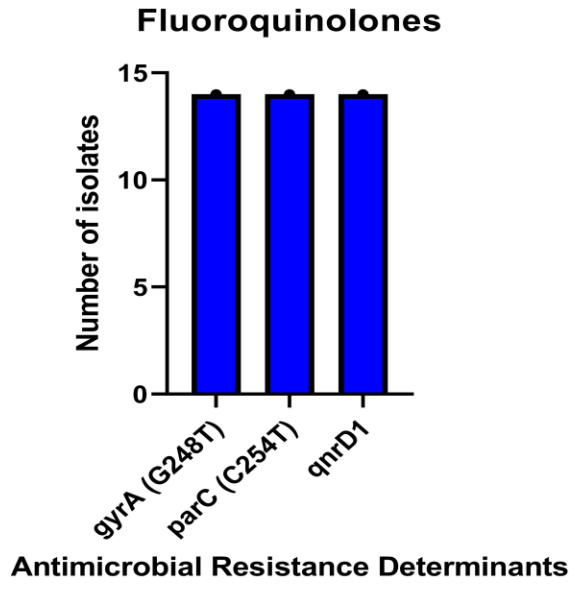
Sample ID	QRDR					Colistin						Nitrofurantoin			
	gyrA	parC	catB9	dfrA1	plasmids	VC_0202	VC_0303	VC_1320	VC_1577	VC_1578	VC_1579	VCA0637	VC0715	VSP-II	ICEVchInd5
S1	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S2	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S3	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S4	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S5	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S6	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S7	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S8	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S9	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S10	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S11	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S12	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S13	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)
S14	S83I (G248T)	85L (C254T	catB9	dfrA1	/	wt	wt	G265A	wt	wt	wt	C13T	C505T	VSP-II (Del VC_0495-VC_0512)	ICEVchInd5 (Del strAB_sul2_floR)

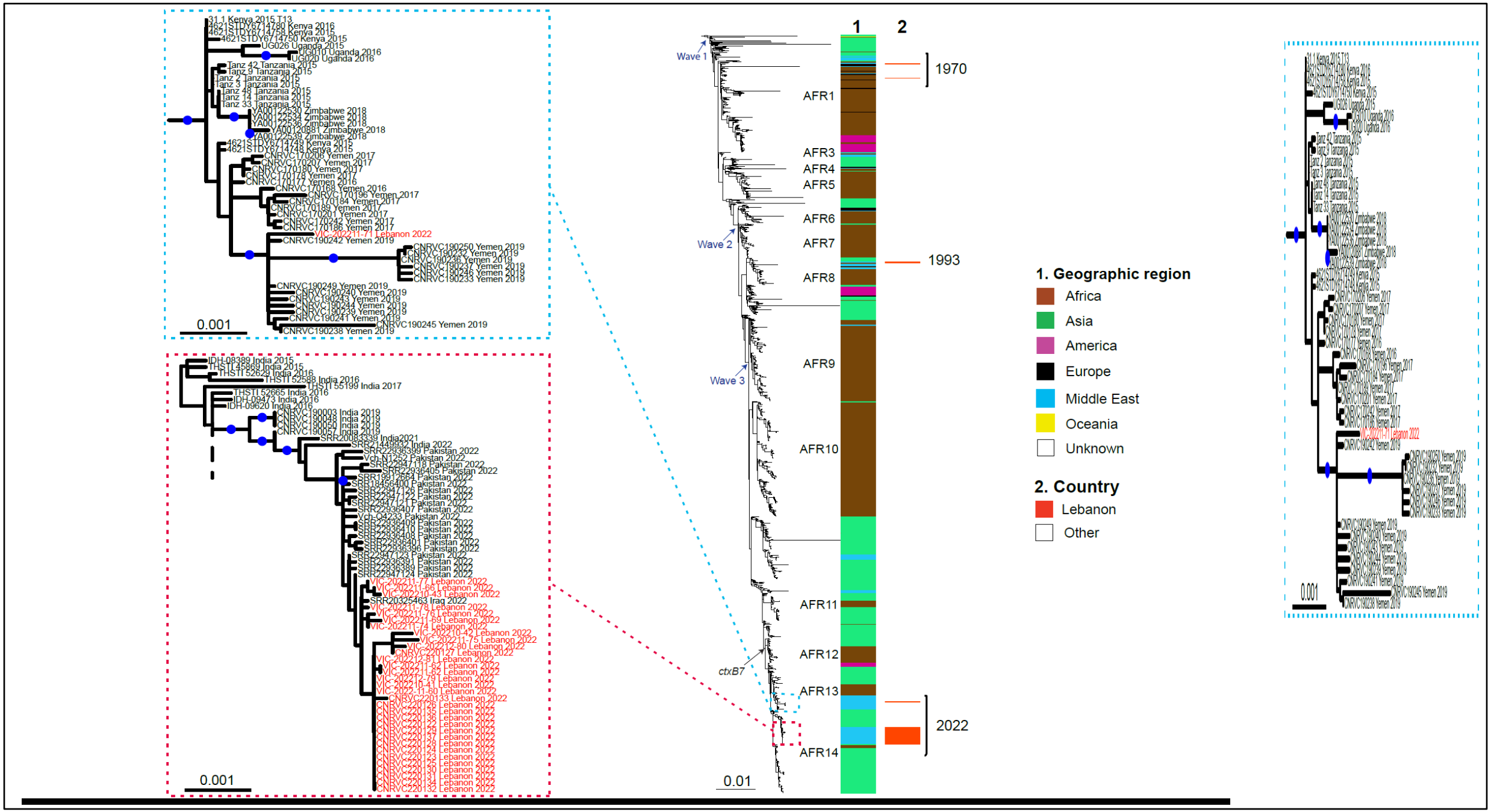
Antimicrobial Resistance Determinants










1
2
Wave 1
Wave 2
Wave 3
ctxB7
AFR1
AFR3
AFR4
AFR5
AFR6
AFR7
AFR8
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AFR10
AFR11
AFR12
AFR13
AFR14
1970
1993
2022
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0.001
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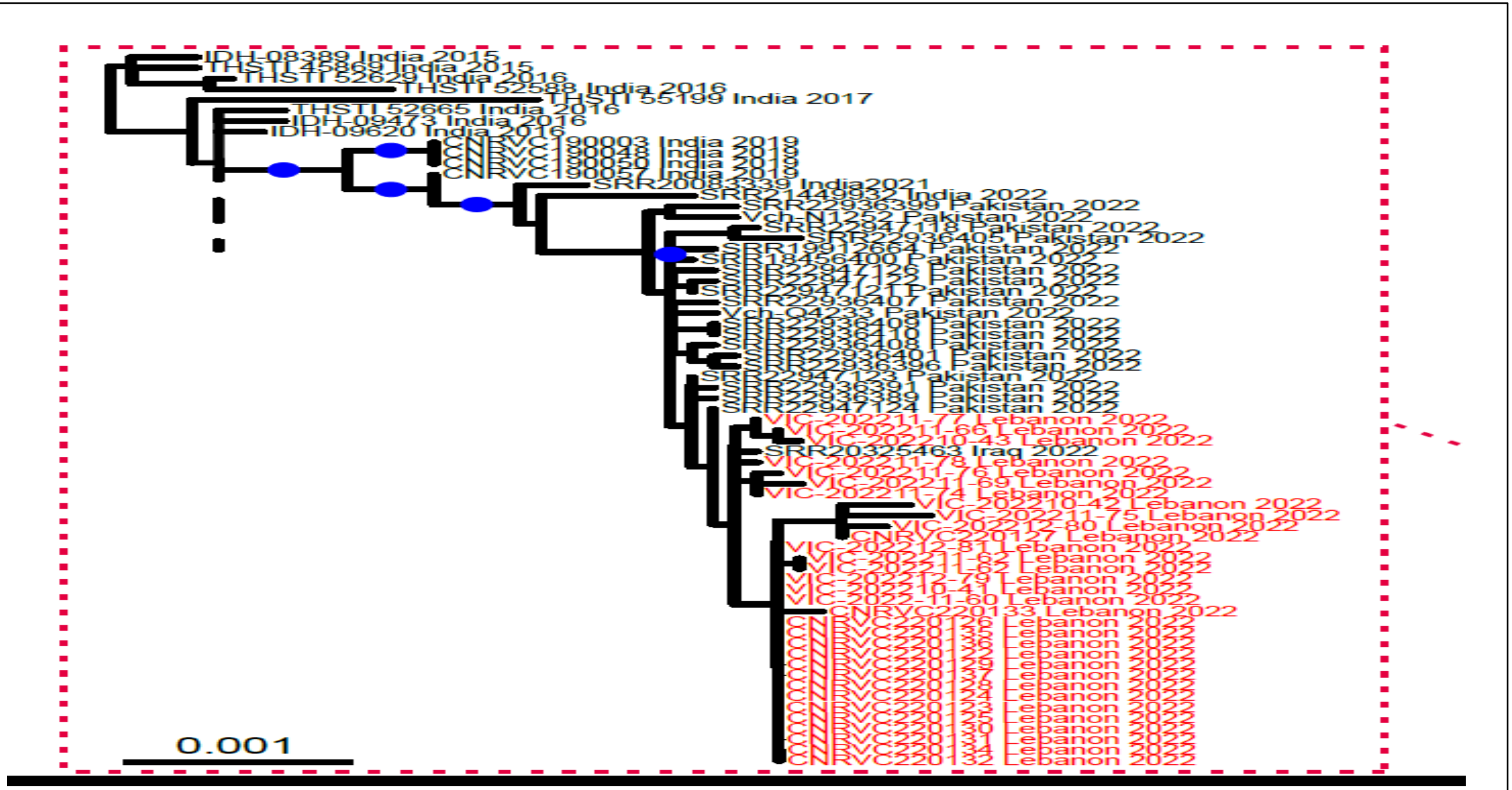
America
Europe
Middle East
Oceania
Unknown
2. Country
Lebanon
Other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THSTI 45869 India 2015
THSTI 52629 India 2016
THSTI 52588 India 2016
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THSTI 52665 India 2016
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IDH-09620 India 2016
CNRVC190003 India 2019
CNRVC190048 India 2019
CNRVC190050 India 2019
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CNRVC220132 Lebanon 2022
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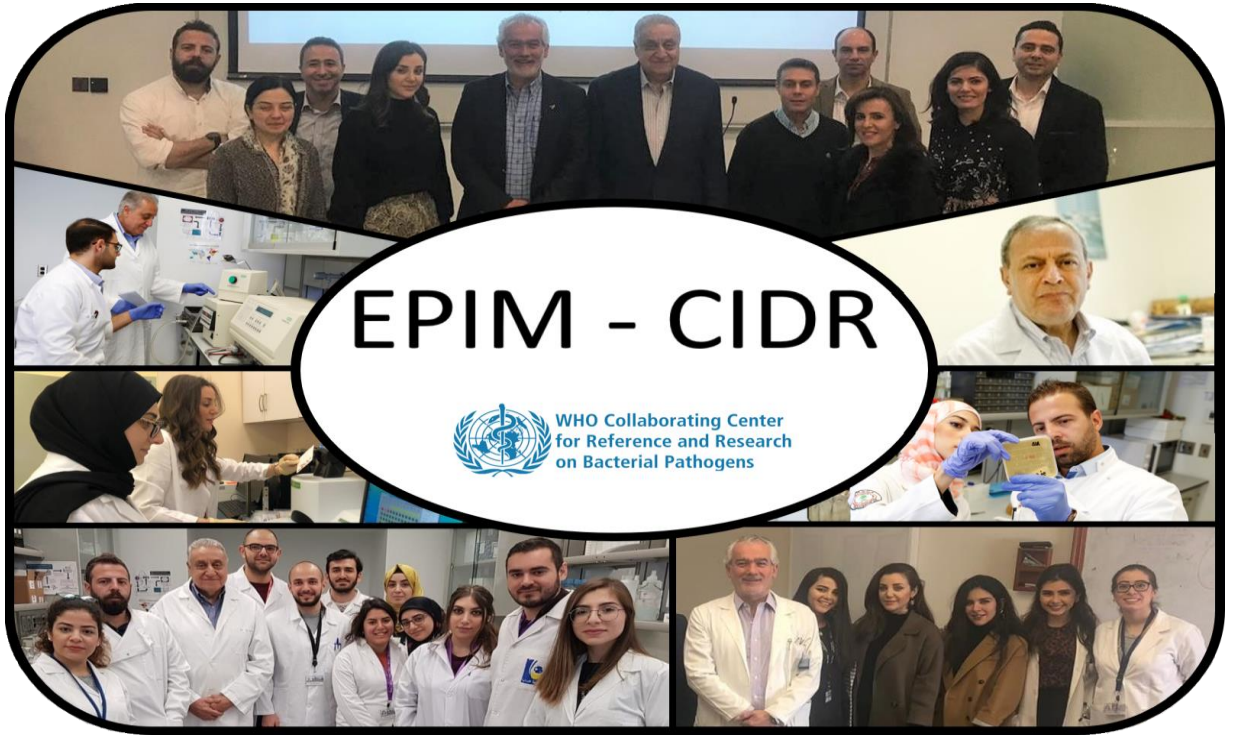
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THANK YOU