

Supplementary Information

Surveillance of *Vibrio cholerae* in a non-sewered sanitation refugee camp setting using culture methods: Dzaleka camp, Malawi

Brandie Banner Shackelford ^{a,b*}, Petros Chigwechokha ^c, Ernest Chilalika ^a, Lucious Ziba ^d, Christopher Misomali ^d, Mphatso Kanjiru ^d, Patrick Buleya ^d, Ruth Lusungu Nyirenda ^c, Marlene K. Wolfe ^b, Rochelle H. Holm ^{e,*}

^aThe United Nations High Commissioner for Refugees, Chilanga Drive, Area 10, Plot 441/442, P.O. Box 30230, Lilongwe, Malawi

^bThe Gangarosa Department of Environmental Health, Rollins School of Public Health, 1518 Clifton Rd. NE, Emory University, Atlanta, GA 30322, United States

^cDepartment of Biological Sciences, Malawi University of Science and Technology, P.O. Box 5196, Limbe, Malawi

^dThe Public Health Institute of Malawi, P/Bag 65, Lilongwe, Malawi

^eCenter for Healthy Air Water and Soil, Christina Lee Brown Envirome Institute, School of Medicine, University of Louisville, 302 E. Muhammad Ali Blvd., Louisville, KY 40202, United States

Corresponding authors:

Brandie Banner Shackelford

The United Nations High Commissioner for Refugees, Chilanga Drive, Area 10, Plot 441/442, P.O. Box 30230, Lilongwe, Malawi

The Gangarosa Department of Environmental Health, Rollins School of Public Health, 1518 Clifton Rd. NE, Emory University, Atlanta, GA 30322, United States

brandie.shackelford@emory.edu

Rochelle H. Holm

Center for Healthy Air Water and Soil, Christina Lee Brown Envirome Institute, School of Medicine, University of Louisville, 302 E. Muhammad Ali Blvd., Louisville, KY 40202, United States

rochelle.holm@louisville.edu

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Table S1. Number of public latrine users at sample sites in Dzaleka camp, Malawi. Forty-nine latrine counting periods were conducted. The site with the highest use per stance was Yetu Community Radio, with a mean of 10.6 latrine users per hour. The site with the lowest use per stance was the Old Transit Centre, with a mean of 1.8 users per hour. Across sampling sites, there was an average of 5.5 users per stance per hour of counting which indicates that weekly sampling campaign included a high level of fresh feces.

Site	Description	Publicly accessible pit latrine stances at site	Unique days counting was conducted	Average number of users per stance per hour counting was conducted
PT1	Reception Centre	10	7	2.6
PT2	Dzaleka Health Centre	4	7	3.0
PT3	Yetu Community Radio	2	8	10.6
PT4	Old Transit Centre	5	8	1.8
PT5	Tuesday Market	6	7	6.9
PT6	Bars	1	7	7.3
PT7	Primary School	11	5	6.7

Table S2. Description of pit latrine sampling sites (n = 7) in Dzaleka camp, Malawi.

Site	Description	# publicly accessible latrine stances	Closest water source^a	Site frequented by members of host community
PT1	Reception Centre	10	Tap water within 20 meters of latrines ^b	No
PT2	Dzaleka Health Centre	4	Unchlorinated tap water within 70 meters of latrines	Yes
PT3	Yetu Community Radio	2	Unchlorinated tap water within 10 meters of latrines	No
PT4	Old Transit Centre	5	Handpump borehole within 80 meters of latrines	No
PT5	Tuesday Market	6	Handpump borehole within 200 meters of latrines	Yes
PT6	Bars	1	Handpump borehole within 100 meters of latrines	Yes
PT7	Primary School	11	Handpump borehole within 50 meters of latrines	Yes

^a Dzaleka Camp has handpump and motorized boreholes. All water tap water in Dzaleka camp is provided intermittently for a few hours per day due to water rationing.

^b The source of the water supply for the reception center was changed during the study. In March 2024 it was switched to a chlorinated source.

Table S3. Details of weekly sampling and analysis of wastewater and environmental surveillance for *Vibrio cholerae* in Dzaleka camp, Malawi.

Date/Site	PT1	PT2	PT3	PT4	PT5	PT6	PT7	Site of pump truck sample
5 March 2024	X				X	X	X	None
11 March 2024	X	X	X	X	X	X	X	None
18 March 2024	X	X	X	X	X	X	X	None
25 March 2024	X	X	X	X	X	X	X	PT1
2 April 2024	X	X	X	X	X	X	X	PT1
8 April 2024	X	X	X	X	X	X	X	PT1
15 April 2024	X	X	X	X	X	X	X	PT4
22 April 2024	X	X	X	X*	X	X	X	PT5
29 April 2024	X	X	X	X	X	X	X	PT1
6 May 2024	X	X	X	X	X	X	X	PT1
13 May 2024	X	X	X	X	X	X	X	PT1
20 May 2024	X	X	X	X	X	X	X	PT7
27 May 2024	X	X	X	X	X	X	X	PT7
3 June 2024	X	X	X	X	X	X	X	PT1
10 June 2024	X	X	X	X	X	X	X	PT1
18 June 2024	X	X	X	X	X	X	X	PT4
24 June 2024	X	X	X	X	X	X	X	PT5
1 July 2024	X	X	X	X	X	X	X	PT2
9 July 2024	X	X	X	X	X	X	X	PT6

*Collected two samples from two different latrine blocks at one site (PT4) on April 15, 2024.

Table S4. Confirmation analysis method used weekly in the Dzaleka wastewater surveillance for *Vibrio cholerae* at the Public Health Institute of Malawi.

Day of sample	Confirmation analysis method
5 March 2024	VITEK MS
11 March 2024	VITEK MS
18 March 2024	VITEK MS
25 March 2024	VITEK MS
2 April 2024	API 20E
8 April 2024	VITEK MS
15 April 2024	VITEK MS
22 April 2024	VITEK MS
29 April 2024	VITEK MS
6 May 2024	VITEK MS
13 May 2024	VITEK MS
20 May 2024	VITEK MS
27 May 2024	VITEK MS
3 June 2024	VITEK MS
10 June 2024	VITEK MS
18 June 2024	API 20E
24 June 2024	API 20E
1 July 2024	API 20E
9 July 2024	API 20E

Table S5. Results of VITEK MS pathogen identification (n = 107) from the analysis conducted at the Public Health Institute of Malawi that had full agreement among replicates.

Number of samples with identification	Pathogen(s)
60 (41%)	<i>Escherichia coli</i> , <i>Providencia rettgeri</i>
37 (26%)	<i>Klebsiella pneumoniae</i>
26 (18%)	<i>Klebsiella oxytoca</i>
11 (7.8%)	<i>Enterbacter cloaceae</i>
10 (7.0%)	<i>Proteus mirabilis</i>
8 (5.6%)	<i>Proteus vulgaris</i> , <i>Raoultella ornithinolytica</i>
7 (4.9%)	<i>Serratia marcescens</i>
6 (4.2%)	<i>Pantoea spp</i>
5 (3.5%)	<i>Providencia alcalifaciens</i>
4 (2.8%)	<i>Acinetobacter iwoffi</i> , <i>Citrobacter freundii</i> , <i>Enterobacter aerogenes</i> , <i>Shewanella algae</i>
3 (2.1%)	<i>Aeromonas punctata</i> , <i>Enterococcus faecalis</i> , <i>Myroides spp</i> , <i>Vibrio alginolyticus</i>
2 (1.4%)	<i>Enterobacter gergoviae</i> , <i>Enterococcus hirae</i> , <i>Morganella morganii</i> , <i>Serratia odorifera 1</i> , <i>Serratia odorifera</i> , <i>Staphylococcus sciuri</i> , <i>Staphylococcus spp</i> , <i>Staphylococcus xylosus</i> , <i>Vibrio parahaemolyticus</i>
1 (0.7%)	<i>Bacillus altitudinis</i> , <i>Bacillus subtilis</i> , <i>Comamonas testosteroni</i> , <i>Cronobacter spp</i> , <i>Cryptococcus laurentii</i> , <i>Enterobacter asburiae</i> , <i>Gardnerella vaginalis</i> , <i>Klebsiella aerogenes</i> , <i>Klebsiella variicola</i> , <i>Ornithobacterium rhinotracheale</i> , <i>Photobacterium damsela</i> , <i>Pseudomonas oryzae</i> , <i>Serratia fonticuli</i> , <i>Serratia liquefaciens</i> , <i>Serratia rubida</i> , <i>Vibrio metschnikovii</i> , <i>Yersinia frederiksenii</i>

Supplementary Text. Suggestions for operationalizing WES in refugee camps.

Preparatory considerations/actions

- **Acknowledge the political sensitivity of refugee health data and actively implement mechanisms to protect data.** We had all stakeholders sign nondisclosure agreements for the project period and only shared data in password-protected files.
- **Select pathogens based on their potential to directly influence public health and water, sanitation, and hygiene (WASH) responses, rather than solely contributing to knowledge.**
- **Foster relationships with local academic and government partners, prioritizing those with technical expertise in WES.** In our experience, academic and government partnerships were critical to securing institutional review board approval, developing sampling and laboratory protocols, procuring supplies, and conducting research team training.
- **Identify which supplies can be locally procured and which needs to be imported as soon as possible.** We were able to locally procure many supplies, including fabricating a sampling device with a local welder.
- **Engage refugees and asylum seekers about the purpose and importance of surveillance.** They can also help identify the most frequently used public latrines to increase the anonymity and representativeness of the samples.
- **Secure dedicated funding for WES.** Given the declining scope of humanitarian funds, WES may not be feasible without dedicated funding. Seek earmarked funding specifically for conducting WES from funders with innovation and/or research agendas.

During sampling and analysis

- **Recruit incentivized refugees for sample collection.** It is critical to ensure adequate protection through training as well as access to vaccination and PPE. The team should receive training on donning and doffing PPE, maintaining sample integrity, and site cleanup after collection.
- **Share password-protected data and discuss its implications in weekly meetings with stakeholders, including laboratory partners, camp management, health center staff, and WASH partners.** The minimum metadata should include site identifiers, sample collection date/time, sample delivery date/time, culture, and confirmation results in a password-protected Excel file.