Highly Resistant Cholera Outbreak Strain in Zimbabwe

Tapfumanei Mashe, Daryl Domman, Andrew Tarupiwa, Portia Manangazira, Isaac Phiri, Masunda Kudzai, Prosper Chonzi, Elisabeth Njamkepo, Masindi Ramudzulu, Sekesai Mtapuri-Zinyowera, et al.

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Mashe Tapfumanei*, MSc
Ministry of Health and Child Care, Zimbabwe

Daryl Domman*, Ph.D
University of New Mexico Health Sciences Center, Albuquerque, New Mexico, USA

Tarupiwa Andrew, MSc
Ministry of Health and Child Care, Zimbabwe

Manangazira Portia, M.D., M.P.H
Phiri Isaac, M.D., M.P.H
Ministry of Health and Child Care, Zimbabwe

Masunda Kudzai, M.D., M.P.H
Chonzi Prosper, M.D., M.P.H
Beatrice Road Infectious Diseases Hospital, Harare, Zimbabwe

Elisabeth Njamkepo, Ph.D
Institut Pasteur, Paris, France

Masindi Ramudzulu, B.Sc (Hons)
National Institute for Communicable Diseases, South Africa

Mtapuri-Zinyowera Sekesai‡, Ph.D
Ministry of Health and Child Care, Zimbabwe

Anthony Marius Smith‡, Ph.D,
National Institute for Communicable Diseases, South Africa

François-Xavier Weill‡, M.D., Ph.D
Institut Pasteur, Paris, France

Corresponding author:
F-X.W., Unité des Bactéries Pathogènes Entériques, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France, francois-xavier.weill@pasteur.fr, Tel +33 1 45688345

*These authors contributed equally; ‡these authors jointly supervised this work

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TO THE EDITOR: From 6 September 2018 to 26 March 2019, Zimbabwe experienced a large cholera outbreak with 10,730 suspected cholera cases and 68 deaths (Fig. S1). Antimicrobial susceptibility data available on 65% (241/371) of the confirmed cases showed that the *Vibrio cholerae* O1 serotype Ogawa isolates were multidrug resistant, with unexpectedly high-level resistance to ciprofloxacin (96.7%, 233/241) and ceftriaxone (99.6%, 240/241). Details of the methods are provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org. We sequenced the whole-genomes of 11 *V. cholerae* O1 isolates – including ten collected during the cholera outbreak in Zimbabwe, and one originating from a South African patient with travel history to Zimbabwe (Fig. S1, Table S1) – to investigate the determinants of antimicrobial resistance as well as their phylogenetic relationships to >1,200 global seventh cholera pandemic *V. cholerae* El Tor (7PET) genomes.[1,2] Phylogenomic analyses show that the 11 studied genomes belonged to sublineage T13 of the 7PET lineage (Figs 1A and 1B, Table S2). Sublineage T13 was recently introduced from South Asia into East Africa and from there to Yemen (Figs 1B and 1C).[2] The T13 isolates were previously shown to have a narrow antimicrobial resistance (AMR) pattern, mostly due to a ~10-kb deletion in the SXT/R391 genomic island (called ICE*Vch*Ind5), resulting in the loss of four AMR genes.[2] However, the 2018 Zimbabwean isolates differed from previous T13 isolates by having 14 additional antimicrobial resistance (AMR) genes carried on a ~160 kb IncA/C2 plasmid, leading to a broader resistance profile (Fig. S2, Tables S3-S4). In particular, the isolates were intermediately resistant or resistant to tetracycline (presence of the *tet(A)* gene), intermediately resistant or resistant to ciprofloxacin (mutations of the *gyrA* and *parC* genes and presence of the *aac6'-Ib-cr* gene) and produced a CTX-M-15 extended spectrum beta-

lactamase. Antibiotics such as tetracyclines, macrolides, and fluoroquinolones are commonly used in the treatment of moderate-to-severe cholera cases – as an adjunct to rehydration therapy – to shorten the duration and volume of diarrhea, thereby limiting bacterial transmission.[3] However, beta-lactams are not. Resistance to extended-spectrum cephalosporins (ESCs) is uncommon in 7PET isolates, and only ESC-resistant (ESC^R) 7PET isolates from sporadic or small outbreak cases were observed in Africa before 2018.[1] It is noteworthy that a large typhoid outbreak (> 3000 suspected cases) caused by a ciprofloxacin-resistant strain – requiring the use of ESCs – occurred in Harare between October 2017 – February 2018.[4,5] This might have contributed to the ESC^R patterns seen among the cholera outbreak strain several months later.

The 2018 Zimbabwean isolates were susceptible to azithromycin, which was consequently used to treat severe cholera cases during the outbreak. However, azithromycin resistance genes, such as *mph(A)* or *mph(E)* were sporadically identified in CTX-M-15-producing 7PET isolates collected in Zimbabwe (Table S4), the Democratic Republic of the Congo[1], and Kenya[2] from 2010 to 2015. The acquisition of an IncA/C2 plasmid carrying both *mph* and *tet* genes by this T13 7PET strain would jeopardize the oral antibiotic treatment of cholera.

We emphasize the need for cross-border collaboration and continued laboratory surveillance to stemming this highly-drug resistant T13 cholera strain in Africa.

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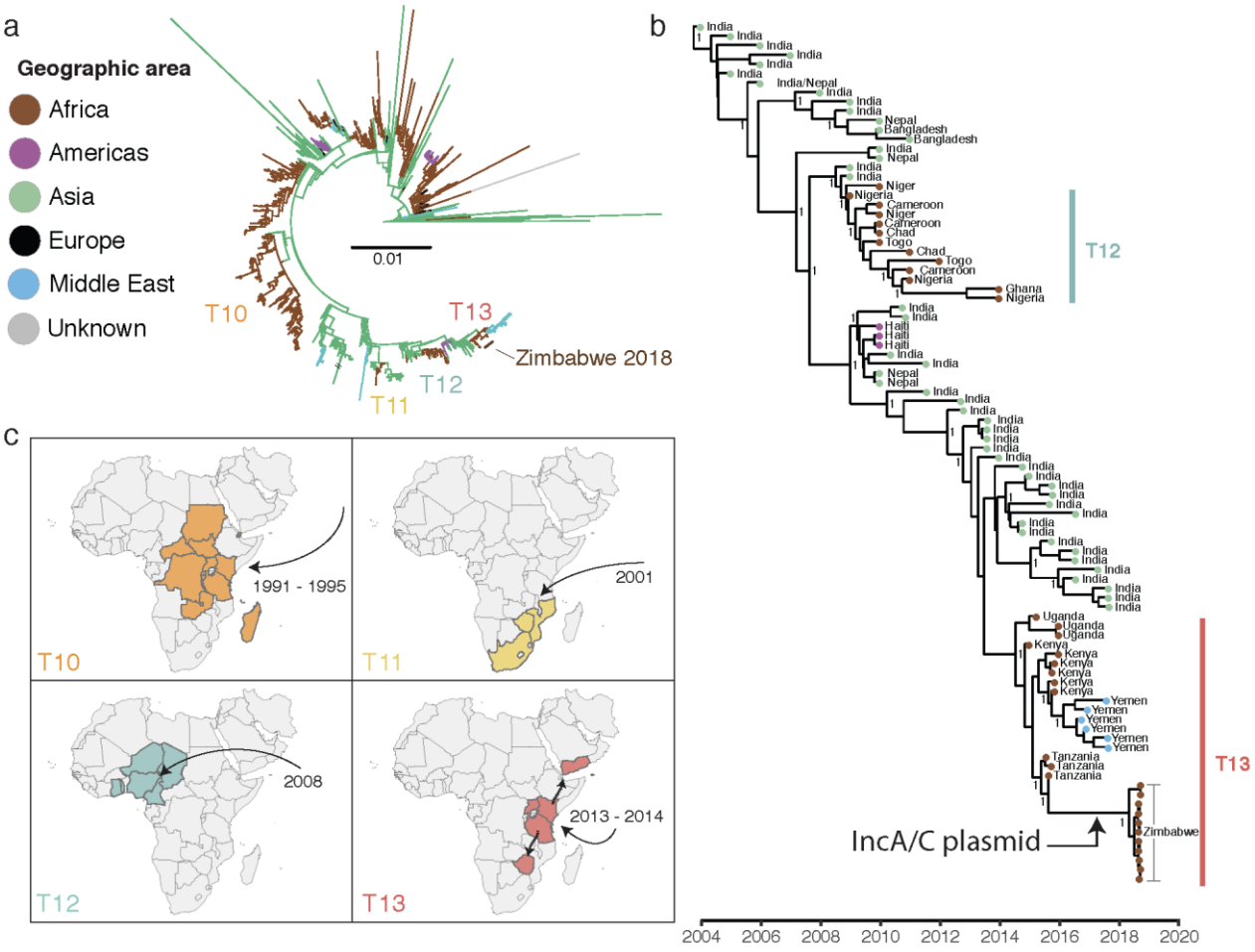

Legend to Figure

Phylogenetic relatedness of the *V. cholerae* O1 El Tor isolates from the 2018 outbreak in Zimbabwe. **a**, Maximum likelihood phylogeny of 1,213 genomic sequences. A6 was used as an outgroup. The scale bar denotes substitutions per variable site (SNVs). Branches are colored according to geographic location, inferred by stochastic mapping of the geographic origin of each isolate onto the tree. The inferred introduction events into Africa are indicated by the letter 'T'. One branch was artificially shortened and as denoted by the hash mark. **b**, Maximum clade credibility tree produced with BEAST for a subset of 92 representative isolates of the distal part of genomic wave 3 (i.e., those with the *ctxB7* allele). Geographic location of the isolates is indicated in the same colors as in panel **a**. Selected nodes supported by bootstrap values ≥ 0.95 are shown. Acquisition of the multidrug-resistant plasmid is shown **c,** The geographical distribution

of selected 7PET sublineages T10 - T13. The date ranges shown for introductions are the 95% credible interval estimate of the MRCA in years. Dashed lines do not represent precise routes of transmission.