LETTER

Reply to Frerichs et al.: Chasing the genetic diversity, not source attribution

Frerichs et al. (1) view the prevalence of *Vibrio cholerae* non-O1/O139 skeptically in the early weeks of the Haitian epidemic (2). Unexpected findings spawn skeptics. Setting aside differences in sampling time frames, differences in recoveries might readily be traced to comprehensive sampling and methodologies. Using a combination of thiosulphate-citrate-bile salt-sucrose and taurocholate tellurite gelatin agar medium to substantiate detection of both sucrose fermenting and nonfermenting *V. cholerae* reported in this region (3), we picked multiple single-colony isolates (average of 4, depending on availability of well-separated colonies and colony morphologies) on each original plate, which were screened systematically, using a battery of specific biochemical, serological, and molecular methods. Sensitive and discriminating, but more expensive and labor- and time-intensive, our approach is seldom used in settings like Haiti National Public Health Laboratory. Our approach uncovered non-O1/O139 in the numbers reported, which less fastidious conventional screening would underestimate. We advocate for such an approach to become standard.

Although Frerichs et al. (1) note our non-O1/O139 *V. cholerae* findings, they fail to grasp the impact. We showed, early in the cholera epidemic, that substantial genomic diversity was observed in circulating populations. We reported an unexpected prevalence of *V. cholerae* non-O1/O139 that may have helped contribute to reported cholera cases in these early weeks; nowhere did we state non-O1/O139 strains "played a notable role in the cholera epidemic origin" as Frerichs et al. (1) assert. They do fail to appreciate subtleties of virulence factors associated with *V. cholerae*. Although non-O1/O139 strains typically lack filamentous phage CTX, satellite phage RS1, and toxin-linked cryptic plasmid (TLC), they often produce extracellular products that play important roles in disease. These include heat-stable and heat-labile enterotoxin, El Tor-like hemolysin, Kanagawa hemolysin, Shiga-like toxin, and hemagglutinins, rendering clinical non-O1/O139 strains highly enterotoxic, hemolytic, and/or proteolytic.

Addressing the study by Hasan et al. (2), Frerichs et al. (1) disagree that "a definitive statement of source attribution cannot yet be made." Precise ascription, however, requires accurate measure of the global genomic complexity (γ-diversity) of *V. cholerae*. We stated that "a qualified reference database (including recent strains from Nepal, India, and Cameroon, and related *V. cholerae* strains from concurrent epidemics) representative of global phylogenetic diversity, as well as well-documented population diversity within the heterogeneous clade and its near neighbor comparators, is critical" (2). Referring to the study by Hendriksen et al. (4), Frerichs et al. (1) imply that cholera was brought to Haiti by a contingent of Nepalese United Nations peacekeeping troops. Data from the study by Hendriksen et al. (4) are indeed consistent with such acknowledgment, although they did not conclude, as Frerichs et al. (1) imply, that the original vector harboring the pathogen was necessarily from Nepal. In their own words, "It is possible that this genetic group will be discovered in countries other than Nepal and Haiti" (4). Actively engaged in examining geographic diversity of *V. cholerae*, we encourage collaboration of the community, mindful that there are three categories of outcomes when interpreting microbial forensic results: inclusion, exclusion, and inconclusive (5). Only when the γ-diversity of *V. cholerae* is more robustly determined, and population samplings within different geographical locations are probed uniformly, will the necessary statistics and epidemiological information, not yet in hand, help attribute "definitively" the source of the Haitian epidemic strain.

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