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Phenotypic, Genotypic, and Antibiotic Sensitivity Patterns of Strains Isolated from the Cholera Epidemic in Zimbabwe▽

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This paper details the phenotypic, genotypic, and antibiotic sensitivity patterns of 88 *Vibrio cholerae* strains from Zimbabwe. Of the 88 strains, 83 were classified as "altered El Tor" and 5 as "hybrid El Tor" strains. All of the strains were susceptible to tetracycline, doxycycline, ciprofloxacin, and azithromycin by disc diffusion, but susceptibility to tetracycline and azithromycin diminished when observed using the MIC method.

Cholera is endemic in Zimbabwe, with small and larger outbreaks occurring since 1992 (16). In August 2008, a new cholera epidemic was reported in Zimbabwe, which affected all 10 provinces and 56 of the 62 districts. Over 7 months, more than 90,000 suspected cholera cases were reported, with more than 4,000 of these patients dying. Responding to a request from the WHO, the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), sent a multidisciplinary team to assess and evaluate the cholera situation in Zimbabwe and provide assistance to control the epidemic.

Based on recently determined phenotypic and genotypic characteristics, the El Tor biotype has been reclassified as "altered El Tor," which refers to an atypical El Tor strain containing El Tor type CTX prophage (*rstR2*) that produces cholera toxin (CT) of the classical type, and "hybrid El Tor," which is characterized by a typical El Tor strain with classical CTX prophage (*rstR1*) that produces CT of the classical type (1, 2, 4, 11). At the time when ICDDR,B was contacted about the 2008 cholera epidemic in Zimbabwe, it was not known which El Tor biotype of *Vibrio cholerae* O1 was involved. Tetracycline and doxycycline have long been the antibiotics of choice for treating severe cholera around the world, but ciprofloxacin has also been widely used following the emergence of tetracycline and doxycycline resistance (7, 14). Recently, however, the emergence of *V. cholerae* with reduced susceptibility to ciprofloxacin has been reported (8).

The present study was carried out to determine the phenotypic and genotypic characteristics, as well as the antibiotic sensitivity patterns, of *V. cholerae* O1 strains isolated during the 2008 epidemic in Zimbabwe.

Isolation and identification of *V. cholerae* O1 were carried out according to previously described procedures (10). All *V. cholerae* O1 strains were then preserved in Luria-Bertani (LB) broth with 30% glycerine at −70°C for further analysis. Multiplex PCR assays were carried out with the boiled template of *V. cholerae* O1 to detect the *ctxA*, *tcp* (El Tor and classical), *ace*, *rfbO1*, *sodB*, and *zot* genes using specific primers following previously described procedures (13). Mismatch amplification mutation assay PCR (MAMA PCR) and pulsed-field gel electrophoresis (PFGE) were also conducted following standard procedures. Disc diffusion assays were carried out, and MICs were determined and interpreted following CLSI guidelines as described previously (5, 12).

A total of 160 rectal swabs were collected, of which 88 (55%) yielded *V. cholerae* O1 (Table 1). The genotypic characteristics of the isolated strains varied according to place of origin (Table 1). With regard to the Harare strains, 4% and 96% of strains possessed *rstR1* and *rstR2*, respectively. The strains containing *rstR1* only belonged to pulse type B, with the remaining strains belonging to pulse type A. All of the strains possessed *ctxA*, *tcpA* (El Tor), *rfbO1*, *ace*, *zot*, and *sodB* genes, and MAMA PCR showed that all of the strains contained the *ctxB* gene for classical toxin (data not shown).

The degree of genetic diversity between strains of different origins was determined using Diversity Database software (version 2.2; Bio-Rad) and Bio Numeric software (Applied Maths, Sint-Martens-Latem, Belgium). The similarity between strains was determined using the Dice coefficient, and cluster analysis was carried out using the unweighted-pair group method using average linkages (UPGMA).

All of the "altered" strains isolated from Zimbabwe, Zambia, and Bangladesh clustered together (cluster A), with a similarity matrix of >90 (Fig. 1). The hybrid strains of Zimbabwe and Mozambique were also found to be closely related to each other, with a similarity matrix of >92 (cluster B) (Fig. 1).

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TABLE 1. Genotypic characteristics of strains isolated from Zimbabwe

Isolation site	Total no. of isolates	No. (%) of isolates positive for:		No. (%) of isolates of PFGE pulse type:	
		rstR1	*rstR2*	A	B
Harare	47	2 (4)	45 (96)	45 (96)	2 (4)
Chitungwiza	7	0	7 (100)	7 (100)	0
Bindura	17	0	17 (100)	17 (100)	0
Chinhoyi	7	0	7 (100)	7 (100)	0
Kadoma	6	0	6 (100)	6 (100)	0
Binga	4	3 (75)	1 (25)	1 (25)	3 (75)
Total	88	5	83	83	5

However, reference strains of classical and El Tor biotypes produced two separate clusters, C and D, respectively. All strains, irrespective of isolation site, were resistant to ampicillin and trimethoprim-sulfamethoxazole but susceptible to tetracycline, doxycycline, ciprofloxacin, and azithromycin. The ranges of MIC also varied in isolates from different origins (data not shown).

The causative agent in the Zimbabwe cholera epidemic in 2008 and 2009 was *V. cholerae* O1 biotype El Tor, serotypes Inaba and Ogawa. Out of 88 El Tor strains, 83 were "altered El Tor" and 5 were "hybrid El Tor." The isolation rates of *V. cholerae* O1 varied from place to place, as did the prevalences of serotypes. The altered and hybrid El Tor strains were also isolated from the neighboring countries of Zambia and Mozambique, respectively (1, 9). However, it is not known whether the altered and hybrid El Tor strains in Zimbabwe had been transported by patients from the neighboring countries or whether the strains isolated in those countries had an origin independent from the Zimbabwe strains. Since altered El Tor strains were isolated from Zambia only, it could be hypothesized that these strains were transmitted from Zambia; similarly, the hybrid strains could have been transmitted from Mozambique, where the hybrid strains had been isolated. As a result, Zimbabwe experienced a cholera epidemic caused by both altered and hybrid strains, possibly due to cross-border transmission from Zambia and Mozambique. Dendrogram results showed that there is no difference in clonality of the altered strains isolated in Zimbabwe, Zambia, and Bangladesh, suggesting that the same clone of altered strain is spreading globally (Fig. 1). As the hybrid strains from Mozambique and Zimbabwe are the same clone, the Zimbabwean hybrid strains might have originated from the same Mozambique clone.

The sensitivity patterns of ampicillin and trimethoprim-sulfamethoxazole were found to be similar for the strains isolated in Bangladesh and Mozambique (6, 10). A preliminary report described the occurrence of *V. cholerae* strains with reduced susceptibility to ciprofloxacin (MIC of 0.25 to 5 μg/ml) in Zimbabwe (3), while a study from Bangladesh reported that cholera caused by strains with a reduced susceptibility to ciprofloxacin was associated with therapy failure (15). To date, this appears to be the first detailed description regarding susceptibility of *V. cholerae* O1 strains from Zimbabwe to azithromycin and tetracycline using MIC. Altered El Tor strains, but

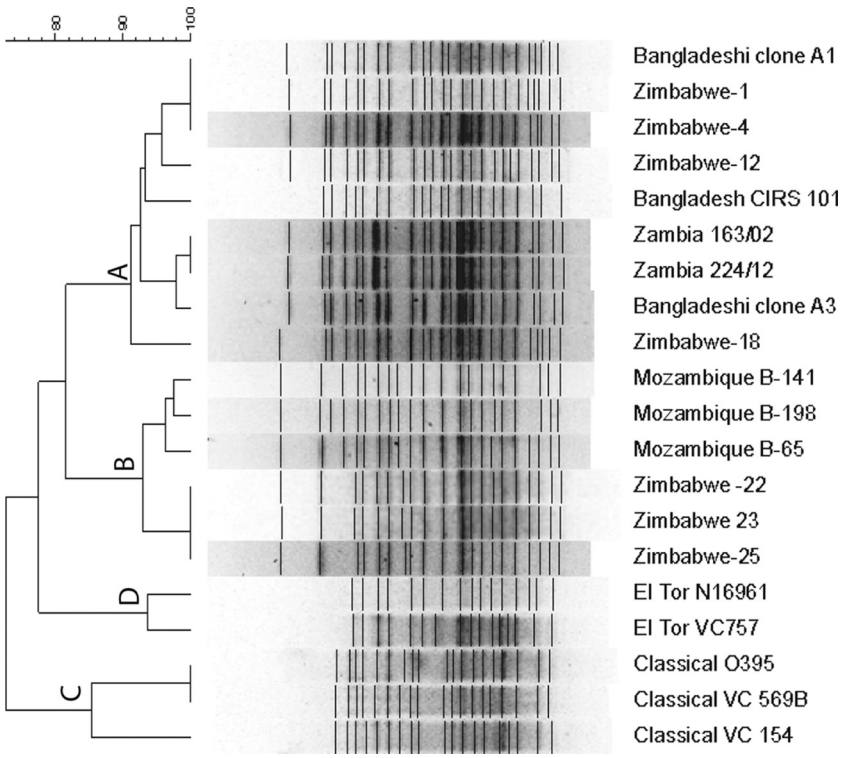

FIG. 1. Dendrogram of *V. cholerae* strains isolated from Zimbabwe, Zambia, Mozambique, and Bangladesh. A, altered cluster; B, hybrid cluster; C, classical cluster; D, El Tor cluster. The scale at the upper left indicates similarity values (where 0 represents total dissimilarity and 100 represents total similarity).

not hybrid El Tor strains, showed a diminished susceptibility to azithromycin. In terms of tetracycline, a diminished susceptibility irrespective of altered and hybrid El Tor strains was also observed by MIC, suggesting that in Zimbabwe, tetracycline may not be viable as a therapeutic option in the near future. Longitudinal surveillance of antimicrobial susceptibility using quantitative methods (MIC) is recommended for early detection of emerging resistance.

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