imported to Kuwait from Iraq. We characterized isolates from 2 recent cholera cases of the strain assumes urgency in light of the spread of vari

The ongoing seventh pandemic is caused by El Tor biotype. The isolates were resistant to nalidixic acid but susceptible to ciprofloxacin, norfloxacin, ofloxacin, tetracycline, meropenem, ampicillin, ceftriaxone, trimethoprim/sulfamethoxazole, chloramphenicol, erythromycin, azithromycin, streptomycin, neomycin, and gentamicin by disk diffusion test. Tetracycline susceptibility confirmed favorable response to doxycycline.

The first case was in a 19-year-old Kuwaiti man who visited Najaf and Karbala in Iraq in September 2015; the second case was in a 52-year-old Kuwait woman who visited the same 2 locations in October 2015. Both had watery diarrhea 3–4 times daily and vomiting; they returned to Kuwait and were admitted to Al Amiri Hospital (Sharq, Kuwait). They gave histories of drinking local water in Iraq, had moderate dehydration, and were treated with intravenous rehydration solution and a single doxycycline dose (500 mg). Diarrhea resolved after 2–3 days.

Fecal specimens collected at admission from both patients grew yellow colonies on thiosulfate bile salt sucrose agar (Eiken, Tokyo, Japan); these colonies were confirmed as *V. cholerae* O1 Inaba serotype by biochemical reactions and agglutination with specific antiserum (Denka Seiken, Tokyo, Japan). The woman’s isolate was designated as Kuwait 36 and the man’s as Kuwait 37. The isolates were positive for chicken cell agglutination and Voges-Proskauer tests and were polymyxin B resistant, characteristics of El Tor biotype. The isolates were resistant to nalidixic acid but susceptible to ciprofloxacin, norfloxacin, ofloxacin, tetracycline, meropenem, ampicillin, ceftriaxone, trimethoprim/sulfamethoxazole, chloramphenicol, erythromycin, azithromycin, streptomycin, neomycin, and gentamicin by disk diffusion test. Tetracycline susceptibility confirmed favorable response to doxycycline.

We studied the genotype of *ctxB* using a double-mismatch amplification mutation PCR (i.e., mismatches in both primers). PCR with classical *ctxB*-specific primers *ctxBF*/*ctxBRvCla yielded an amplicon of 191 bp, but not with Haitian *ctxB* specific primers *ctxBF3/*ctxBRvCla, indicating that the isolates had a *ctxB* of classical biotype (genotype 1) (5,6). Mismatch amplification mutation assay PCR (MAMA-PCR, i.e., mismatch in only 1 primer) with Haitian-specific *tcpA* primers *tcpAF*/*tcpARev* produced an amplicon of 167 bp but not with El Tor *tcpA*–specific primers *tcpAF1/*tcpAEIRev*, suggesting these isolates had the Haitian variant *tcpA* (2). MAMA-PCR for *rtxA* with El Tor–specific primer pair *rtxA*F/*rtxA*R yielded a 187-bp amplicon but no amplicon for Haitian variant primer pair *rtxA*F/*rtxA*R2, suggesting the occurrence of *rtxA* of El Tor variety (2). The isolates possessed El Tor type *rstR* because they produced a 500-bp amplicon with primer pair *rstR*/*rstA*3R (7). The isolates were positive for *rstC*, a repeat sequence activator found in El Tor biotype, because they yielded an amplicon of 238 bp with primer pair *rstC1/*rstC2 (8). *rstB* is required for CTXφ phage integration. The Haitian strain has a GTA deletion at positions 77–79. MAMA-PCR with primer pair *rstB*F/*rstBR*1 produced a 160-bp amplicon, suggesting the absence of deletion in El Tor type *rstB* (2). The isolates had CTXφ integrated in the large chromosome with RS element downstream because they produced a 766-bp amplicon with CII F/CII R primers.
(9). PCR sequencing with primers Zot F/ctxA R indicated the presence of 4 heptad (TTTTGAT) repeats in the ToxR binding region of ctxAB promoter, similar to El Tor bio-type (2). Both isolates possessed vibrio seventh pandemic islands I and II, typical of El Tor biootype as assessed by PCRs with a variety of primers (10). Clonal relationship studied by pulsed-field gel electrophoresis suggested that isolates from the Kuwaiti patients were similar to each other and closer to Indian isolates of 2004 (Figure). Cholera is endemic to India; many El Tor variants circulate there (2).

We showed that the strain causing cholera in Iraq did not have the typical El Tor characteristics but instead had mixed characteristics of El Tor, classical, and Haitian strains. Altered strains of V. cholerae O1 might have implications for disease severity and vaccine efficacy (1). El Tor variants seem to be sweeping the world. We wonder whether they could replace the archetypal El Tor strain and become the causative agent of the eighth pandemic of cholera.

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References

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Figure. Comparison of PFGE patterns of NotI-digested chromosomes of Vibrio cholerae O1 isolates from Kuwait with those of isolates obtained from various years (indicated by last 4 digits) from Kolkata, India. The digested chromosomes were separated on CHEF MAPPER (Bio-Rad, Hercules, CA, USA) and dendrogram constructed and analyzed by BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). PFGE, pulsed-field gel electrophoresis.