JOURNAL OF CLINICAL MICROBIOLOGY, July 1997, p. 1917 Vol. 35, No. 7
0095-1137/97/$04.00+0

Instability of a Shiga Toxin Type 2 Gene in *Enterobacter cloacae*

Strains of *Escherichia coli* and other species of bacteria producing Shiga toxins (previously referred to as Shiga-like toxins or verotoxins) are recognized causes of gastrointestinal disease in humans. In 1996 we reported the isolation of a strain of *Enterobacter cloacae* producing Shiga toxin type 2 (Stx2) from the feces of a patient with hemolytic-uremic syndrome (2). Stx2-producing *E. coli* OR:H9 was also isolated from this patient. The presence of an *stx2* gene in the *Enterobacter* isolate, designated 95MV2, was confirmed by PCR and by DNA hybridization, and cell lysates were cytotoxic for Vero cells. The *stx2* gene was cloned and sequenced and found to be very similar, although not identical, to the *stx2* gene in the *E. coli* OR:H9 isolate (2). Recently, however, we have had cause to retest subcultures of a stock of 95MV2, which had been preserved in glycerol at −80°C. Such cultures were not highly cytotoxic for Vero cells. The preserved stock was plated on MacConkey agar, and colonies were picked into wells of a microtiter tray. Lysates were prepared, spotted onto a nylon membrane, and then subjected to dot blot hybridization analysis with an *stx2*-specific DNA probe. Lysates from only 8 of 48 colonies tested reacted (weakly) with the probe. Subcultures from the probe-positive wells were confirmed biochemically to be *E. cloacae*, but these were now negative for *stx2* even when tested by PCR. Thus, it appears that the *stx2* gene is unstable in 95MV2 in vitro.

Instability of *stx* genes in *E. coli* and in other species appears to be a very common phenomenon. Karch et al. (1) reported that 15 of 45 consecutive *stx*-positive *E. coli* isolates from patients with various gastrointestinal disorders became negative for *stx* by the Vero cell assay, hybridization, and PCR after subculture. Also, Schmidt et al. (4) reported complete loss of *stx* genes and toxigenicity from five of seven *Citrobacter freundii* strains tested after a single subculture on agar plates or after two passages in broth culture. Interstingly, the remaining two *C. freundii* strains remained *stx* positive even after repeated subculture, indicating that in vitro stability is strain dependent. During investigation of a large outbreak of hemolytic-uremic syndrome caused by a combination of Stx-producing *E. coli* strains (principally O111:H$^-$) (3), we observed that many *stx* probe-positive colonies (usually strains belonging to serotypes other than O111 or O157) had also lost their toxin genes when subcultures were retested. We have also observed what appeared to be transient expression of *stx2* by a strain of *Serratia marcescens* that we isolated from a patient who also was infected with Stx2-producing *E. coli* O157 (unpublished observations).

Clearly, the in vitro instability of *stx* genes in enteric bacteria poses problems for clinical microbiologists, particularly when cultures have to be sent to a reference laboratory for confirmation of Stx production. Such instability may reduce the frequency of detection of toxigenic strains in fecal specimens and may also result in overestimates of the rate of false-positive reactions obtained with Stx-specific screening tests such as enzyme-linked immunosorbent assays and PCR. For this reason, screening tests should be carried out either directly on fecal samples or on primary cultures, and confirmatory tests should carried out on isolates after the minimum possible number of subcultures.

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