

TITLE: TRANSFER OF *stx2* GENE AFTER CONJUGATION ASSAY IN CLASS 1 INTEGRON POSITIVE *Escherichia coli* - CONJUGATION OR TRANSDUCTION?

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ABSTRACT:

Shiga toxin-producing *Escherichia coli* (STEC) are strains that harbor lambda prophage containing *stx* genes responsible for toxin-encoding Stx. Dissemination mechanisms of the *stx* genes and the toxin expression are related to the lytic cycle induction of the phage. This induction is usually caused by bacterial SOS response. It is known that the conjugation represents one of the triggers for the SOS response. In order to verify integron transfer by conjugation in class 1 integron-positive *E. coli* (n=12) isolated from calves' feces, we performed conjugation assay. We used the overnight culture (18h) at 37°C of the strains donors and recipient in LB broth. Subsequently, cultures of donor and recipient strains were transferred to the same tube at 1:10 (v/v) ratio and incubated at 37°C for 18h. After 100µL of the donor + recipient cultures were plated onto Mueller Hinton agar with sodium azide (100µg/mL), tetracycline (100µg/mL) and sodium azide with tetracycline (100µg/mL). The plates were incubated at 37°C for up to 72h to visualize the transconjugant colonies. We verify genes transfer by PCR. Two transconjugant strains obtained from two integron positives STECs showed *stx2* gene and cytotoxic effect in Vero cells. This result indicates the Stx2 transfer during method execution. Total DNA of the transconjugants and donor strains showed integron genes (*int11*, 5'CS-3'CS, *sul1*, and *qacEΔ1*) besides *stx2* and *tetA*. We also performed PCR from phage DNA and *stx2* and *int11* were detected but not *tetA*. Therefore, we think that in this assay *stx2* gene was transfer by transduction through lytic cycle induction generated by the high population density of bacteria in initial culture, but the integron and antimicrobial resistance genes related to them can have been transferred by conjugative elements as well as plasmids and conjugative transposons. Our results also indicate that conjugation and transduction can occur simultaneously and the dissemination of *stx2* gene may be more common than recorded in literature.

Keywords: STEC, tetracycline, phage

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