Creation of a CTX-M-14/CTX-M-15 Gene Fusion to Determine if an Intrinsic Structural Feature of CTX-M-15 Causes Upregulation of Its Expression

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Introduction: Bacteria develop resistance to β-lactam antibiotics by synthesizing a β-lactamase to counteract this. It is vital to examine the genetic elements within the β-lactamases that cause the proliferation of the β-lactamases which could be a target for the novel antibiotic. Clinically important β-lactamases are those encoded on plasmids such as the extended-spectrum β-lactamases (ESBLs) of which the most prominent are the CTX-M-14 and CTX-M-15 enzymes. CTX-M-15 mRNA levels in E. coli are up-regulated compared to CTX-M-14 levels. By fusion of these two genes, the location of an element causing up-regulation of CTX-M-15 can be assessed. Hypothesis: If structural element causing upregulation of CTXM15 is localized towards one half of the gene, and genetic fusions are created using compatible halves of CTXM15 and CTXM14, then gene fusion containing up regulation element of CTXM15 should show elevated mRNA expression. Methods: Two singleplex PCRs were used to amplify the 5' half of CTX-M-15 and the 3' half of CTX-M-14 (fusion). The reverse fusion was created by amplifying the 5' half of CTX-M-14 and the 3' half of CTX-M-15. These amplified products were ligated into pCR2.1 vector, subcloned into the pACYC184 vector, and transformed into a wild type E. coli. mRNA expression was evaluated by real-time reverse transcriptase PCR. Results: In, CTX-M-15/CTX-M-14 fusion, the mRNA expression was up-regulated 31-fold vs 7.5 fold for a reverse fusion, CTX-M-14/CTX-M-15. Element within the 5' end of CTX-M-15 is responsible for up-regulated mRNA levels. Conclusion: Element located within the 5' half of CTX-M-15 cause an up-regulation of mRNA levels. Further research is needed to identify the specific element in the CTX-M-15 gene that is responsible for the up-regulation.

Awards Won:

Fourth Award of $500