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Rapid detection and discrimination of closely related Enterobacteriaceae CTX-M group 1 variants, *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$, using an internally controlled multiplex loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) assay

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Abstract

Cefotaximases (CTX-Ms) are a class of plasmid-encoded extended-spectrum beta-lactamase (ESBL) enzymes found in Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae* that confer resistance to third-generation cephalosporin antibiotics. CTX-M enzymes are classified into five groups; CTX-M-1, 2, 8, 9 and 25. The rapid emergence and dissemination of CTX-M group 1 variants *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$, typically associated with animal and human infection, respectively, is a global public-health concern and highlights the requirement for effective diagnostic tools. However, *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$ variants are almost identical in nucleotide sequence and difficult to differentiate using conventional molecular diagnostics. Loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) is a recently developed technology that enables rapid real-time multiplex pathogen detection with single-base specificity and portable on-site testing applications. In this study we have developed an internally controlled multiplex LEC-LAMP assay for the differential detection of *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$ variants in a single reaction. Analytical specificity and sensitivity of the *bla*$_{CTX-M-1/15}$ LEC-LAMP assay was established using clinical and environmental *E. coli* isolates from Ireland and Central Germany. The *bla*$_{CTX-M-1/15}$ LEC-LAMP assay demonstrated specific differential detection of both variants at high bacterial load concentrations of 10^6 genome copies, and low-level detection for each variant of 10 genome copies per reaction in approximately 15-20 min. This assay will be further validated using *bla*$_{CTX-M}$ positive bovine and porcine faecal samples, and evaluated for on-site agricultural faecal sample testing in combination with portable instrumentation and a rapid bacterial DNA extraction protocol.