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Optimisation of a molecular methodology for the detection of virulence factors of enterotoxigenic *Escherichia coli* for the diagnosis of swine colibacillosis

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The most common bacterial pathogen causing enteric infections in pigs is enterotoxigenic *Escherichia coli* (ETEC). Since pigs represent the largest livestock category in the European Union, ETEC-associated diseases, better known as swine colibacillosis leading to acute diarrhea and eventual death of the animal, result in significant costs to the pig industry. These diseases are traditionally prevented or treated with antibiotics, and this has had a huge impact on the emergence of resistant bacteria, correlating with the emergence of resistant infections in humans. Recognition of this problem has led the authorities to set ambitious goals for the reduction of this type of drug in animal husbandry, leading to the creation of a national project, APTAcoli, which aims to select aptamers (consisting of small single-stranded oligonucleotides capable of binding to target molecules with great affinity and specificity, due to the specific secondary and/or tertiary structures they can acquire) as an alternative in the treatment of colibacillosis. The present experimental study, which is on the APTAcoli agenda, focused on the optimization of a molecular methodology - Multiplex PCR - for the detection of the main virulence factors of ETEC to be used in an epidemiological study to characterize fecal samples from pigs in Portuguese farms. After using different optimization techniques, the results were two multiplex PCR amplification sets, one for amplification of the main toxigenic factors of ETEC (STa, STb, LT and STx2e) and another for amplification of the main adhesion factors (F4, F5, F6, F18 and F41).

Keywords: ETEC, Escherichia coli, Swine colibacillosis, Multiplex PCR

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