Macrolide resistance rapid detection qPCR assay from nasopharyngeal specimens DNA in Bordetella pertussis

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Bordetella pertussis remains a significant global public health concern, with recurring outbreaks and increasing incidence worldwide. Macrolides are still the first-line treatment for pertussis; however, the emergence of resistant strains associated with the A2047G point mutation in the 23S rRNA gene has been reported with increasing frequency. Detection of macrolide resistance by the disk-diffusion method depends on bacterial growth and requires standardization for *B. pertussis*, hindering timely detection of resistance. In this context, real-time PCR (qPCR) offers a rapid and sensitive alternative, allowing direct detection of the A2047G mutation in *B. pertussis* DNA without the need for culture. This study aimed to evaluate a novel qPCR assay for the detection of macrolide resistance in *B. pertussis*-positive DNAs. Between January 2024 and December 2025, a total of 1,508 DNAs were analyzed, as part of ongoing public health surveillance. The detection of the A2047G mutation, associated with erythromycin resistance, was performed using a TaqMan®-based qPCR assay. Allele-specific primers and fluorescent probes targeting wild-type and mutant alleles were used on the QuantStudio platform, with a total turnaround time of 1 hour and 34 minutes. Over two years, allelic status was successfully determined in 1,476 of the 1,508 samples (97.9%). In 2024, 766 DNA samples were analyzed, of which 741 (98.9%) were wild-type and eight (1.1%) presented the A2047G mutation, while in 2025 the mutation was detected in only one sample (0.1%) among the 742 DNAs analyzed. Of the total tested in both years, 32 samples (2.1%) presented inconclusive or invalid results, with 30 having an undetermined allele type and two having insufficient DNA quantity, demonstrating consistent performance. These findings demonstrate a high success rate in determining macrolide susceptibility in DNA of clinical samples of *B. pertussis*, with approximately 98% valid results. The assay showed high sensitivity and specificity, reliably distinguishing wild-type alleles from mutant A2047G alleles directly from *B. pertussis*-positive DNAs. This qPCR approach allows for rapid resistance detection in routine surveillance. Its accuracy, simplicity, and short response time make it a powerful first-line screening tool for national pertussis surveillance and early detection of antimicrobial resistance, which can guide public health policies and evidence based actions.

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