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Oral Session
Molecular detection of resistence: dream or reality?

APPLICATION OF REAL-TIME PCR FOR THE DETECTION OF MACROLIDE RESISTANCE IN BELGIAN M. PNEUMONIAE STRAINS

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Objectives: Since the first report (Matsuoka et al. 2004) was published on macrolide resistant *M. pneumoniae* (MR-MP) prior to 2003, an increasing number of papers were published on macrolide resistance in MP worldwide. The main objective of this study was to validate an assay for the detection of MR-MP and to apply this assay retrospectively on clinical samples and strains present in the biobanks at the University Hospital Antwerp and Leuven University Hospitals, for the presence of macrolide resistance.

Methods: A real-time PCR (Peuchant et al, 2009), designed to characterize MR-MP based on melting temperatures of the 23S rDNA was validated and then retrospectively applied to 110 MP strains isolated before resistance to macrolides was reported and to 99 samples archived (2001-2013) MP positive samples. The assay was validated for its specificity, sensitivity, accuracy, and reproducibility using reference strains and six well-characterized MR-MP strains or DNA-extracts from France and Leuven.

Results:

The test to differentiate macrolide sensitive MP from MR-MP resulted in the expected melting temperatures (Tm) for both the sensitive and resistant genotypes in both channels. No Tm was obtained with the 23S rDNA amplicons of the other 17 bacterial species used to test the specificity of the assay in both channels. The calculations of the analytical sensitivity showed that the assay has a 95% hit rate of 3.786 colour changing units per PCR (CCU/PCR) and a 25% hit rate when there are 0.084 CCU/PCR.

A coefficient of variation of 0.15% and 0.32% was obtained with strong positive and weak positive spiked samples on channel 640 and of 0.06% and 0.05% with strong positive and weak positive spiked samples on channel 705 when calculating the intrarun variability (repeatability).

Interrun variability (reproducibility) experiments resulted in a coefficient of variation of 0.15% with the strong positive and 0.38% with the weak positive spiked samples in channel 640 and of 0.20% with the strong positive and 0.13% with the weak positive spiked samples on channel 705.

None of the archived MP strains was found to be macrolide resistant. For 85/99 MP PCR-positive archived clinical samples a result was obtained, while no conclusion could be made for 14% (Ct value >35 in the P1 assay). In 3 (3,5%) specimens a resistant genotype was found.
Conclusion: To our knowledge, this is the first report on MR-MP in Belgium with the first identified case in 2001. Based on the results of these limited archived samples, no conclusions can be drawn on the evolution of macrolide resistance in MP. However, up to 4% of the MP positive samples in both biobanks were shown to be macrolide resistant. This is comparable to studies carried out in different European countries. Epidemiological surveillance of MR-MP might become a necessity.