

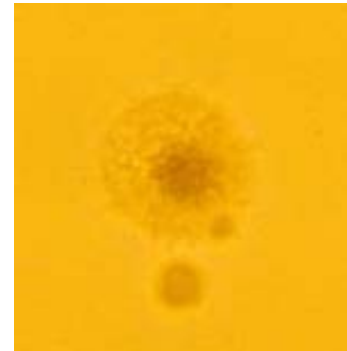


Application of real-time PCR for the detection of macrolide resistance in Belgian *M. pneumoniae* strains

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Introduction (1)

- One of the smallest self-replicating bacteria with a genome size of only 816 kbp and the absence of a cell wall
- Worldwide occurrence
- Epidemics every 4-5 years
- Common cause of CAP and other RTI
- Occasional cause of encephalitis and other neurologic syndromes
- Detection by using PCR and/or serology
- Macrolides are first choice for treatment especially in children
- Tetracyclines and fluoroquinolones not recommended for children due to the risk of severe adverse events



Introduction (2)

- First report of macrolide resistant *M. pneumoniae* in Japan (Matsuoka M. et al. 2004).
- Macrolide resistance also found in
 - USA up to 8.2% (Tsai et al. 2013, Yamada et al. 2012)
 - Europe 1.0%-26.0% (Chironna et al. 2011, Dumke et al. 2010, Ferguson et al. 2013, Peuchant et al. 2009, Uldum et al. 2012)
 - Asia up to 90% (Cao et al. 2010, Liu et al. 2009, Xin et al. 2009)
- Macrolide resistance due to point mutations in domain V from the 23S rDNA:
 - A2058G
 - A2059G
 - A2058C
 - C2611G

Introduction (3)

- The Belgian Reference Centre for Respiratory Pathogens was set up officially on October 1, 2010 as a collaboration between the University Hospital of Antwerp and the University Hospital of Leuven. (Muyldermans et al. 2012. Arch. Public Health: 70:16.)
- Some of the aims:
 - confirmation of diagnosis,
 - study antibiotic susceptibility of *M. pneumoniae*,
 - typing of *M. pneumoniae*,
 - participation in (inter)national surveillance,
 - evaluation of new detection techniques,

Objectives

- To validate an assay for the detection of macrolide resistant *M. pneumoniae* directly in clinical specimens
- To apply this assay retrospectively on strains and clinical samples present in the biobanks at the University Hospital Antwerp and Leuven University Hospitals to get an idea about the presence of macrolide resistant *M. pneumoniae* in Belgium

Materials and Methods (1)

- *M. pneumoniae* strains:
 - 101 *M. pneumoniae* archived strains:
 - 3 reference strains
 - 76 strains isolated <1996 from different sources
 - 12 Belgian isolates from children with LRTI (1992-1995)
 - 10 isolates from a Belgian CAP-study (2000-2003)
- Other *Mycoplasma* reference strains:
 - *M. hominis*, *M. orale*, *M. salivarium*, *M. buccale*, *M. arthritidis*, *M. pirum*, *M. lipophilum*
- Other reference strains of respiratory pathogens
 - *S. pneumoniae*, *H. influenzae*, *S. pyogenes*, *E. coli*, *S. aureus*, *E. faecalis*, *P. aeruginosa*, *C. pneumoniae*, *L. pneumophila* serotype 1, *B. pertussis* and *M. catarrhalis*

Materials and Methods (2)

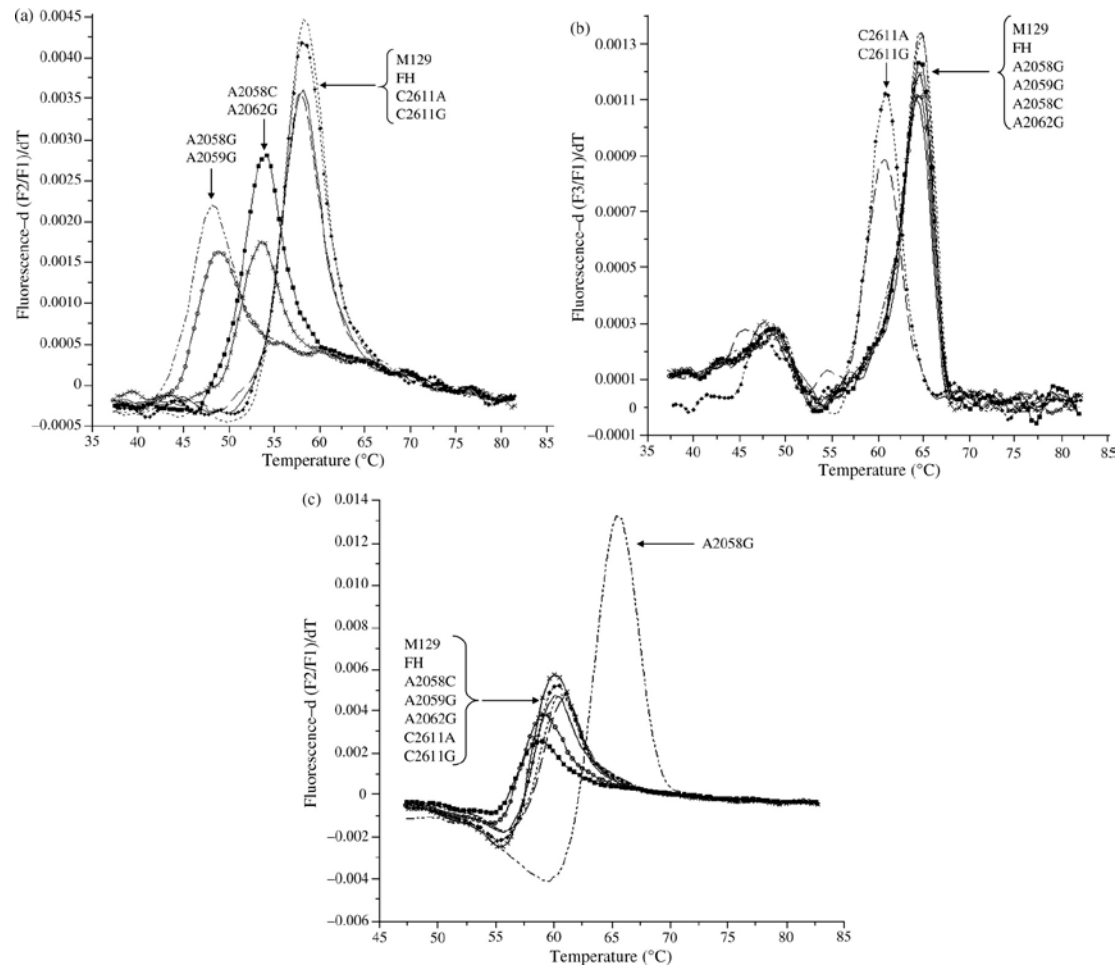
- *M. pneumoniae* positive NA extracts from respiratory specimens
 - 28 DNA extracts from the GRACE-study which performed sampling during three consecutive winter seasons in Europe from 2007-2010
 - 47 *M. pneumoniae* positive archived DNA extracts from the biobanks at the UZA and KUL (2001-2013).
- 24 *M. pneumoniae* positive archived specimens from the biobank at the UZA.(2001-2013)

Materials and methods (3)

- Nucleic acid extraction:
 - NucliSens EasyMag, specific protocol A
- Real-time PCRs applied:
 - *M. pneumoniae* identification (Ursi et al. 2003. J. Microbiol. Meth. 55:149-153)
 - *M. pneumoniae* macrolide R (Peuchant et al. 2009. J Antimicrob Chemother. 64:52-58), real-time duplex PCR combined with melting curve analysis



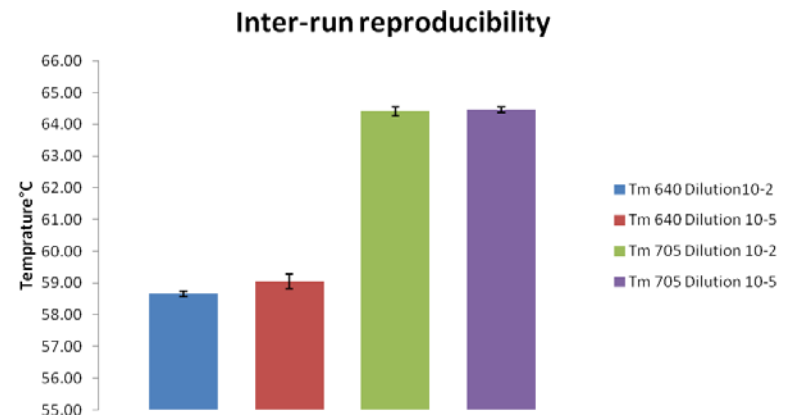
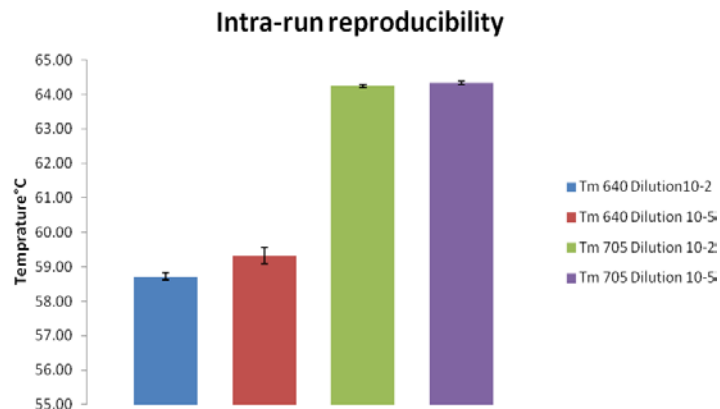
Melting curve analysis obtained with the anchor-probe7/sensor-probe7 (a) and with the anchor-probe8/sensor-probe8 (b) by the duplex real-time PCR, and with the anchor-probe1/sensor-probe1-2058G by the simplex real-time PCR (c) for the M129 (continuous line) and FH (small dashes) wild-type strains and mutant isolates harbouring the A2058G (large and small dashes), A2058C (filled squares), A2059G (open circles) A2062G (crosses), C2611A (filled diamonds) and C2611G (large dashes) mutations.



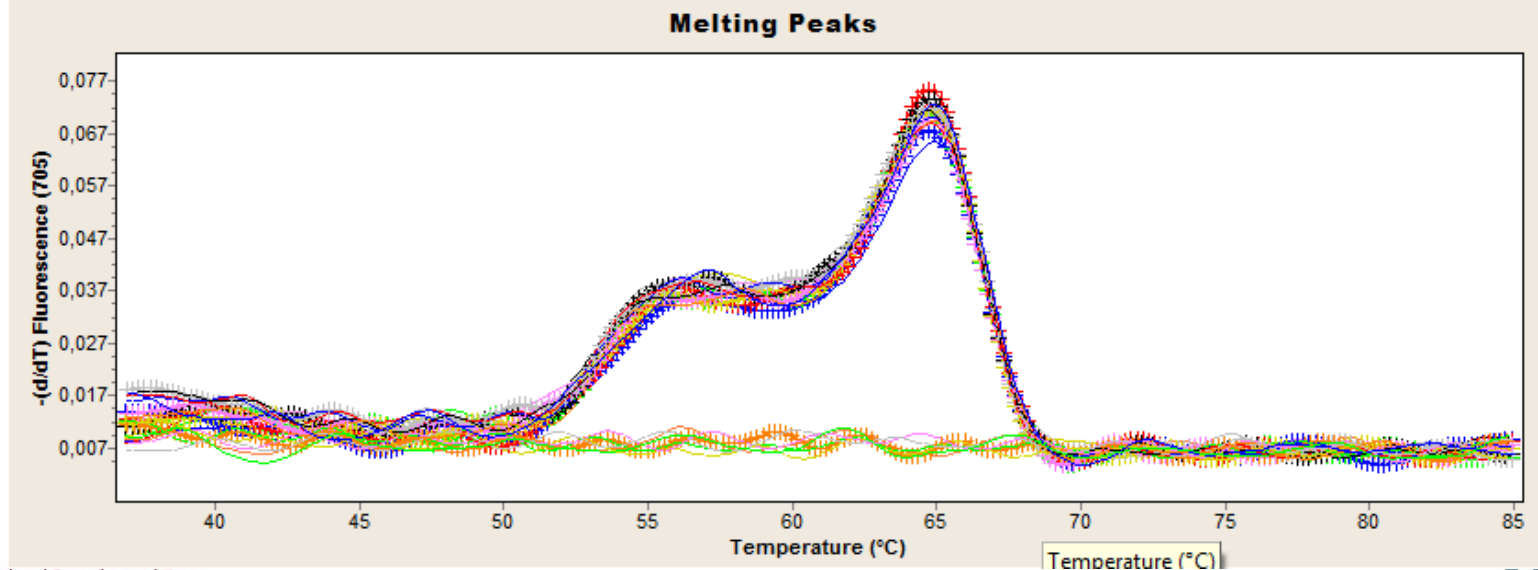
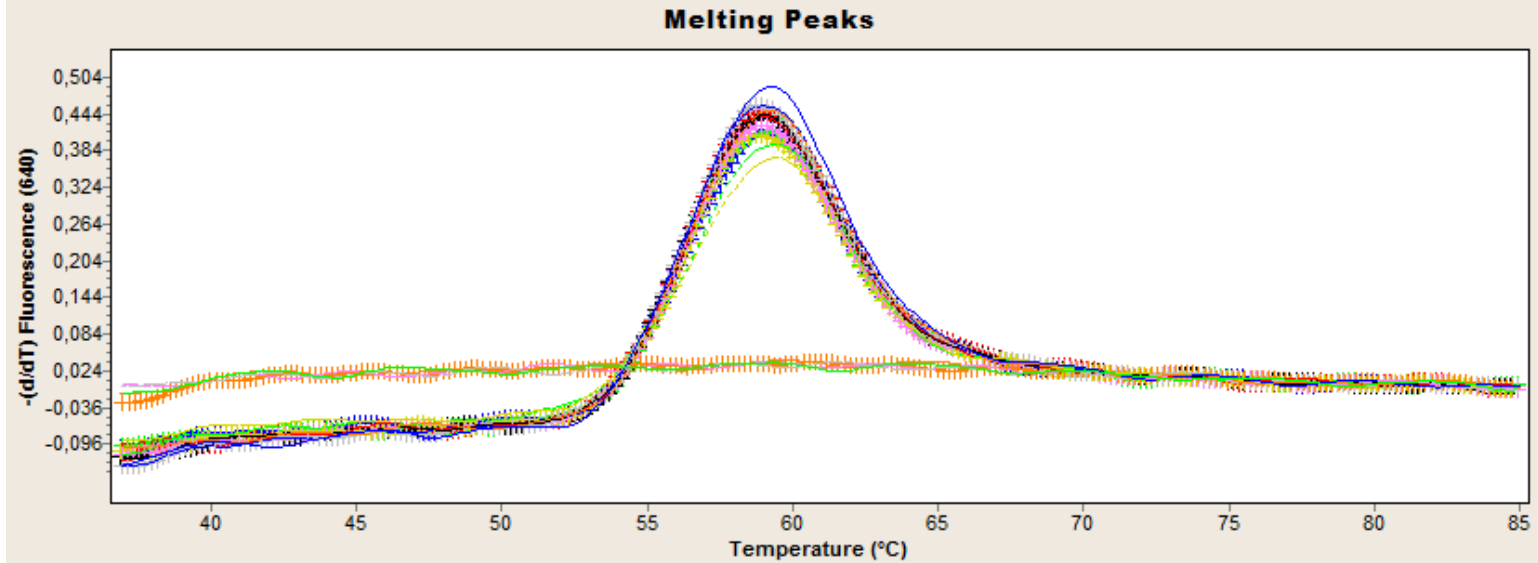
Peuchant O et al. J. Antimicrob. Chemother. 2009;64:52-58

Results (1)

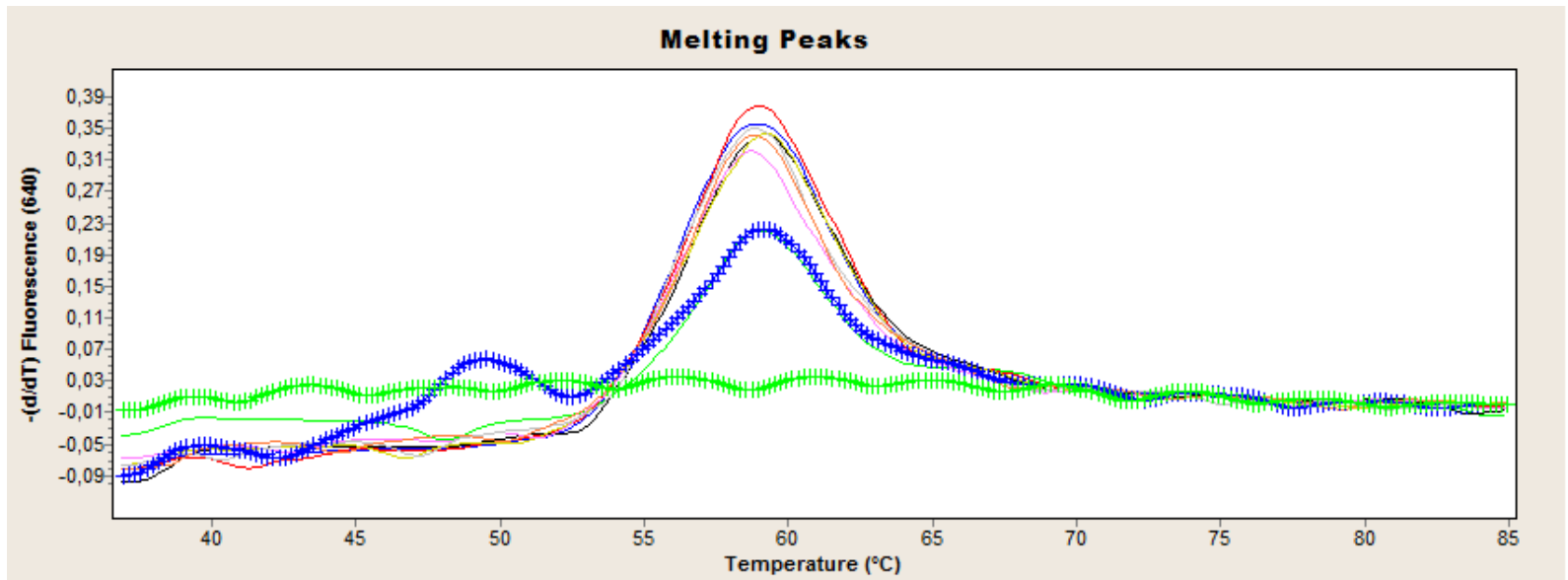
- Specificity:
 - amplicons and melting curves were only obtained with DNA from *M. pneumoniae*
- Sensitivity:
 - a 95% hit rate of 3.786 CCU/PCR and a 25% hit rate of 0.084 CCU/PCR
- Intra and interr run reproducibility:



Results (2)



Results (3)



Results (4)

- Results in function of Ct-value P1 PCR

Ct P1 PCR	Nr of extracts	PCR result macrolide S/R/NI
<35	58	S
≥35	10	NI

Results (5)

- Prevalence of macrolide resistant *M. pneumoniae*: strains: no macrolide resistant strain among the 101 strains tested
- Results GRACE extracts: no macrolide resistance detected
- Belgian archived extracts and specimens; 4% macrolide resistant *M. pneumoniae* detected
- Mutations found: A2059G (49.1°C) and A2058G (48.28°C)

Conclusions

- The evaluated assay is applicable to a broad range of strains and specimens if the Ct value of the P1 gene is ≤ 35 .
- 2001, Belgium: the first macrolide R *M. pneumoniae* case detected
- Up to 4% of the *M. pneumoniae* positive samples were shown to be macrolide resistant. This is comparable to studies carried out in different European countries.
- The mutations found are those frequently reported in the literature
- Based on the results of the limited archived samples, no conclusions can be drawn on the evolution of macrolide resistance in *M. pneumoniae* in Belgium.
- Epidemiological surveillance in Belgium of *M. pneumoniae* and macrolide resistant *M. pneumoniae* is of importance.

Acknowledgement and disclosures

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- No disclosures