

# Survey of macrolide-resistant *Mycoplasma pneumoniae* in children with community-acquired pneumonia in Switzerland

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## Introduction

*Mycoplasma pneumoniae* is a leading cause of community-acquired pneumonia (CAP) in children and macrolides are recommended for this entity [1]. Extensive macrolide use led to the rapid, worldwide emergence of macrolide-resistant *M. pneumoniae* (MRMP) [2] with rates of over 90% in Asia (China, 2012 [3]) and up to 26% in Europe (Italy, 2010 [4]). The first two cases of MRMP in Switzerland were reported in adults in 2012 [5]. We aimed to assess the presence of MRMP in children with CAP.

## Methods

*Mycoplasma pneumoniae* strains detected by real-time polymerase chain reaction (RT-PCR) in respiratory specimens (pharyngeal swab, sputum, nasopharyngeal or tracheal secretion, bronchoalveolar lavage, or pleural aspirate) were investigated for macrolide resistance by sequence analysis of the *M. pneumoniae* 23S rRNA gene. All respiratory specimens were collected from both in-patients and out-patients ≤16 years of age with clinically diagnosed CAP at the University Children's Hospital of Zurich between January 2011 and December 2013. Isolation of *M. pneumoniae* chromosomal DNA and RT-PCR targeting the P1 adhesion protein gene (P1 gene) was performed on all respiratory specimens as previously described [6, 7]. Amplification of the partial *M. pneumoniae* 23S rRNA gene (domain V) followed by sequence analysis for the detection of resistance mutations at positions 2063, 2064, and 2617 was conducted on *M. pneumoniae* RT-PCR-positive samples according to Matsuoka et al. [8]. Clinical data was gathered from medical records.

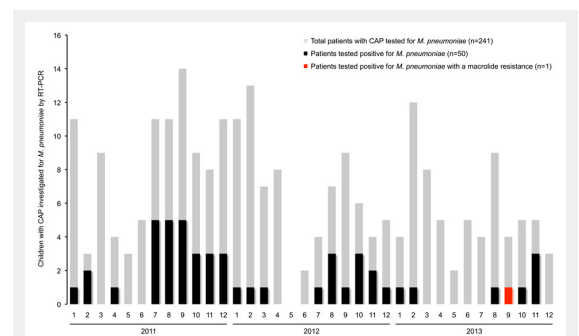
### Abbreviations

CAP	=	Community-acquired pneumonia
DNA	=	Deoxyribonucleic acid
MRMP	=	Macrolide-resistant <i>Mycoplasma pneumoniae</i>
rRNA	=	Ribosomal ribonucleic acid
RT-PCR	=	Real-time polymerase chain reaction

## Results

During the 3 years, respiratory specimens from 50 out of 241 CAP patients (20.7%; figure) tested positive for *M. pneumoniae*. The median age of the 50 patients was 9.1 years (range, 1.3–15.9). The clinical diagnosis of CAP was radiologically confirmed in 93% (43/46). Macrolides were administered in 2% of patients before and in 26% after detection of *M. pneumoniae*.

Sequencing of the partial 23S rRNA gene amplicons from these *M. pneumoniae* RT-PCR-positive respiratory samples (96% pharyngeal swabs) revealed one sample with an A2063G mutation, which confers high-level macrolide resistance [2]. Mutations at position 2064 or 2617 were not found. The resistant strain was detected in a pharyngeal swab specimen of an otherwise healthy 7-year-old boy seen in September 2013 with a mild and self-limiting *M. pneumoniae* CAP (no hospitalisation nor antimicrobial treatment). The patient had not received prior antimicrobial treatment.



Figure

Case distribution of children with community-acquired pneumonia (CAP) investigated for *Mycoplasma pneumoniae* by real-time polymerase chain reaction (RT-PCR) in respiratory specimens (n = 241) during the study period between 2011 and 2013.

## Discussion

Our survey documents the first existence of MRMP in a child with CAP due to *M. pneumoniae* in Switzerland. We detected the A2063G mutation that represents the most common cause of high-level macrolide resistance in *M. pneumoniae* [2]. At least four mutations in the 23S rRNA gene of *M. pneumoniae* have been reported *in vitro*, whereby mutations at positions 2063, 2064, and 2617 (corresponding to *Escherichia coli* 23S rRNA gene positions 2058, 2059, and 2611) were also found *in vivo* [2]. The point mutation results in modification of the peptidyltransferase loop of domain V of 23S rRNA, which reduces the binding affinity of macrolides to the 23S rRNA component of the large subunit (50S) of the bacterial ribosome.

The frequency of MRMP among our paediatric CAP patients (2.0%, 1/50) is remarkably low compared to that recently detected in children in Japan (87.1%, 176/202) [9] and China (97.0%, 32/33) [3] although study designs and cohorts differ, which limits comparison. The high resistance rate in Asia is linked with broad macrolide use [2]. This may be also true for the alarming resistance rate observed in the neighbouring country Italy (25.6%, 11/43) [4], where selective antibiotic pressure has been demonstrated to lead to *de novo* macrolide resistance during treatment. However, the emergence of MRMP in our study parallels the lower occurrence of MRMP in other neighbouring countries, e.g., Germany (1.2%, 2/167) [10] and France (9.8%, 5/51) [11]. There was no macrolide treatment history in our patient. This suggests MRMP did not emerge in the patient himself but rather was acquired from the community. Importantly, children have recently been recognised to carry *M. pneumoniae* in their respiratory tract in up to 21% [12]. This influences current diagnostic procedures, because neither PCR nor single sample serology is a reliable indicator of infection [1, 12], and, in turn, may suggest that MRMP is indeed considerably less frequent in Switzerland compared to in countries with higher prescription numbers of macrolides, as e.g., Asia [3, 9].

Limitations of our survey include the retrospective design (missing *M. pneumoniae* CAP cases not tested for *M. pneumoniae* and/or managed by general practitioners only), the patient cohort (both in-patients and out-patients) that was geographically confined to the region of Zurich, Switzerland, and the lack of control samples from asymptomatic children. Moreover, RT-PCR for *M. pneumoniae* from respiratory specimens other than pharyngeal swabs is less validated.

MRMP can potentially have clinical consequences, i.e., longer duration of symptoms and hospitalisation, more severe CAP, and increased frequency of extrapulmonary manifestations [1]. Thus, further surveys of MRMP in children should be considered also in Switzerland to prevent missing changes in MRMP in the local community. Our results are compatible with the rather judicious prescription of macrolides in Switzerland [13]. To this effect current guidelines confine macrolide treatment for CAP in children at any age as second choice if there is no response to first-

line empirical  $\beta$ -lactam antibiotics or in the case of very severe CAP [14].

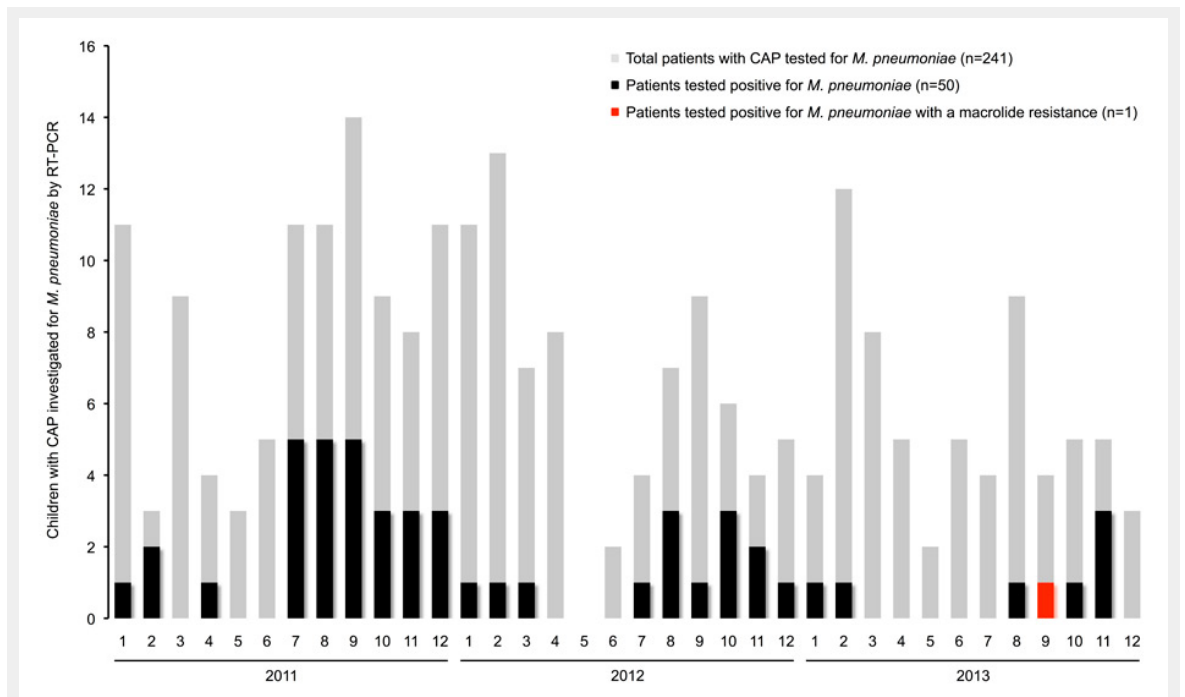
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## Figures (large format)



Figure

Case distribution of children with community-acquired pneumonia (CAP) investigated for *Mycoplasma pneumoniae* by real-time polymerase chain reaction (RT-PCR) in respiratory specimens (n = 241) during the study period between 2011 and 2013.