

Assessment of Genotypic Macrolide Resistance among *Mycoplasma pneumoniae* Infections in Children in Singapore

Dear Editor,

Mycoplasma pneumoniae belongs to the class *Mollicutes* and is a small bacterium without a cell wall that is a common cause of community-acquired pneumonia in adults and children. Due to its unique physical structure, *M. pneumoniae* is resistant to cell wall-targeting agents, such as beta-lactam antibiotics. Therefore, the antibiotics of choice for the treatment of suspected *M. pneumoniae* infections, particularly for children, are the macrolides, with their main class representative being erythromycin. Alternatives for treatment include the tetracyclines and fluoroquinolones.

Reduced susceptibility or resistance of *M. pneumoniae* to macrolide antibiotics has been described; this is the consequence of point mutations in domain V (peptidyltransferase region) of the 23S rRNA gene.^{1,2} The most common mutation is the substitution of adenine with guanine (A2063G) at base position 2063 (*M. pneumoniae* numbering, equivalent to base position 2058 in *Escherichia coli*).³ This mutation has been found associated with a 10³-fold to >10⁵-fold elevation of the minimum inhibitory concentration (MIC) of erythromycin as compared to susceptible strains.⁴ Less common mutations occur at positions 2063 (A→T, A→C), 2064 (A→G, A→C), 2067 (A→G) and 2617 (the latter not studied in this work). These mutations reduce the affinity of macrolides for the ribosome,^{4,5} thereby reducing their efficacy. Macrolide resistance due to *erm* genes or enzymes that inactivate macrolides have not been detected thus far in *M. pneumoniae*.²

Studies conducted from 2011 onwards in Japan, South Korea and China have demonstrated alarmingly high levels of macrolide resistance (ranging from 63% to 98%) in *M. pneumoniae* strains.² On the other hand, macrolide resistance in *M. pneumoniae* from Europe, North America and Australia has been less common (ranging from 0% to 26%).² The prevalence of macrolide resistance in *M. pneumoniae* in Singapore or its neighbouring countries in South-east Asia is currently unknown. Thus, we sought to determine the prevalence of genotypic macrolide resistance in *M. pneumoniae* among clinical specimens submitted for diagnostic purposes at a paediatric hospital in Singapore. We used PCR primers published by Wolff et al⁶ to amplify

a region that encompasses the mutations at positions 2063, 2064 and 2067 that account for more than 99% of macrolide resistance in published works.²

Materials and Methods

The microbiology laboratory at KK Women’s and Children’s Hospital receives throat swabs and other respiratory tract specimens for a diagnostic *M. pneumoniae* polymerase chain reaction (PCR) test. This PCR assay uses a combination of 2 primers and a dual-labelled probe that targets the *M. pneumoniae* community-acquired respiratory distress syndrome (CARDS) toxin gene, as described by Winchell et al.⁷ We selected consecutive nucleic acid extracts submitted between 1 January 2013 and 31 March 2014 that were positive for *M. pneumoniae* and had a threshold cycle (Ct) of less than 30 in the diagnostic PCR assay. This cutoff was chosen based on preliminary experiments (data not shown) that indicated that such samples yielded sufficient amounts of DNA for sequencing reactions. A total of 327 samples were positive for *M. pneumoniae*, and 200 samples had a Ct value of <30. Using primers designed by Wolff and coworkers⁶ targeting the domain V of the 23S rRNA gene of *M. pneumoniae*, we amplified a 217 base-pair fragment (from base position 1937 to 2154) (Taq Core PCR Kit, Qiagen GmbH, Germany). We purified the DNA fragment by gel extraction (Qiaquick Gel Extraction Kit, Qiagen GmbH, Germany) and dispatched the purified fragment for DNA sequencing to identify the known point mutations associated with resistance to macrolides. Multiple sequence alignment was performed on the 200 positive samples, using the wild-type *M. pneumoniae* M129 23S rRNA gene (GenBank accession number NC_000912; Gene ID 876745) as a reference (Fig. 1).

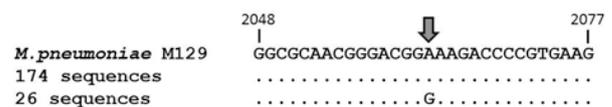


Fig. 1. Sequence alignment of domain V region of 23S rRNA genes of *M. pneumoniae* from base position 2048 to 2077. Two representative clinical samples are shown, along with their observed frequency in this study, compared to the wild-type strain M129 (GenBank accession NC_000912). Dots represent identity to the wild-type sequence. Position 2063 is indicated by an arrow.

Results

The median age of the patients from whom the samples originated was 6 years, and the age range was 0 to 15 years. There was an almost equal distribution of specimens from male and female patients. More than 90% of the patient specimens were throat swabs, with the rest made up of nasopharyngeal swabs, nasal swabs, pleural fluid and bronchioalveolar lavage. The A2063G point mutation was detected in 26 of the 200 nucleic acid extracts that were investigated. All other sequences were identical to the reference sequence of a macrolide-susceptible strain (NC_000912; Gene ID 876745). Thus, the prevalence of genotypic macrolide resistance in this cohort was 13%. None of the other mutations at base positions 2064 and 2067 that were previously associated with macrolide resistance were found.

Conclusion

To our knowledge, this is the first report on the prevalence of genotypic macrolide resistance in *M. pneumoniae* from Singapore or any other country in its direct vicinity within South-east Asia. The prevalence among Singaporean samples, collected in 2013 and 2014, was 13% based on sequencing results of the 217 base-pair region. This is within the range of 0% to 26% reported from Europe, North America and Australia.² Thus, the prevalence of genotypic macrolide resistance in Singapore is lower than might be expected from the high rates reported in East Asia. All 26 mutant sequences from Singapore contained the A2063G substitution. This is consistent with other studies that demonstrated the presence of the A2063G mutation in the vast majority (93.9%) of macrolide-resistant *M. pneumoniae*^{2,3,8} compared to mutations at positions 2064 (2.8%), 2067 (0.05%) and 2617 (0.1%), which are significantly less common. There is a possibility that we may have missed mutations at base position 2617, but this would be an extremely infrequent event based on the literature.² The fact that all mutant strains possessed the same point mutation at base 2063 is likely a reflection of the fact that this is the most common mutation. An alternative explanation would be the clonal transmission of mutated strains, but this appears less likely, and it would require molecular typing to establish whether this was the case. In addition, our study established an estimate of the prevalence of macrolide resistance among respiratory samples submitted to our laboratory, but was not aimed at assessing pathogenicity, especially in view of the fact that it is known that *M. pneumoniae* can colonise asymptomatic children.⁹

Most infections with *M. pneumoniae* tend to take a mild-to-moderate clinical course, even if untreated or treated with ineffective antibiotics, and severe infections are uncommon.¹⁰ Infections with macrolide-resistant *M.*

pneumoniae (MRMP), when treated with macrolides, have been reported to be associated with a longer time to defervescence compared to infections with macrolide-susceptible strains.^{10,11} Genotypic macrolide resistance was found associated with treatment failure in a case of severe pneumonia reported from Hong Kong.¹² The correlates for severe illness in *M. pneumoniae* infections are not fully understood, and lung injury may be related to the host cell-mediated immune response.¹³ The authors of a recent review concluded that no change away from primary macrolide therapy is required in countries that have a low incidence of MRMP infections, and a replacement with fluoroquinolones or tetracyclines should be considered in regions where MRMP are common.¹⁰ A change of antibiotics should also be considered if symptoms persist or if there is clinical deterioration, and primary treatment with other antibiotics should be considered when infections are severe, even in the early phase.¹⁰

One limitation of our study is that these data reflect the situation among the Singaporean paediatric population, but not necessarily among the adult population. In addition, we did not perform phenotypic tests for macrolide resistance because we did not perform cultures. However, previous studies by other researchers have shown that other mechanisms of macrolide resistance are rare.^{2,14}

Susceptibility testing for *M. pneumoniae* is not routinely performed in diagnostic laboratories, but the knowledge of local macrolide resistance is useful to guide empiric therapy. Our findings indicate that the prevalence of macrolide resistance in *M. pneumoniae* strains from paediatric patients in Singapore is relatively low. That would imply that a change away from primary macrolide treatment for *M. pneumoniae* infections is not indicated at this point in time. There is a possibility that MRMP may spread rapidly¹⁵ and thus, periodic surveillance for resistance to macrolides appears necessary to detect emerging resistance.

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