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The fall of the Macrolide: Macrolide-resistant *Mycoplasma pneumoniae*

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Abstract

Mycoplasma pneumoniae (M. pneumoniae) is a common cause of community-acquired pneumonia in children. It has always been considered to be a benign and self-limiting condition. Even when treatment is required, macrolide has always been considered to be the first line antibiotic, particularly in children under the age of eight. Since macrolide-resistant *M. pneumoniae* (MRMP) was first described in Japan in 2001, there has been a substantial increase in the number of cases of MRMP, both in Asia and in European countries. This has resulted in a problem with antibiotic selection, particularly in younger children. In this article, I will review the local epidemiology and discuss the diagnosis and treatment options of MRMP in Hong Kong.

Keywords: Antibiotics ressitance, Community-acquired pneumonia, Macrolide-resistance, *Mycoplasma pneumoniae*

Introduction

Mycoplasma pneumoniae (M. pneumoniae) is a major cause of community-acquired pneumonia (CAP) in children, accounting for 10-30%[1-4] of the etiological agents worldwide. Yet, the epidemiology of *M. pneumonia*-associated CAP in Hong Kong remains unknown for several reasons. First, there is no systemic surveillance of agents causing CAP in place in Hong Kong. Secondly, serology was the mainstay of diagnosis until a few years ago, and the data provided by serology only reflects the trend of disease and it does not provide an accurate measurement of the magnitude of the disease, nor does it provide any data on antibiotic resistance. Thirdly, *M. pneumoniae* has always been perceived as a benign disease and it was unable to attract the attention of the health care administrators to implement a cumbersome surveillance system and put in resources to study the epidemiology, until recently.

The aetiology of acute respiratory tract infections was studied in an university hospital in the New Territories East cluster of Hong Kong from 2005 to 2006 and *M. pneumoniae* accounted for 1.5% of the non-bacterial pathogens causing acute respiratory tract infections in children under the age of five.[5]

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In Hong Kong public hospitals managed by the Hospital Authority (HA), the Chief Infection Control Officer (CICO) office has recently published the HA data on *M. pneumoniae* requiring hospitalisation[6] where the data was extracted by CDARS search. Although the data is not a systemic study of the epidemiology of *M. pneumoniae* and the denominator remains unknown, there is an apparent increasing trend of *M. pneumoniae* infection starting from the year of 2013. The reason can be due to a genuine increase in the incidence of *M. pneumoniae* signifying an epidemic, or it can be due to a more widespread use of Mycoplasma PCR resulting in the uncovering of more cases that were missed by serology in the past. However, comprehensive data on genotypic resistance was not available. In this article, I aim to review the epidemiology, clinical features, laboratory testing and treatment options of Macrolide-resistant *M. pneumoniae* (MRMP) in Hong Kong.

Mechanism of resistance of MRMP

Macrolide inhibits the 50s rRNA by binding to the 23s rRNA, therefore inhibiting protein synthesis. Mutation in the domain V of the 23s rRNA has been associated with elevated MIC towards macrolides, with mutation at the position A2063 and A2064 resulting in a high level resistance, whereas mutation at A2067 and C2617 results in a lower level of resistance.[7] The most common point mutation is A2063G, and only this mutation has

been described in Hong Kong.[8,9] In a recent large community outbreak in Yamagata in Japan, the pathogenic organism carried an uncommon mutation A2063T transversion, which is associated with high-level resistance to clarithromycin.[10]

Epidemiology of *Mycoplasma pneumoniae* and MRMP in Hong Kong

MRMP was first reported in Japan in 2001.[11] Since then, there has been reports in China,[12-15] Taiwan,[16,17] Korea,[18] the United States of America[19,20] and various European countries, including Scotland,[21] Spain[22] and Germany.[23] In China, the prevalence of MRMP is exceptionally high, constituting over 90% of all isolates of *M. pneumoniae*.[13] The first imported case of MRMP in Hong Kong was reported in the Hong Kong West Cluster in an adult returning from Xi'an in 2009.[24] The first locally acquired case of MRMP in Hong Kong has been reported in the New Territories West cluster in 2010.[25]

There is currently no territory wide surveillance of Mycoplasma in Hong Kong, therefore the true epidemiology of *M. pneumoniae* and the prevalence of MRMP in Hong Kong remains unknown. There are two local publications providing information on the local situation of MRMP. The first study evaluated different molecular methods to detect genotypic resistance in *M. pneumoniae* in both adult and paediatric subjects.[9] Pyrosequencing identified mutation at the position A2063G in 78.8% of the *M. pneumoniae* PCR positive cases, where Sanger sequencing and melting curve analysis only identified the genotypic mutation in less than 40% of the PCR positive cases. The difference is mainly due to the ability of pyrosequencing to identify low-frequency MRMP quasispecies. Another local study evaluated the antibiotics treatment efficacy against MRMP in the paediatric age group only.[8] Among the paediatric CAP cases with a positive Mycoplasma PCR, 70% were MRMP. Only A2063G mutation was identified in both studies. Both publications only studied a selected group of patients and this may not represent the true epidemiology of MRMP in Hong Kong.

M. pneumoniae has always been considered a disease of school aged children, and the Infectious Disease Society of America (IDSA) guideline even recommended that testing should only be considered in school-aged children as this group has a higher pre-test probability.[26] A recent study has demonstrate a high rate of *M. pneumoniae*-associated CAP in younger children, where 18% were infant age group 0-1 years and 30% were between 2-11 years.[9]

Laboratory diagnosis of MRMP

M. pneumoniae infection cannot be diagnosed based on clinical signs or symptoms alone.[27] Infection is a result of the interaction between the host and the infective microorganism, therefore the combination of clinical features of lower respiratory tract infection, radiological changes, microbiological evidence of the presence of an infective organism and demonstration of host immune response towards the offending microbiological agent by serological means would be the most definitive and reliable way[28] to diagnose an infection. However this approach is not realistic and not always possible particularly in the busy ward setting.

Serology remained the only available means to diagnose *M. pneumoniae*-associated CAP in Hong Kong until recently, where Mycoplasma PCR has become readily available and has replaced serology to become the mainstay of diagnosis of *M. pneumoniae*-associated CAP nowadays. *M. pneumoniae* PCR has become a routine test in most of the HA hospitals in Hong Kong[29] since 2010. Mycoplasma PCR has been shown to be a promising alternative to serological diagnosis, it is rapid,[30,31] sensitive and specific and has been demonstrated to be superior to serology in the acute phase of *M. pneumoniae* infection.[32] By using the real-time PCR targeting the P1 adhesin gene, a large number of specimens can be processed at the same time.[31] PCR does not only shorten the time to diagnosis, it also allows more rapid initiation of appropriate antimicrobial agents, therefore enhancing parent's satisfaction and more rapid discharge. The shortened total hospital journey can in-turn save the hospital cost and reduce the workload of healthcare workers.

Mycoplasma IgM is available in selected public hospitals and Mycoplasma particulate agglutination is available in the Government Public Health Laboratory Service in Hong Kong. However the value of Mycoplasma IgM in assisting diagnosis during the acute phase is questionable, due to the low sensitivity, high false positive rate[33] and the delayed result, and serological test by particulate agglutination may not always provide clinically useful result during acute phase as a paired titre is required. The delayed positive result does not provide timely information to guide clinical management in real life situations. Moreover serological diagnosis does not provide any information on genotypic

resistance. But serological diagnosis still remains an important epidemiological tool to monitor the trend of *M. pneumoniae* in the local population.

PCR may be able to detect *M. pneumoniae* in seronegative cases, however it is unable to differentiate delayed serological response and Mycoplasma carrier.[31] The Mycoplasma PCR may remain positive up to 7 months after disease onset.[32] None the less, Mycoplasma PCR is becoming the choice laboratory diagnosis worldwide.

North American[26] and European guidelines[34] does not have any recommendation on the laboratory diagnosis of MRMP. Sequencing of the domain V of the 23s rRNA gene remains the mainstay of identification of macrolide-resistance in *M. pneumoniae* in Hong Kong,[8,9,24,25] the test is available in selected specialised centers, University hospitals and the Government Public Health Laboratory Service in Hong Kong. Since the result of the resistance genotype may not be readily available, empirical initiation of alternative antimicrobial agents may sometimes be required. This may not be a problem in children older than 8 years of age, however for children under this age, initiation of a tetracycline group of antibiotics may be problematic. Resistance genotype testing results can back-up the treatment decision and aid clinicians in the counseling process, and this would require good communication and arrangement with specialised clinical microbiology laboratory.

Clinical features

There have been various reports of failure of macrolides for the treatment of MRMP in terms of persistence of symptoms and prolonged duration of fever.[7,18,35-37] Alternative antibiotics, such as quinolones and tetracyclines have therefore been used.[17,25] Several case series in Japan have demonstrated the efficacy of minocycline and doxycycline for treatment of MRMP in children,[38-40] this will be discussed in the next section in detail. But the main argument against using these agents are that if the patient, parents and clinicians can tolerate the fever and cough, these symptoms will eventually subside as most Mycoplasma infection are benign and self-limiting, so why is there a need to use tetracyclines and quinolones?

Earlier studies did not show any increase in severity and complications in patients infected with MRMP[41] but current evidence suggests otherwise. Recent reports have suggested that MRMP infection was associated with more extra-pulmonary complications,[42] more critical care admissions[21] and more ARDS.[43] The overall rate of *M. pneumoniae* requiring intensive care admission in Hong Kong was 2.2% by CDARS search.[29]

The role of *M. pneumoniae* in persistent cough has been well studied[44] but there is no study on MRMP. There may also be a possible association between asthma and *M. pneumoniae* infection.[45]

Treatment of MRMP

There is currently no consensus on whether or not we should treat *M. pneumoinae*-associated CAP with antibiotics. Meta-analysis failed to show the benefit to treat *M. pneumoniae*-associated CAP[46] or to refute treatment.[47] Studies are extremely heterogeneous and the benefit of treating *M. pneumoinae*-associated CAP has not been consistently demonstrated, but the most important reason is the lack of high quality double blind RCTs.[46] Despite the lack of good evidence to support treatment of *M. pneumoniae* infection, this has not been a great concern to paediatricians, at least before the appearance of MRMP.

There are at least three reasons to support the treatment of MRMP:

1. ***Prevent the development of complications***
   As mentioned in the above section, there is increasing evidence to demonstrate that MRMP infection is associated with more severe clinical manifestations and extra-pulmonary complication. Although there is currently no evidence to suggest that early treatment can prevent the occurrence of complications, it would be logical to treat MRMP early to prevent the progression of the infection.

2. ***Infection control issues***
   It is common to see Mycoplasma outbreaks in schools and institutes. Patients infected with Mycoplasma can appear to be quite well (hence the name "walking pneumonia") despite harboring a high Mycoplasma load in the respiratory tract. The use of tetracycline has been demonstrated to be able to achieve a significant reduction of Mycoplasma load after 48 hours of treatment with tetracycline, and by day five of doxycycline treatment, all study subjects would be rendered culture negative. Therefore treatment of children infected with MRMP can prevent school and community outbreaks.

3. Prevent any possible long-term damage to the airway by M. pneumoniae

There is growing evidence to demonstrate the role of *M. pneumoniae* in contributing to the development of asthma.[48] Although the causal relationship has not been adequately demonstrated yet, it is a reasonable choice to treat MRMP in-order to prevent any possible long-term damage to the airway. In-vitro studies have demonstrated that MRMP is not suppressed by macrolide,[13,49] but in real-life clinical situations, fever eventually subsides even if macrolide is continued,[16,36] it just takes longer to achieve defervescence. Despite the disappearance of symptoms, a Japanese study has demonstrated persistently high Mycoplasma load in the airway by the end of macrolide treatment,[40] and the persistence of Mycoplasma in the airway can possibly continue to exert damage to the respiratory epithelium even in the absence of clinical symptoms.[48]

Neither IDSA guideline nor BTS guideline have any recommendation on the treatment of MRMP.[26,34] The Japanese guideline for management of respiratory infection in children published in 2007 has recommended the switching to tetracycline antibiotics if fever persists for more than 48 hours after macrolide antibiotic initiation.[50] In-vitro studies have demonstrated that the tetracyclines and quinolones have low MIC value against MRMP.[7,14,49,51] Several case series in Japan have suggested the use of minocycline and doxycycline for treatment of MRMP in children.[38-40] The discussion below will mainly concentrate on the use of tetracyclines and quinolones to treat MRMP, the use of corticosteroid will not be discussed in this review article.

1. Tetracyclines

Recent prospective study in Japan has demonstrated that minocycline or doxycycline was significantly more effective than tosufloxacin (a respiratory fluoroquinolones available in Japan) in achieving defervescence within 24 hours and decreasing the number of *M. pneumoniae* DNA copies three days after initiation.[38] More than 80% of the patients in the doxycycline group were able to achieve defervescence within 24 hours. The group receiving macrolide had poor clinical response in terms of delayed defervescence and poor microbiological response. Another study in Japan demonstrated low clinical and bacteriological efficacies of macrolides against MRMP.[40] Minocycline was able to achieve defervescence within 48 hours in 87% of the patients, where only 69% of those in the tosufloxacin, 41% in azithromycin and 48% in clarithromycin group.

A local study reviewing 34 children with MRMP-associated CAP demonstrated the ability of doxycycline to achieve rapid defervescence.[8] All patients infected with MRMP treated with doxycycline were able to achieve defervescence within 24 hours, and most of the patients achieved defervescence within 12 hours, which was significantly more effective than macrolides. The mean duration of defervescence in the doxycycline group, irrespective of MRMP or MSMP, was within 24 hours. Minocycline is not licensed in Hong Kong, the United States or the European Union[38] for the treatment of *M. pneumoniae* infection. Therefore doxycycline is the only available choice to be used in these countries. The main concern for using tetracyclines in children is the potential to cause teeth staining. The tetracyclines have the affinity to bind to calcium and resulting in the formation of tetracycline-calcium orthophosphate complex.[52] This complex is coloured and the rate of binding depends on the rate in mineralisation, i.e. the higher the rate, the more rapid the binding. Since

Table 1. Antibiotics available for the treatment of *M. pneumoniae* in Hong Kong

	Licensed for treatment of *M. pneumoniae* in Hong Kong	Licensed for treatment of respiratory tract infection	Bioavailability	Other comments
Erythromycin	✓	✓	45%	
Clarithromycin	✓	✓	50%	
Azithromycin	✓	✓	37%	
Doxycycline	✓	✓	90-100%	Lowest affinity to bind to calcium
Minocycline	×	×	90-100%	Only licensed for acne in Hong Kong
Tetracycline	×	×	60-80%	
Tigecycline	×	✓	IV only	
Ciprofloxacin	✓	✓	70%	
Levofloxacin	✓	✓	99%	
Moxifloxacin	✓	✓	86%	

the formation of enamel in permanent teeth is completed by the age of eight, the use of tetracyclines before this age would therefore result in permanent staining.[52] Among the three commonly used tetracyclines, doxycycline causes the least teeth-staining in children, possibly because it has lower affinity to bind to calcium,[53] yet it is still not widely recommended to be used below eight years of age. The American Academy of Pediatrics (AAP) recommends that the use of tetracyclines in children under the age of eight is justified when the benefit is greater than risk,[54] and doxycycline is choice of treatment because the calcium binding affinity is the lowest. In one report, the investigator concluded that doxycycline does not cause teeth-staining in children under the age of eight.[55]

For children >8 years old, doxycycline can be used for the treatment of MRMP (both clinical macrolide non-responder or laboratory confirmed case) without any problem. For children ≤8 years old infected with MRMP (clinical macrolide non-responder or laboratory confirmed case), doxycycline can be considered when the benefit exceeds risk, for example in life-threatening pneumonia or in children experiencing severe extra-pulmonary complications. For severe MRMP cases where oral antibiotics cannot be tolerated, intravenous minocycline can be used (available in some HA hospitals).

2. Fluoroquinolones

Despite the in-vitro susceptibility of fluoroquinolone, the clinical and microbiological response was inferior when compared with tetracyclines (minocycline and doxycycline) in all three studies mentioned above.[8,38,40] Two studies in Japan demonstrated persistence of Mycoplasma in the airway despite fluoroquinolone treatment[38,40] and this can result in continuous airway damage[48] and spread of mycoplasma to susceptible individuals. Fluoroquinolone is not recommended for the treatment of CAP in Hong Kong due to the high prevalence of tuberculosis in the Hong Kong IMPACT guideline published in 2012, however this recommendation is mainly for adults and no local paediatric recommendation is available at this moment. Therefore Fluoroquinolone can be considered as an option for MRMP (clinical macrolide non-responder or laboratory confirmed case) associated CAP in children ≤8 years old, particularly if the parents are not willing to accept the risk of doxycycline.

Unanswered questions remain:

1) What is the optimal timing to initiate tetracyclines in the absence of genotypic results to guide treatment?
2) Will the use of tetracyclines really shorten the duration of hospitalisation?
3) Can early treatment prevent the development of complications?
4) Can long-term carriage of *M. pneumoniae* really result in asthma?

Conclusion

Mycoplasma PCR has greatly shortened the time to establish the diagnosis of *M. pneumoniae* infection, enabling the detection of genotypic resistance and allows us to have a better understanding of the epidemiology of MRMP in Hong Kong. Local studies suggest an apparent increase in cases of MRMP in Hong Kong and there is a need to reconsider our choice of empirical antibiotics in children with CAP. The tetracyclines (particularly doxycycline) has been consistently demonstrated to be able to achieve rapid defervescence and satisfactory microbiological response where fluoroquinolones were inferior to the tetracyclines but can act as an alternative.

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