Clinical Characteristics and Predictor of Macrolide Resistant Mycoplasma Pneumoniae in Paediatric Pneumonia

Tammy Cheung[1], David Shu-yan Lam[1], and David Lung[2]

[1]Tuen Mun Hospital
[2]Hospital Authority

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Abstract

The aim is to look at the rate of macrolide resistance in a group of children admitted with mycoplasma pneumonia at a local hospital and to compare and analyze clinical features of macrolide-resistant Mycoplasma pneumoniae (MRMP) and macrolide-sensitive Mycoplasma pneumoniae (MSMP) to facilitate early recognition of likely resistance and to guide the choice of appropriate antibiotics for treatment. Paediatric patients with pneumonia with a real time mycoplasma PCR positive result were analyzed. Mutations associated with macrolide resistance were identified by direct DNA sequencing of the domain V of the 23S rRNA gene and patient. MRMP was identified in 43% of the patients tested within March 2013 to August 2013. No single clinical characteristic or laboratory markers were reliable in differentiating between MSMP and MRMP. A clinical parameter of non-defervescence at 72 hours was identified as a good clinical indicator for likely macrolide resistance. 96% of MSMP patients achieved defervescence by 72 hours. There were 5 patients that developed pleural effusion and 80% of them belonged to the MRMP group. All of the 15 patients treated with doxycycline, were able to achieve rapid defervescence within 24 hours. The overall length of stay was longer in the MRMP group. Macrolide resistance in Hong Kong appears to be lower than previous published data. With limited laboratory support to identity resistance, clinical parameters help facilitate and support the decision for alternative treatment options such as doxycycline to prevent complications and prolong length of stay in a common and treatable condition in the paediatric population.

Clinical Characteristics and Predictor of Macrolide Resistant *Mycoplasma Pneumoniae* in paediatric pneumonia

Authors:

[1]CHEUNG Tammy Wing Yee

[2]LAM Shu Yan

[3]LUNG David Christopher

[1,2] Department of Paediatrics & Adolescent Medicine, Tuen Mun Hospital, Hong Kong

[3] Department of Clinical Pathology, Tuen Mun Hospital, Hong Kong

Corresponding author

Tammy Wing Yee CHEUNG

Department of Paediatrics & Adolescent Medicine, Tuen Mun Hospital, Ching Chung Koon Road, New Territories, Hong Kong

Telephone: +852 2468 5392

Email: cwy531@ha.org.hk

Abstract

Objective

The aim is to look at the rate of macrolide resistance in a group of children admitted with mycoplasma pneumonia at a local hospital and to compare and analyze clinical features of macrolide-resistant *Mycoplasma pneumoniae* (MRMP) and macrolide-sensitive *Mycoplasma pneumoniae* (MSMP) to facilitate early recognition of likely resistance and to guide the choice of appropriate antibiotics for treatment.

Patient and method

Paediatric patients with pneumonia with a real time mycoplasma PCR positive result were analyzed. Mutations associated with macrolide resistance were identified by direct DNA sequencing of the domain V of the 23S rRNA gene and patient.

Result

MRMP was identified in 43% of the patients tested within March 2013 to August 2013. No single clinical characteristic or laboratory markers were reliable in differentiating between MSMP and MRMP. A clinical parameter of non-defervescence at 72 hours was identified as a good clinical indicator for likely macrolide resistance. 96% of MSMP patients achieved defervescence by 72 hours. There were 5 patients that developed pleural effusion and 80% of them belonged to the MRMP group. All of the 15 patients treated with doxycycline, were able to achieve rapid defervescence within 24 hours. The overall length of stay was longer in the MRMP group.

Conclusion

Macrolide resistance in Hong Kong appears to be lower than previous published data. With limited laboratory support to identity resistance, clinical parameters help facilitate and support the decision for alternative treatment options such as doxycycline to prevent complications and prolong length of stay in a common and treatable condition in the paediatric population.

Keywords : mycoplasma pneumonia; paediatrics; macrolide resistance; community acquired pneumonia

Introduction

Pneumonia remains an important disease with significant morbidity and mortality in children across both developed and developing countries worldwide. It is estimated to be responsible for nearly 20% of all paediatric admissions in the developed world [1-3].

M. pneumoniae has been reported to cause up to 40% of community-acquired pneumonia (CAP) in children and about 18% of infections in patients requiring hospitalization.[4]

The current international recommended antibiotic treatment for children with CAP is to use macrolide to cover atypical bacterial pathogens if there is no response to first-line empirical beta-lactam antibiotics, or if the causative agent is compatible to atypical pathogens.[5,6]

Macrolide resistance has been increasingly observed in a number of countries across several continents since the first report in Japan in 2001 [7]. Yet there has been a wide variation in the resistant rate among different regions worldwide. In Europe and North America, the resistance rate is relatively lower with rates ranging from 2% to 23% in Germany and Italy, to 12% in Canada. In Asian countries, recent studies reported 30% in Japan to an alarmingly high rate of 90% in China. [7-15]

There is currently no territory wide surveillance of Mycoplasma in Hong Kong and hence the true epidemiology of *M. pneumoniae* remain unknown. Previous local publication in Hong Kong has shown up to a high 75% resistance rate in Hong Kong. However, the studied group was of a highly selective population, which involved patients not responsive to first line treatment previously. [16]

Although the use of antibiotic treatment of *M. pneumoniae*associated respiratory diseases is questioned by some physicians, most experts suggest that antibiotics should be systematically used, especially those involving the lower respiratory tract[17].

Beta-lactam antibiotics are generally considered the drugs of choice for treating respiratory diseases because they are active against most respiratory bacterial pathogens, but are ineffective against *M. pneumoniae* because they target the cell wall, which is lacking in*M. pneumoniae* .

In contrast, macrolides and tetracyclines which act as protein synthesis inhibitors, and quinolones which inhibit DNA synthesis and replication, are usually highly effective against *M. pneumoniae,* and are the drugs of choice for the treatment of infections due to atypical bacteria. Although macrolides are generally considered to be the first line of treatment, with recent emerging resistant strains of Mycoplasma, alternative antibiotics such doxycycline and levofloxacin has become increasingly used. However, doxycycline and levofloxacin have limited paediatric use due to their respective potential adverse effects such as teeth staining, enamel hypoplasia, depressed bone growth and articular problems, and considered for treatment when benefits appear to outweigh risks. Therefore we aim to look for clinical indicators to identify cases with likelihood of resistance, as molecular methods are not widely available in public hospitals in Hong Kong. This can guide the clinical decision of early treatment with doxycycline and therefore shorten the length of hospitalization and the overall burden on the health care system.

This study looks at a larger sample of patients beyond the previously published selected group of patients admitted with *M. pnuemoniae*to a large regional hospital in Hong Kong. The aim is to extend the data surveillance for a clearer perspective on the resistance rate in our local community and to compare characteristics of macrolide resistant strains of Mycoplasma to look for any associated clinical parameters predicting resistance with implications in guiding treatment with antibiotics alternative to those in the macrolide group.

Methods

Children under the age of 18 years old admitted to Tuen Mun Hospital, a local regional hospital serving a population of over one million in the northwest region of Hong Kong, with a diagnosis of CAP and subsequently tested positive for Mycoplasma PCR isolated from nasopharyngeal aspirates or sputum, gastric lavage samples in the period of March 2013 to August 2013 were retrospectively studied.

The diagnosis of pneumonia was based on fever, acute respiratory symptoms (tachypnoea, chest retractions, abnormal findings on ausculation) or both, with evidence of parenchymal infiltrates on chest radiography.

Based on the direct DNA sequencing of the domain V of the 23S rRNA gene, mutations associated with macrolide resistance were identified and divided the patients into MRMP and MSMP groups. All patients screened positive had a mutation at the position 2063 of the gene. Their medical records were reviewed and demographic data such as age and sex, previous use of antibiotics prior to admission and travel history 2 weeks prior to admission were recorded with the investigator blinded to their respective MSMP or MRMP status.

Clinical parameters including cough, shortness of breath, respiratory rate, rash, and oxygen supplementation required were compared.

Laboratory findings including white blood cell count (absolute neutrophil and lymphocyte count) and C-reactive protein levels (CRP), erythrocyte sediment rate (ESR), alanine aminotransferase (ALT), cold agglutinin levels were compared between the two groups. Radiological findings on chest radiographs were also analyzed. A total of 74 patients were included in the subgroup analysis performed with characteristics within the episode of admission such as total duration of fever and time to defervescence (defined as core body temperature less than 38.5°C for 3 consecutive readings with no recurrence of fever within 24 hours without the use of antipyretics).

All statistical analysis was performed using the SPSS software, version 21. Categorical variables were assessed by two-tailed Fisher's exact test and chi-square test, Mann–Whitney U-test was used for continuous variables.

ROC curves and area under curve was constructed to look at clinically predictive factors for MRMP. P value <0.05 was considered statistically significant.

Approval was obtained with IRB prior to conducting this study.

Mycoplasma Polymerase Chain Reaction (PCR) testing and sequencing

Respiratory tract specimens, including nasopharyngeal aspirates, sputum samples or gastric lavage were tested for *M. pneumoniae* by real-time PCR targeting the P1 adhesion protein gene.[16] Samples tested positive for *M. pneumoniae* were then subjected to real time PCR for the detection of A2063G mutation by the method previously described. The DNA extract prepared from the clinical specimens were analyzed by real time PCR to detect the point mutation from A to G at the position of 2063 (A2063G).(22) Isolates with A2063G mutation is considered to be a strain of genotypic resistant MRMP and negative strains are considered to be wild type (absent of genotypic resistance and assumed to be MSMP in the current study). For strains with no amplification or ambiguous results, the DNA extract is subjected to direct DNA sequencing of the domain V of the 23S rRNA gene to identify A2063G mutation associated with macrolide resistance using techniques previously described by the microbiological department of Tuen Mun Hospital. [16]

Results

Incidence

During the studied period of March to August 2013, a total of 293 patients admitted to hospital for CAP were sampled. A total of 111 patients positive for Mycoplasma infection via PCR, obtained from sputum, gastric lavage or nasopharyngeal aspirates were analyzed. All included in this study were tested by direct DNA sequencing for domain V of the 23S rRNA gene to identify the mutation associated with macrolide resistance, of which 48 patients (43%) had A2063G point mutation detected (MRMP group) and no genotypic resistance identified in 63 patients (57%) (MSMP group).

Patient characteristics

Table 1 illustrates and compares the characteristics of the patients in MSMP and MRMP groups. The patient demographics were comparable in both groups, the male to female ratio was 1:1.5, the mean age in both groups were 8 years old (s.d±4years in MSMP and s.d±3years in MRMP). None of the children had chronic respiratory conditions defined as conditions requiring long-term treatment for respiratory disease. Clinical features on presentation such as fever duration prior to admission, presenting symptoms of fever, cough, rash and tachypnoea (according to age adjusted ranges) were comparable and no significant differences between the two groups were observed. The average duration of achieving normal respiratory rate with treatment were slightly longer in the MRMP group (52hours) compared to the MSMP group (41hours), but they were not statistically significant (P=0.648).

Laboratory and clinical features

Laboratory findings (previously mentioned including WCC, CRP, ESP, ALT, CA) were compared. Both groups did not show a significant difference in raised or lowered WCC, CRP levels were elevated in both groups (90% in MSMP and 94% in MRMP). There were an equal number of patients with raised ALT in both groups. CA was not routinely taken in this group of patients. Only 1 patient with MSMP had a raised CA titre ([?]64) and 3 out of 6 patients with MRMP had a raised titre.

Overall there were no significant laboratory differences between patients with MSMP and MRMP.

Given the large variation of resistance in different parts of the world, other characteristics such as travel history within the last two weeks particularly to China were studied in the two groups. Twenty four percent of all patients with Mycoplasma associated pneumonia had a history of travel two weeks prior to admission and the majority visited China. In the MSMP group, 10 patients out of 12 with travel history (83%) had travelled to China and comparatively, 10 out of 14 patients (71%) in the MRMP group with travel history had travelled to China. But these results were statistically insignificant.

The presenting clinical condition and complications were compared. All patients presented with chest radiograph with changes compatible with pneumonia, none had lobar collapse and 5% of all patients (n=5) diagnosed with Mycoplasma associated pneumonia had evidence of pleural effusion. There was higher incidence of developing this complication in patients with MRMP although not statistically significant. One patient in MSMP group had pleural effusion (20%) and the remaining 4 patients with effusion had MRMP (80%).

Both MSMP and MRMP had similar percentage of patients requiring oxygen within hospitalization. The overall duration of oxygen use was shown to be greater in the MRMP group than in the MSMP group. The mean duration of oxygen use in MSMP was shorter at 60 hours (s.d+-39 hours), compared to the mean duration of 102 hours (+- of 76 hours) in MRMP, but not statistically significant.

Information on the outcome such as the total duration of fever, prior to admission, time of defervescence (TTD), length of stay and comparing outcomes with the use of doxycycline were analyzed.

Co-infection was detected in 8 patients and the associated pathogens include Influenza A, Parainfluenza, *Streptococcus pneumoniae* and*Haemophilus influenzae* . They were excluded from the subgroup analysis for TTD. Another 2 patients were admitted for concomitant conditions contributing to the febrile episode, which was acute lymphoblastic leukaemia and hemorrhagic bullous myringitis and were excluded. One patient was excluded due to early self-discharge hence insufficient data for analysis.

Other patients excluded from the subgroup analysis were those with prior use of macrolide before admission and those never used macrolide or switched from macrolide to doxycycline within the course of admission were excluded from the TTD analysis. After exclusion, a total of 74 patients were eligible for this sub-group analysis. Comparing patients with MSMP and MRMP, there was a statistical significance in mean total duration of fever after admission, which were 1.6 days (+-1 day) and 2.8days (+-2.3 days) respectively. TTD between patients with MSMP and MRMP at different cut offs were compared. In the MSMP group, 40% of the patients achieved defervescence within 24hours and 83% within 48 hours (both not statistically significant). The results show a statistically significant 96% of the MSMP patients had achieved defervescence by 72 hours and by comparison, only 71% of the patients with MRMP had achieved defervescence at this cut off point. (P=0.005).

ROC Curve

The aim of this analysis to identify the optimal TTD cut off which best predicts and represents likely macrolide resistance. The overall defervescence pattern in patients with MSMP and MRMP are shown in figure 1. The sensitivity and specificity of using different cut off values for TTD were plotted on a ROC curve as shown in figure 2. The sensitivity and specificity of TTD using cut off at 24hrs, 48 hrs and 72 hours were compared. In order to achieve a best indicatory cut-off to confirm positive resistance, we aim for a higher specificity with regards to TTD as we aim to avoid over-treating patients due to the potential side effects of doxycycline. By setting a cut-off at 48 hours (46-49 hours) 81% specificity and 38% sensitivity was achieved. With the cut off at 72 hours (68-74 hours) a high specificity of 96% was achieved with a sensitivity of 29%. TTD at hours beyond the 72 cut-off produced an even higher specificity (98-100%) and lower sensitivity (around 10%). However, the presence of two outliers with prolonged febrile illness, possibility due to co-infection and may have skewed the results. Also the average length of stay was 3 days in MSMP and 4 days in MRMP, hence cut off beyond 86 hours was not considered reliable or practical.

The area under the ROC curve (AUC) for TTD was 0.59 with a 95% confidence interval lower bound of 0.413 and upper bound of 0.775. This supports the time to defervescence indicates a fair discriminative ability to predict likelihood presence of MRMP. For the ease of clinical practice, the TTD was rounded off to the nearest 24hours which means the optimal cut off to use is at 72 hours.

As per current literature and recommendations for children diagnosed with CAP, beta-lactam antibiotics and a macrolide group antibiotic,[12] clarithromycin, were used as first choice on admission. During the course of hospitalization, 15 patients changed the choice of antibiotic treatment to doxycycline and 10 of

those patients were in the MRMP group. In the 5 patients with MSMP, 1 patient switched to doxycycline due to the development pleural effusion. Two out of the remaining 3 patients had significant shortness of breath and required oxygen and the remaining patient had prolonged fever of more than 1 week prior to admission. All patients with MSMP that switched to doxycycline were older than 12 years old. Four patients with MRMP treated with doxycycline were excluded from this subgroup analysis due to co-infection or other febrile conditions mentioned previously. Patients in both MSMP and MRMP group with doxycycline use achieved rapid defervescence compatible with previous publications. The mean TTD after use of doxycycline use in MSMP was 18 hours (+-13 hours) and 15 (+-12 hours) in MRMP group. However due to limited sample size, this was not statistically significant. (P=0.61)

A significant difference was noted between the two groups in the length of stay. The mean length of stay was 3 days (+-2 days) and 4 days (+-4 days) in patients with MSMP and MRMP respectively (P=0.004).

Discussion

CAP is a frequent cause of admission and disease burden in children worldwide and *M. pneumoniae* has been shown to be an important causative agent. Macrolides are generally considered an effective first choice antibacterial treatment for this condition. With a large variation in resistance rates worldwide, it is of our interest to look at the resistance rate of our local population of children comparably to nearby regions.

According to our series of patients, the resistance rate is around 43%. This is not as high as the 75% previously published group of patients from 2010 to 2013, when Mycoplasma PCR and genotype testing for point mutations were limited to selected ill patients not responding to first line treatment. Our observed resistance rate is more representative of the pattern in our local population as the sample size has increased, and patients admitted with pneumonia were more readily tested for Mycoplasma PCR and resistant strains.

Although the observed resistance rate is more representative of the local population, there are several limitations of this study. The sample population is limited to hospitalized patients with Mycoplasma associated pneumonia, and to one regional hospital of Hong Kong. With the diagnosis of CAP, the subsequent testing for atypical pathogens such as *M. pneumoniae* was based on the attending doctor's clinical decision and suspicion. Therefore collaborating with other hospitals for territory-wide and routine testing would produce more comprehensive results and further accuracy in the local resistance rates with the larger data samples. Another technical limitation in identifying resistance strains is that all our patients were tested for specific resistance genotype (A2063G) by real time PCR, the position relevant to most cases of macrolide resistance since gene sequencing would be too time consuming.

A number of prior studies have shown that it is not possible to predict the presence of *M. pneumoniae* resistance solely on the basis of presenting manifestations or routine laboratory investigations. Similarly, chest radiography cannot be used to predict atypical bacterial infection, limited in terms of sensitivity, specificity and inter-observer variability. It is well known that a comprehensive overview of world-wide resistance is hampered by the difficulty of cultivating *M. pneumoniae* strains. Previously, serology remained the only available means to confirm mycoplasma infection.

Currently, the use of molecular methods such as real-time PCR offers the possible rapid identification of *M. pneumoniae* strains independent of serological studies. Information on resistance can guide appropriate treatment improving the clinical outcome, preventing complications and improving the length of stay. Although informative, genotype sequencing for identifying resistance is offered in selective laboratories. Therefore , we aim to identify clinical parameters or patient characteristics to guide the decision in optimizing and using alternative antibiotic therapies.

Use of alternative antibiotics as tetracyclines and quinolones for the treatment of Mycoplasma associated pneumonia have not been favoured particularly in younger age groups of children due to their potential bone growth suppression, teeth staining and enamel hypoplasia effects during tooth development or cartilage erosion in some animal studies.

Our results have shown a clinical parameter facilitating early recognition of likely MRMP to prompt earlier

change in antibiotic choice. Clinically and practically on a daily basis, the use of '72 hours non-defervescence' can be used as a cut off tool to facilitate recognition of likely MRMP and to support the clinical decision for earlier change of antibiotic therapy during the course of admission. Patients with MRMP treated with doxycycline achieved rapid defervescence within 24 hours. This is valuable information to avoid development of potential complications and shorten the length of stay. This could be particularly useful in clinical decisions for younger groups of patients, balancing the risks of the disease and treatment side effects when considering the use of doxycycline.

Previous literature has mentioned that children with *M. pneumoniae* treated with ineffective antibiotics have similar outcomes to those observed in patients infected by susceptible strains. This may indicate that mycoplasma pneumonia itself may be a self-limiting disease. [14] This seems to indicate that there may not be a need to change current macrolide use systematically in the case of mild to moderate disease, but alternatives should be considered if for persistent symptoms , clinical deterioration or evolving complications. In our study, 4 patients with MRMP developed pleural effusion. This suggests there may a difference in the rate of developing complications in mycoplasma pneumonia, particularly if the strain is macrolide resistant, as an ineffective antibiotic treatment may lead to a prolonged clinical course.

According to our data, by 72 hours, 96% of MSMP patients achieved defervescence.

Conclusion

CAP is a common condition in paediatric patients requiring admission with *M. pneumoniae* a common causative pathogen. The current recommended treatment for children with CAP suggests to add-on macrolide to cover atypical bacterial pathogens on top of beta-lactam antibiotic coverage if there is no response to first-line empirical therapy, or if causative agent is compatible with atypical pathogens. However recent reported resistance worldwide have demonstrated a large variation in prevalence and we look at the local data for resistance trends in Hong Kong. With a large and more comprehensive sample of data in this extended study, we have observed a local resistance rate of 43% that is significantly lower than the 75% previously published.

Patients in the MRMP group appeared to have a higher rate of developing complications and requiring additional clinical support. Clinical parameters and radiological changes did not show significant factors to help differentiate clinically those likely with MRMP. TTD with a cut off at 72hours showed a discriminative ability to identify those likely with macrolide resistant mycoplasma pneumonia supporting the clinical decision in early switch to doxycycline therapy particularly balancing the risks of adverse effects in the younger population of children. This would overall shorten the length of stay and decrease the burden of such a common condition in the paediatric population to the overall health care system in Hong Kong.

M. pneumoniae infection is considered a treatable disease with significant burden to the paediatric population and therefore necessary to give careful consideration to the appropriate antimicrobial therapy. With increasingly reported resistance of *M. pneumoniae* in Asian regions, there is a need for more widespread use of molecular methods to confirm *M. pneumoniae* infection and to identify resistance in order to provide further comprehensive data from our local population for clearer perspective on MRMP in Hong Kong.

Conflicts of interest: None

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Table 1.

Characteristics of children with Mycoplasma Pneumoniae (n=111)

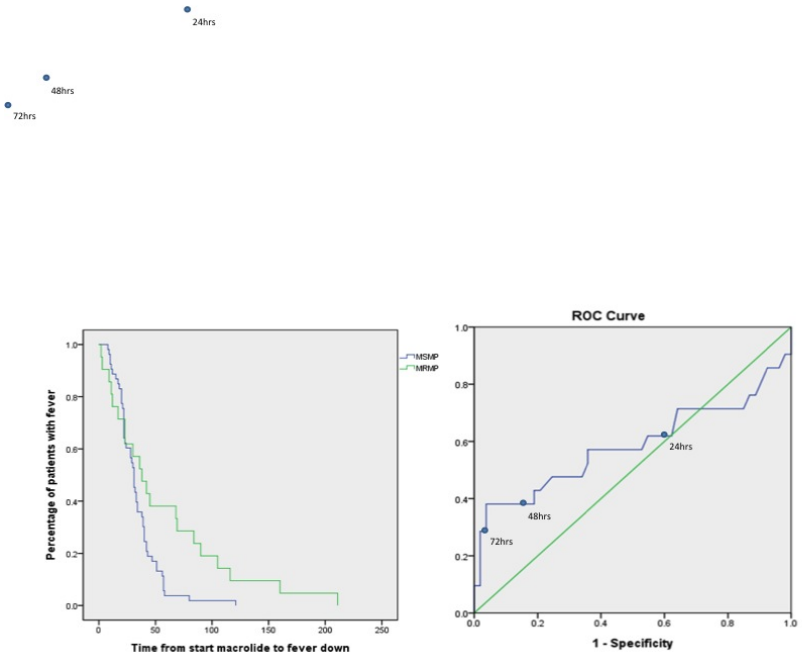
Characteristic	**MSMP group**	**MSMP group**	**MRMP group**	**MRMP group**	**P value**
	n	%	**n**	%	

Characteristic	**MSMP group**	**MSMP group**	**MRMP group**	**MRMP group**	**P value**
Total patients	63	57	48	43	
Mean age, years	8	±4	8	±3	
Male:female	1:1	1:1	1:1.5	1:1.5	
Clinical symptoms	Clinical symptoms	Clinical symptoms	Clinical symptoms	Clinical symptoms	Clinical symptoms
Fever	60	100	42	100	
Cough	60	100	42	100	
Tachypnoea*	31	49	22	46	0.724
Rash	2	3	2	4	
Admission characteristics	Admission characteristics	Admission characteristics	Admission characteristics	Admission characteristics	Admission characteristics
Days of fever prior to admission *(mean±sd)*	6	±2	6	±1	0.63
Use of antibiotics prior to admission	43	68	33	69	0.956
duration of antibiotics use *(hrs, (mean±sd)*	3	±2	3	±1	0.131
Travel history	12	19	14	29	0.230
Travel history China	10	16	10	21	0.492
Complications	Complications	Complications	Complications	Complications	Complications
Use of oxygen	7	11	6	13	0.822
hours of oxygen use *(mean±sd)*	60	±39	102	±76	0.295
Pleural effusion	1	2	4	8	0.164
Laboratory Findings	Laboratory Findings	Laboratory Findings	Laboratory Findings	Laboratory Findings	Laboratory Findings
Neutorphil count *(mean±sd)*	8	±2	9	±3	0.955
C reactive protein *(mean±sd)*	48	±45	32	±29	0.082
Raised Alanine transaminase	4	12	4	24	0.21
Cold agglutinin *(mean±sd)*	64		129	193	0.857
Fever characteristics (n=74)	Fever characteristics (n=74)	Fever characteristics (n=74)	Fever characteristics (n=74)	Fever characteristics (n=74)	Fever characteristics (n=74)

Characteristic	MSMP group	MSMP group	MRMP group	MRMP group	P value
total duration of fever *(mean±sd)*	39	±23	68	±55	0.075
Time from macrolide to fever down *(mean±sd)*	33	±20	57	±55	0.257
TTD 24hrs	21	40	8	38	0.903
TTD 48 hrs	44	83	13	62	0.068
TTD 72hrs	51	96	15	71	*0.005*
Use of doxycycline	5	8	10	21	*0.049*
Time from doxycycline to fever down *(hrs, (mean±sd)*	18	±13	15	±12	0.61
Length of stay *(days, (mean±sd)*	3	±2	4	±3	*0.004*

**tachypnoea defined as respiratory rate outside of normal range adjusted for age*

Figure 1. Figure 2.



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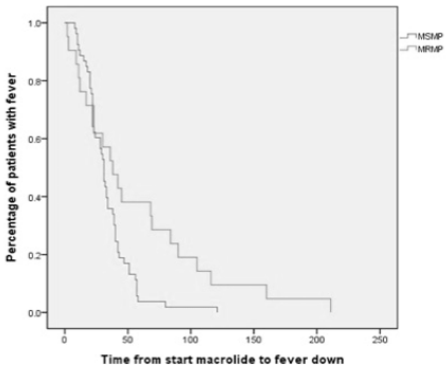

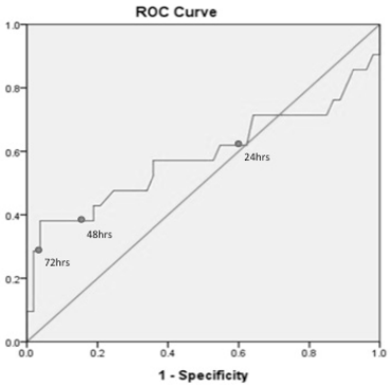

Figure 1.

Figure 2.

Graphs showing the defervescence pattern in MSMP and MRMP and the ROC curve of TTD.

Majority of MSMP patients overall achieved defervescence around 72 hours.