



Bacterial co-infection in *Mycoplasma pneumoniae* infected cases.

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ABSTRACT

Bacterial co-infections in Mycoplasma pneumoniae pneumonia are not rare. The present study makes an effort to investigate and understand the impact of such bacterial co-infections. For this purpose 26 cases with Mycoplasma pneumoniae infection were screened. Streptococcus pneumoniae was found to be the most co-infecting bacteria (46.1%). The co-infected pathogen plays an influential role by altering the severity of infection. Course of the disease as well as clinical outcomes are also heavily dependent on the secondary pathogen other than Mycoplasma pneumoniae itself.

KEYWORDS : *Mycoplasma pneumoniae, Streptococcus pneumoniae, co-infection, culture.*

Introduction:

The major bacterial causes of community acquired pneumonia are *Streptococcus pneumoniae* and *Mycoplasma pneumoniae*, together accounting for up to 60% cases. *Mycoplasma pneumoniae* infection occurs in all age groups. More severe clinical pictures are generally associated with chronic lung disease and age extremes. Disease burden of *Mycoplasma pneumoniae* is about 20 to 40 infections or co-infections per 100 patients with lower respiratory tract infections. The severity of infection is highly variable and this condition may lead to severe sequelae.¹

Co-infection of *M. pneumoniae* with various viruses or bacteria has been reported in 52% cases.² In the similar way co-infections with *S. pneumoniae* in *M. pneumoniae* infections is observed to be 51.4%.³ Also *M. pneumoniae* and *S. pneumoniae* co-infection was estimated to have an incidence of 10%. The condition can prolong the course of the disease as well as make management more difficult.² In Taiwan, a prospective study on the etiology of hospitalized children with CAP demonstrated a high incidence (41%) of mixed infections and 37% with *M. pneumoniae* infection. Furthermore, concurrent viral-bacterial infection was identified in approximately 60% cases with *M. pneumoniae* infection.⁴ Studies suggest that infections with *M. pneumoniae*, like respiratory viruses, may predispose to secondary bacterial infection.

Methodology:

Patients (n=150) belonging to either the in-patient or out-patient department of a tertiary care centre were included. The respiratory samples like sputum or broncho-alveolar lavage were collected in sterile wide mouthed container and transported to the microbiology lab for processing. Each sample suspected of atypical pneumonia was also processed to rule out other bacterial co-infection apart from routine PPLO broth inoculation for *Mycoplasma pneumoniae* isolation. Following were the details of sample processing-

Routine Microscopy:

Grams Stain: Gram stain was performed to grade the samples as per Bartlett's grading system for sputum. A score of zero led to the exclusion of the sample. The types of inflammatory cells present in the sample were observed. Also the bacterial cells present were graded as occasional, few, moderate and many.

ZN Stain: The technique was carried out to screen for the presence of

acid fast bacilli. A positive smear led to the exclusion of the sample from the study.

Culture:

Mycoplasma pneumoniae: The samples were inoculated into Pleuro Pneumonia Like Organism broth. This inoculated PPO broth bottles were placed in a desiccator (candle jar) and kept in the incubator. The optimum conditions for *Mycoplasma pneumoniae* growth, 5% CO₂ and 37°C were maintained. Change in colour of the broth from orange to yellow indicates positive growth.

Other Bacteria: Samples were inoculated into Blood agar, MacConkey's agar and chocolate agar plates and incubated at 37°C for 24 hours as per Mackie and McCartney.⁵

Identification:

Mycoplasma pneumoniae: PCR was done using primers specific for a 375bp fragment of P1 cytoadhesion gene of *Mycoplasma pneumoniae*.^{6,7} The samples were also subjected to a second PCR to confirm the presence of *M. pneumoniae*. For this PCR *Mycoplasma pneumoniae* species specific primers were used which targets a 277bp fragment of the 16S rRNA gene.^{8,9}

Other Bacteria: The colony characteristics were noted from Blood agar, MacConkey's agar and chocolate agar plates down after 24 hours. Grams staining was done with smears made from the colony to observe the type of bacteria. Various biochemical tests were put as per Mackie and McCartney to identify the bacteria isolated in culture.⁵

Results:

A total of 26 out of 150 cases included in the study were found to have infection with *M. pneumoniae*. Among the 26 cases with *M. pneumoniae* infection 12 cases were also infected with *Streptococcus pneumoniae* (46.1%). Hence, *Streptococcus pneumoniae* was found to be the most common co-infecting bacteria.

Co-infection with more than one bacterium was also seen among the cases with *M. pneumoniae* infection. Majority of the cases had co-infection with only one bacterium (17 out of 26 cases) but co-infection with two bacteria (5 out of 26 cases) and three bacteria (1 out of 26 cases) were also present. Only 3 out of 26 cases (11.5%) had *M. pneumoniae* as the sole causative bacteria for infection.

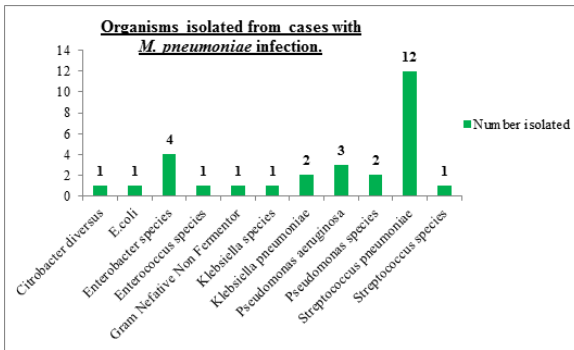


Fig: Bar diagram showing other organisms isolated from cases with *M. pneumoniae* infection p- value 0.000014 (significant).

Number of other organisms isolated	Number of Cases	Total number of Cases	Percentage
One	17	26	65.3%
Two	5	26	19.5%
Three	1	26	3.8%
Nil	3	26	11.5%

Table: Number of other organisms isolated from cases with *M. pneumoniae* infection, p-value 0.00012 (significant).

Discussion and Conclusion:

The study emphasises on ruling out the presence of any bacterial co-infection, in the cases with *M. pneumoniae* infection. All the 26 cases infected with *M. pneumoniae* were checked for bacterial co-infection and it was found that 23 cases had the presence of co-infecting bacteria. The remaining 3 cases had *M. pneumoniae* as the sole etiological agent of infection. Majority of the cases (17 cases) had only one co-infecting bacteria. The rest of the cases had mixed infection with two (5 cases) or three (1 case) bacteria. We have found *Streptococcus pneumoniae* as the most common co-infecting bacteria. 12 out of 26 cases (46.1%) with *M. pneumoniae* infections had co-infection of *Streptococcus pneumoniae*.

The observations made from this study allow us to arrive at the view that *Streptococcus pneumoniae* is the most common bacteria found to be associated in *M. pneumoniae* infected cases (46.1%). Vervloet LA et al.¹ observed 51.4% *M. pneumoniae* infected cases to have *S. pneumoniae* co-infections. The incidence of *S. pneumoniae* co-infection observed in their study was 10%. These observations are in accordance with our findings as well. The study of Toikka P et al.² is also similar to the current study. They found that 9 out of 17 cases (52.94%) with *M. pneumoniae* infection had co-infection with *Streptococcus pneumoniae*.

Our findings are also comparable with Chiu CY et al.¹⁰, wherein 9 out of 59 cases (15.25%) with *M. pneumoniae* infection were reported to have co-infection with *Streptococcus pneumoniae*. They found that in comparison with cases infected with *M. pneumoniae* alone, coinfection of *S. pneumoniae* was more likely to occur with a longer duration of fever and hospital stay. Esposito S et al.¹¹ in their study found 14 out of 196 cases (7.14%) to have co-infection of *S. pneumoniae* and *M. pneumoniae*.

The next most common organism co-infecting *M. pneumoniae* cases found was *Enterobacter species* (15.3%), followed by *Pseudomonas aeruginosa* (11.5%). These findings can be compared with Basil MV et al.¹² who found 6.25% cases with *M. pneumoniae* infection had co-infection with *Pseudomonas species*. Although there are not many studies stating the co-infection of *M. pneumoniae* and *Enterobacter species*, such mixed infection can increase the intensity of infection.

In this study, we have seen that 65.3% (17 out of 26) of the cases with *M. pneumoniae* infection had one, 19.5% (5 out of 26) had two and 3.8% (1 out of 26) had three co-infecting bacteria. Dey AB et al.¹³ in their study, on cases with *M. pneumoniae* infection, isolated co-infecting bacteria in 50% (blood) and 68% (respiratory tract secretion) cases. Chiu CY et al.¹⁰ also observed that 20% of cases with pneumonia had three or more microorganisms. These observations concord with our results as well as, sheds light on the importance of ruling out co-infecting bacteria in cases with *M. pneumoniae* infections.

The findings obtained in the current study along with the supporting literature reports indicate that severe bacterial infection may either follow or coincide *M. pneumoniae* respiratory infection, by facilitating alterations in local respiratory immunity or structure and function.^{14,15}

The influence on the clinical outcomes of *M. pneumoniae* co-infection may be heavily dependent on the co-infected pathogen other than *M. pneumoniae* itself. Pneumonia caused by *S. pneumoniae* usually accompanies intense inflammation followed by parapneumonic effusion and empyema.^{16,17} Attributing to the increase of macrolide-resistant *M. pneumoniae* in recent years, a potentially unfavorable outcome such as necrotizing pneumonitis, lung abscess, and acute respiratory distress syndrome caused by *M. pneumoniae* is, however, occasionally encountered.^{18,19,20,21} Therapeutic agents active against mycoplasma could be a critical component of managing severe cases of *S. pneumoniae* and *M. pneumoniae* coinfection.^{14,15} A co-infection of *M. pneumoniae* with other bacteria is frequently seen, hence, a typical bacterial pathogen should always be considered in cases *M. pneumoniae* infections.

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