Mycoplasma pneumoniae Macrolide Resistance in Children in Central Ohio Detected by Sequencing

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Abstract

Background: Mycoplasma pneumoniae (Mp) is one of the most common causes of lower respiratory track infections in school aged children, with estimates of up to 40% of cases of community-acquired pneumonia (CAP). Macrolides are the drug of choice for treating Mp infections, but in the last 2 decades there has been emergence of resistance worldwide. Reported rates in USA vary from 3.5% up to 13.2%, but rates in Ohio are unknown. Currently there is no well-established, standardized method for detecting macrolide resistance and antibiotic treatment is empiric because of these limitations. Our goal was to determine the frequency of macrolide resistance in children with Mp respiratory infections in central Ohio.

Methods: A convenience sample of Mp positive PCR identified by our laboratory-developed assay, obtained from both inpatient and outpatient settings, were selected. These were then cultured using Remel's SP4 glucose broth and then incubated at 35° C until isolates grew, or for a maximum of 4 weeks. All samples that yield positive cultures were then sequenced for the domain V of the 23S rRNA (nucleotide 1937-2154, accession no. X68422) using Sanger methods and sequences were compared with corresponding region of wildtype reference strain (ATCC 15322).

Results: For the period of October 2015-March 2017, culture was attempted in 433 PCR-positive samples, and 347 (80%) yield an isolate. Sequencing was performed in those 347 samples and was successful in 334 (96%). A macrolide resistance mutation was detected in 10 samples (3%). From the 10 mutations detected, 7 (70%) were the A2063G mutation, and 3 (30%) were A2064G. We also detected a deletion in A2065 of unknown significance in 3 samples (1%).

Conclusions: In this pediatric population, culture was successful in 80% of PCR positive samples, thus providing isolates for sequencing to be able to assess our local resistance. Our overall Mp resistance was 3%, suggesting that empiric treatment with macrolides is still appropriate in Central Ohio. Additional studies are needed to correlate the presence of these resistance mutations with phenotypic susceptibility testing and clinical outcomes.

Background

- Mycoplasma pneumoniae (Mp) is one of the most common causes of lower respiratory tract infections in school aged children, with estimates of up to 40% of cases of community-acquired pneumonia (CAP).
- Macrolides are the drug of choice for treating Mp infections.
- Emergence of resistance worldwide has been described in the last 20 years.
- Reported resistance rates in USA vary from 3.5% up to 13.2%, Ohio rates are unknown.
- Currently there is no well-established, standardized method for detecting macrolide resistance.
- Treatment is therefore empiric.

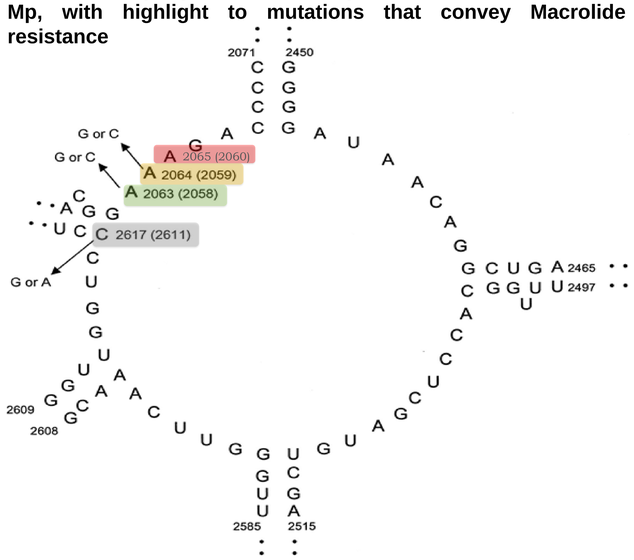
Objectives

- Our goal was to determine the frequency of macrolide resistance through sequencing of Mp isolates obtained from children with respiratory infections in central Ohio.

Methods

- A convenience sample from all Mp positive PCRs samples available in the Microbiology laboratory from both inpatient and outpatient settings was selected for analysis.
- These samples were cultured using Remel's SP4 glucose broth.
- Incubated at 35° C until isolates grew, or for a maximum of 4 weeks.

FIGURE 1. Peptidyltransferase loop of domain V of 23SrNA of Mp, with highlight to mutations that convey Macrolide resistance



- All samples that yield positive cultures were then sequenced for the domain V of the 23S rRNA (nucleotide 1937-2154, accession no. X68422) using Sanger methods.
- Sequences were compared with corresponding region of wildtype reference strain (ATCC 15322).
- We targeted mutations A2064G and A2063G. These have been the only two mutations associated to macrolide resistance described in the United States.

Results

- Sample were obtained from October 2015 to March 2017.
- Culture was attempted in 433 PCR-positive samples collected.
- From those, 347 (80%) yield a Mp isolate.

FIGURE 2. Yield of Positive Culture

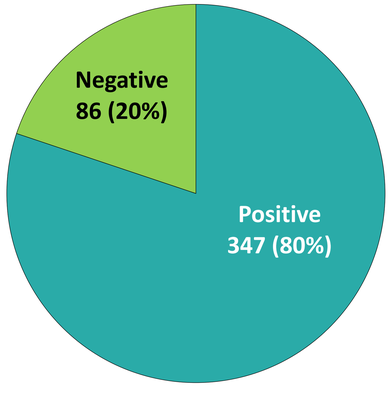
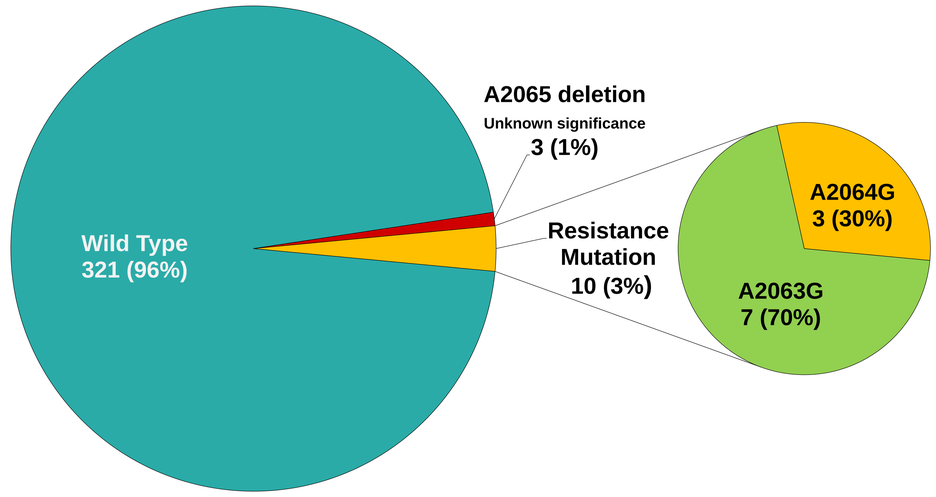


FIGURE 3. Sequencing Results



- Sequencing was successful in 334 samples (96%).
- A macrolide resistance mutation was detected in 10 samples (3%).
- 7 (70%) were the A2063G mutation (2% of total samples).
- 3 (30%) were A2064G (1% of total samples).
- We also detected a deletion in position A2065 of unknown/unclear significance in 3 samples (1%).

Conclusions

- The overall rate of detection of Mp resistance genes was 3%.
- This suggest that macrolides are still appropriate as initial empiric therapy for Mp infections in Central Ohio.
- As described in other USA studies, A2063G is the most common identified mutation.
- Culture was successful in 80% of PCR positive samples.
- This provides viable Mp isolates to perform sequencing.
- Additional studies are underway to correlate the presence of these resistance mutations with in vitro susceptibility testing, as well as clinical outcomes.