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Case Report

Nanopore sequencing enables rapid diagnosis and drug resistance profiling in refractory *Mycoplasma pneumoniae* pneumonia: a case report

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Abstract: Objective: To evaluate the clinical value of nanopore sequencing technology (NST) for the early diagnosis and resistance guidance of *Mycoplasma pneumoniae* (MP) infection in children. Methods: We analyzed four pediatric patients with MP pneumonia. Sputum specimens were subjected to NST, and results were compared with serological MP-IgM testing, nucleic acid detection, and chest CT. Results: In all four cases, MP-IgM yielded false-negative or weakly positive results within the first 5 days. In contrast, NST provided a definitive diagnosis within 24 hours of admission, detecting MP with sequence reads ranging from 14 to 3024 and simultaneously identifying the macrolide-resistant A2063G mutation in the 23S rRNA gene. This led to a confirmed diagnosis 3-7 days earlier than serological methods. Blood cultures were negative in all cases. Conclusion: NST overcomes the limitations and delay of traditional methods, enabling rapid and accurate diagnosis of MP infection and concurrent detection of resistance mutations. Its integration into clinical practice may significantly improve the management of pediatric MP pneumonia.

Keywords: *Mycoplasma pneumoniae*, children, nanopore sequencing, drug resistance, early diagnosis

Introduction

Mycoplasma pneumoniae (MP) is a major cause of community-acquired pneumonia (CAP) in children, responsible for approximately 10% to 40% of cases globally [1, 2]. As a cell wall-deficient pathogen exhibiting characteristics of both bacteria and viruses, MP can lead to a broad spectrum of clinical outcomes - from mild respiratory infections to severe pneumonia, acute respiratory distress syndrome (ARDS), and even extrapulmonary complications such as hemolytic anemia and encephalitis [3, 4].

In recent years, the rising prevalence of macrolide-resistant MP (MRMP) has become a serious clinical concern, largely due to the widespread use of macrolide antibiotics. For instance, a 2023 study in southern China reported a resistance rate as high as 93.02%, with the A2063G mutation in the 23S rRNA gene accounting for 71.0% of cases. This high resistance rate complicates treatment and underscores the need for accurate and timely diagnosis.

Conventional diagnostic methods face significant limitations. Culture-based detection is time-consuming (taking 7-14 days) and has low sensitivity (positivity rate <30%) [5-8]. Serological tests for MP-IgM often fail to detect early-stage infections, as antibodies may take 1-2 weeks to reach detectable levels, leading to missed diagnoses [9]. While conventional PCR can confirm MP presence, it does not simultaneously identify resistance genes [10]. These shortcomings contribute to an early MRMP diagnosis rate of less than 40%, frequently resulting in treatment delays and inappropriate antibiotic use [9].

Nanopore sequencing technology (NTS) has emerged as a promising solution, offering unbi-

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ased pathogen detection, high sensitivity, and rapid results (within 24 hours). Its ability to simultaneously identify pathogens and resistance mutations makes it particularly valuable in managing complex infections such as MRMP [11, 12]. In this study, we analyzed clinical data from four pediatric cases of MRMP infection to systematically evaluate the utility of NTS in diagnosing and managing MP infections in children.

Case presentation

Table 1 summarizes the key demographic, clinical, and diagnostic characteristics of the four pediatric cases. All patients presented with fever and cough and failed to respond to initial empiric antibiotic therapy. Notably, conventional *Mycoplasma pneumoniae* IgM testing was negative or equivocal at initial presentation in all cases. Critically, nanopore sequencing of sputum specimens uniformly identified and confirmed the presence of the A2063G mutation in the 23S rRNA gene (conferring macrolide resistance) in all four children (**Figures 1**, **2**). This definitive diagnosis guided a successful shift to targeted tetracycline-class antibiotics (doxycycline or minocycline), leading to clinical improvement and eventual discharge in all instances.

Discussion

The diagnosis and treatment of *Mycoplasma pneumoniae* pneumonia (MPP) in children, especially macrolide-resistant MPP (MRMP), remain challenging in pediatric clinical practice. The inherent limitations of traditional detection methods, the high prevalence of resistant strains, and the occult nature of complex infections often lead to delayed diagnosis, inappropriate treatment strategies, and poor outcome. In recent years, NTS has fundamentally transformed this landscape. In particular, the rapid turnaround of nanopore sequencing (within 12-24 hours) demonstrates significant value in overcoming diagnostic blind spots, optimizing treatment strategies, and improving prognosis [12].

In this study, all four pediatric MRMP cases tested negative or weakly positive for serum MP-IgM within ≤5 days of symptom onset, consistent with literature reports that MP-IgM reaches detectable levels only 1-2 weeks after infection [9]. In contrast, NTS provided a definitive diagnosis within 24 hours of admission, shortening the time to diagnosis by 3-7 days and creating a valuable window for early intervention. Furthermore, traditional culture methods yielded negative results in all cases, confirming their limited sensitivity (reported positivity rate <30%), while NTS detected MP with as few as 14 reads, demonstrating high sensitivity (reported sensitivity of 77.2%, far exceeding the 29.3% of culture methods) [8, 13].

NTS can directly detect resistance-conferring mutations in the MP 23S rRNA gene (e.g., A2063G) with a sensitivity of 92.8% [8]. Studies in Wuhan have shown that 96% of MRMP strains carry the A2063G mutation [14]. In this study, Case 3 was initially misdiagnosed with bacterial pneumonia due to negative MP-IgM and treated ineffectively with cefixime. NTS detection of MP and the A2063G mutation corrected the diagnosis, preventing further misuse of antibiotics. All four cases in this study carried this mutation, providing molecular evidence for early resistance identification and overcoming the limitation of conventional methods that "detect infection but not resistance".

It is important to note that three patients under 10 years old received tetracyclines based on an individualized assessment confirming macrolide-resistant infection with poor response to prior macrolides. This approach is supported by recent studies indicating the safety of short-term doxycycline use in children under 8 [15]. Treatment courses were strictly limited to ≤10 days to minimize the risk of tooth discoloration, adhering to the principle of "individualized treatment after risk-benefit evaluation". With nationwide data showing MRMP prevalence reached 93.02% in 2023-2024, such precision prescribing is essential to reduce ineffective treatment and antimicrobial pressure, forming a core component of antimicrobial stewardship [2, 16].

Persistent use of macrolides in MRMP infection can lead to prolonged illness and increased pulmonary complications (e.g., plastic bronchitis, lung consolidation) [17, 18]. NTS enables early (within 24 hours) confirmation of resistance, facilitating timely treatment adjustment and significantly improving outcomes. In all four cases, azithromycin was discontinued within 24 hours after nanopore sequencing identified

Table 1. Clinical characteristics of the four pediatric cases with macrolide-resistant *Mycoplasma pneumoniae* pneumonia

Clinical Characteristic	Case 1	Case 2	Case 3	Case 4
Demographics				
Sex/Age	Female/8 years	Male/6 years	Female/10 years	Female/10 years
Symptoms & History				
Major Symptoms	Fever, Cough	Fever, Cough	Fever, Cough	Fever, Cough, Chest tightness
Peak Temperature	39.4°C	39.4°C	39.6°C	39.6°C
Initial Empiric Therapy	Azithromycin (Oral)	Amoxicillin (Oral)	Cefixime (Oral)	Cefprozil (Oral)
Response to Initial Therapy	Poor	Poor	Poor	Poor
Laboratory & Imaging Findings				
CRP on Admission	22.4 mg/L	21.67 mg/L	35.3 mg/L	104.4 mg/L
Chest CT Findings	Large consolidation in RLL	Consolidation in right lung	Complete consolidation in RUL	Multiple patchy opacities in both lungs
Conventional MP-IgM Result	Negative	Negative	Weak Positive/Negative	Negative/Positive
Nanopore Sequencing Diagnosis				
MP Sequence Count	534	3024	107	14
Detected Resistance Mutation	A2063G (100%)	A2063G (100%)	A2063G (100%)	A2063G (100%)
Final Diagnosis & Management				
Final Diagnosis	Macrolide-Resistant MP Pneumonia	Macrolide-Resistant MP Pneumonia	Macrolide-Resistant MP Pneumonia	Macrolide-Resistant MP Pneumonia
Targeted Therapy	Doxycycline (Oral)	Doxycycline (IV)	Doxycycline (Oral)	Minocycline (Oral)
Outcome	Discharged after improvement	Discharged after 10-day therapy	Discharged after 10-day therapy	Discharged after 9-day therapy

Abbreviations: MP, *Mycoplasma pneumoniae*; CRP, C-reactive protein; RLL, right lower lobe; RUL, right upper lobe; IV, intravenous.

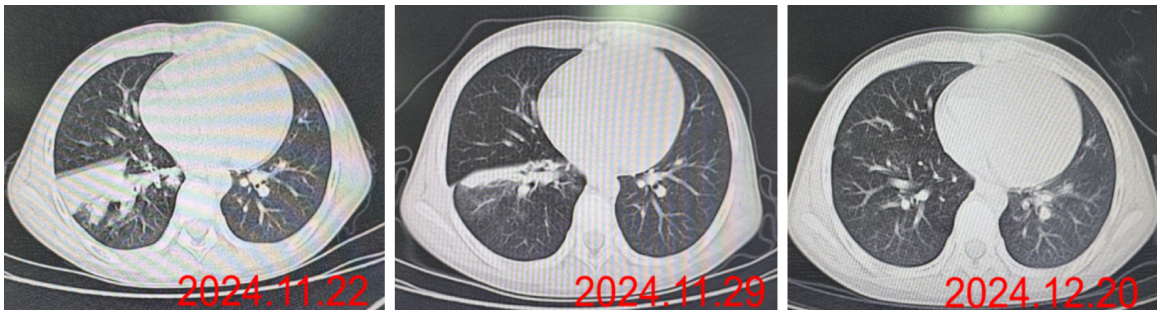

Figure 1. Lung CT manifestations of Case 1 before and after treatment.

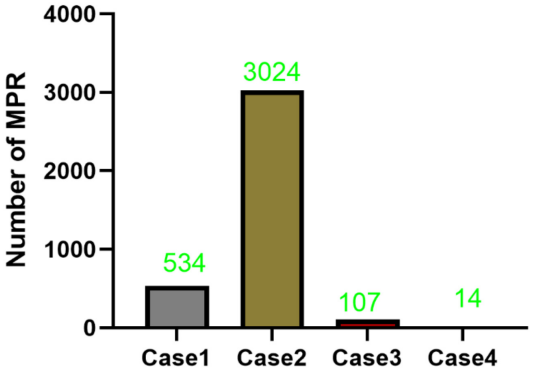

Figure 2. Metagenomic sequencing of *Mycoplasma pneumoniae* reads of four children. MPR: Mycoplasma pneumoniae reads.

the A2063G mutation, and therapy was switched to doxycycline or minocycline. The mean time to defervescence was shortened to 1.5-2 days, with notable resolution of lesions on follow-up CT. A study from Chongqing reported that MRMP patients switched to effective antibiotics had hospital stays shortened by 3.2 days compared to those continued on macrolides [19]. Moreover, in severe cases with co-infections, combination therapy guided by nanopore sequencing reduced rates of severe disease progression by 25%, underscoring the positive effect of rapid and accurate diagnosis on prognosis.

In this study, the number of *Mycoplasma pneumoniae* sequences (3024, 534, 107, 14) with significant differences in four cases was obtained by nanopore sequencing, which has important implications. First, the number of sequences was positively correlated with the presumed pathogen load and the severity of imaging findings, confirming its clinical relevance. Especially critical, even in the case of negative traditional serological tests or low pathogen load, nanopore sequencing can still maintain extremely high detection sensitivity, and successfully achieve accurate identification of pathogens and analysis of drug-resistant mutations. This not only highlights the significant advantages of this technology compared to traditional methods in early diagnosis, but also shows that the number of sequences provided can be used as a semi-quantitative indicator, which provides a new and valuable reference for clinical evaluation of infection load and severity.

In summary, NTS with its rapid turnaround (within 24 hours), high sensitivity, and unbiased detection capability-provides a new paradigm for the diagnosis and treatment of resistant MPP and severe pneumonia. By overcoming the limitations of traditional diagnostics, correcting misdiagnoses, and revealing complex infections, it plays a central role in enabling early precision diagnosis, optimizing antimicrobial stewardship, guiding intensive therapy, and improving prognosis. Its core value makes it a critical tool for managing pediatric respiratory infections in the post-COVID-19 era.

Clinical implications and limitations

This case series underscores the significant clinical utility of Nanopore sequencing in enabling the rapid and accurate diagnosis of macrolide-resistant *M. pneumoniae* pneumonia, thereby guiding critical therapeutic decisions in a timely manner. However, the generalizability of our findings was constrained by the limited sample size and the single-center nature of this report. Future large-scale, prospective studies are warranted to validate its diagnostic efficacy and cost-effectiveness in routine clinical practice.

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Disclosure of conflict of interest

None.

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