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CASE REPORT AND REVIEW OF THE LITERATURE

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Macrolide-resistant *Mycoplasma pneumoniae* pneumonia in transplantation: Increasingly typical?

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Abstract

Mycoplasma pneumoniae is one of the most common bacterial causes of pneumonia. Macrolide-resistant *M pneumoniae* (MRMP) was documented in 7.5% of isolates in the United States. Resistance portends poor outcomes to macrolide therapy, yet patients respond well to fluoroquinolones or tetracyclines such as minocycline. However, MRMP may be under-appreciated because *M pneumoniae* generally causes relatively mild infections in non-immunosuppressed adults that may resolve without effective therapy and because microbiological confirmation and susceptibility are not routinely performed. We report two cases of pneumonia due to MRMP in kidney transplant recipients. Both patients required hospital admission, worsened on macrolide therapy, and rapidly defervesced on doxycycline or levofloxacin. In one case, *M pneumoniae* was only identified by multiplex respiratory pathogen panel analysis of BAL fluid. Macrolide resistance was confirmed in both cases by real-time PCR and point mutations associated with macrolide resistance were identified. *M pneumoniae* was isolated from both cases, and molecular genotyping revealed the same genotype. In conclusion, clinicians should be aware of the potential for macrolide resistance in *M pneumoniae*, and may consider non-macrolide-based therapy for confirmed or non-responding infections in patients who are immunocompromised or hospitalized.

KEYWORDS

macrolide, *Mycoplasma pneumoniae*, resistance, resistant, transplantation

1 | INTRODUCTION

Infections due to *Mycoplasma pneumoniae* occur both endemically and epidemically worldwide and are one of the most common bacterial causes of pneumonia. Resistance to macrolides, traditionally the treatment of choice, emerged in Japan in 2001. Subsequently, macrolide resistance spread throughout East Asia, with >80% of isolates in China resistant by 2006. In Europe, prevalence is substantially lower than in Asia and varies from country to country, with recent reports ranging from very low levels of 1% in Slovenia and 1.6% in Denmark to 3.6% in Germany, 9.8% in France, 19% in the United Kingdom, 26% in Italy, and 30% in Israel.[1] In the United States, Waites et al collected 360 clinical specimens from hospitals in 8 states between 2015 and 2018 and found 7.5% of isolates to be resistant. However, resistance varied geographically, ranging from <2% in the west to more than 20% in the southeast and northeast. 33.3% of patients with macrolide resistance had a history of immune deficiency or malignancy.[2] Importantly, resistance appears to reasonably correlate with outcome, with defervescence of fever within 48 hours seen in 77%-85% of patients receiving fluoroquinolones or minocycline versus only 28% in patients receiving macrolides.[3] Despite this compelling data, macrolide-resistant *M pneumoniae* (MRMP) may be under-appreciated as a threat for several reasons. First, *M pneumoniae* generally causes

relatively mild infections in healthy patients that may resolve even with ineffective therapy. In addition, *M pneumoniae* microbiological diagnosis and susceptibility testing are not routinely performed.[2]

In January 2018, a 23-year-old man with a history of autism spectrum disorder, developmental delay, and epilepsy was admitted to Michigan Medicine with necrotizing pneumonia complicated by acute respiratory distress syndrome (ARDS) requiring extracorporeal membrane oxygenation (ECMO). Broad-spectrum antimicrobial therapy, consisting of intravenous (IV) vancomycin, tobramycin, piperacillin-tazobactam, and azithromycin (500 mg daily), was initiated. A multipathogen nucleic acid amplification test for respiratory pathogens (RPAN) (BioFire® FilmArray® Respiratory Panel, BioFire Diagnostics) was positive for only *M pneumoniae*, and a bronchoalveolar lavage (BAL) performed the same day noted purulent secretions but yielded no growth on routine bacterial culture. After 7 days of therapy with azithromycin, given the severity of infection and knowledge of the possibility for MRMP, levofloxacin 750 mg IV daily was added at the recommendation of the infectious diseases service. The BAL sample was sent to the Diagnostic Mycoplasma Laboratory at the University of Alabama at Birmingham (UAB) for detection of macrolide resistance. He was extubated after 4 days of levofloxacin therapy, completed a 14-day course of levofloxacin, and transferred to inpatient rehabilitation. Molecular testing performed directly on the BAL specimen confirmed macrolide resistance.[3,4]

Given the above case, we were alert to the possibility of MRMP. Recently, we cared for two immunocompromised patients—with no known contact with each other—who presented within 1 week of each other with pneumonia due to *M pneumoniae* that failed macrolide therapy and responded to alternative antimicrobials. As such, we wish to raise the awareness of MRMP with clinicians, especially in immunocompromised patients.

The University of Michigan Investigational Review Board granted exempt status for this study.

2 | CASE 1

In January 2020, a 30-year-old woman presented to Michigan Medicine complaining of abdominal pain, nausea, vomiting, and fever. She had a history of end-stage renal disease due to reflux nephropathy and underwent kidney transplantation in 2005 (complicated by rejection), and a second transplant in 2012. Her maintenance immunosuppression consisted of mycophenolic acid 540 mg twice daily, prednisone 5 mg once daily, and tacrolimus (5 mg every morning and 4 mg every evening). She also had a history of B-cell lymphoma in 2006 that was in remission. On admission, she did not endorse cough or dyspnea, and a chest x-ray was unremarkable. A nasopharyngeal RPAN was positive for only *M pneumoniae*. She completed 5 days of azithromycin (500 mg IV X 1 followed by 250 mg IV daily X 4 days), improved, and was discharged. Five days after discharge, she reported productive cough, dyspnea, sputum production, fevers, chills, nausea, vomiting, and abdominal pain. After 3 days of these symptoms (8 days after her discharge), she returned to our emergency department. She was febrile to 38.9°C and a CT scan showed extensive multifocal pneumonia. RPAN was again positive for only *M pneumoniae*. Sputum culture indicated the presence of oropharyngeal commensal flora. She was started on IV vancomycin, piperacillin-tazobactam, and doxycycline (100 mg twice daily), and her fever defervesced within 24 hours. Transplant infectious diseases were consulted, and her antimicrobials were replaced by levofloxacin 750 mg IV every 48 hours (renally adjusted dose). Her nasopharyneal sample was sent to the UAB Diagnostic Mycoplasma Laboratory which identified MRMP. She was discharged 4 days after admission to complete a 10-day course of oral levofloxacin 750 mg every 48 hours. She was seen at Nephrology Transplant Clinic 12 days later and denied diarrhea, fever, or cough.

3 | CASE 2

In January 2020, a 39-year-old woman presented to Michigan Medicine with cough, shortness of breath, fevers, chills, nausea, and vomiting. She had a history of Hirschsprung disease, cloacal atresia status post-multiple surgeries, end-stage renal disease with a kidney transplant in 2013, and Epstein-Barr virus (EBV)-related spindle cell tumor of the liver in 2017. Her maintenance immunosuppression consisted of prednisone 10 mg daily and tacrolimus 0.5 mg twice daily. In December of 2019, she received 3 weekly doses of HLA restricted EBV-directed cytotoxic T cells to treat her EBV-related malignancy. Upon presentation in the emergency department, she was febrile to 39.3°C and a chest x-ray demonstrated a right upper lobe pneumonia. A nasopharyngeal RPAN was negative. She was started on piperacillin-tazobactam, vancomycin, oral azithromycin (500 mg X 1 followed by 250 mg daily) and admitted. Her therapy was transiently de-escalated to ampicillin-sulbactam and azithromycin, but was quickly broadened back to piperacillin-tazobactam, vancomycin, and azithromycin given continued fevers. A sputum culture resulted as oropharyngeal commensal flora. Given continued cough, shortness of breath, and fevers, a chest CT was ordered on day 5 of hospitalization and demonstrated multiple patchy consolidations and centrilobular nodules involving the right lung (Figure 1). Transplant infectious diseases were subsequently consulted and recommended continuation of piperacillin-tazobactam. She continued to have fevers (39.3°C on day 7 of hospitalization), and a bronchoscopy with BAL was performed on day 7. The BAL grew mixed oral flora and rare *Candida albicans*, but a RPAN performed on the BAL fluid was positive for only *M pneumoniae*. A BAL sample was sent to the UAB Diagnostic Mycoplasma Laboratory and identified MRMP. On day 8 of hospitalization, oral levofloxacin 750 mg daily was added and her fevers defervesced. She was discharged on day 10 to complete a 10-day course of levofloxacin. She was seen in infectious diseases clinic 7 days after discharge and was improving with resolution of cough and fever.

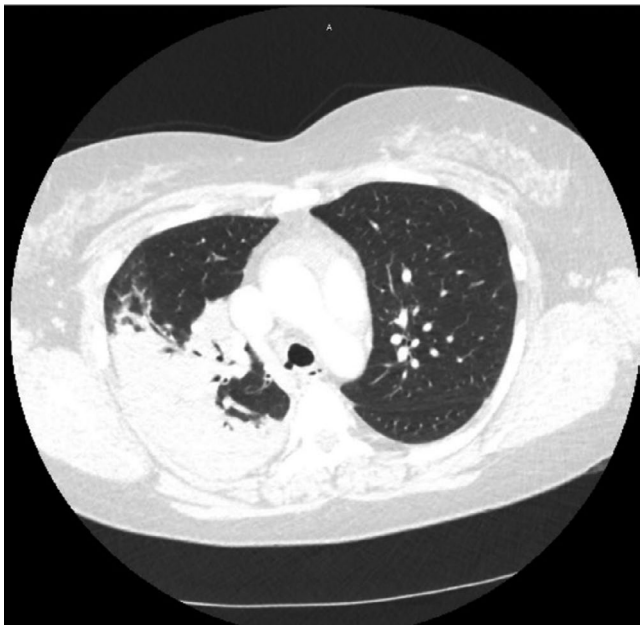

FIGURE 1 Computed Tomography of the chest of Case 2

3.1 | *Mycoplasma* laboratory testing

3.1.1 | *Mycoplasma pneumoniae* PCR and culture

DNA was isolated from the original nasopharyngeal and BAL samples and tested by real-time PCR assays on a Roche LightCycler 480 (Roche Diagnostics) to detect *M pneumoniae* and the point mutations in *23S rRNA* gene known to be associated with macrolide resistance in *M pneumoniae*.[1,5] Specimens were also cultured successfully from both specimens using SP4 broth and agar to obtain *M pneumoniae* isolates.[6]

3.1.2 | Molecular genotyping

Both P1 subtyping and multi-locus variable tandem repeat analysis (MLVA) were performed on the two *M pneumoniae* isolates. Conventional PCRs were used to amplify the *P1* gene and the tandem repeat fragments. Two portions of the *P1* gene were amplified using primer pairs ADH1/ADH2[7] and Mp5f/M16r.[8] The 4-locus MLVA typing scheme (Mpn 13-16) was used as described.[9] PCR was carried out on a Veriti 96-well thermal cycler (Applied Biosystems) with a 25 μL PCR reaction volume containing 0.4 μmol/L of each primer, 2.5 μL of 10X AccuPrime Pfx reaction mix (Thermo Fisher), 0.5 U of AccuPrime Pfx DNA polymerase, and 2 μL of template DNA. Amplicons were sequenced by Sanger sequencing at the UAB Heflin Genomics Center and analyzed using CLC Main Workbench 12 (Qiagen). The amplicons from the real-time PCR detecting the *23S rRNA* mutations were sequenced as well. The assembled sequences were compared with the reference sequences in NCBI, and both isolates were identified to be P1 subtype 2 (P1-2) variant 2c and MLVA type 3-5-6-2. The isolate from case 1 carried a point mutation A2063G (*M pneumoniae* numbering) in *23S rRNA* gene, while A2064G was found in case 2.

4 | DISCUSSION

We report two cases in solid organ transplant patients illustrating the clinical impact of infection due to MRMP. These cases also represent, to our knowledge, the first cases of MRMP in the state of Michigan. There are several interesting characteristics of our experience. First, both patients were clearly failing macrolide therapy, as manifested by continued high fevers. However, the response to alternative therapy was dramatic, with a resolution of fevers within 24 hours of converting to doxycycline and levofloxacin, respectively. Although quinolone therapy represented the definitive therapy in our patients, adult[10] and pediatric[11] data from Japan confirm that tetracyclines such as minocycline are also efficacious for the treatment of macrolide-resistant *M pneumoniae* pneumonia. Our second case is also notable in that although the patient had a negative RPAN from a retropharyngeal swab on admission, RPAN testing was subsequently positive for *M pneumoniae* from a BAL sample. There are several possible explanations for this, including inadequate collection of the nasopharyngeal specimen, but in this case, deep respiratory sampling was necessary to procure a diagnosis and identification of optimal therapy. There is some evidence to suggest that lower respiratory specimens such as sputum and BAL may contain larger numbers of organisms making it easier for them to be detected.[1]

The *M pneumoniae* isolates from both cases have the same genotypes: P1-2 variant 2c and MLVA type 3-5-6-2. However, this does not necessarily mean that there was transmission from one patient to another. First, these genotypes are the most common types identified in a recent surveillance in 9 locations across the United States.[2,12] Although Michigan was not included in the surveillance, we expect the general strain type distribution to be similar to other geographic areas and it is certainly possible both patients could have independently acquired the same strain from different sources. Second, the macrolide-resistant point mutations in the two isolates are different, suggesting different evolving routes for resistance development.

Diagnosis of *M pneumoniae* infection was achieved by PCR, now the diagnostic method of choice.[1] Serologic testing was used for many years prior to the availability of molecular methods, despite significant shortcomings and the likelihood for both false-positive and false-negative results. Serum samples taken during the acute phase of illness may not be indicative of current infection, either because it is too soon for antibody detection or because some individuals have persistently elevated antibodies. In addition, IgM may not be produced during reinfection in persons older than 40 years. Serology is unreliable in immunosuppressed persons who may be unable to mount a humoral immune response, whether it is innate or iatrogenic, so it was not a consideration in the present cases.[1]

In conclusion, clinicians should be aware of the potential for macrolide resistance in *M pneumoniae* and should maintain a high

index of suspicion in patients who fail to defervesce promptly on macrolide therapy. As a result of our experience, we no longer treat immunocompromised patients with *M pneumoniae* pneumonia with macrolides. Continued vigilance and surveillance are necessary to track the evolving epidemiology of MRMP.

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CONFLICT OF INTEREST

All authors have nothing to declare.

AUTHORS' CONTRIBUTIONS

DK, JR, TG, and GE managed the patients and collected the data. AR performed the PCR assays for detecting *M pneumoniae* and macrolide resistance. LX obtained sequencing to identify mutations conferring macrolide resistance and also performed and analyzed the P1 and MLVA subtyping. DC performed culture to obtain and identify *M pneumoniae* isolates. All authors wrote the manuscript.

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REFERENCES

1. Waites KB, Xiao L, Liu Y, Atkinson TP. *Mycoplasma pneumoniae* from the respiratory tract and beyond. *Clin Microbiol Rev*. 2017;30:747-809.
2. Waites KB, Ratliff A, Crabb DM, et al. Macrolide-resistant *Mycoplasma pneumoniae* in the United States as determined from a national surveillance program. *J Clin Microbiol*. 2019;57:e00968-e1019.
3. Miyashita N, Akaike H, Teranishi H, et al. Macrolide-resistant *Mycoplasma pneumoniae* pneumonia in adolescents and adults: clinical findings, drug susceptibility, and therapeutic efficacy. *Antimicrob Agents Chemother*. 2013;57:5181-5185.
4. Xiao L, Atkinson TP, Hagood JA, Makris C, Duffy LB, Waites KB. Emerging macrolide resistance in *Mycoplasma pneumoniae* in children: detection and characterization of resistant isolates with real-time PCR. *Pediatric Infectious Disease Journal*. 2009;28:693-698.
5. Li X, Atkinson TP, Hagood J, Makris C, Duffy LB, Waites KB. Emerging macrolide resistance in *Mycoplasma pneumoniae* in children: detection and characterization of resistant isolates. *Pediatr Infect Dis J*. 2009;28:693-696.
6. Waites KB, Duffy LB, Xiao L. Detection of human mycoplasmas and ureaplasmas from clinical specimens by culture and PCR. In: Laber A ed. *Clinical Microbiology Procedure Handbook*, (4th ed.). Washington, DC: ASM Press. Chapter 3.15, 2016.
7. Sasaki T, Kenri T, Okazaki N, et al. Epidemiological study of *Mycoplasma pneumoniae* infections in Japan based on PCR-restriction fragment length polymorphism of the P1 cytadhesin gene. *J Clin Microbiol*. 1996;34:447-449.
8. Dumke R, Luck PC, Noppen C, et al. Culture-independent molecular subtyping of *Mycoplasma pneumoniae* in clinical samples. *J Clin Microbiol*. 2006;44:2567-2570.
9. Sun H, Xue G, Yan C, et al. Multiple-locus variable-number tandem-repeat analysis of *Mycoplasma pneumoniae* clinical specimens and proposal for amendment of MLVA nomenclature. *PLoS ONE*. 2013;8:e64607.
10. Tashiro M, Fushimi K, Kawano K, et al. Comparison of efficacy of antimicrobial agents among hospitalized patients with Mycoplasma pneumonia pneumonia in Japan during large epidemics of macrolide-resistant *M pneumoniae* infections: a nationwide observational study. *Clin Infect Dis*. 2017;65:1837-1842.
11. Kawai Y, Miyashita N, Kubo M, et al. Therapeutic efficacy of macrolides, minocycline, and tosufloxacin against macrolide-Resistant *Mycoplasma pneumoniae* pneumonia in pediatric patients. *Antimicrob Agents Chemother*. 2013;57:2252-2258.
12. Xiao L, Ratliff A, Crabb D, et al. Molecular characterization of Mycoplasma pneumoniae isolates in the United States from 2012-2018. *J Clin Microbiol*. 2020 (in press).

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