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Review Article

Research Progress in Mechanisms of Drug-Resistance of Macrolide Antibiotics Resistance in *Mycoplasma pneumoniae*

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ABSTRACT

Aim/Background: This review aims to enhance understanding of *Mycoplasma pneumoniae* (MP) infection and Macrolide-Resistant *Mycoplasma pneumoniae* (MRMP) pneumonia, emphasizing their epidemiological characteristics and resistance mechanisms. MP is a major cause of Community-Acquired Pneumonia (CAP) in children, with potential extra-pulmonary manifestations. The objective is to guide early diagnosis and timely treatment, addressing the challenges posed by MP's resistance to β-Lactam and vancomycin drugs. **Materials and Methods:** The review examines the current status of macrolide antibiotics, such as erythromycin, azithromycin, clarithromycin, josamycin, and roxithromycin, highlighting their mechanisms of action and interference with microbial protein synthesis. It also explores laboratory testing methods for identifying MRMP and assessing resistance mechanisms. **Results:** The review synthesizes knowledge on MRMP infection characteristics and epidemiology, highlighting the global increase in MRMP cases, which limits treatment options for pediatric patients. It discusses resistance mechanisms and the urgent need for effective clinical treatments due to rising macrolide resistance. **Conclusion:** This review underscores the critical need for a deeper understanding of MP infection and the alarming rise of macrolide resistance. It emphasizes judicious use of macrolide antibiotics and advocates for ongoing research to develop alternative treatment strategies. The findings highlight the necessity for enhanced surveillance, accurate laboratory testing methods, and updated clinical guidelines to manage MRMP pneumonia effectively, particularly in the pediatric population.

Keywords: *Mycoplasma pneumoniae*, Pneumonia, Macrolide Antibiotics, Drug Resistance, Mechanism.

INTRODUCTION

Characteristics of *Mycoplasma pneumoniae* (MP) Infection and Macrolide Resistant *Mycoplasma pneumoniae* (MRMP) Infection

Mycoplasma is a pivotal pathogen in respiratory tract infections, often accompanied by diverse extrapulmonary manifestations that lead to functional damage, particularly in the nervous and cardiovascular systems.[1-4] Upon Mycoplasma infection, the symptoms exhibited by patients vary significantly,[5] and the mode of infection remains unclear. In addition to variations in the types and loads of infection, the physical conditions and lifestyle habits of patients may also contribute to clinical differences.[6] Research studies have indicated that *Mycoplasma pneumoniae* (MP) adheres to the surface of respiratory mucosal epithelial cells after primary infection, resulting in airway hyperreactivity.[7]

C-Reactive Protein (CRP), an acute-phase reaction protein synthesized by the liver, serves as a common indicator for the acute phase of systemic inflammatory responses. Elevated CRP levels signify the presence of disease or rheumatic immune conditions and are routinely examined through blood tests. Interleukin (IL), a cytokine facilitating interaction between white blood cells and immune cells, plays a crucial role in inflammation. Studies suggest that various cytokines such as IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, TNF-α, and IFN-γ contribute significantly to the pathogenesis of MP infection.[8,9]

As lung tissue damage increases, alterations in certain cytokines like IL-6, IL-8, IL-12, TNF-α, IFN-γ, etc., become more pronounced. These changes serve as regular detection parameters during the course of MP infection, providing valuable insights into the infection severity and the diagnosis of treatment

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outcomes for mycoplasma pneumonia. When MP invades the respiratory tract, the body initiates the production and secretion of pro-inflammatory agents such as TNF-α, IL-6, and IL-8 to clear the pathogen. Furthermore, the metabolizing products and toxins of chlamydia stimulate the release of various inflammatory cytokines from immune cells, underscoring the critical role of cell-mediated immune responses in the pathogenesis of MP infection.[10]

Current research indicates that cases of Macrolide-Resistant *Mycoplasma pneumoniae* (MRMP) infection tend to manifest with more persistent fever, severe cough on imaging, and prolonged antibiotic usage time.[11-15] In comparison to Macrolide-Sensitive *Mycoplasma pneumoniae* (MSMP) infection, MRMP infection may result in a gradual decrease in bacterial load in the body. The heightened immune indicators associated with MRMP in children may stem from prolonged stimulation of the body by drug-resistant pathogens.[12]

Serum cytokine responses and local immune abnormalities in MRMP-infected patients are more pronounced than those in MSMP-infected patients. These cytokines have been shown to correlate with the severity of *Mycoplasma pneumoniae* Pneumonia (MPP) in children. Consequently, the likelihood of severe pulmonary lesions and extrapulmonary complications following MRMP infection is higher.[13] However, there remains controversy regarding whether MRMP may lead to a faster progression and a higher incidence of extrapulmonary complications in actual clinical studies.[14,15]

A recent study investigates molecular traits, diagnosis, and treatment of Macrolide-Resistant *Mycoplasma pneumoniae* (MRMP) in children. It identifies genetic mutations, diagnostic strategies, and suggests corticosteroids for severe cases, particularly in China where MRMP prevalence is notable.[16]

Epidemiological Characteristics

MPP is a respiratory-transmitted disease that spreads through close contact and respiratory droplets, displaying characteristics of regional epidemics. Typically, outbreaks occur in relatively closed and densely populated areas every 3 to 7 years, with each episode lasting approximately 18 months.[17-19] The prevalence of these outbreaks is notably higher during the autumn and winter seasons. Various studies have indicated that the epidemic patterns are influenced by regional climate and other factors, with variations observed among different geographical areas.[20]

Following an outbreak in 2005, a new epidemic surfaced around 2011, garnering detection and reports in numerous countries, particularly in northern Europe, the United States, East Asia, and parts of the Middle East. In 2012, Lenglet *et al.* in Sweden published an epidemiological analysis of MP in Europe around 2011, as conducted by the European Center for Epidemic Disease and Prevention.[21] Epidemiological surveys were carried out in countries within the European Union or the European Economic Community, with 20 participating nations. Among these, seven countries, namely Denmark, Finland, the Netherlands, Norway, Sweden, the United Kingdom, and the Czech Republic, reported the prevalence of MP in 2011.

The primary epidemic season was observed in autumn, and the outbreak was mainly concentrated in northern Europe, with no significant prevalence noted in the southern regions.

MPP is predominantly self-limited, and its pathogenicity is closely linked to the immune characteristics of the body. Children and adolescents are particularly susceptible to MPP due to their immature immune systems, making them prone to clinical symptoms. Preschool children, in particular, constitute the primary population affected by MPP.[22]

In 2013, He *et al.* in China reported on the situation of hospitalized children with MPP. The target gene for detection was the gene-specific 16S rRNA coding gene.[23] The detected positive rate of MP was 20.23%, with the distribution across age groups as follows: 13.6% in children under 1 year old, 18.1% in children under 3 years old, 30.3% in children under 6 years old, and 21.2% in children over 6 years old, illustrating susceptibility across various age groups. Other studies have indicated that some asymptomatic individuals can carry the pathogen, exhibiting opportunistic disease characteristics. However, there is no consensus on this issue both domestically and internationally.[23]

A systematic review and meta-analysis analyze global trends in Macrolide-Resistant *Mycoplasma pneumoniae* (MRMP) infections. It reveals increasing MRMP proportions with regional variations, identifying prevalent variant types. The study emphasizes the need for strategies to prevent MRMP spread and mitigate its disease burden worldwide.[24] Another systematic review explores the epidemiology of Macrolide-Resistant *Mycoplasma pneumoniae* (MRMP) infections across Europe from 2010 to 2021. Despite variations among countries, overall MRMP spread in Europe remains low. However, increased transmission occurred during epidemic waves, notably in Italy and Scotland. Conversely, Finland and the Netherlands reported no MRMP infections. The study underscores the importance of ongoing surveillance to maintain low infection rates and advocates for a coordinated pan-European surveillance program to mitigate antimicrobial resistance spread effectively.[25] Recent study investigates the impact of macrolide resistance on pediatric community-acquired pneumonia caused by *Mycoplasma pneumoniae*. While clinical severity remains comparable between macrolide-resistant and sensitive infections, macrolide resistance prolongs febrile periods, hospital stays, antibiotic courses, and delays defervescence post-treatment. The risk of persistent fever post-treatment and the need for second-line therapies are significantly elevated in macrolide-resistant cases. These findings highlight the diagnostic and therapeutic challenges posed by

macrolide-resistant *Mycoplasma pneumoniae* infections in pediatric populations.[26]

Mechanism of Action of Macrolide Antibiotics

Macrolide antibiotics are a class of drugs with macrolide ring structure, among which erythromycin is the first natural macrolide antibiotic discovered (Figure 1). The second generation of macrolide antibiotics consists of semi-synthetic derivatives of erythromycin, such as azithromycin, roxithromycin, clarithromycin, and others. The third generation introduces ketolactones, with telimycin being a representative example.[27] The mechanism of action of macrolide antibiotics includes antibacterial action and non-antibacterial action (Table 1).

Antibacterial effect

Mechanism of Protein Synthesis Inhibition

In every cell, protein synthesis is an indispensable process. The ribosome serves as the cellular site for protein synthesis, comprising a 30S small subunit and a 50S large subunit. The 30S small subunit facilitates the interaction between the messenger Ribonucleic Acid (mRNA) codon and the transfer Ribonucleic Acid (tRNA) anticodon, crucial for ensuring the accuracy of translation. The 50S subunit is involved in the initiation, elongation, and termination phases of protein synthesis, composed of 23S rRNA, 5S rRNA, and proteins. The 23S rRNA can be further divided into six structural domains,[41,42] with domain V closely associated with peptidyl transferase activity.

Ribosomes guide protein synthesis through four key steps: initiation, extension, termination, and the ribosome cycle. Macrolide antibiotics are capable of targeting the 23S rRNA of bacterial ribosomes. By interfering with ribosomal function, these antibiotics disrupt protein synthesis, inhibit bacterial growth, and consequently exhibit antibacterial properties.[43]

Amino acids come together to form peptide bonds at the Peptidyl Transferase Center (PTC) of the 50S subunit of the ribosome, leading to the synthesis of polypeptide chains that exit through the newly generated Peptides' Exit Tunnel (NPET).[27,42]

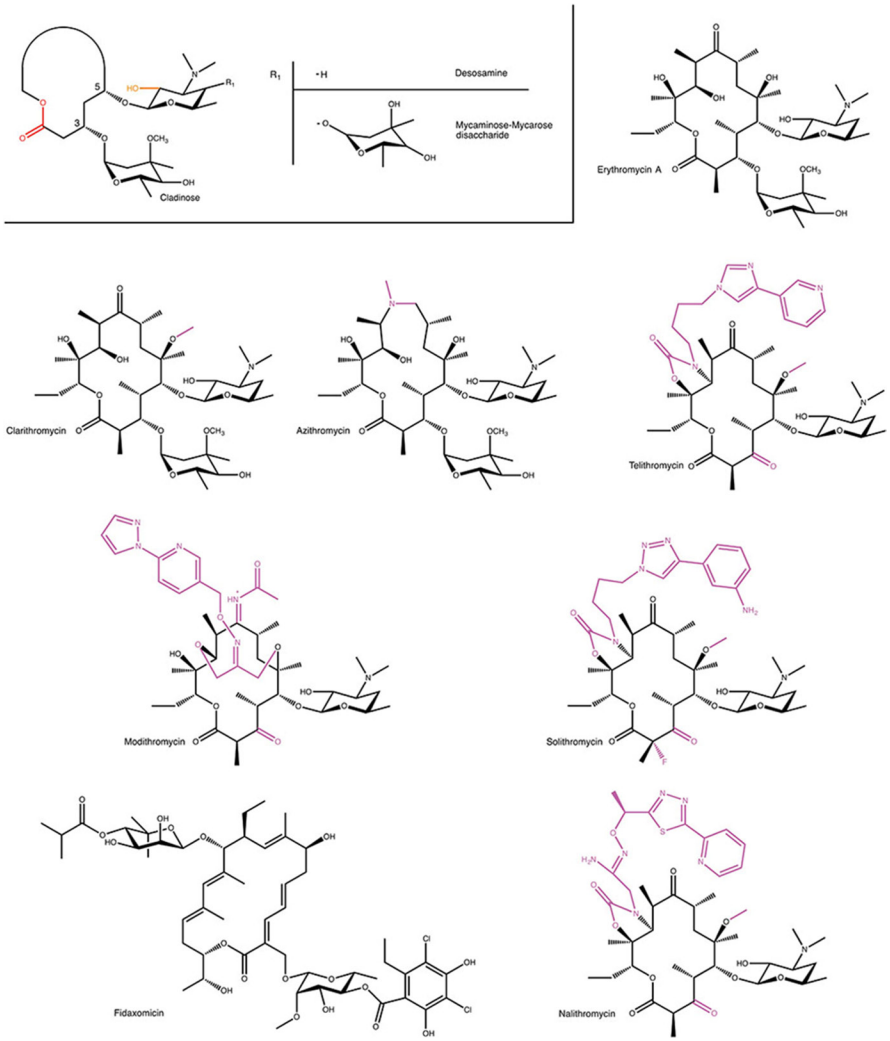

Figure 1: Examples of macrolides used in the clinic and in clinical development.

Table 1: The activity profiles of different macrolide antibiotics.

Group	Molecule	Origin	Target Pathogens	References
First generation	Erythromycin	Semi-synthetic derivative of erythromycin.	**Gram-positive bacteria:** *Staphylococcus aureus, Streptococcus pneumoniae*, and *S. pyogenes.* **Gram-negative bacteria:** *Neisseria meningitis, N. gonorrhoeae*, and *Bordetella pertussis*.	28,29
Second generation	Clarithromycin	Semi-synthetic conversion of erythromycin.	**Gram-positive bacteria:** *S. aureus, S. pneumoniae*, and *S. pyogenes.* **Gram-negative bacteria:** *Mycoplasma pneumoniae, Legionella pneumophila*, and *Chlamydia pneumoniae, Helicobacter pylori, Pseudomonas aeruginosa*.	30
	Roxithromycin	Semi-synthetic derivative of erythromycin.	**Gram-positive bacteria:** *S. aureus, S. pyogenes, S. pneumoniae, Listeria monocytogenes.* **Gram-negative bacteria:** *N. meningitidis, B. pertussis, Haemophilus influenzae*.	31
	Flurithromycin	Fluorinated derivative of erythromycin A.	*H. pylori, Bacteroides forsythus*.	32,33
	Dirithromycin	Semi-synthetic derivative of erythromycin.	**Gram-positive bacteria:** *S. aureus, S. pneumoniae*, **Gram-negative bacteria:** *H. influenzae, L. pneumophila, Moraxella catarrhalis*, and *M. pneumoniae*.	31
	Azithromycin	Derivative of erythromycin.	**Gram-positive bacteria:** *S. aureus, S. Pneumoniae.* **Gram-negative bacteria:** *H. influenzae, M. catarrhalis, C. trachomatis, Pseudomonas aeruginosa*, and *H. pylori*.	34,35
Third generation	Telithromycin	Semi-synthetic derivative of erythromycin.	**Gram-positive bacteria:** *S. pneumoniae.* **Gram-negative bacteria:** *M. pneumoniae, C. pneumoniae, H. influenzae* and *L. pneumophilia*.	36
	Cethromycin	Derivative of erythromycin.	**Gram-positive bacteria:** Macrolide-resistant *S. pneumoniae, S. pyogenes.* **Gram-negative bacteria:** *H. influenzae*.	37,38
	Josamycin	*S. narbonensis* var. josamyceticus	**Gram-positive bacteria:** *S. aureus, S. pneumoniae,* and *S. Pyogenes.* **Gram-negative bacteria:** *H. influenzae, M. catarrhalis, M. genitalium, N. gonorrhea, N. meningitides.*	39
	Tylosin	S. fradiae, H. influenzae.	*H. influenzae*, Gram-positive pathogens and mycoplasma.	40

Binding to the Peptide Exit Tunnel

Previous studies have proposed that macrolide antibiotics can bind to NPETs, reduce the tunnel diameter, impede the elongation of new peptides, and exert inhibitory effects on all proteins synthesized within ribosomes.[42,44,45]

Selective Inhibition of Protein Synthesis

However, an increasing number of studies have revealed that macrolide antibiotics do not act as global inhibitors of translation but function as modulators of translation.[46,47] They can prevent the aggregation of specific amino acid sequences and inhibit peptide bond formation, showcasing the context specificity of macrolide antibiotics in selectively interfering with protein synthesis. Moreover, additional research suggests that macrolide antibiotics can induce coding errors and impact translation outcomes.[48]

Non-antibacterial effect

Anti-inflammatory Properties

The non-antibacterial effects of macrolide antibiotics primarily involve inhibiting inflammatory responses, reducing airway mucus secretion, and modulating immune balance. Macrolide antibiotics can curb excessive and detrimental inflammatory responses by influencing inflammatory cells, inflammatory factors, and airway epithelium. Neutrophils, crucial players in the inflammatory response and major antibacterial effectors, engage in pathogen elimination through degranulation, phagocytosis, production of reactive oxygen species, and the release of

Neutrophilic Extracellular Traps (NETs). However, sustained inflammation may prompt neutrophils to release excessive NETs, causing harm to the host.[49-51] Studies have identified inhibitory or stabilizing effects of macrolides on neutrophils, with a concentration-dependent impact on NETs release.[52]

Pro-inflammatory factors, including Granulocyte Colony-Stimulating Factor (GM-CSF), Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Interleukin-8 (IL-8), play pivotal roles in promoting inflammatory responses. Nonetheless, macrolides can impede the expression of these inflammatory factors.[53,54] Azithromycin, for instance, demonstrates inhibitory effects on various pro-inflammatory pathways, characterized predominantly by regulation rather than complete inhibition. Additionally, studies have indicated that azithromycin can safeguard respiratory mucosal epithelium and mitigate damage caused by airway inflammation.[55]

Reduction of Airway Mucus Secretion

A significant feature of chronic airway inflammation is the excessive secretion of airway mucus. Macrolides play a role in diminishing this hypersecretion by regulating chloride ion channels, inhibiting goblet cell hyperplasia and hypertrophy, and suppressing the expression of mucus secretion genes.[56,57] Additionally, there are studies suggesting that macrolides can reduce airway mucus secretion by inhibiting sodium ion channels.[58]

Immune Modulation

Currently, extensive research is underway regarding the immune-regulatory effects of macrolide antibiotics in pulmonary diseases such as asthma, Chronic Obstructive Pulmonary Disease (COPD), bronchiectasis, and cystic fibrosis.[54,59] Their primary mechanisms of action involve inhibiting microbial adhesion, blocking toxic factors, suppressing biofilm formation, and hindering cell quorum sensing. For instance, azithromycin has demonstrated the ability to inhibit the adhesion of Pseudomonas aeruginosa to epithelial cells and regulate the pH value of epithelial cells. While these drugs appear to expedite the restoration of immune balance and may have a positive impact on enhancing antibacterial abilities, prolonged use may lead to drug resistance and other potential harm.

Current situation of resistance of MP to macrolide antibiotics

The prevalence of Macrolide-Resistant *Mycoplasma pneumoniae* (MRMP) exhibits substantial variation across different regions, with notably high prevalence in Asian countries such as China, Japan, and South Korea, while showing relatively lower prevalence in North America, Europe, and Africa. The status of macrolide resistance in MP both domestically and internationally is a matter of concern.

Regional Variations in MRMP Prevalence

North America and Europe

In the United States, the prevalence of MRMPs ranges from 3% to 10%.[60] European countries generally report low detection rates of macrolide-resistant strains, with the notable exception of Italy, where the prevalence reaches 26%.[61] In France, the detection rate of macrolide-resistant strains is 8.3%,[62] and in Germany, the macrolide resistance rate from 2016 to 2018 was approximately 3%.[63] All MP strains in South Africa from 2012 to 2015 were found to be sensitive Figures 2 and 3.[64] The relatively low prevalence in these regions may be attributed to stricter antibiotic usage policies and better implementation of antimicrobial stewardship programs.

Asia

In Japan, the detection rate of MRMPs in children ranged from 50% to 90% between 2008 and 2015.[65] This high prevalence can be linked to the overuse and misuse of macrolide antibiotics, which creates selective pressure for resistant strains. However, following the introduction of the 2011 Japanese MP Treatment guidelines, which allowed the use of fluoroquinolones in some MRMP-infected children, the MRMP detection rate decreased to 43.6% by 2013.[65]

China exhibits similarly high MRMP prevalence rates, with regional variations. For instance, the detection rate of MRMPs in children in Beijing, Shanghai, Xinjiang, Yunnan, and Harbin ranged from 66.7% to 86.7%.[66] In Beijing, the MRMP detection rate in children from 2010 to 2012 was 94.3%, slightly higher than that in adults (83.8%).[67] These high rates may result from the widespread and inappropriate use of macrolides, coupled with less stringent antibiotic regulation policies. However, certain areas have reported reductions in MRMP detection rates, likely due to increased awareness and improved antibiotic stewardship.[66]

South Korea has also reported high MRMP detection rates; with a significant increase from 62.9% in 2011 to 87.2% in 2015.[68] This surge highlights the critical need for stricter regulation and judicious use of antibiotics to control the spread of resistance.

Possible Reasons for Regional Differences

The epidemiology of MRMP varies widely across different regions due to a complex interplay of factors including antibiotic usage patterns, treatment guidelines, and healthcare infrastructure. Addressing these variations requires region-specific strategies to improve antibiotic stewardship, enhance surveillance, and develop tailored treatment guidelines to effectively manage and control the spread of MRMP globally.

Antibiotic Usage and Stewardship

The high prevalence in Asia like China and Japan may be linked to the widespread and often inappropriate use of antibiotics,

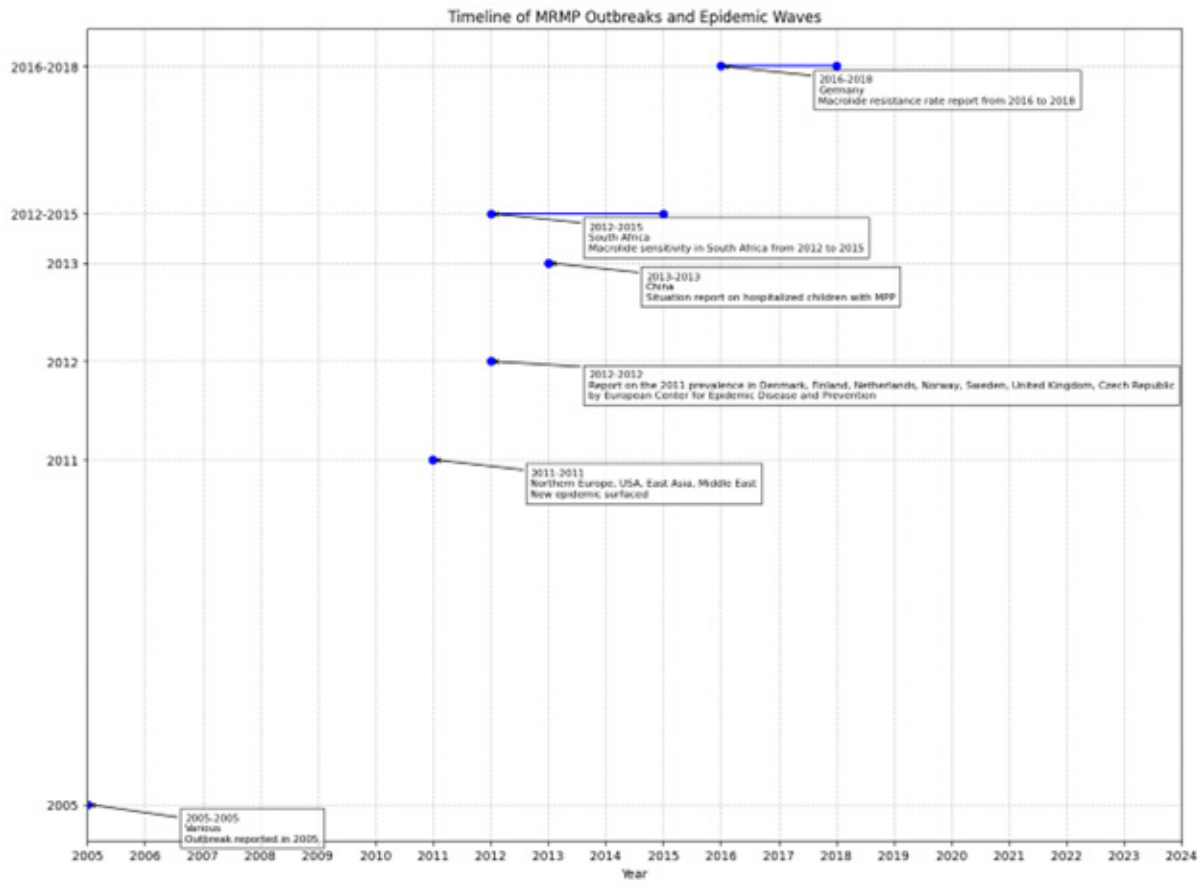

Figure 2: A timeline or bar graph showing the development of macrolide resistance in different regions over the years. This can highlight the prevalence rates and significant milestones or guidelines changes.

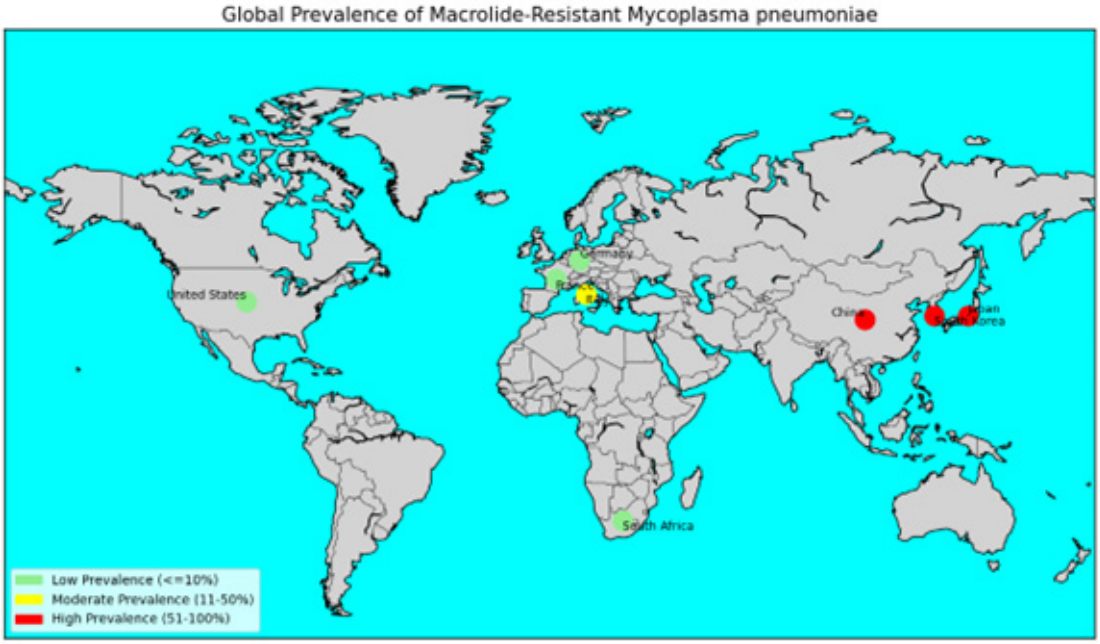

Figure 3: A world map highlighting the prevalence rates of MRMP in different countries, with color-coding to indicate low, moderate, and high prevalence regions.

including over-the-counter availability without prescriptions, leading to higher selection pressure for resistant strains. Stricter antibiotic stewardship programs and guidelines on antibiotic use may contribute to the lower prevalence of MRMP in North America and Europe (excluding Italy). These regions typically have more regulated antibiotic prescription practices, reducing the development and spread of resistance.[69,70]

Treatment Guidelines

In Japan, the observed decline in MRMP rates following the introduction of new treatment guidelines in 2011, which included the use of fluoroquinolones for children with MRMP, highlights the impact of clinical guidelines on resistance patterns.[65]

Surveillance and Reporting

The apparent absence of MRMP in South Africa might reflect both the true epidemiological situation and differences in surveillance intensity or diagnostic capabilities compared to other regions.

Cultural and Societal Factors

In some Asia regions, cultural practices and healthcare-seeking behaviors may contribute to the higher use of antibiotics, thus driving resistance. Additionally, densely populated areas with frequent close contact, such as schools and daycare centers, facilitate the spread of resistant strains.

Resistance mechanism of macrolide antibiotics

MP belongs to the class Mollicutes within the Mycoplasma genus. It lacks a cell wall, rendering it naturally resistant to antibiotics targeting cell walls, such as β-lactam antibiotics. Antibiotics that act on protein synthesis, such as macrolides and tetracycline, as well as fluoroquinolone antibiotics that target topoisomerases during DNA synthesis and replication, exhibit a strong inhibitory effect on it. Clinical treatment of atypical pneumonia can yield satisfactory results. However, the use of tetracycline antibiotics in children can impede bone development, leading to enamel dysplasia and tooth yellowing. Similarly, quinolones may impact the growth and development of children and adolescents. As a result, 14 and 15-membered ring macrolide antibiotics have emerged as the preferred choice for treating MPP due to their notable efficacy and minimal side effects.

The primary mechanisms through which bacteria develop resistance to macrolide antibiotics include: (1) modification of the antibiotic ribosomes by target methylation enzymes (encoded by ERM genes); (2) mutations in the target genes involved in the action of antibiotic ribosomes; (3) enhancement of the active efflux mechanism (encoded by mefE and mefA genes); and (4) production of modifying enzymes that deactivate drugs (encoded by ereA and ereB genes). Currently, in research on MP resistance mechanisms, only mutations in the target genes of antibiotic ribosome action have been conclusively identified, with other related resistance genes not yet discovered in MP studies.

Presently, there is a more in-depth exploration of 23S rRNA V and II region gene mutations, as well as mutations in the ribosomal protein L4 and L22 genes, in the research on the mechanisms of MP resistance.

Gene mutations in drug targets

Mutations in the transpeptidase ring

The 23S rRNA of the bacterial ribosomal 50S subunit is implicated in antibiotic resistance,[71] with the mutation site identified as 550 base pairs between the transpeptidase loop at positions 2059 and 2617.[72]

Lucier, utilizing ribosome binding assays and ribosomal sequence detection, confirmed that the binding site between 14-membered macrocyclic lactones and ribosomes is situated at positions 2063, 2064, 2607, and 2611 on the 23S rRNA of MP.[73] For macrolides with 15-membered and 16-membered rings, the binding sites are roughly analogous to those of 14-membered ring macrolides, specifically at positions 2062, 2063, 2064, 2607, and 2616 of 23S rRNA.[74,75] Single nucleotide polymorphisms at these binding sites can lead to a reduced ribosomal affinity, resulting in the ineffective attachment of macrolide molecules to the ribosomal peptidyl transferase loop.[72]

A2063G and A2064G mutations confer high levels of macrolide resistance, with Minimum Inhibitory Concentrations (MICs) of ≥128 mg/mL and 6256 mg/mL, respectively. The A2063T mutation leads to moderate levels of erythromycin resistance, with an MIC of 32 mg/mL.[72] Mutations at A2067G, C2617G, and C2617A result in lower levels of macrolide resistance.

In a study by Zhao *et al.*, examining MP infection samples in Beijing, China from 2007 to 2012, the A2063G mutation rate in the 23S rRNA gene V domain of drug-resistant MP was found to be 89.92%.[76] Sun *et al.* further tested samples from MP infection in Beijing, China from 2003 to 2015, revealing an A2063 mutation rate of 99.3%.[77] MaZ *et al.*, investigating MP infection samples in Shenzhen, China, found that the proportion of A2063G mutations was 63%.[78] In central Slovenia, Kogoj *et al.* detected drug-resistant MP and observed that A2063G mutations were the most common, followed by A2063C, A2063T, A2067G, C2617G, and C2617A mutations.[79] Loo *et al.*, in a study involving samples from children infected with MP in Singapore, concurred that the A2063G mutation was the most prevalent.[80] Consequently, the A2063G mutation stands out as the most common mutation in drug-resistant MP worldwide.

Cao B *et al.* comprehensively reviewed over 20 domestic and international studies on drug resistance in MP spanning from 2000 to 2015.[81] They outlined the order of point mutation

frequencies as follows: A2063G, A2064G, A2063T, A2063C, A1290G, C2617A, A2067G.

The frequency of point mutations on 23S rRNA varies at different stages. Suzuki Y *et al*. examined MP samples from Yamagata, Japan, between 2004 and 2013. [82] They observed an increase in the frequency of A2063T mutation in 2009, followed by a surge in the frequency of A2063G mutation in 2010. Consequently, it remains essential to continue monitoring the mutation sites of drug-resistant MP.

Mutations in ribosomal proteins

Ribosomal proteins L4 and L22 are situated in the peptide efflux channel of the ribosome's 50S subunit, exhibiting spherical domains and slender tentacles on their surfaces. The latter extends into the core of the ribosome, serving as the inner wall of the peptide efflux channel.[83] In the early assembly process of Dayaki, L4 and L22 play a "scaffolding" role.

As per the Escherichia coli induced resistance test, alterations in the tunnel caused by mutations in L4 or L22 can be transmitted to the Peptidyl Transferase Center (PTC) region, impacting the peptide elongation rate.[83] The specific mechanism involves the reduction of peptidyl transferase activity due to L4 mutation, thereby increasing the likelihood of code shifting, mistranslation, and crossing of termination codons.[84] This mutation also narrows the peptide efflux channel, preventing erythromycin from entering and binding to the target.[85, 86] The specific mechanism of the L22 protein is not yet clear and requires further exploration.

Pereyre *et al*. reported that L4 and L22 amino acid mutations can be induced in vitro, characterized by H70R and H70L substitution of the L4 protein, and 1-3 glycine insertions at 60 sites; replacing P112R and A114T of L22 or deleting 111IPRA114.[74]

Matsuoka discovered a C62A mutation in the L22 protein in drug-resistant strains.[52] Sindley *et al*. also detected the H70L substitution of the L4 protein (i.e., A209T) in MP-sensitive strains, suggesting that it is not related to drug resistance.[87] The mutations associated with drug resistance are C58A and G81T mutations in the L4 protein and C62A and T65A mutations in the L22 protein.

Modification of ribosome targets

This process, known as RNA methylation modification, can be catalyzed by Erm-encoded rRNA methyltransferase, leading to dimethylation at A2058 (E. coli number, corresponding to the A2063 site of MP) on 23S rRNA. This induces changes in ribosomal targets, ultimately rendering macrolide drugs ineffective against MP.[88] ErmB is a common gene responsible for methylation,[89] and its encoded ribosomal glycosylase can demethylate the 23S rRNA A205 of MP. This demethylation causes macrolide antibiotics to lose their inhibitory effect on MP, resulting in high levels of resistance.[90] Genetic testing on MP resistant to roxithromycin found a positive rate of 93.3% (70/75 cases) for the ermB gene. It is believed that the target changes mediated by the ermB gene are the mechanism behind Mycoplasma pneumonia's resistance to roxithromycin.

While target modification has been reported in other bacteria, it is rare in MP. It is essential to expand the sample size for MP ERM gene detection and conduct tests in multiple regions to gain a more comprehensive understanding.

Increased active efflux of drugs

Active drug efflux is a common cause of drug resistance, and MP can alter the composition of the cell membrane to create a specialized membrane protein. This protein facilitates the pumping of drugs out of the cell through various efflux pumps, reducing intracellular drug concentration, preventing its action on the target site, and impacting the antibacterial effectiveness of antibiotics. This efflux mechanism is not specific to particular drugs but is effective against multiple antibiotics and mechanisms of action, playing a significant role in mediating multidrug resistance.

Among prokaryotes, there are primarily five efflux transporter protein families: the ATP-Binding Cassette (ABC) family, Major Facilitation factor Superfamily (MFS), Small Multidrug-Resistant family (SMR), Multidrug and Toxic compound Efflux family (MATE), and drug-Resistant Nodular cell Differentiation family (RND).[91] The ABC family relies on ATP hydrolysis for energy supply, while the other families depend on proton pump function. The ABC family is strongly associated with mycoplasma resistance, although research on this topic is currently limited.

The ABC transporter family is one of the largest transporter protein families discovered to date, present in almost all organisms. It relies on the hydrolysis of ATP to provide energy and can transport substrates across membranes, playing a crucial role in various physiological processes. When its substrate is a drug, it can lead to drug resistance, and is therefore also known as multidrug-resistant proteins. So far, hundreds of ABC transporters have been discovered in clinical practice. Currently, the known ABC transporters belong to types IV and V. Type IV can be divided into eight subfamilies, ABCA to ABCH, according to genotype. Among them, ABCB and ABCC subfamilies are highly correlated with drug resistance.[92]

A study used reserpine as an efflux pump inhibitor and explored the antibacterial effect of tolamycin on *Mycoplasma hypermania* based on *in vitro* pharmacokinetic/pharmacological analysis.[93] It was found that *Mycoplasma hypermania* can reduce drug sensitivity by regulating the expression of different ABC transporters. Due to their crucial role in antibiotic resistance, efflux pumps have been identified as effective antibacterial targets, and the development of related efflux pump inhibitors will help improve microbial resistance.

Table 2: Summary for the Laboratory Testing Methods.

Method	Description	Advantages	Limitations
MP Isolation Culture	Gold standard for diagnosing MRMP infection. Involves culturing MP bacteria under strict conditions.	Provides accurate results.	Hindered by strict growth conditions and prolonged culture cycles, unsuitable for routine examinations.
In vitro Drug Sensitivity Testing	Gold standard for treating MRMP infection. Determines the effectiveness of drugs against cultured MP bacteria.	Guides tailored treatment.	Time-consuming, influenced by various factors.
Polymerase Chain Reaction (PCR)	Rapid detection method widely used in clinical practice. Amplifies MP DNA for quick and sensitive diagnosis.	Speedy results, high sensitivity.	Not as comprehensive as culture methods, may require specialized equipment and expertise.

Li *et al.* sequenced clinical isolates and found that four nonsynonymous mutant SNPs clustered in the macB gene, which encodes the macrolide-specific efflux pump protein of the ATP-binding cassette transporter family.[94] The application of efflux pump inhibitors, such as reserpine and carbonyl cyanide chlorophenylhydrazone, can reduce the MIC value of MP. In some strains of MP, the MIC value can even be restored to the level of sensitive strains. Therefore, it is believed that the efflux pump protein encoded by the macB gene is associated with drug resistance.

The non-specific characteristic of active efflux leading to drug resistance results in bacteria with this resistance mechanism developing resistance to antibiotics of different structural categories or mechanisms of action. Significant progress has not yet been made in the clinical response to infections caused by bacteria with active efflux resistance mechanisms. Efflux pumps have been identified as effective antibacterial targets, and the development of related efflux pump inhibitors will help improve microbial resistance. Therefore, further research is still needed on the efflux pump protein genes mentioned above.

Drug inactivation (antibiotic enzymatic hydrolysis)

Pathogens possess the ability to synthesize specific enzymes that modify or break down antibiotics, resulting in alterations to their structure and rendering them inactive. These enzymes comprise β-Lactamases, tetracycline hydroxylase, esterases, acetyltransferases, nucleotide transferases, and others.[95] Macrolide antibiotics face inactivation through the actions of phosphotransferases, esterases, formyl reductases, and glycosyltransferases produced by pathogens.

Research into Macrolide Phosphotransferase (MPHs) commenced in 1989. MPHs facilitate the transfer of γ-phosphate groups from ATP or GTP to macrolide substrates, substituting the hydroxyl group of macrolide and thereby deactivating the drug. MPHs present in 15 subtypes, from MPH (A) to MPH (O), with MPH (A) utilizing GTP as the phosphate group donor, leading to bacterial resistance to 14-membered and 15-membered macrolide antibiotics. Similarly, MPH (B) subtypes, which also prefer GTP as the donor, confer resistance to 14-membered, 15-membered, and 16-membered macrolide antibiotics.[96]

Erythromycin esterases (EREs) play a role in breaking macrolide bonds and cleaving the macrolide ring. Among the EREs, ERE (A) stands out as the most prevalent and earliest discovered member, and ERE (C) serves as its homologous analogue. These two members share high sequence homology and similarity, and their modes of action align closely. In a study, it was observed that erythromycin forms a tight bond with the active center of ERE (C).[97] This binding prompts the closure of the active center during their interaction, reopening after the reaction to prepare for subsequent catalytic reactions.

Both ERE (A) and ERE (C) demonstrate ring-opening effects on 14-membered cyclic ketorolactone, 15-membered cyclic azithromycin, and 16-membered cyclic doxorubicin. In contrast, ERE (B) and ERE (D) exhibit effects on 14-membered cyclic non-ketorolactone antibiotics and 15-membered cyclic azithromycin.

Laboratory Testing Methods

Table 2 provides a concise overview of each method's description, advantages, and limitations in managing MRMP infection.

Conventional methods

Table 3 provides a concise summary of the description, advantages, and limitations of MP culture and serological testing methods for diagnosing MRMP infection.

Molecular biology detection techniques

Table 4 provides a concise overview of the molecular biology detection techniques used for detecting *Mycoplasma pneumoniae* (MP) and its resistance to macrolide antibiotics. Each method has its advantages and limitations, and further exploration is needed to identify clinically applicable detection methods for guiding rational treatment plans.

Table 3: Summary for the Conventional Methods.

Method	Description	Advantages	Limitations	References
MP Culture (Solid and Liquid)	WHO-endorsed gold standard diagnostic method. Utilizes solid and rapid liquid culture techniques.	Established gold standard encompasses both solid and liquid culture.	Solid culture method takes 2-4 weeks, exhibits low sensitivity, limited specificity, susceptible to false positives due to contamination.	-
Serological Testing	Widely adopted for speed and cost-effectiveness. Requires acute and recovery phase serum samples.	Quick and cost-effective.	Requires 2-3 weeks for meaningful results, influenced by antibody production, patient factors, potential missed diagnoses.	98

Clinical Treatment

This type of disease is mainly self-limited. During the treatment process, targeted antibiotics are usually used to shorten the course of the disease and reduce the incidence of complications (Figure 4). Antibiotic selection should consider the patient's age, with restrictions on antibiotics like tetracyclines and quinolones. In treating MPP in children over 8 years old, minocycline and doxycycline are commonly used. Azithromycin remains the preferred treatment for pediatric patients, considering MRMP, due to its relatively high intracellular concentration, which can meet or exceed the resistance MPMIC value (usually about 64-128 μg/mL).[108,109]

If clinical symptoms worsen, fever persists, and pulmonary imaging deteriorates after more than 7 days of macrolide antibiotic treatment, refractory *Mycoplasma pneumoniae* may be considered. In such cases, changing antibiotics or adding other medications is recommended. Despite the increasing resistance of macrolide antibiotics, tetracycline and fluoroquinolone antibiotics maintain good antibacterial activity and clinical efficacy against MP. However, due to concerns about adverse effects, such as cartilage dysplasia with fluoroquinolones and tooth yellowing and enamel dysplasia with tetracycline, their use in children is limited.

Japan has approved oral fluoroquinolone antibiotic tosufloxacin as a second-line treatment for children with community-acquired pneumonia. Studies have shown minimal side effects, such as mild diarrhea, and no joint symptoms observed with fluoroquinolones. The Guangdong Pharmaceutical Association's Expert Consensus on the Application of Fluoroquinolone Antibiotics in Children (2017) provides guidance for their use in clinical practice. Fluoroquinolones can be considered in critically infected patients after consultation with infectious disease experts and clinical pharmacists. Approval from the hospital's drug management committee, informed consent from the patient and family, strict dosage control, careful monitoring for adverse reactions and post-medication follow-up are essential. So far, no clinical isolates have been found to be resistant to tetracycline or fluoroquinolones in MP strains. Although foreign studies have induced MP isolates to exhibit resistance to quinolones and tetracycline,[110] caution is advised to prevent the improper use of these antibiotics, especially fluoroquinolones, which may pose a risk of developing clinical resistance.

The use of glucocorticoids becomes an option in cases of refractory MP caused by MRMP. Children with MRMP often exhibit more abnormal immune indicators, such as T cell subsets and cytokines, compared to those with MSMP. In contrast to the use of antibiotics alone, glucocorticoids play a role in inhibiting cellular immune responses, alleviating lung injury, and mitigating systemic inflammatory response syndrome induced by excessive inflammation.[111,112]

A study by Miyashita *et al.* in 2015 identified the critical value of lactate dehydrogenase for glucocorticoid use in MP patients as 302-364 IU/L.[113] The dynamic changes of lactate dehydrogenase were emphasized as sensitive biological indicators predicting the clinical efficacy of refractory MP infections. In cases of refractory MP pneumonia in children, specific conditions, such as persistent high fever (>7 days), C-reactive protein ≥110 mg/L at initial diagnosis, neutrophil ratio in peripheral blood white blood cell count >0.78, lymphocyte a0.13, serum LDH >478 IU/L, serum ferritin e328 g/L, and chest CT showing uniformly dense consolidation shadows across the entire lung, should raise awareness. When these predictive indicators are present, conventional-dose hormone therapy may be ineffective, necessitating high-dose corticosteroid shock therapy or exploring additional treatment options.

While Intravenous Immunoglobulin (IVIG) can neutralize toxins produced by MP infection, inhibit cytokine and inflammatory factor production, and reduce excessive inflammatory responses, its efficacy in MRMP treatment lacks strong support from multicenter randomized controlled trials and evidence-based medicine. Therefore, careful consideration of the pros and cons is essential, and medication choices should be tailored to the individual patient's situation in clinical applications.

Table 4: Summary for the Molecular Biology Detection Techniques.

Technique	Description	Advantages	Limitations	Referennces
Traditional Polymerase Chain Reaction (PCR)	Amplifies DNA segments of interest using specific primers and DNA polymerase.	High specificity - Rapid detection speed.[99,100]	Requires specialized equipment and expertise.[101,102]	99-102
Real-time Quantitative PCR (qPCR)	Quantifies DNA or RNA levels in real-time during amplification process.	High sensitivity - Quantification of target nucleic acid.[99,100]	Limited by primer design and specificity. [101,102]	99-102
Loop-mediated Isothermal Amplification (LAMP)	Isothermal amplification technique that amplifies DNA at a constant temperature.	Rapid detection - Visible results without specialized equipment.[99,100]	Limited multiplexing capability - Risk of non-specific amplification.	99,100
RNA Amplification Technology	Amplifies RNA fragments to detect RNA-based pathogens.	Differentiates between active infection and carrier status.[103]	RNA extraction may be challenging - Limited to RNA-based pathogens.	103
Real-time Fluorescence Nucleic Acid SAT (RNA SAT)	Extracts specific nucleic acids through magnetic bead method, ensuring high specificity.	High specificity - Rapid detection.[103]	Limited usage in *Mycoplasma pneumoniae* detection.	103
Sequencing Techniques (Sanger sequencing, etc.)	Determines nucleotide sequence of DNA fragments.	Identifies specific mutations or variants.	Time-consuming and costly - Requires specialized equipment and expertise.	-
Commercial Reagent Kits	Analyzes melting curves to detect resistance mutations.	High sensitivity and specificity.[104]	Limited to specific mutations covered by the kit.	104
Real-time PCR High-Resolution Melting (PCR HRM)	Analyzes DNA melting curves to detect genetic variations.[105]	Rapid detection of resistance mutations.	Limited multiplexing capability - May require confirmation with sequencing techniques.	105
Cyclic Probe Method	Utilizes chimeric cyclic probes composed of RNA and DNA for detection.	Rapid detection and discrimination of drug-resistant mutant strains. [106,107]	Limited to specific mutations targeted by the probes.	106,107
Unified Testing Standards	Development of standardized testing protocols.	Ensures consistency and reliability in clinical practice.	Challenges in establishing consensus due to diversity in testing methods and targets.	-

Challenges in the Study of MRMP

Rising Global Incidence

There's a concerning increase in MRMP cases worldwide, particularly affecting pediatric patients, which limits treatment options and poses challenges for managing Community-Acquired Pneumonia (CAP).

Limited Treatment Options

Macrolides are often the first-line treatment for MP infections, but the emergence of resistance reduces their efficacy, leading to treatment failure and prolonged illness.

Complex Resistance Mechanisms

Understanding the diverse mechanisms of macrolide resistance in MP, including mutations in antibiotic targets, efflux pumps, and enzymatic inactivation, presents a significant challenge for researchers and clinicians.

Diagnostic Challenges

Accurate and timely diagnosis of MRMP infections is crucial for appropriate treatment, but laboratory testing methods for identifying MRMP and assessing resistance mechanisms may not be widely available or standardized.

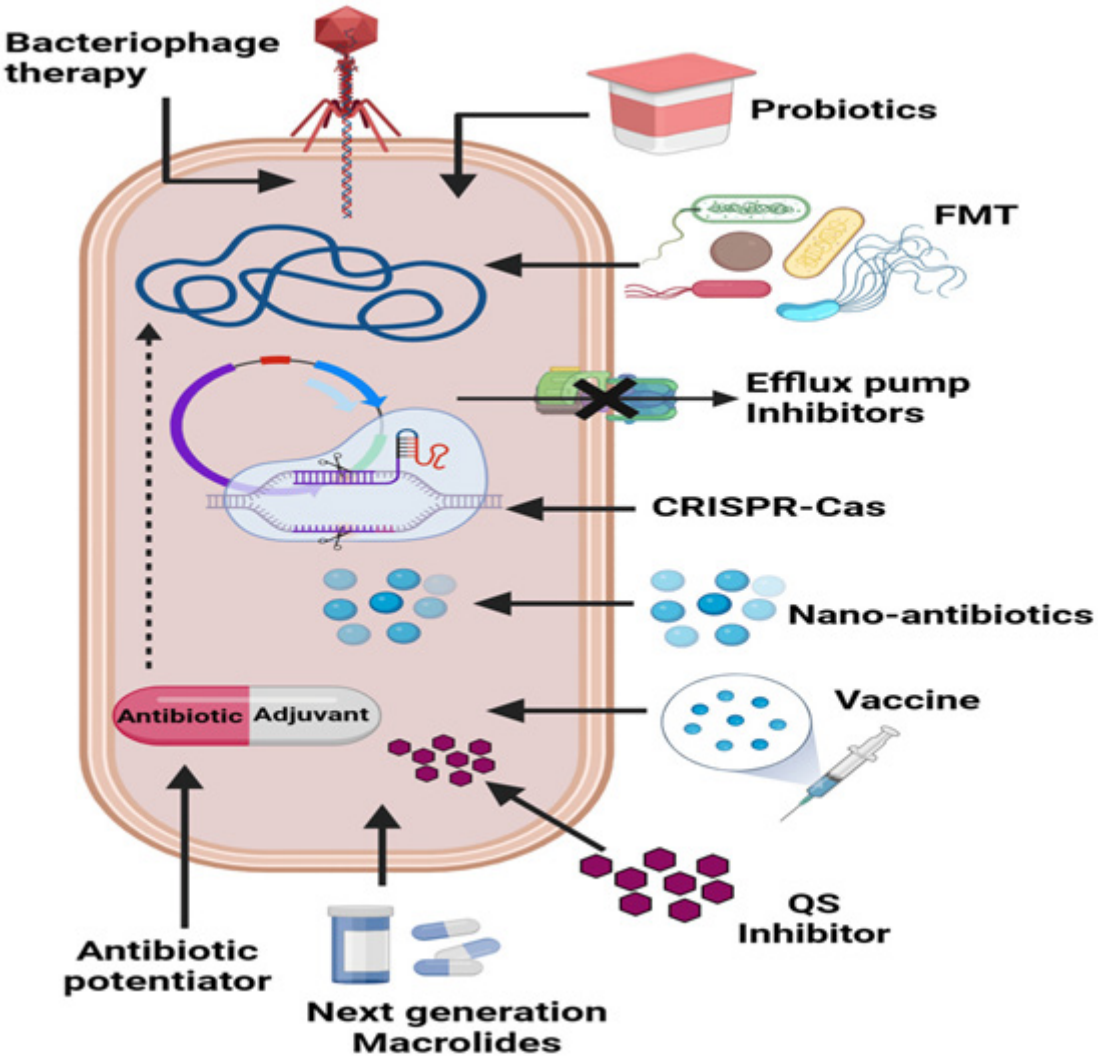

Figure 4: Schematic diagram of clinical treatment strategy.

Future Directions in the Study of MRMP

Alternative Treatment Strategies

Research efforts should focus on developing alternative treatment strategies for MRMP infections, such as novel antibiotics or combination therapies that target multiple resistance mechanisms to overcome macrolide resistance.

Enhanced Surveillance

Improved surveillance systems are needed to monitor the prevalence and spread of MRMP strains globally, including the development of standardized protocols for MRMP detection and reporting.

Development of Rapid Diagnostic Tests

There is a need for rapid and accurate diagnostic tests for MRMP infections that can be readily implemented in clinical settings, enabling prompt identification and treatment of resistant strains.

Optimization of Antibiotic Use

Promoting judicious use of macrolide antibiotics and implementing antimicrobial stewardship programs can help reduce the selective pressure driving the development of macrolide resistance in MP.

Updated Clinical Guidelines

Regular updates to clinical guidelines for the management of CAP, particularly in pediatric patients, should incorporate the latest evidence on MRMP epidemiology, resistance mechanisms, and treatment strategies to ensure optimal patient outcomes.

Addressing these challenges and pursuing these future directions will be essential for effectively managing MRMP infections and reducing the burden of macrolide resistance in *Mycoplasma pneumoniae*.

CONCLUSION AND PERSPECTIVES

Macrolide resistance in *Mycoplasma pneumoniae* (MP) primarily arises from mutations in antibiotic targets, efflux proteins, and enzymatic inactivation.

Mutation A2063G in the transpeptidase loop is prevalent in drug-resistant strains, but other mutation sites vary regionally and temporally.

Controversy exists regarding the role of H70L replacement of ribosomal L4 protein (A209T) in drug resistance, warranting further investigation.

Understanding the mechanisms of target modification, active efflux genes, and enzymatic inactivation is crucial for developing effective antibacterial strategies.

Beyond the major resistance mechanisms, additional factors like reduced drug absorption rates contribute to clinical resistance.

Further research is necessary to inform judicious drug selection, impede the emergence of drug-resistant strains, and improve patient outcomes in clinical practice.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Meixuan Jia (First Author) and Qingtao Li (Co-First Author) contributed equally as first authors to this review article, focusing on the mechanisms of drug-resistance of macrolide antibiotics in MP. They were involved in the conception, design, and drafting of the manuscript. Lijun Zhang (Second Author) and Xiaona Qian (Third Author) played significant roles in acquiring and analyzing relevant literature and data, contributing valuable insights to the review. Zhihui Yan (Corresponding Author) and Liyuan Tian (Co-Corresponding Author) served as corresponding authors, making equal contributions by providing critical revisions and final approval of the manuscript. They also offered guidance throughout the research process, ensuring the accuracy and coherence of the content presented in the article.

SUMMARY

- Review focus: *Mycoplasma pneumoniae* (MP) infection and macrolide-resistant *Mycoplasma pneumoniae* (MRMP) pneumonia.
- Objective: Guide early diagnosis and timely treatment amid challenges of MP's resistance to β-Lactam and vancomycin drugs.
- Examination: Current status of macrolide antibiotics, their mechanisms, and emergence of MRMP due to widespread and inappropriate use.
- Method: Identifying MRMP and assessing resistance mechanisms
- Highlight: Global increase in MRMP cases limiting treatment options for pediatric patients. • Resistance mechanism: MP's strategies against macrolides, emphasizing challenges of misuse.
- Conclusion: Emphasizes need for deeper understanding, judicious macrolide use, research for alternative treatments, enhanced surveillance, accurate testing methods, and updated clinical guidelines for effective management, especially in pediatric patients.

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