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Review Article

A Review- Molecular Mechanisms of Azithromycin Resistance in *Neisseria gonorrhoeae*: Current Evidence and Future Therapeutic Strategies

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ABSTRACT

Neisseria gonorrhoeae has become one of the most concerning bacterial pathogens due to its growing resistance to antimicrobial drugs, which is a major challenge for global sexual health and the management of gonorrhea. The gradual decrease in susceptibility to azithromycin, along with the appearance of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, has greatly reduced the effectiveness of previously recommended treatments. This review provides an overview of current knowledge regarding the molecular mechanisms behind azithromycin resistance, recent progress in genomic surveillance, and emerging therapeutic strategies aimed at combating resistant gonococcal infections. Azithromycin resistance is caused by multiple genetic changes that lower the drug's effectiveness. Important factors include mutations in domain V of the 23S rRNA gene, such as A2059G and C2611T, which disrupt the binding of macrolides to the bacterial ribosome. Changes in the rplD and rplV genes, which code for ribosomal proteins L4 and L22, also contribute to resistance by altering the structure of the 50S ribosomal subunit. Another key mechanism involves the overexpression of the MtrCDE multidrug efflux pump, which can result from mutations in the mtrR gene, changes in the promoter region, or the acquisition of mosaic mtr loci via horizontal gene transfer. Additionally, the spread of resistant strains is facilitated by adaptive evolution, genetic recombination, and clonal expansion of successful international lineages. Advances in whole-genome sequencing, comparative genomics, and genome-wide association studies have improved the identification of resistance factors, transmission routes, and molecular epidemiology of N. gonorrhoeae. At the same time, the reduced effectiveness of azithromycin has driven the development of alternative treatments, including new antibiotics like zoliflodacin and gepotidacin, efflux pump inhibitors, optimized combination therapies, and vaccine-based prevention strategies. A thorough understanding of the molecular basis and evolutionary dynamics

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of azithromycin resistance is vital for strengthening antimicrobial stewardship, improving surveillance, and guiding the development of effective next-generation treatments and prevention strategies against gonococcal infections.

INTRODUCTION

Neisseria gonorrhoeae, the bacteria that causes gonorrhea, is one of the most common sexually transmitted infections globally and continues to pose a serious threat to public health. The World Health Organization reports that millions of new cases of gonorrhea occur each year, especially in countries with lower and middle incomes. If left untreated, gonorrhea can lead to serious health problems, including pelvic inflammatory disease, ectopic pregnancy, infertility, chronic pain, epididymitis, and an increased risk of contracting HIV. Managing gonorrhea has become more challenging because the bacteria can quickly develop and spread resistance to antibiotics, making many treatments less effective. In the past, gonorrhea was effectively treated with antibiotics such as sulfonamides, penicillins, tetracyclines, and fluoroquinolones. [30]

However, the rise of resistance to these drugs has led to changes in treatment guidelines. As a result, azithromycin, a broad-spectrum antibiotic with good absorption and activity inside cells, became an important part of dual therapy, often used along with extended-spectrum cephalosporins like ceftriaxone. This approach was intended to improve treatment success and prevent the development of resistance to cephalosporins. However, reports of azithromycin-resistant strains of N. gonorrhoeae appearing in many parts of the world have raised concerns about the long-term effectiveness of current treatments. The resistance to azithromycin in N. gonorrhoeae involves several complex and interconnected factors. One well-known cause is mutations in the 23S rRNA gene, particularly at positions A2059G and C2611T, which change the way the drug binds to the bacterial ribosome and reduce its effectiveness. Other factors include mutations in genes that code for ribosomal proteins L4 and L22, which affect the structure of the ribosome. Additionally, the overexpression of the MtrCDE efflux pump, often due to mutations in the mtrR gene or its promoter, allows the bacteria to remove azithromycin and other drugs more efficiently. Recent studies have also found that N. gonorrhoeae can acquire resistance genes through horizontal gene transfer from other Neisseria species, showing how genetic exchange plays a role in the development of resistance. Advances in technologies like whole-genome sequencing, comparative genomics, and genome-wide association studies have significantly improved our understanding of the genetic factors that contribute to azithromycin resistance. These tools have helped identify new mutations linked to resistance, track the spread of resistant strains, and explain how resistance evolves and spreads globally. The emergence of strains that are resistant to both azithromycin and cephalosporins has raised major concerns about the possibility of gonorrhea becoming untreatable. To address the growing threat of antimicrobial resistance, researchers are exploring various new strategies. Some promising antibiotics, such as zoliflodacin and gepotidacin, have shown effectiveness against resistant strains. Other approaches under study include drugs that block efflux pumps, antimicrobial adjuvants, optimized combination therapies, phage-based treatments, and vaccine development. These innovations may offer important solutions for maintaining treatment effectiveness and controlling the spread of resistant strains.

Because azithromycin resistance is becoming more common and the resistance mechanisms are increasingly complex, it is crucial to understand the molecular factors involved. This understanding is key for monitoring resistance, making informed treatment decisions, and developing new drugs. This review aims to evaluate current evidence on the molecular basis of azithromycin resistance in N. gonorrhoeae, summarize recent findings from genomic and epidemiological studies, and discuss new treatment strategies that may help in the fight against multidrug-resistant gonococcal infections. [7,8,9]

Mechanism of Action of Azithromycin:

Azithromycin is a type of second-generation macrolide antibiotic known as an azalide. It is chemically related to erythromycin, but it has a modified structure that includes a methyl-substituted nitrogen atom in the lactone ring. This change makes the drug more stable in acidic conditions, improves its ability to enter tissues and cells, increases its accumulation inside cells, and enhances its overall effectiveness. These properties help explain why azithromycin is widely used in clinical settings. It has been used over time to treat a variety of bacterial infections, including those caused by Neisseria gonorrhoeae, the bacteria responsible for gonorrhea. The way azithromycin fights bacteria is by stopping them from making proteins.

In bacterial cells, protein production takes place in structures called ribosomes, which consist of two parts: the 30S and 50S subunits. The 50S subunit includes 23S and 5S ribosomal RNA along with several ribosomal proteins that help with forming peptide bonds and extending the growing protein chain. Azithromycin specifically attaches to a part of the 23S rRNA in the 50S subunit, which is part of the tunnel where new proteins exit the ribosome and is crucial for the ribosome to function properly. Once inside the cell, azithromycin binds to specific nucleotides in the area of the 23S rRNA that is responsible for forming peptide bonds. This binding takes place in a region known as the nascent peptide exit tunnel. By occupying this location, azithromycin physically blocks the passage of newly made protein chains. This action interferes with the process by which the ribosome moves the protein-building components through the structure, stopping the extension of the growing chain. As a result, the bacteria cannot make the proteins they need for survival, growth, and other essential functions.

The effectiveness of azithromycin depends heavily on how well it can bind to the macrolide-binding pocket in the 23S rRNA. The binding is stabilized by hydrogen bonds and hydrophobic interactions, which block the ribosome from carrying out protein synthesis. Because making proteins is essential for bacterial growth and replication, this interference significantly reduces the ability of bacteria to multiply. Usually, azithromycin works by stopping bacteria from growing (bacteriostatic), but in some cases, especially when the drug concentration is very high or when bacteria are highly sensitive, it can kill the bacteria (bactericidal). A key factor in the effectiveness of azithromycin is its unique way it moves through the body.

When taken by mouth, azithromycin quickly spreads into tissues and accumulates in various types of cells, including epithelial cells, macrophages, white blood cells, and fibroblasts. The concentration of the drug inside these cells is often much higher than in the bloodstream. This ability to reach and stay in tissues helps the drug get to the sites where infections occur and helps it work over a longer period. Additionally, azithromycin has a long time it takes to be removed from the body, which helps maintain steady drug levels and allows for simpler dosing schedules. The ability of azithromycin to be effective against N. gonorrhoeae depends on its ability to maintain a stable connection with its target, the 23S rRNA, and to reach high enough levels inside the cells. Any changes that affect how it binds to the ribosome or how it accumulates inside cells can greatly reduce its effectiveness. Because of widespread use of macrolides, selective pressure has led to the development of various genetic changes that reduce the drug's effectiveness. These include mutations in the 23S rRNA gene, structural changes in ribosomal proteins, reduced activity of systems that remove drugs from cells, and the transfer of resistance genes between bacteria through genetic exchange. All of these factors contribute to the emergence and spread of strains of Neisseria gonorrhoeae that are resistant to azithromycin. [1,2]

Epidemiology of Azithromycin Resistance in Neisseria gonorrhoeae:

The spread of azithromycin-resistant Neisseria gonorrhoeae across the world is a major challenge for controlling gonorrhea and managing the use of antibiotics. Gonorrhea is still one of the most commonly reported bacterial sexually transmitted infections (STIs), with the World Health Organization (WHO) estimating more than 80 million new cases every year globally. The high health burden, along with the strong ability of N. gonorrhoeae to change its genetic makeup, has allowed antimicrobial resistance (AMR) to evolve and spread quickly, making current treatments less effective. In the past, azithromycin was used together with ceftriaxone in dual therapy regimens because it had a wide range of antimicrobial effects, good absorption in the body, and was effective against other infections like Chlamydia trachomatis. This combination was the main treatment for gonorrhea in many countries for more than a decade. However, since the early 2000s, there has been an increase in azithromycin resistance worldwide, leading to worries about the effectiveness of treatments and the possible development of strains resistant to multiple drugs. Surveillance data from international monitoring programs, such as the WHO Global Gonococcal Antimicrobial Surveillance Programme (GASP), the Gonococcal Isolate Surveillance Project (GISP) in the United States, the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP), and national systems in Australia, Canada, Japan, and China, have shown a steady rise in the number of azithromycin-resistant infections over the past twenty years. Although resistance levels vary between regions, there is a clear trend of decreasing susceptibility across many parts of the world. Early reports of azithromycin resistance usually described bacteria showing only slight resistance.

Over time, strains with moderate and high resistance became more common. Particular concern arises from isolates showing high-level azithromycin resistance (HL-AziR), which is defined by minimum inhibitory concentrations (MICs) of 256 mg/L or more. These strains have been found in many countries, including the UK, the US, Argentina, Canada, China, Australia, and several in Europe. The presence of these highly resistant strains indicates that Neisseria gonorrhoeae continues to evolve under the pressure of antibiotic use and suggests that these strains can spread widely through connected sexual networks.

The variation in resistance rates between regions is linked to differences in how antibiotics are prescribed, how well resistance is monitored, sexual behavior patterns, access to healthcare, and how mobile people are.

Countries where macrolide antibiotics are used a lot have often reported higher rates of azithromycin resistance, showing a strong link between antibiotic use and the development of resistance. International travel and globalization have helped resistant strains move across borders, allowing successful strains to spread quickly around the world. Studies on the genetics of resistant strains have often shown the movement of related resistant clones across countries, highlighting that resistance is a global issue.

The spread of azithromycin resistance is also affected by the genetic flexibility of N. gonorrhoeae. Unlike many other bacteria, N. gonorrhoeae can easily acquire genes for resistance from other bacteria in the human mouth through gene transfer. This process allows the emergence of new types of resistance, which might then spread among sexually active people. Whole-genome studies have shown that azithromycin resistance can arise independently in different lineages and then be transferred locally or globally through successful resistant clones.

Some groups have been more affected by the rise and spread of resistant strains. Men who have sex with men (MSM) are often the group where azithromycin-resistant isolates are commonly found. Factors like having many sexual partners, interconnected sexual networks, not showing symptoms, and using more antibiotics may help resistant strains persist and spread within these communities. Similar trends have been seen in people with repeated gonorrhea infections and in urban areas with high rates of STIs. The pharynx is now understood to be a key environment for the development and spread of antimicrobial resistance in N. gonorrhoeae. Pharyngeal infections are often symptomless and may not be diagnosed for a long time, allowing bacteria to stay in the body and exchange genes with other bacteria found in the mouth. This environment supports gene transfer and the development of resistance mutations, especially those involving the mtr gene areas and other factors related to azithromycin resistance. As a result, pharyngeal infections are seen as important places where resistant strains can develop and spread.

Recent studies using genome data have provided important information on the structure of azithromycin-resistant N. gonorrhoeae and how it spreads. Whole-genome sequencing has helped identify different groups of resistant bacteria and map out how resistant strains spread in local, national, and global settings. These studies show that resistance spreads through both new mutations and the expansion of successful resistant clones. Additionally, genomic monitoring has helped detect new strains that are resistant to both azithromycin and extended-spectrum cephalosporins, raising concerns about the effectiveness of treatment options in the future. The increase in azithromycin resistance has directly influenced recommendations for treating gonorrhea internationally. In response to rising resistance, public health organizations like the Centers for Disease Control and Prevention (CDC) and the World Health Organization have updated treatment guidelines and stopped using the combination of azithromycin and ceftriaxone as standard. Now, treatment usually focuses on using ceftriaxone alone, with improved monitoring of antibiotic resistance to keep treatments effective and prevent further resistance. The continued growth of azithromycin-resistant N. gonorrhoeae highlights the need for global coordination, better diagnosis, responsible use of antibiotics, and the development of new treatments. Ongoing tracking of resistance patterns, along with using genetic data to study how resistant strains spread, will remain essential for understanding how they move, finding new resistant strains, and making informed decisions for the future. [4,7,30]

Molecular Mechanisms of Azithromycin Resistance:

1. Mutations in the 23S rRNA Gene ~

The most extensively studied mechanism involves mutations within domain V of the 23S rRNA gene. Mutations such as A2059G and C2611T reduce azithromycin binding affinity to the ribosome and are strongly associated with resistance. [1,16]

Key effects include:

- Reduced antibiotic binding
- Impaired inhibition of protein synthesis
- High-level resistance when multiple alleles are mutated

Recent genomic analyses have demonstrated that high-level azithromycin-resistant strains frequently multiple copies of mutated 23S rRNA alleles. [1,2]

2. Overexpression of the MtrCDE Efflux Pump ~

The MtrCDE multidrug efflux pump represents another major resistance determinant. [14]

Functions:

- Actively exports azithromycin from bacterial cells
- Reduces intracellular drug concentration
- Enhances bacterial survival

Mutations within the mtrR regulatory gene or promoter region increase expression of the MtrCDE system. Recent WHO reference strain characterization has highlighted mosaic MtrRCDE alleles as emerging contributors to azithromycin resistance. [14,15]

3. Mosaic mtr Loci and Horizontal Gene Transfer ~

Genetic recombination with commensal Neisseria species has generated mosaic resistance determinants. [3,11]

Consequences include:

- Enhanced efflux activity
- Increased minimum inhibitory concentrations
- Accelerated dissemination of resistance

Horizontal gene transfer contributes significantly to the rapid evolution of resistant gonococcal populations. [3,11]

4. Ribosomal Protein Alterations ~

Mutations in ribosomal proteins L4 and L22 can alter ribosomal architecture and reduce macrolide binding. [21]

Although less common than 23S rRNA mutations, these alterations contribute to intermediate resistance phenotypes and may act synergistically with other mechanisms. [10,21]

5. Biofilm Formation ~

Biofilm production enhances bacterial persistence and antimicrobial tolerance. [20]

Mechanisms include:

- Reduced antibiotic penetration
- Altered metabolic activity
- Increased genetic exchange

Biofilms may facilitate the emergence and maintenance of resistant gonococcal populations. [20]

Genomic Evolution and International Spread

The genomic evolution of azithromycin-resistant Neisseria gonorrhoeae is driven by a combination of spontaneous mutations, homologous recombination, horizontal gene transfer, and clonal expansion. Unlike many bacterial pathogens, N.gonorrhoeae exhibits remarkable genetic plasticity, enabling rapid adaptation to antimicrobial selective pressures. This adaptive capacity has facilitated the emergence of resistant lineages and contributed to the global dissemination of azithromycin resistance.

Whole-genome sequencing (WGS) studies have demonstrated that azithromycin resistance evolves through the sequential accumulation of genetic alterations affecting antimicrobial targets and drug transport systems. Mutations within the 23S rRNA gene, particularly A2059G and C2611T, represent major evolutionary events associated with increased resistance. These mutations frequently arise in combination with alterations in ribosomal proteins and overexpression of the MtrCDE efflux pump system, resulting in progressively elevated resistance levels. The coexistence of multiple resistance determinants within a single genome enhances bacterial survival under antimicrobial pressure and promotes the selection of highly resistant strains. A major contributor to gonococcal genomic evolution is horizontal gene transfer. The human pharynx serves as an important ecological niche where N. gonorrhoeae coexists with commensal Neisseria species, facilitating the exchange of genetic material through natural transformation and homologous recombination. This process has led to the emergence of mosaic resistance loci, particularly within the mtr operon, which are associated with enhanced efflux-mediated azithromycin resistance. Such recombination events accelerate the acquisition of novel resistance determinants and increase genetic diversity within gonococcal populations.

Genomic epidemiological investigations have revealed that azithromycin resistance has emerged independently in multiple geographic regions, followed by the international spread of successful resistant clones. Several studies have identified phylogenetically related resistant lineages circulating across North America, Europe, Asia, and Oceania, indicating frequent intercontinental transmission. Increased global travel, migration, and interconnected sexual networks have facilitated the rapid dissemination of these resistant strains across national boundaries.

The application of whole-genome sequencing has significantly improved understanding of transmission dynamics by enabling high-resolution analysis of strain relatedness and evolutionary history. Comparative genomic studies have demonstrated that both de novo mutation events and clonal expansion contribute to resistance dissemination. In many cases, resistant strains possessing favorable fitness characteristics persist and spread within populations despite the potential biological costs associated with resistance acquisition. Compensatory mutations may further enhance bacterial fitness, promoting long-term maintenance of resistant lineages.

The international spread of azithromycin-resistant N. gonorrhoeae has become a major public health concern due to the increasing detection of strains exhibiting resistance to multiple antimicrobial classes. The convergence of azithromycin resistance with reduced susceptibility to extended-spectrum cephalosporins raises concerns regarding the future emergence of extensively drug-resistant gonococcal infections. Consequently, genomic surveillance has become an essential component of global antimicrobial resistance monitoring programs, providing critical insights into resistance evolution, transmission pathways, and the emergence of high-risk clones.

Continued integration of genomic epidemiology with international surveillance initiatives is essential for the early detection of emerging resistant lineages and for informing evidence-based strategies aimed at limiting the global spread of antimicrobial-resistant N. gonorrhoeae. [5,19]

Clinical Implications

1. Impact on Treatment Efficacy:[7,8]

- Reduces the effectiveness of azithromycin-containing treatment regimens.
- Increases the likelihood of microbiological and clinical treatment failure.
- Limits the role of azithromycin as a companion drug in dual therapy.
- Contributes to the emergence of persistent and recurrent gonococcal infections.

2. Consequences for Patient Outcomes:

- Prolonged infection duration due to incomplete bacterial eradication. [7,8]
- Increased risk of transmission to sexual partners. [7,8]
- Higher incidence of complications, including:
- Pelvic inflammatory disease (PID) [7,8]
  - Tubal infertility
  - Ectopic pregnancy
  - Chronic pelvic pain
  - Epididymitis in males
- Enhanced susceptibility to HIV acquisition and transmission. [7,8]

3. Impact on Treatment Guidelines

- Rising resistance rates have prompted revisions of international treatment recommendations. [7,30]
- Discontinuation of routine azithromycin–ceftriaxone dual therapy in several countries. [7,30]
- Increased reliance on ceftriaxone-based monotherapy. [7,8]
- Greater emphasis on evidence-based antimicrobial stewardship. [7,8]

4. Challenges Associated with Multidrug Resistance

- Co-occurrence of azithromycin resistance and reduced cephalosporin susceptibility threatens current treatment options. [19,22]
- Emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) gonococcal strains. [10,19]
- Increased risk of future untreatable gonorrhea. [19,24]

5. Implications for Diagnostic and Surveillance Programs

- Necessitates continuous antimicrobial susceptibility testing. [7,30]
- Supports implementation of molecular diagnostics targeting resistance-associated mutations. [12,13]
- Highlights the importance of whole-genome sequencing (WGS) for: [12]
  - Resistance detection
  - Outbreak investigation
  - Transmission tracking
  - Global surveillance

6. Public Health Implications

- Facilitates dissemination of resistant strains through sexual networks. [18]
- Increases healthcare costs associated with diagnosis, treatment, and follow-up. [7,8]
- Challenges STI control programs worldwide. [7,30]
- Requires strengthened surveillance systems such as:
  - WHO Global Gonococcal Antimicrobial Surveillance Programme (GASP) [30]
  - European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) [30]

Future Therapeutic Strategies~

1. Development of Novel Antimicrobial Agents:

Zoliflodacin: [25]

- First-in-class spiropyrimidinetrione antibiotic. [25]
- Targets bacterial DNA gyrase (GyrB subunit). [25]
- Exhibits potent activity against multidrug-resistant (MDR) and azithromycin-resistant N.gonorrhoeae. [25]
- Demonstrates a mechanism distinct from macrolides and cephalosporins, reducing cross-resistance. [25]
- Currently considered one of the most promising candidates for future gonorrhea treatment. [25]

Gepotidacin: [26]

- Novel triazaacenaphthylene antibacterial agent. [26]
- Inhibits bacterial DNA replication through interaction with DNA gyrase and topoisomerase IV. [26]
- Effective against isolates resistant to azithromycin, fluoroquinolones, and cephalosporins. [26]
- Represents a potential oral treatment option for uncomplicated gonorrhea. [26]

2. Combination Antimicrobial Therapy :~

- Utilization of multiple antibiotics with different mechanisms of action. [25,26]
- Reduces the probability of resistance development during treatment. [25,26]
- May improve bacterial eradication rates. [25,26]
- Potential combinations include:
  - Ceftriaxone plus novel antimicrobial agents. [25,26]
  - Zoliflodacin-based combination therapies. [25]
  - Gepotidacin-containing regimens. [26]
- Requires continuous evaluation through clinical trials and surveillance studies. [25,26]

3. Efflux Pump Inhibition Strategies :~

- Targeting the MtrCDE multidrug efflux pump system. [14]
- Inhibition of efflux activity may restore azithromycin susceptibility. [14,15]

- Potential to enhance intracellular antibiotic concentrations. [14]
- May be used as adjuvant therapy alongside existing antimicrobials. [14]
- Represents a promising approach for overcoming low- and moderate-level resistance. [14,15]

4. Precision Medicine and Molecular Diagnostics :~

- Rapid molecular detection of resistance-associated mutations. [12,13]
- Identification of:
  - 23S rRNA mutations (A2059G, C2611T) [1,16]
  - mtrR regulatory mutations [15]
  - Mosaic *mtr* alleles [3]
- Supports individualized antimicrobial therapy. [12,13]
- Reduces inappropriate antibiotic use. [12,13]
- Facilitates targeted treatment based on resistance profiles. [12,13]

5. Genomic Surveillance and Resistance Monitoring :~

- Integration of whole-genome sequencing (WGS) into routine surveillance programs. [12,19]
- Early detection of emerging resistant lineages.
- Monitoring of global transmission pathways. [19,24]
- Identification of novel resistance determinants. [23]
- Supports evidence-based treatment guideline updates. [12,19]

6. Vaccine Development :~

- Considered the most sustainable long-term strategy for gonorrhea control. [28]
- Potential benefits include:
  - Prevention of primary infection. [28]
  - Reduction of transmission rates. [28]
  - Decreased antimicrobial consumption. [28]
  - Reduced selective pressure for resistance development. [28]
- Current research focuses on:
  - Outer membrane proteins. [28]
  - Lipooligosaccharide-associated antigens. [28]
  - Conserved surface-exposed proteins. [28]
  - Cross-protective meningococcal vaccine antigens. [28]

7. Alternative Antimicrobial Approaches :~

Antimicrobial Peptides:

- Broad-spectrum activity against gonococcal isolates. [27]
- Reduced likelihood of conventional resistance development. [27]
- Potential use as topical or systemic therapeutics. [27]

Anti-Virulence Therapies:

- Target bacterial colonization and pathogenicity rather than bacterial survival. [27]
- Lower selective pressure for resistance emergence. [27]
- May inhibit adhesion, invasion, or immune evasion mechanisms. [27]

Bacteriophage-Based Approaches:

- Exploitation of phage-derived enzymes and antibacterial proteins. [27]
- Potential alternative for multidrug-resistant infections. [27]
- Requires further investigation for clinical applicability. [27]

8. Antimicrobial Stewardship Programs :~

- Rational prescribing of antibiotics. [7,8]
- Restriction of unnecessary macrolide use. [7,8]
- Optimization of treatment regimens. [7,8]
- Continuous monitoring of resistance trends. [7,30]
- Essential for preserving the efficacy of existing and future antimicrobials. [7,8,30]

Challenges and Research Gaps:

Challenges:

- The quick spread of azithromycin-resistant strains of Neisseria gonorrhoeae around the world.
- A growing number of cases showing high-level resistance to azithromycin, linked to specific mutations in the 23S rRNA gene (A2059G and C2611T). [1,5]
- The complicated relationship between different resistance mechanisms, such as mutations in target sites, overexpression of the MtrCDE efflux pump, and changes in ribosomal proteins.
- The rise of gonococcal strains that are resistant to multiple drugs (MDR) and even more than that (XDR), which leaves few treatment choices.
- The widespread transfer of genetic material between Neisseria species, including commensal ones, making it harder to control resistance.
- There is not enough access to quick and accurate tests for detecting antibiotic resistance in everyday medical settings.
- Many low- and middle-income countries do not have strong systems for tracking antimicrobial resistance.
- There are more reports of treatment failures and reduced effectiveness of the previously recommended combination therapy regimens.
- Despite extensive research over many years, there is still no effective vaccine available for Neisseria gonorrhoeae.
- Resistant strains continue to evolve due to international travel and connected sexual networks.

Research Gaps:

- There is limited knowledge about how high-level azithromycin resistance develops over time.
- There is not enough understanding about how different resistance mutations interact with each other.
- More research is needed to study the costs and compensatory changes that occur in resistant strains.
- The novel genetic factors involved in azithromycin resistance have not been fully identified.
- There is not enough data on how pharyngeal infections act as reservoirs for resistance and spread it.
- Better research is needed to connect genetic markers of resistance with how well treatment works in real patients.
- There is a lack of large-scale studies that combine whole-genome sequencing with tracking disease spread.
- There is not enough evidence on the long-term effectiveness and potential for resistance of new drugs like zoliflodacin and gepotidacin.
- Research on drugs that block efflux pumps as additional treatments is limited.
- There is a need to find and confirm vaccine targets that can provide long-lasting protection.
- There is a demand for fast, easy-to-use tests that can detect resistance-causing mutations in the lab.
- The use of artificial intelligence and genetic tools for predicting resistance and tracking outbreaks is still limited. [14,21]

CONCLUSION

Azithromycin resistance in *Neisseria gonorrhoeae* has become a major issue worldwide. This resistance is caused by several genetic changes, such as mutations in the 23S rRNA gene, increased activity of the MtrCDE efflux pump, changes in ribosomal proteins, and the transfer of resistance genes between bacteria. These developments have led to the quick spread of resistant strains across different countries, making treatments that rely on azithromycin less effective. [16]

The growing number of resistant bacteria has led to major updates in how gonorrhoea is treated and shows the importance of constantly tracking antimicrobial resistance.

New techniques in genomic epidemiology have helped researchers better understand how resistance develops and spreads, offering useful methods to detect dangerous bacterial lineages.

To manage azithromycin-resistant *N.gonorrhoeae* in the future, it will be necessary to improve surveillance systems, promote responsible use of antibiotics, develop faster diagnostic tests, and create new treatments like zoliflodacin and gepotidacin. Ongoing research into resistance mechanisms and the development of vaccines are also key to controlling gonorrhoea effectively in the long term.

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