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Review Article

# The Role of Crucial Genes in the Multiplication Cycle, Immune Evasion, and Development of Novel Treatments in Intracellular Bacteria Chlamydia: A Focus on *Chlamydia trachomatis*

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**Abstract**

**Background:** *Chlamydia* species are known to cause significant health challenges in humans, including respiratory tract infections and sexually transmitted diseases. Among them, *Chlamydia trachomatis* is one of the most prevalent sexually transmitted pathogens worldwide. Although *C. trachomatis* exhibits minimal documented antibiotic resistance, the development of advanced therapeutic strategies, such as peptide-based drugs and vaccines, presents promising avenues for mitigating resistance and enhancing treatment outcomes.

**Methods:** This study investigated the role of key genes involved in *Chlamydia* biology, including *TNT*, *FtsK*, *Euo*, and *ClpX*, through a comprehensive review of scientific literature from reputable databases, such as Google Scholar. Diagnostic methods, such as polymerase chain reaction, ligase chain reaction, and nucleic acid sequence-based amplification, were highlighted for their accuracy in detecting pathogenic factors, such as the type III secretion system.

**Results:** The analysis demonstrated that *Chlamydia* possesses sophisticated immune evasion mechanisms by targeting neutrophils, dendritic cells (DCs), and macrophages. The reproductive cycle of *C. trachomatis* and its genetic components were found to be intricately linked to its pathogenicity. Furthermore, understanding immune evasion strategies and key genes related to the bacterial lifecycle provides valuable insights into disease progression and potential therapeutic targets.

**Conclusion:** The findings of this study highlight the significance of unraveling the complex biology of *Chlamydia* species. A comprehensive understanding of its genetic makeup, immune evasion strategies, and pathogenic mechanisms is essential for developing novel preventive measures, effective therapies, and accurate diagnostic techniques. Future research in these areas is crucial for mitigating the public health impact of *Chlamydia* infections.

**Keywords:** *Chlamydia trachomatis*, Immune evasion, Antibiotic therapy, Virulence gene

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## Introduction

Since its discovery in 1907, *Chlamydia* has been extensively characterized, leading to significant advancements in understanding its biology and pathogenicity. This evolution has led to the development of various infections, including lung infections (e.g., *Chlamydia pneumoniae*), sexually transmitted infections (STIs), and abortions. However, due to the remarkable adaptability of this bacterium, novel genetic variants have emerged over time. While adaptability presents challenges in treatment and control, it also underscores the organism's resilience and biological complexity (1,2). *Chlamydia* exhibits a tropism for various epithelial tissues, leading to diverse clinical manifestations (3). The biphasic developmental cycle of *Chlamydia* involves two distinct forms, namely, elementary bodies (EB) and reticulate bodies (RB). *Chlamydia* relies on eukaryotic host cells for survival and replication (4). The *Chlamydia* genus includes medically significant species, such as *C. trachomatis*, which is a major human pathogen (5). Additionally, other *species* in this

family (*C. abortus*, *C. caviae*, *C. felis*, and *C. psittaci*) cause zoonotic infections (6). *Chlamydia* commonly leads to asymptomatic infections in both men and women. These infections may go undiagnosed and prevent treatment with appropriate medications, resulting in substantial tissue damage (7). *C. trachomatis* can cause various infections and is associated with several health concerns, including epididymitis in men and fertility problems in women (8). Additionally, vertical transmission from the mother to the child can result in severe symptoms, underscoring the importance of early diagnosis and prompt treatment. Routine screening for asymptomatic *Chlamydia* infections is crucial for preventing complications and limiting transmission within high-risk populations (9,10). Other bacterial complications include ectopic pregnancy and pelvic inflammatory disease (1,2). Chronic *Chlamydia* infections can result in irreversible tissue damage and sustained inflammation in affected tissues (11).

The genetic composition of the outer membrane of *C. trachomatis*, which affects various tissues and induces diverse symptoms, divides *C. trachomatis* into distinct serological variants. Serotypes A, B, and C primarily infect ocular epithelial cells, leading to trachoma, a major cause of blindness worldwide (12). Genital infections caused by *C. trachomatis*, primarily associated with serotypes D–K, represent the most common bacterial STIs worldwide (13). These serotypes lead to conjunctivitis, urinary tract infections, and pneumonia in children, as well as urogenital and extragenital infections in adults (14,15). Serotypes L1, L2, and L3 of *C. trachomatis* are responsible for lymphogranuloma venereum, an STI characterized by invasive infection of the lymphatic system (16). A summary of this information is displayed in Figure 1.

Studying *C. trachomatis* through immunology, epidemiology, and bioinformatics facilitates the recognition of important processes, elucidates its infection cycles, and highlights complex host-pathogen interactions. Moreover, *C. trachomatis* exhibits a distinct dependence on specific host species and tissue tropism. This characteristic underscores its role in infertility, host immune responses, and vaccine development against human infections (17,18). In 2024, Poston reviewed recent advancements in vaccine development and protective adjuvants. These findings are critical for enhancing vaccine efficacy, and the proposed strategies hold potential for future research applications (19). Additionally, Chiarelli et al demonstrated that *C. trachomatis* undergoes a complex developmental cycle with three distinct cell types (EB, IB, and RB). Asymmetric division suggests that RBs exhibit stem cell-like properties, generating IBs as a renewable intermediate stage during each replication cycle (20).

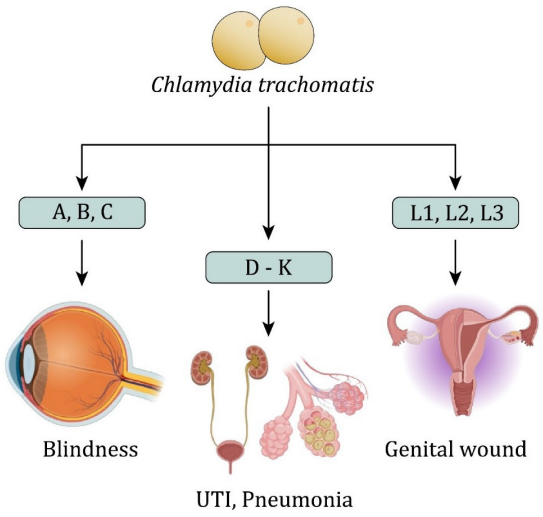


**Figure 1.** *Chlamydia trachomatis* Serovars Associated With Distinct Human Infections. *Note*. UTI: Unitary tract infection. Serovars A, B, and C are etiological agents of trachoma, which can result in visual impairments. In addition, serovars D–K are implicated in urogenital infections, including UTIs and pneumonia. Moreover, serovars L1, L2, and L3 are causative agents of lymphogranuloma venereum, characterized by genital ulceration and lymphatic involvement.

Despite significant advancements in our understanding of the *Chlamydia* life cycle and immune evasion mechanisms, critical gaps persist in the identification of specific targets for therapeutic interventions. Bridging these gaps will facilitate the development of novel diagnostic and therapeutic strategies. This review explores the diverse immune evasion strategies employed by *Chlamydia* spp., the virulence-associated genes contributing to pathogenicity, and the most effective detection and treatment approaches.

## Structure, Cycle of Growth, and Persistent Infection in *Chlamydia*

*Chlamydia* spp. exhibit a distinct two-phase developmental cycle for persistence and propagation, consisting of EB and RB, each fulfilling a specific function. In this cycle, the Euo regulatory protein plays a pivotal role in facilitating the transition to the EB form. The progression of the cycle is regulated by *Euo* expression levels, although the primary formation of RB remains unaffected (20-22). The EB form does not proliferate; rather, it enters host cells approximately eight hours post-infection and rapidly transforms into the RB form. Approximately twenty hours post-infection, RBs replicate via binary fission; subsequently, they transit into EBs, leading to an increased EB population upon host cell lysis (23-25). Given the absence of a homologous FtsZ protein and the lack of peptidoglycan recognition, investigations have been conducted to identify alternative essential proteins; (1) $Bac_{ACT}$, an analogous of bactophilin, plays a significant role in preserving cellular morphology, as its reduced expression may lead to structural alterations in *Chlamydia* (26,27). (2) The *Chlamydia* proteins MreB and Pbp2 are crucial for cell division. Pbp2, a high-molecular-weight penicillin-binding protein, is essential for bacterial cell proliferation, whereas MreB regulates the maintenance of *Chlamydia*'s rod shape. The interaction among MreB, Pbp2, and Ftsk suggests their cooperative role in *Chlamydia* cell division. During bacterial proliferation, Ftsk is required for the proper segregation and formation of RBs and EBs. The findings suggest that

MreB may functionally compensate for the absence of FtsZ during *Chlamydia* cell division (27,28). (3) Among the two key proteins regulating *Chlamydia* survival and developmental cycle, ClpX functions as an adenosine triphosphate-dependent protease (29).

Tunnel nanotubes (TNTs) facilitate the spread of *Chlamydia* from infected cells. Nanotubes are transient cellular structures that enable material exchange and intercellular communication. These TNTs mediate the transfer of RBs from infected to adjacent non-infected cells. TNTs play a crucial role in the dissemination of *Chlamydia*, enabling pathogens to move across distances of up to 50 µm in size. *C. trachomatis* can spread directly from one cell to another through TNTs, even when the extracellular entry into host cells is inhibited (30). TNT-associated proteins play essential roles in RB cell division, highlighting the complexity of the obligate intracellular replication strategy of *Chlamydia* (Figure 2).

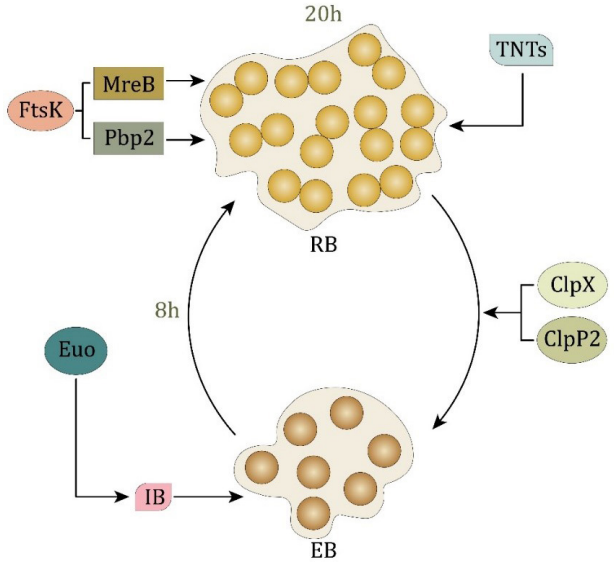


**Figure 2.** The Biphasic Life Cycle of *Chlamydia* Alternating Between EB and RB. *Note*. EB: Elementary body; RB: Reticulate body; TNT: Tunnel nanotubes. EB represents the infectious, metabolically inactive form that transits into the replicative RB form approximately 8 hours post-infection. This transformation is regulated by *Euo* and inhibited by IB. RBs undergo proliferation and are subsequently reconverted into EBs, a process influenced by *FtsK*, *MreB*, and *Pbp2* approximately 20 hours post-infection. Proteins ClpX and ClpP2 facilitate RB maintenance, whereas TNTs contribute to intercellular communication and dissemination

Both *C. pneumoniae* and *C. trachomatis*, which are implicated in chronic chlamydial infections, are associated with high-morbidity diseases, particularly respiratory infections and reactive arthritis (31). A key contributor to chronic chlamydial infections is the metabolic activity of the host, which modulates chlamydial metabolism. This metabolic alteration can lead to persistent infections by inducing specific adaptive changes that enhance bacterial survival and proliferation (32,33). Additionally, the metabolic activity of the bacterium in its EB form contributes to the maintenance of its infectious potential and prolonged persistence in the host, both of which play a role in chronic chlamydial infections (34). Systemic *C. trachomatis* infection has been associated with systemic diseases, including asthma, Alzheimer's disease, and reactive arthritis (35). The role of ClpX and ClpP2 in *Chlamydia* development highlights their potential as therapeutic targets. However, further studies are necessary to elucidate the precise mechanism of action.

## Various Factors in the Increase in *Chlamydia* Pathogenicity

The most important pathogenicity factors of *C. trachomatis* are categorized based on their roles in bacterial survival, reproduction, and interaction with the host (Table 1 and Figure 3). The stages of the life cycle involve essential genes that regulate bacterial adaptation within the host cells. The regulatory elements of gene expression control pathogenicity by modulating important molecular signaling pathways. The type III secretion system is an important virulence determinant that enables bacterial effectors to manipulate host cellular functions, suppress immune responses, and facilitate intracellular survival. Specific virulence factors, including sctQ and copN, contribute to bacterial replication and host cell modulation.

## Diagnosis and Intervention of *Chlamydia* Infection

### *Multiple Methods for the Diagnosis of an Infection Caused by Chlamydia*

#### *Conventional Techniques*

*C. trachomatis* can be diagnosed through conventional diagnostic methods, such as cytological analysis, antigen

**Table 1.** Pathogenicity Factors of *Chlamydia trachomatis* and Their Roles

| Specific Pathogenicity Factors | Details | Reference(s) |
|---|---|---|
| Life cycle stages | A significant portion of C. trachomatis pathogenicity is attributed to essential genes for each life cycle stage. The survival and proliferation of bacteria within host cells depend on these genes. | (36-43) |
| Regulatory elements of gene expression | The pathogenicity of this bacterium is influenced by several factors. Understanding the regulation of gene expression is crucial for understanding molecular processes underlying pathogenicity. | |
| T3SS | The most significant pathogenic factors in C. trachomatis are the secretion system and the variables affecting it. These elements modify the host cell functions to the advantage of the bacterium. Through T3SS, bacteria inject effector proteins into target cells, altering host signaling pathways and inhibiting immune responses. These components are crucial for the pathogenesis of infection. | |
| Specific virulence factors | Certain C. trachomatis factors significantly influence the pathogenicity. These components interact with host cells to promote microbial survival and replication. Various genes affect these classifications and have distinct functions. For example, sctQ genes play distinct roles in T3SS copN | |

*Note*. T3SS: Type III.

detection, cell culture, and serological assays (Figure 4). Nucleic acid amplification tests are the most sensitive, whereas cell culture is the gold standard for specificity (44).

### *Molecular Procedure*

*Chlamydia* is detected, quantified, and identified through molecular diagnostic techniques, such as nucleic acid sequence-based amplification (NASBA), ligase chain reaction (LCR), and polymerase chain reaction (PCR). Compared to conventional PCR, real-time (RT)-PCR offers enhanced sensitivity in detecting genetic variations and is widely used for pathogen identification (45). These molecular techniques (Figure 4) are critical for the sensitive and accurate identification of *Chlamydia* infections. Advanced methods, such as PCR, LCR, NASBA, and RT-PCR, enable medical practitioners to identify and diagnose *Chlamydia* infections with high specificity and accuracy. Physicians can accurately detect *Chlamydia* infections using molecular diagnostic techniques, thereby facilitating early disease management and treatment. Moreover, the most accurate and advised technique for identifying *C. trachomatis* infections is the nucleic acid amplification test, particularly when the infection affects the lower genital tract, anus, throat, or ocular epithelium (46). High accuracy, sensitivity, and stability were achieved in the concurrent identification of three animal *Chlamydia* species, including *C. psittaci*, *C. abortus*, and *C. pecorum*, via multiplex RT TaqMan-MGB PCR processes (47).

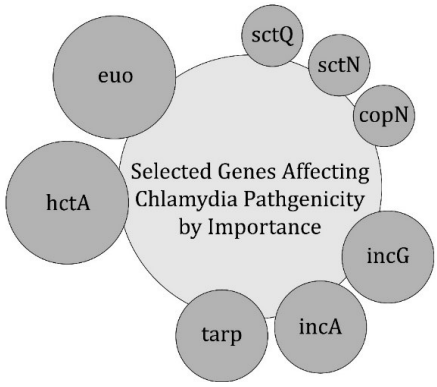


**Figure 3.** Selected Genes Influencing the Pathogenicity of Chlamydia trachomatis, Categorized According to Their Relative Importance. *Note*. T3SS: Type III secretion system; *C. trachomatis*: *Chlamydia trachomatis*. Genes such as *euo* and *hctA* regulate bacterial development and the transition between different stages of the life cycle. Tarp plays a crucial role in host cell invasion. Sct, sctN, and copN are components of the T3SS, which facilitates bacterial manipulation of host cell functions. The inclusion of membrane proteins (IncA and IncG) contributes to the intracellular survival and evasion of the immune system. This schematic diagram illustrates the key molecular determinants of *C. trachomatis* pathogenesis

### *Urine Testing for Identifying Chlamydia Infections*

Several studies have reported the effectiveness of urine tests in detecting *C. trachomatis* infections (Figure 4). With an overall sensitivity of 94.6% and a relative accuracy of > 99.9%, the *Chlamydia* rapid test showed high accuracy in detecting bacteria in urine samples, yielding a total accuracy of 97.9%. These findings indicate that this technique offers a rapid and accurate approach to diagnosing sexually transmitted *Chlamydia* infections (48). Similarly, reactive arthritis (ReA) associated with *C. trachomatis* has been reliably detected in its early stages through PCR analysis of urine samples using specific primers; *C. trachomatis* DNA was identified in 36% of ReA patients (49).

## ***Expansion of Effective Drug Therapies and Novel Treatment Approaches***

This overview presented conventional, alternative, and novel therapeutic strategies for *C. trachomatis* infection (Table 1). Conventional antibiotics, such as doxycycline and azithromycin, primarily target bacterial proteins or DNA synthesis and are approved for clinical use. Alternative therapies, including plant extracts and host defense peptides, are being investigated for their antimicrobial properties. Novel therapeutic approaches, such as spider venom and ezrin-derived peptides, are currently in the research phase and have demonstrated potential for innovative treatment strategies. Table 2 summarizes the mechanisms of action, current status, and references for each category, providing insights into both the established and emerging treatment options.

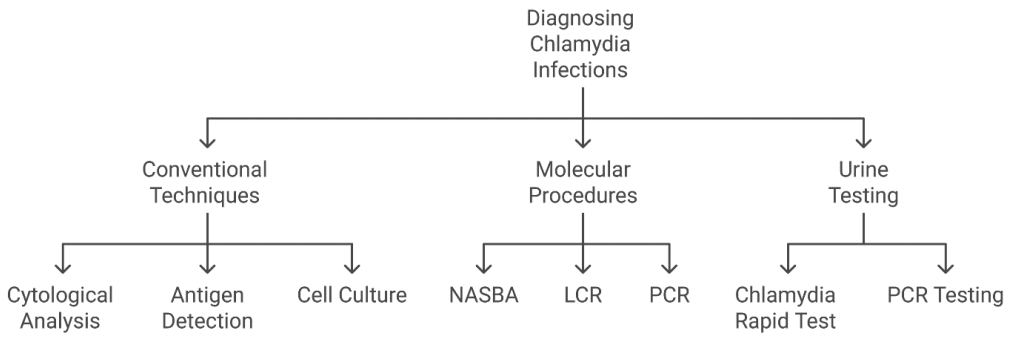


**Figure 4.** Overview of Diagnostic Techniques for *Chlamydia trachomatis* Infection. *Note*. PCR: Polymerase chain reaction; LCR: Ligase chain reaction; NASBA: Nucleic acid sequence-based amplification. These methods are categorized into three primary groups, including conventional techniques (e.g., cytological analysis, antigen detection, and cell culture, which have been traditionally employed but frequently demonstrate lower sensitivity), molecular procedures (e.g., PCR, LCR, and NASBA, which offer high sensitivity and specificity through the detection of nucleic acids), and urine testing (comprising PCR-based methods and rapid tests, providing non-invasive, efficient, and user-friendly diagnostic options).

**Table 2.** Conventional, Alternative, and Novel Therapeutic Strategies for *Chlamydia trachomatis* Infection

| Category | Medication/Agent | Mechanism of Action | Current Status | Reference(s) |
|---|---|---|---|---|
| Conventional drugs | Doxycycline | Inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit. | Approved for use | (50) |
| | Azithromycin | Binds to the 50S ribosomal subunit, preventing bacterial protein synthesis. | Approved for use | (51) |
| | Ofloxacin | Inhibits bacterial DNA gyrase, interfering with DNA replication. | Approved for use | (52) |
| | Erythromycin | Inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. | Approved for use | (50) |
| | Trimethoprim-sulfamethoxazole | Inhibits bacterial folic acid synthesis, essential for DNA and RNA production. | Approved for use | (50) |
| Alternative therapies | Plant extracts | Recent studies have identified bisabolane sesquiterpenes isolated from Ligularia narynensis, which demonstrated anti-chlamydial activity against C. abortus in host cells, suggesting that specific plant-derived compounds may serve as potential therapeutic candidates. A comprehensive study assessed the antimicrobial properties of various plant extracts against C. trachomatis and identified Artemisia inculta as a potent inhibitor of Chlamydia infection in vitro. These findings highlight its potential as a promising candidate for alternative therapeutic strategies. | Experimental | (53,54) |
| | Host defense peptides | Mimics natural peptides such as LL-37, disrupting bacterial membranes and modulating immune responses. | Experimental | |
| Novel therapies | Spider venom peptide | Lycotoxin-Pa2a has antimicrobial properties and reduces inflammation; it has the potential for gene therapy. | In the research phase | (55) |
| | (HDPs | HDPs are capable of compromising the integrity of Chlamydia EB, thereby preventing their attachment and entry into host cells. Research indicates that peptides such as LL-37 and melittin possess potent anti-chlamydial properties, significantly lowering infection rates. Considering the constraints of existing antibiotic treatments, especially with the rise of resistance, HDPs offer a promising strategy for developing novel therapies against Chlamydia infections. | In the research phase | (56,57) |
| | Ezrin peptides (human ezrin peptide one: HEP-1) | Ezrin-derived peptides, particularly HEP-1, have been recognized as promising therapeutic strategies for managing Chlamydia infections. HEP-1, a 14-amino acid peptide, enhances adaptive immunity and has demonstrated effectiveness against various STIs, including Chlamydia, in clinical applications in Russia since 2001. Its mechanism of action involves stimulating immune responses while downregulating pro-inflammatory cytokines such as IL-6, which play a critical role in infection pathogenesis. | In the research phase | (58) |
| Limitations(s) | Gene and peptide therapy | These therapeutic strategies exhibit potential; however, their clinical translation necessitates rigorous validation of efficacy and safety. Moreover, gene therapy delivery remains challenging due to limitations in cellular uptake and the stability of therapeutic molecules. | - | (59,60) |
| | Plant extracts | The safety profiles of numerous plant-derived compounds remain inadequately characterized, with some demonstrating potential cytotoxicity even at therapeutically relevant concentrations. Furthermore, the lack of well-controlled clinical trials assessing the efficacy of these botanical extracts against Chlamydia trachomatis significantly hinders their integration into mainstream medical practice. | - | (61,62) |

*Note*. EB: Elementary body; IL: Interleukin; HDPs: Host protection peptides; STIs: sexually transmitted infections.

### *Challenges in Developing Gene- and Peptide-Based Treatments for Chlamydia*

Several key challenges exist in the development of peptide and gene therapy for *Chlamydia*, including accurate antigen recognition and genetic constraints.

1. Accurate antigen recognition: Bioinformatic studies have identified unique antigen targets in the outer membrane proteins of *C. trachomatis*, suggesting a potential candidate for T-cell activation in *Chlamydia* treatment. These findings also provide insights into novel vaccine strategies aimed at preventing *Chlamydia* transmission (63).
2. Genetic constraints: *C. trachomatis* is challenging to genetically manipulate due to its complex and obligatory intracellular life cycle. Although significant progress has been made, including the use of suicide vectors and targeted mutagenesis techniques, several obstacles remain to be addressed (64,65).

### *The Increasing Threat of Drug Resistance to Chlamydia trachomatis Treatment*

Although antibiotic resistance in *Chlamydia* has been reported, it does not appear to be widespread. Resistance to azithromycin in *C. trachomatis* has been rarely reported, although it has been observed in laboratory settings under specific conditions (66). Resistance to tetracyclines and macrolides remains uncommon; however, heterotypic resistance has been observed in certain cases, occurring under specific conditions and high bacterial loads (67). Growing concerns have emerged regarding treatment efficacy, as increasing antibiotic resistance to *Mycoplasma hominis* and *C. trachomatis* is increasing (68). In *C. trachomatis*, efflux pumps significantly contribute to antibiotic resistance by actively expelling antibiotics, thereby reducing their intracellular concentration and efficacy. Studies have identified compounds, such as selenocompounds, that inhibit these efflux pumps and

increase antibiotic efficacy against *C. trachomatis* (69). Furthermore, certain isoflavones, notably biochanin A, have been shown to inhibit efflux pumps in *C. trachomatis*, thereby enhancing bacterial susceptibility to antimicrobial agents. This highlights the potential of efflux pump inhibitors as adjunctive treatments for resistance (70). Bacterial antibiotic resistance is largely mediated by the multiple transferable resistance (Mtr) efflux system. This system, designated *MtrCDE*, comprises three essential components. (1) MtrC is a periplasmic membrane fusion protein, and (2) MtrD is an inner membrane transporter. In addition, MtrE is an outer membrane channel. These elements function synergistically to form a complex that actively extrudes deleterious substances, thereby reducing the intracellular concentration of antimicrobial agents and facilitating drug resistance (71). Fluoroquinolone resistance typically involves mutations in *gyrA*, while macrolide resistance, particularly to azithromycin, is associated with mutations in the *23S rRNA* gene. Additionally, mutations in *rpoB*, such as H471Y, are linked to rifampin resistance. Fosfomycin resistance arises due to modifications in the *murA* gene, altering the antibiotic's binding site (72,73). The integration of exogenous genomic elements, such as the *tet* gene, into bacterial chromosomes is strongly linked to antibiotic resistance. This phenomenon has been observed in related species, such as *Chlamydia suis*, and can be transmitted to *C. trachomatis* through horizontal gene transfer (74). The spread of antibiotic resistance has been exacerbated by the excessive and inappropriate use of these agents in both clinical and agricultural settings, coupled with inadequate diagnostic methodologies. Furthermore, the practice of prophylactically administering antibiotics to asymptomatic individuals who have been in contact with infected persons has contributed to unnecessary exposure to these antimicrobial compounds. Implement targeted prescription protocols and "assess-then-postpone" strategies to minimize unnecessary antibiotic administration (75). The emergence of antibiotic resistance in STIs, including *Chlamydia*, may lead to increased healthcare costs and a higher disease burden, particularly in disadvantaged communities (76).

### *Future Prospects for Developing a Vaccine Against Chlamydia Infection*

Recent advancements in *C. trachomatis* vaccine development have been observed. Table 3 elucidates the key research areas, including the successful completion of the first clinical trial, advancements in multi-epitope immunization strategies, and insights into immune response optimization. Animal model studies have demonstrated promising results, particularly for *Chlamydia* protease-like activity factor (CPAF)-targeted vaccines in porcine models. Furthermore, research on attenuated live vaccines suggests their potential as a viable strategy for long-term protection against *Chlamydia* infection. These findings contribute to ongoing efforts to develop an efficacious and durable *Chlamydia* vaccine.

## Immune System Avoidance of *Chlamydia*

### *Evasion From Neutrophils and Dendritic Cells*

Neutrophils and dendritic cells (DCs) are critical components of the early immune response that employ phagocytosis and antigen presentation to combat

**Table 3.** Vaccine Development Against *Chlamydia* Infection

| Research Area | Key Findings | Reference(s) |
|---|---|---|
| First clinical trial | The Phase 1 clinical trial of the CTH522 Chlamydia vaccine focused on investigating its safety and ability to trigger an immune response in humans. This randomized, double-blind, and placebo-controlled study evaluated the vaccine with two distinct adjuvants (CAF01 liposomes and aluminum hydroxide). The findings indicated that both formulations were well-tolerated and exhibited no significant safety concerns. However, the CAF01-adjuvanted vaccine demonstrated a stronger immunogenic profile compared to the aluminum hydroxide formulation. These results highlight the potential of the CTH522:CAF01 combination for further clinical advancement. The trial primarily assessed safety, while the secondary focus was on humoral immunity, measured by anti-CTH522 IgG seroconversion. The vaccine elicited a strong immune response, significantly increasing IgG levels. | (77,78) |
| Multi-epitope immunization | The exploration of multi-epitope vaccines, incorporating a diverse range of antigens from human papillomavirus, Chlamydia trachomatis, and herpes simplex, offers a novel approach to enhance both humoral and cellular immune responses, particularly for sexually transmitted diseases. | (79) |
| Immune response optimization | Immune response analysis indicates that effective vaccination requires the activation of a balanced Th1 and Th2 immune response. These findings suggest a pathway for the development of safer and more effective Chlamydia vaccines, aligning immune system activation with vaccine efficacy. | (80) |
| Animal model research | Animal model studies, particularly in porcine models, have revealed that vaccines targeting the CPAF protein, when combined with the TriAdj adjuvant, can induce robust immune responses, potentially accelerating the transition of vaccine candidates from preclinical to clinical stages. | (81) |
| Attenuated live vaccines | A genetically modified strain of Chlamydia muridarum has demonstrated enhanced protective capabilities against genital Chlamydia challenges, with a reduced ability to infect the genital tract, suggesting that live attenuated vaccines may serve as an effective strategy for preventing Chlamydia infections. | (82) |
| Vaccine efficacy and challenges | The initial trials of the CTH522 vaccine candidate have been completed, confirming its safety and immunogenicity in Phase I/II studies. However, further research is required to evaluate its efficacy in infection prevention. Preclinical studies on multi-epitope vaccines, designed using computational immunology approaches, have shown promise in eliciting strong immune responses in animal models. Live-attenuated vaccines demonstrate significant potential; nonetheless, they encounter substantial regulatory hurdles for approval. | (83-86) |

*Note*. IgG: Immunoglobulin G; Th: T-helper; CPAF: *Chlamydia* protease-like activity factor.

pathogens. However, *C. trachomatis* uses multiple mechanisms to evade these defense systems. The bacterium produces nucleases and proteases to degrade neutrophil extracellular traps and secretes CPAF to inhibit FPR2-mediated neutrophil recruitment (87-89). Furthermore, *C. trachomatis* modifies Rab proteins in DCs, which disturbs effective antigen presentation via major histocompatibility complex (MHC)-I and MHC-II pathways. Certain strains induce apoptosis in both neutrophils and DCs through the caspase 3/7 pathway, leading to impaired immune signaling and clearance (90-92).

### *Manipulation of Mast Cells, Eosinophils, and Natural Killer Cells*

Mast cells and eosinophils play critical roles in allergic and inflammatory responses. *C. trachomatis* infection induces an excessive interleukin (IL)-4 response, resulting in host tissue damage and immune dysregulation (93-95). Further, the bacterium alters NK cell function by modulating MHC-I expression and evading NKG2D receptor-mediated cytotoxicity, allowing immune evasion (96,97).

### *Immune Evasion Strategies of Chlamydia in Macrophages*

MQs are crucial for intracellular pathogen clearance through cytokine signaling and nitric oxide (NO) production. *C. trachomatis* modulates MQ metabolic pathways by inducing a shift toward glycolysis and regulating NO synthesis via inducible nitric oxide synthase and signal transducer and activator of transcription 1 pathways (98,99). Moreover, the bacterium promotes pyroptosis through gasdermin activation, leading to the excessive release of IL-1β and IL-18, which may contribute to persistent infection (100,101) (Figure 5).

## Conclusion

*Chlamydia*, particularly *C. trachomatis*, is one of the most prevalent sexually transmitted bacterial pathogens. It primarily targets the epithelial cells of the human reproductive tract. Concerns associated with chlamydial infections include pelvic inflammatory disease, infertility, and trachoma. The public health and individual consequences of these complications are significant.

Therefore, effective strategies for the prevention and management of chlamydial infections are crucial. This study investigated the defense mechanisms employed by *C. trachomatis* to evade the human immune system and its replication cycle. Furthermore, this study explored the potential of novel antibiotic- and peptide-based therapies to address the emerging issue of antibiotic resistance in *C. trachomatis*. By understanding the intricate mechanisms of chlamydial pathogenesis, this study attempted to facilitate the development of more effective treatment options. This study also discussed how preventive medicine measures could help interrupt transmission dynamics to eradicate this infection. To develop more sophisticated treatments for *Chlamydia* infections, further research is needed on the development of vaccines targeting key virulence factors. This study underscores the importance of targeting virulence factors such as TNTs in developing next-generation therapies. Future research should focus on translating these findings into clinical implications.


### Authors' Contribution

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**Resources:** Mohammad Ebrahim Karimbakhsh.


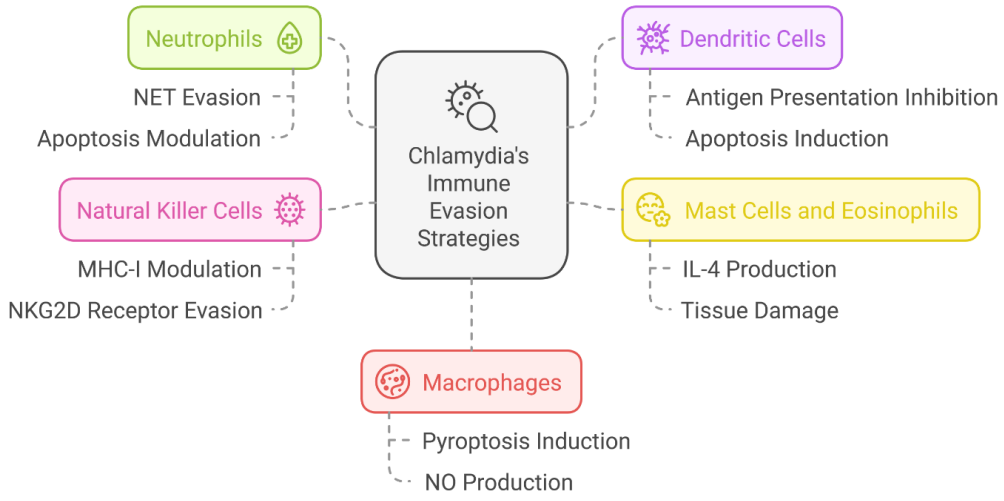


**Figure 5.** *Chlamydia trachomatis* Employing Diverse Immune Evasion Strategies to Establish Persistent Infection. *Note*. NET: Neutrophil extracellular trap; NK: Natural killer; NO: Nitric oxide; MHC: Major histocompatibility complex; IL: Interleukin; *C. trachomatis*: *Chlamydia trachomatis*. These mechanisms involve the modulation of key immune cells: (1) neutrophils, by evading NETs and modulating apoptosis, (2) dendritic cells, by inhibiting antigen presentation and inducing apoptosis, (3) NK cells, by modulating MHC-I expression and evading NKG2D receptor-mediated recognition, (4) macrophages, by inducing pyroptosis and regulating NO production, and (5) mast cells and eosinophils, by stimulating IL-4 production and contributing to tissue damage. These strategies enable *C. trachomatis* to evade immune detection and establish chronic infections

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## Competing Interests

The authors declare that they have no conflict of interests.

## Ethical Approval

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

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