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How reliable is chlamydia trachomatis screening?

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HOW RELIABLE IS CHLAMYDIA TRACHOMATIS SCREENING?

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Assinatura do Estudante
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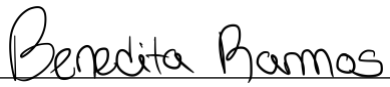

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Assinatura do Coorientador
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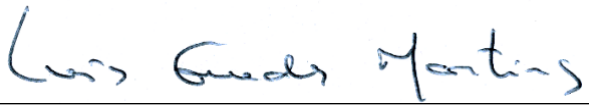

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Resumo

Enquadramento: A infeção por *chlamydia trachomatis* (*CT*) é a infeção bacteriana sexualmente transmissível mais prevalente no mundo, com 131 milhões de novos casos por ano. Pode infetar o epitélio geniturinário sendo maioritariamente assintomática. No entanto, pode levar a complicações graves a longo prazo, principalmente em mulheres. Os métodos de deteção da infeção evoluíram e os Testes de Amplificação de Ácido Nucleico (TAANs), tornaram-se o teste de escolha, principalmente em países desenvolvidos. Com o aumento da consciencialização acerca do impacto da infeção por *CT* na saúde pública, vários países desenvolveram estratégias de controle específicas, incluindo o rastreio sistemático.

Objetivo: O objetivo deste artigo foi realizar uma revisão abrangente e atualizada das características clínicas da infeção por *chlamydia trachomatis*, discutir a viabilidade do rastreio e apresentar o estado de arte dos programas de rastreio de *CT* em Portugal.

Métodos: Foi realizada uma revisão da literatura utilizando a base de dados PubMed - foram aplicados os termos Mesh < chlamydia infection AND screening>, < chlamydia infection AND mass screening>, bem como subtítulos relevantes e palavras-chave relacionadas com <chlamydia infection>: (epidemiology, microbiology, physiopathology, diagnosis, complications, vaccine, treatment) - foram recuperados 665 artigos publicados de 2012 a 2022. Destes, 76 artigos foram escolhidos devido à sua relevância e caráter inovador. Por meio de pesquisa cruzada e consulta de diretrizes de diferentes países e organizações internacionais sobre a gestão da doença 15 artigos foram adicionados; no total, 92 artigos foram incluídos.

Conclusão: A infeção sintomática por *CT* ocorre em cerca de 20% dos casos; a longo prazo, 17% das infeções complicam com doença inflamatória pélvica (DIP). A DIP pode levar a dor pélvica crónica (36%), infertilidade por fator tubário (0,5-3%) ou gravidez ectópica (7%). Os TAANs são os testes mais específicos e sensíveis disponíveis e devem ser o teste de escolha para deteção da infeção, embora em contextos com recursos limitados seja necessário investir em testes sensíveis, fáceis de utilizar e acessíveis. Até à data, não existem provas consistentes de que o rastreio reduz a incidência da infeção por *clamídia* ou de complicações graves relacionadas. Em Portugal, não existe atualmente nenhuma estratégia organizada para gerir a infeção por *CT*, e a taxa de notificação é extremamente baixa. A

implementação de um rastreio oportunístico de infeção genital a grupos específicos bem como de uma orientação para a gestão dos casos de *clamídia* são uma necessidade urgente, considerando que a redução da morbilidade associada à doença é um objetivo crucial.

Palavras-chave: Chlamydia trachomatis; infeção por clamídia; rastreio; gestão da doença; epidemiologia; diagnóstico; tratamento; vacina; doença inflamatória pélvica; infertilidade; Infeções sexualmente transmissíveis.

Abstract:

Background: *Chlamydia trachomatis (CT)* infection is the most prevalent bacterial sexually transmitted infection worldwide, with 131 million new cases each year. It can infect the genitourinary tract, being usually asymptomatic. However, severe complications can occur mostly in women. Methods of diagnosis have evolved, and Nucleic Acid Amplification Tests (NAATS) have become the test of choice, mainly in high-income countries. With increased awareness of the impact of *CT* infection on public health, several countries had developed specific control strategies, including systematic screening.

Objective: Our aim was to make a comprehensive and updated review of the clinical characteristics of *chlamydia trachomatis* infection, discuss the reliability of its screening, and present the current status of *CT* screening programs in Portugal.

Methods: A literature review was conducted using the PubMed database - Mesh Terms applied were < chlamydia infection AND screening>, < chlamydia infection AND mass screening> as well as relevant subheadings and keywords related to <chlamydia infection>: (epidemiology, microbiology, physiopathology, diagnosis, complications, vaccine, treatment) -665 articles published from 2012-2022 were retrieved. From these, 76 articles were chosen due to their relevance and innovative character. By cross-research and consultation of guidelines from different countries and international organisations regarding disease management, 15 more articles were added; in total, 92 articles were included.

Conclusion: Symptomatic *CT* infection occurs in nearly 20% of patients; if left untreated, complications such as pelvic inflammatory disease (PID) occur in about 17% of cases. PID may lead to chronic pelvic pain (36%), tubal factor infertility (0,5-3%) or ectopic pregnancy (7%). NAATs are the most specific and sensitive tests available and should be the test of choice for detecting infection, although in limited-resource settings, more sensitive, user-friendly, and affordable tests need to be considered. Currently, there is no strong evidence that screening reduces the incidence of *CT* infection or its complications. In Portugal, there is no current organised strategy addressing *CT* infection, and the notification rate is extremely low. Opportunistic testing for specific patient groups and chlamydia case management guidelines are an urgent need, considering that reducing the burden of disease is a crucial goal.

Keywords: Chlamydia trachomatis; chlamydia infection; screening; disease management; epidemiology; diagnosis; treatment; vaccine; pelvic inflammatory disease; infertility; Sexually transmitted infections.

Abbreviations

ACCEPt - Australian Chlamydia Control Effectiveness Pilot
AMR - Antimicrobial Resistance
CE - Conformité Européenne
CPAF - Chlamydial Protease-Like Activating Factor
CPP - Chronic Pelvic Pain
CT - Chlamydia Trachomatis
EB - Extracellular Elementary Bodies
ELISA - Enzyme-Linked Immunosorbent Assay
EP - Ectopic Pregnancy
FDA - Food and Drug Administration
HLA - Human Leukocyte Antigen
HIV - Human Immunodeficiency Virus
HSP60 - 60-kDa Heat Shock Protein
INCs - Inclusion Membrane Proteins
MOMP - Major Outer Membrane Proteins
mRNA - Messenger RNA
MSM - Men Who Have Sex with Men
Natsal - National Surveys of Sexual Attitudes and Lifestyles
NCSP - National Chlamydia Screening Programme
NG - Neisseria Gonorrhoea
nvCT - New Variants of CT
OMPs - Outer Membrane Proteins
PCR - Polymerase Chain Reaction
PID - Pelvic Inflammatory Disease
PMNs - Polymorphic Nuclear Leukocytes
POC - Point-of-Care
RB - Intracellular Reticulate Bodies
RCTs - Randomised Control Trials
STI - Sexually Transmitted Infection
TFI - Tubal Factor Infertility
T3S - Type III Secretion System
TLR1/2 - Toll-Like Receptor
US - United States
USPSTF - US Preventive Services Task Force
V-PCR - Viability-PCR
WHO - World Health Organisation

Index

List of tables

List of figures

1. Introduction

Chlamydia trachomatis infection is the most prevalent bacterial sexually transmitted infection (STI) worldwide. [1, 2] Its pathogen, *chlamydia trachomatis* (*ct*), belongs to the genus *chlamydia,* whose species are intracellular gram-negative bacteria. Various species of *chlamydia* infect humans and have a tropism for the epithelial tissue that makes up the lining of the genitourinary tract, the conjunctiva and the respiratory tract. [3]

There are approximately 131 million new cases of chlamydia trachomatis infection globally, according to the World Health Organisation (WHO). [2] It was the most reported sexually transmitted infection in the United States (US). [4]. According to the European Sexually Transmitted Infections Surveillance Network from the European Centre for disease control (ECDC), the rate of chlamydia in countries with chlamydia control strategies - active screening or widespread opportunistic testing- is above 200 per 100 000. [5] In Portugal, the crude prevalence is 2.4-2.6 per 100,000 inhabitants. [5, 6] In Lisbon and Porto's urban densely populated metropolitan areas, the prevalence is higher (68-200). [6] Portugal was listed as one of the countries with lower rate of *ct* disease; however, it is to note that these differences across Europe reflect disparities in surveillance data collection, access to sensitive diagnostics, differences in national testing policies and their implementation, rather than actual differences in prevalence. [5]

The population with a higher burden of infection are sexually active young people between the ages of 15-24 years [5, 7], mainly women but also men who have sex with men (MSM). [5, 8] For both men and women, the majority of the cases of infection are asymptomatic – more than 80%[9] - or associated with few symptoms, which translates into a reduced testing and detection rate. Therefore, chlamydia infection often remains unnoticed and untreated, although it is still easily transmitted. [4, 7, 8, 10] In men, when symptomatic, the usual presentation is urethritis (nongonococcal) but can also be epididymitis and, rarely, infertility. [4, 11] In women, long-term untreated chlamydia infection can lead to severe complications. [4, 7, 11, 12] In all cases, the transmission of the human immunodeficiency virus (HIV) is facilitated by this STI.[10]

Intending to prevent severe sequelae, many developed high-income countries have implemented national health guidelines for managing chlamydia infection in asymptomatic sexually active people, in most cases women. [11-13] In Portugal, there is no current organised *chlamydia* screening or control

activities set by the national health service. In 2015, *ct* infection entered the list of mandatory notifiable diseases, meaning that it was considered a disease in need of epidemiologic control; data on infection only started to be registered systematically by this year. [14] The current gold standard diagnostic considerations for *ct* are nucleic acid amplification tests (NAATs), which are highly sensitive and specific [7, 10], for use with samples of vaginal swabs (for women) and first-void urine (for men), generally. [7]

The principle of screening the asymptomatic target population is now being questioned. International chlamydia experts affirmed that it is still unclear what the actual effectiveness of screening for ct is in reducing complications of infection and transmission in sexually active, asymptomatic adolescents and adults. [15] Overdiagnosis and consequent overtreatment are linked to problematic antimicrobial resistance development of other pathogens [15, 16], and there have been more reported cases of false negative results on NAATs testing, weakening the strong claim of its high sensitivity and specificity. [10] Moreover, early detection and treatment of infection can potentially disrupt the partial immunity against reinfections, making patients more susceptible to subsequent infections and potential complications.[17]

There also seems to be a change of paradigm, with more governments analysing their current screening policies and commuting their aim. [15, 16, 18] It is urgent to remind that the most important outcome is the reduction of disease, and not the prevalence of the agent, an aspect that can frequently be misleading[19].

Our aim was to make a comprehensive and updated review of the clinical characteristics of chlamydia trachomatis infection, discuss the reliability of its screening, and provide an insightful view of chlamydia control in Portugal.

2. Methods

A literature review was conducted between 2012 to 2022 using the PubMed database. The languages applied as filters were Portuguese, English, Italian, and Spanish. Mesh Terms were applied using combinations of the more commonly adopted terms, < chlamydia infection AND screening>, < chlamydia infection AND mass screening> as well as relevant subheadings and keywords related to <chlamydia infection> : (epidemiology, microbiology, physiopathology, diagnosis, complications, vaccine, therapy) which resulted in 665 articles on the PubMed database. From these, 76 articles were chosen due to their relevance and innovative character. By cross-research and consultation of guidelines from different countries and international organisations regarding disease management, 15 more articles were added, including a few from before 2012. Overall, 92 articles were included.

3. Microbiology and Pathogenic mechanisms

Chlamydia trachomatis is a gram-negative obligate intracellular bacteria, which means it depends on the relationship with the host's cell to replicate and grow. [20]. Its reduced genome and consequent shortage of metabolic enzymes make it unable to generate metabolites, meaning it utilises the host´s ATP and GTP for reproduction. [20, 21] It has a biphasic life cycle, interchanging between extracellular elementary bodies (EB), ready for the infection of the host's cells, and the intracellular reticulate bodies (RB), responsible for growth and replication within a protective parasitophorous vacuole - the inclusion body. [20, 22, 23]

The elementary body, the differentiated form of the reticulate body, has characteristics that enable it to handle the harsh external environment. [21] Its structure is maintained by a set of outer membrane proteins (omp) that form a matrix. [24] When the EB attaches to another cell's receptor, numerous signalling pathways are activated so that the EB internalises the cell. [22] This happens due to a type III secretion system (T3S) encoded in the *ct's* genome responsible for delivering bacterial effector proteins to the host cell. [25] Examples of these are Tarp and TmeA, which can remodel the cell's actin cytoskeleton, enabling the internalisation of the EB.[22] Internalised, the EB are initially in endocytic vacuoles that unite to form an endocytic inclusion. Here, the EB transforms into the RB, which can reproduce by binary fission using metabolites and nutrients of the cytoplasm. [26] When the inclusion body reaches its maximum capacity, and the available ATP and nutrients become scarce, the RB is converted again into EB. Besides, the RB can transform into a persistent, viable, nonreplicative form in adverse circumstances. [26]

The EB exit from the host cell occurs by one of two mechanisms, the lysis of the host cell or the ejection of the inclusion body, similar to exocytosis. [20, 22] This last is best at protecting EB from immune attacks and controlling the release of inflammatory content. It also allows for the persistence of some bacteria in the host cell, if beneficial. [20] The host cell's lysis is promoted by the chlamydial protease-like activating factor (CPAF) and plasmid-encoded factor PGP4. [23] A graphical example of *ct's* life cycle and infection process is pictured in Figure 1.

The application of recent genetic tools has enabled a more in-depth understanding of *ct's* virulence factors and interactions with the host cell. [23] The Tarp effector protein interferes with the cell's

signalling cascade, regulating its ability to proliferate, survive or suffer apoptosis. [22, 23] Other known virulence factors are the inclusion membrane proteins (Incs), which make part of the membrane of the inclusion bodies. They are responsible for homotypic inclusion fusion, connecting sites between the inclusion and the endoplasmic reticulum and regulating traffic in the host cell's membrane. [23]

Regarding immune response evasion mechanisms, besides the specific pathways mentioned above, *ct* relies on its capacity to paralyse polymorphic nuclear leukocytes (PMNs) at the site of infection. This strong suppression of the immune response might explain the mechanism behind asymptomatic infections. The PMNs are the first line of immune defence the EB encounters when liberated from the host epithelial cells. When the secreted effector protease CPAF meets the PMNs, it blocks the downstream activation of these neutrophils. By preventing this, the EBs evade eradication when moving from one host cell to another. [27] This varied concurrence of adaptations allows chlamydia to defy the host's immune response and endure within the cell. [20, 22, 24, 27]

4. CT and the genital tract disease

Chlamydia trachomatis has three biological variants (biovars), which, in humans, pathologically translates as three significant diseases. [20, 23, 24] Currently, there are 19 known *ct* serological variants (serovars), each corresponding to epitopes of the bacteria's major outer membrane proteins (MOMP). These proteins contain four domains that vary in sequence according to each serovar. Serovars A, B, Ba and C are mainly the aetiological agents of trachoma; serovars D–K are associated with urogenital infections, and serovars L1–L3 with lymphogranuloma venereum. [28] Genital tract disease translates as infection of endocervical epithelia in women and urethral epithelia in women and men [3] and will be the main subject of discussion throughout this review.

Clinically, the major problem of *ct* infection is the damage to the infected epithelial cells. [29] This happens due to a provoked proinflammatory immune response. However, the responsible mechanism and components are still not fully defined. [30] Through animal models of repeated infection, adaptive T-cell response was found to be a key mechanism involved in controlling or eliminating infection. However, it may have a double-edged nature and contribute to tissue damage. [31]

The host's disease expression depends on multiple aspects, such as cytokine profile, host genetic factors, human leukocyte antigen (HLA) subtype, chlamydial antigen, infectious load, and course of infection. [32] According to the cellular hypothesis by Stephens, when the *ct* pathogen infects non-immune epithelial cells, several cytokines, growth factors and chemokines are produced. [30] As an example, Wang et al. demonstrated that specific chlamydial lipoproteins could induce the release of proinflammatory cytokines, such as IL-8, by cell lines that express human Toll-Like Receptor (TLR)1/2, a type of receptor that plays a big part on initiating inflammatory reactions and activating the innate immune system. [30] This induced inflammatory activity activates adaptative immunity - regulatory T cells, Th17 cells, and $CD8^+$ T cells - leading to the further production of proinflammatory mediators. These conditions set the tone for an ongoing inflammatory response, ultimately accountable for chlamydial disease, characterised by chronic inflammatory infiltrates and scarring in the epithelia. During recurrent infection by the same or other serovars, which is common, the adaptive response is potent. It may generate damage significant enough to prompt disease sequelae, which will be further addressed. [3]

One of *ct's* most crucial immune evasion mechanisms is its ability to transform into a persistent state, the aberrant reticulate body, that stops producing structural and membrane components but instead

generates 60-kDa heat shock protein (hsp60) [26], and whose infectivity is re-established once the harmful trigger is gone. [33] Studies have identified *in vitro* triggers for aberrant RBs that probably translate to *in vivo* circumstances, such as nutrient depletion, exposure to IFN- γ, antibiotics, [26, 32, 33] iron deficiency, or coinfection with Herpes Simplex Virus (HSV) . [26, 33] Furthermore, according to studies with animal models, the chronic release of chlamydial hsp60 by persistent RBs is most likely responsible for fallopian tube inflammation and functional impairment. Luminal occlusion and infertility might result from this continuous damage and consequent fibrosis. [26, 32]

During the infectious process, *ct* also faces challenges, such as competing with the urogenital commensal microorganisms for nutrients. [20] In women, the cervicovaginal microbiota protects from chlamydia infection and other STIs. The lactobacillus dominant vaginal microbiota has a characteristic acidic vaginal ph (4.5) due to lactic acid production. Studies have demonstrated the association between many lactobacilli and a diminished risk of infection by sexually transmitted pathogens, *ct* included. Even preexposure of host epithelial cells to lactic acid lowered its vulnerability towards infection by *ct*, as demonstrated in a study by Edwards et al. It was possible to conclude that D(-) lactic acid-producing Lactobacillus spp. can regulate host cell gene expression by interfering with a series of epigenetic mechanisms, ultimately reducing cell cycling and consequent proliferation, thus protecting against chlamydia infection. [34] Regarding urine microbiome, it was proven that women positive for *ct* had less microbial biodiversity than controls. The samples also had fewer microorganisms from mycoplasma species - especially *Ureaplasma parvum*. This pathogen might function as a protective factor for the adherence and colonisation of *ct*, working as a competitor. [35]

There is an ongoing scientific debate about to which extent *ct* is linked to adverse reproductive health outcomes[36] and an evidence gap on high-quality studies regarding this matter. [37]

In women, long-term untreated chlamydia infection is associated with severe complications, such as pelvic inflammatory disease (PID), which is responsible for more severe sequelae such as chronic pelvic pain (CPP), tubal factor infertility (TFI), ectopic pregnancy (EP), salpingitis, and neonatal conjunctivitis/pneumonia due to vertical transmission. [4, 7, 11, 12]

PID is a prevalent condition, with approximately 4.4% of sexually active women reporting it. It's a clinical syndrome based on pelvic or lower abdominal pain and signs of tenderness of the cervix, adnexa, or uterus on physical exam. [38] It is heterogenous in its presentation, severity, and aetiology,

one of the most known being *ct* and *Neisseria gonorrhoea (ng)*. [38] Nonetheless, more than half of women with clinical signs, symptoms and histologically confirmed PID do not have infection with either pathogen.[39] According to a recent clinical trial involving women in the US who experienced symptomatic PID, only 25% of them had one of the STIs. [40] So, while still a significant cause of PID, *ct* infection is not the main one. [41]

A mathematical synthesis of evidence from different studies (2013) estimated that the risk of PID disease following infection with chlamydia was about 16% (95% credible interval 6–25). [42] Similarly, a recent systematic review (2016) stated that 17% of *ct* infections developed into PID (symptomatic or asymptomatic). [41]

Contemporary reports of the prevalence of PID sequelae range from 0,5-3% for TFI, 7% for EP, 7% for salpingitis and 36% for CPP. [38, 41] Regarding the impact of repeat infection, two recent studies found that repeat infection (repeat positive tests) increases the risk of developing PID. [36, 43] Davies et al. quantified it as a 20% increase. This wasn't, however, associated with higher risks of EP or TFI. [43]
Regarding the overall impact of *ct* past infection on female infertility, the most recent systematic review of the subject (2022) highlighted a significant correlation between them. Thirteen out of seventeen studies supported this, and four found no association. [44] This association was also corroborated by a Chinese study conducted in 2022.[45]

There appears to be a timeframe in which treating chlamydia, detected through screening, can cease the damage to the fallopian tubes. These findings are coherent with the abovementioned hypothesis, primarily based on animal studies, that *ct* triggers persistent immune responses that can lead to tubal damage during the infection. Nonetheless, understanding the timing of chlamydia disease progression is still somewhat limited. [46] Particularly the timing of fallopian tube damage, as damage to the tissue may have already occurred between the time of infection acquisition and detection. [15] This could justify why in the Davies et al. and Hoenderboom et al. studies, women with a positive *ct* test were more at risk of complications independent of having received antibiotic treatment. [36, 47]

5. *Detection methods* ***of genitourinary infection***

Different types of samples from different anatomical sites can be used to detect infection by *ct*. In women, diagnosis can be made through endocervical or vaginal swabs or first-void urine; in men, urogenital infection is detected by first-void urine or urethral swab. Rectal and oropharyngeal samples can be collected at the respective exposure sites. [7]

Several laboratory tests can be used to detect *ct* infection. The use of cell culture started in the 1970s and was until recently the "gold standard" technique due to its specificity of practically 100%. [48] Specimens can be collected with a swab from the endocervix (1-2cm in) or the urethra (2-3cm in), in women and men, respectively. Sample storage should be done in a suitable transport media (ex: sucrose phosphate glutamate buffer) at ≤4°C and sent to the laboratory within 24 hours. [49] After centrifuging specimens and placing them in a confluent cell monolayer of McCoy, HeLa 229, or Buffalo green monkey kidney cells, [49, 50] they are examined for characteristic intracytoplasmic inclusions (48-72 hours later) using staining methods such as Giemsa, iodine, or fluorescence-labelled antibodies specific to chlamydial antigens - lipopolysaccharide (LPS) or MOMP. MOMP-specific antibodies to stain cell culture guarantee the test's higher specificity. However, this conventional method is laborious, costly, and technically challenging. [51] Because it relies on the viability of microorganisms, inadequate specimen collection, storage, transport, the presence of toxic substances in clinical specimens, and commensal microbial overgrowth can all potentially diminish the sensitivity of culture-based tests, even when conducted by experienced laboratory technicians. [50] This is why its sensitivity to detect *ct* infections ranges from 70 to 85%, and its use in screening and diagnosis is now less common. [51] Nonetheless, it is crucial to maintain culture availability in laboratories since it is the only way to detect changes in the susceptibility to antibiotics and preserve research projects - on mutations and new variants of *ct*- [49], or in cases of suspected sexual assault, where the test with the highest specificity is required. [50]

Culture remained the preferred detection method until, in 2002, more effective implementation and expansion of nucleic acid amplification tests (NAATs) was possible, due to technological development. By this time, screening programs using less invasive specimen collection began to appear. [49]
Most NAATs are based on polymerase chain reaction (PCR) methods and use fluorescence-labelled probes to detect amplification products of *ct*-specific DNA or RNA sequences in real time. [50, 52] With combined automated nucleic acid extraction, results can be generated in a few hours. [50]

Currently, there is still no true gold-standard defined method. [49, 53] However, the CDC's latest recommendations are that the preferred tests for detecting *ct* urogenital infection are NAATs due to their high sensitivity, specificity, and ease of transport. [7, 49]. The NAATs approved by the Food and Drug Administration (FDA) can be used for diagnostic or screening purposes since they have been evaluated in symptomatic and asymptomatic patient studies. [49]

The 2021 CDC STI Treatment Guidelines stated that the best type of sample for detecting *ct* by NAATs is first-void urine in men and vaginal swabs in women. [7] According to the US Preventive Services Task Force (USPSTF) report, the sensitivity of endocervical and vaginal samples is ultimately the same – endocervical samples had a sensitivity of 89%-100% and vaginal samples, collected either by self or by a clinician, where 90%-100% sensitive. Specificity was also high in all cases (95-100%). [4] The sensitivity with urine samples was more variable among studies. [4] In men, the sensitivity for either first-void urine, urethral, or meatal swabs was essentially equal (98-100%, 99% and 92%, respectively) [4], being self-collected meatal swabs comparable to the others. [7] Liquid-based cytology specimens collected for Pap smears can also be used for NAAT. Despite probable lower sensitivity, FDA approved certain NAATs for use with this specimen. [7] Sometimes rectal and pharyngeal specimens can also be collected for either symptomatic infection or screening purposes, and its detection is also best by NAATs. [54] In men, rectal swab screening with NAATs had a high sensitivity (92%), according to the 2021 report from the USPSTF, while one study (in MSM) with pharyngeal specimens' concluded a sensitivity of only 69%. [4] An observational study at the U.S. Army Medical Center concluded that self-collected samples from extra-genital sites were as reliable as clinician-collected samples. [55]

However, there are also downsides to using NAATs assays, one of them being the fact that amplification of DNA occurs from either viable or non-viable (dead) ct. This could result in overestimating quantitative test positivity by using existing NAATs. [51] Other challenges in diagnosing *ct* with NAATs have emerged. New variants of *ct* (nvCT), with mutations in the 23S rRNA gene, have evaded detection by many NAAT assays, culminating in an increase in false negatives. To overcome this, new techniques are being developed using more than one gene from *ct*. [10] NAATs also require specific working conditions, such as continuous power, good infrastructure, clean running water and climate control. In resource-limited settings, samples must be transported to these laboratories for processing, employing in-country sample carrier networks. Often, these services only work partially, leading to delays in

returning results to patients and loss of follow-up. With this in mind, there is a significant demand for more sensitive, easier-to-use, and affordable tests for *ct*, especially in resource-limited settings. [56]

In 2006, the World Health Organization created the ASSURED criteria to guide the development of point-of-care tests for STIs. They advocated the need for near-patient tests that are affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and delivered to end-users (ASSURED). [57] There are a few NAAT-based near-point-of-care (POC) *ct* tests available, which are Conformité Européenne (CE)-marked and FDA-approved for various urogenital specimen types. These assays are rapid, need no sample preparation and have high sensitivity and specificity (> 95%) on genitourinary samples. Among the available, the one with the most evidence is a qualitative in vitro RT-PCR executed on the GeneXpert® Platform to detect *ct* and *ng* DNA. [56, 58] The GeneXpert system was proven reliable in high or low-resource environments, as shown by rural Papua New Guinea and South Africa studies. The results become available 90 minutes after loading the sample. [58] However, it still obliges for equipment and is considered expensive. [9] More POC NAATs are available, but any peer-reviewed data on the performance of these is yet to be published. [56]

In addition, *ct* can also be detected with antigen tests. The antigen detection tests are immediate, cheap, easy-to-use immunochromatographic lateral flow assays. [58] A systematic review of point-of-care antigen tests evaluated the main available antigen tests and concluded that these were not sensitive enough for screening use (sensitivity 37%–63%, despite specificity ≥97%), [59] thus not meeting the ASSURED criteria.[9]

Serological ELISA (enzyme-linked immunosorbent assay) tests have also been developed to detect IgA and IgG antibodies specific for MOMP antigens of *ct*. Despite having high sensitivity (100%), their low specificity (58,3%)[60], due to the cross-reactivity of antibodies against different chlamydia species, the fact that antibodies take several weeks to become detectable, and the potential low antibody titers keep them from being used for the diagnosis or screening of *ct*.[50, 60] The identification of past infection may be made using ELISAs based on the antigen Pgp3, a specific and immunogenic *ct* protein that persists following infection. [61] Serology can also help diagnose chronic and invasive chlamydia infections, such as PID.[50]

In a comparative study using commercially available assays for use in resource-limited environments, neither antigen tests (immunofluorescence or immunochromatography) nor immunoenzyme (ELISA) serological tests were reliable enough to be suggested for diagnosing acute infection. Notwithstanding, antigen tests will probably continue to be the most used in developing countries due to the high cost of NAATs tests.[62] A summary of the available detection method's sensitivity and specificity is made in Table I.

Meanwhile, upcoming techniques are emerging to answer previously detected problems. Microbial viability is not evaluated when using conventional NAATs and is determined based on three criteria: active replication, active metabolism, and membrane integrity. Viability determination might be advantageous in cases where the clinical interpretation of a positive NAAT is unclear, in order to define the actual presence of an infection. There are three approaches to determining microbial viability: cell culture, viability-PCR (V-PCR) and messenger RNA (mRNA) detection. [63] Molecular mRNA has a short half-life (minutes), so their detection, contrary to DNA, indicates existing metabolically active bacteria and determines their life-cycle stage. However, the chemical liability of mRNA makes it challenging to work with. [63] Another approach is using a sample pre-treatment technique (e.g.membrane impermeable DNA intercalating dyes) before running the molecular procedures. [51] This is called V-PCR and inhibits the amplification of bacteria with damaged membranes. [63] A study by Jansen, k. j et al. proved it was rapid and detected that a considerable part of the amplified *ct* DNA was derived from non-viable cells.[51] Despite this, correlation is yet to be proved in *in*-vivo extensive clinical studies. All in all, mRNA and V-PCR are promising future substitutes for cell culture viability assessment.[63]

6. *Treatment of ct infection*

Treatment should be available to all people infected with *ct*, as this is the only way to prevent possible further complications and transmission.

The 2021 CDC STI Treatment Guidelines, and the 2022 Australian guidelines, among others, recommend doxycycline as the first line of therapy. This is due to its evident superiority when treating *ct* infections in all anatomical sites (urogenital, rectal, and oropharyngeal). [7, 64] The other option is Azithromycin, which is more indicated in genital-only *ct* infection and used when patient nonadherence is a problem.[7, 64] Rectal and genital infection can co-occur in women and is not related to reported sexual behaviours. So, the choice of treatment ought to be effective for treating infection in both anatomical sites, which happens less with azithromycin. [7]

The recommended doses are Doxycycline 100 mg orally two times/day for seven days or Azithromycin 1 g orally in a single dose for adults and adolescents. In pregnant women, the recommended treatment is Azithromycin 1 g orally in a single dose or, alternately, amoxicillin 500 mg orally three times/day for seven days due to the existence of more evidence with the use of these medications and the fact that Doxycycline is contraindicated in the 2nd and 3rd trimester. [7, 65]

Test of cure (by NAATs) is only indicated in pregnant women 3-4 weeks after treatment due to possible complications for the neonate. Retesting should also happen three months after treatment. [7, 65] Test of cure is not recommended in other population groups because post-treatment NAAT RNA/DNA-positive results are mainly derived from non-viable molecular remnants of *ct*.[52, 63] This is known because the result was negative when the traditional culture method was used. Few viable *ct* were found by culture method, which can be justified by a remaining seven-day period of infectiousness after treatment. [52]

Sexual partners who have come into contact with the infected person 60 days before the beginning of the partner's symptoms or who are the last person they had sexual contact with must be offered testing and evaluation. [7, 64, 66] Partner notification and treatment are essential to avoid reinfection and stop transmission chains. [67] The CDC states that expedited partner therapy should be considered if an evaluation is impractical or inaccessible. [7] On the other hand, 2022 Australian guidelines state that treatment should only be offered if an infection is detected. [64]

7. Screening programs

The current recommendation on screening differs among countries and guidelines and is, to date, a subject of discussion. In the following section, we will expose the current recommendations in the countries that have explored this health problem the most, emphasising their considerations for screening young women.

The 2021 US Preventive Services Task Force recommends screening annually women < 25 years and older women in the case of a higher risk of infection (e.g. new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has an STI). [7, 13] For pregnant women, routine screening is recommended in the first prenatal visit if <25 years of age and older if at higher risk of infection, as specified above. Pregnant women who persist with a higher risk for *ct* infection should also be screened in the third trimester to avoid ct infection in the neonate or maternal postnatal complications. [7]

In England, the National Chlamydia Screening Programme (NCSP), created in 2003, remains the main guideline. [66] In 2021, the English changed their aim and the current recommendations are opportunistic screening (active offer of a chlamydia test) to asymptomatic women and other people with womb or ovaries below 25, along with rapid test results, treatment, and partner notification. [66, 68]

The 2022 Australian STI guidelines propose that asymptomatic *ct* testing should be done on four occasions: to people who request it, to the ones with a higher risk of STIs (new sexual partner(s), people living in or travelling to areas of higher prevalence); that have a known exposure to any STI or history of an STI within the past 12 months; or are a member of or have partner/s from priority sub-populations (e.g. MSM, sex workers, pregnant people, trans and gender diverse people, etc.) [64]

In the US, England and Australia a test at three months is recommended to detect re-infection [7, 64, 66, 68] since it might be the basis of chronic inflammation and severe sequelae development. The detection and treatment of recurrent infection can be even more beneficial than the screening of asymptomatic population.[41]

The Netherlands decided to forgo the implementation of a national screening program for STIs, which was based on a national study- mentioned below. [9, 69, 70] Ireland also decided not to go through with an

opportunistic screening programme after a modelling study was considered expensive to implement and unlikely to be considered cost-effective by legislators.[71]

Sweden and Denmark have widespread opportunistic chlamydia screening but no organised programme, which they discontinued. They reported that their STI control strategies had shifted from promoting testing to intensifying primary prevention activities to control STIs.[9, 69]

When it comes to screening of sexually active young men, there is a lack of evidence to recommend it; however, in high prevalence settings (e.g., STD and adolescent clinics, correctional facilities) or population groups (e.g.MSM), some state that screening should be considered, as long as resources permit and such screening do not deter chlamydia screening efforts for women. [7, 13, 19]

A recent network modelling study proved that screening of men (15-24 years) could reduce the prevalence of ct among women by 8,1% in high-prevalence communities. Other studies have supported testing asymptomatic young men in high-prevalence populations because it can be cost-effective, by preventing cases in women. Some hypothesise that there needs to be an intervention in this population group because men are a pool of infection for women. [72] In their 2021 report, the USPSTF concluded that screening men for *ct* infection was considerably related to a lower risk of developing PID in women of young age. [4] On the other hand, in 2021, England abandoned screening of men below 25 years as part of their National Chlamydia Screening Programme. [68]

The CDC states that pharyngeal or rectal testing should also be offered at least annually to young MSM.[7] In Australia, rectal screening is only offered in clinics of high prevalence (such as sexual health clinics) and to higher-risk populations, such as MSM. Nevertheless, screening for rectal infections with *ct* is becoming more of an object of study as more data show a higher frequency of infections with escalating missed diagnoses. [73] Studies suggest that, in women, recurrent infection may be a result of autoinoculation, regardless of rectal symptoms or intercourse. [74, 75]

In Portugal, there are no currently organised chlamydia control activities. The national health plan from 2011-2016 recommended opportunistic testing every two years in all women who came to planned parenthood consultations in primary care centres or when abortion was requested. [76] However, this recommendation didn't seem to be adequately implemented, and even though this disease is of mandatory notification, it is still under reported. [5] Also, in primary care centres, there is difficulty in requesting NAATs. So, treatment is usually offered to symptomatic people, especially women, often in a presumptive way.

In Table II there is a summary of the information regarding chlamydia control activities taken by the countries considered relevant to this discussion. The countries of Malta, Slovakia and Slovenia are other examples of countries which do not have any organised chlamydia control activities. Other European countries were also included in the category of "Opportunistic testing for selected asymptomatic individuals" in the article by van der Broek et al., however, weren't considered relevant to the present review. [69]

8. *Where is the controversy of screening?*

Monitoring and measuring Chlamydia incidence and prevalence is challenging. While there exists solid proof from surveillance data that *ct* infection is still widespread amongst young sexually active adults, there is not enough strong evidence that screening, as practised in some developed countries, has significantly lowered the prevalence of infection in the target population. [77]

With the NCSP, there was a significant increase in testing coverage in young women - 4% in 2000 to 35% in 2012. However, the expected decrease in chlamydia prevalence was not visible. Two cross-sectional population-based British National Surveys of Sexual Attitudes and Lifestyles (Natsal) showed similar prevalence in people aged 18 to 24 years both from 1999– 2001 (Natsal-2) and 2010-2012 (Natsal-3) (3.1% vs 3,2% in women and 2.9% vs 2.6% in men). [78] The ECDC review (2014) and the Cochrane review (2016) analysed the main randomised control trials (RCTs) on the effects of *ct* screening on transmission and infection complications compared to standard care. The evidence on the impact of chlamydia screening in preventing chlamydia transmission was of low quality. [46, 79] On the Australian Chlamydia Control Effectiveness Pilot (ACCEPt) from 2018, which was not included in the reviews mentioned above, they inferred that there was no clinically relevant difference between the intervention and control clusters after three years. Through these results, they concluded that sizeable reductions in chlamydia prevalence might not be achievable. In addition to that, the trial showed that attaining high enough levels of testing to make a change in prevalence is extremely difficult.[80] Accordingly, the Netherlands Chlamydia screening implementation program(2008-2011) had a participation rate lower than expected (16%), which declined over the three screening rounds (to 10%), despite efforts to promote screening (personal postal invitations, self-collection sample kits, etc.). They saw no difference in positivity in the intervention or control clusters. This could be a result of the low participation rate. Still, van den Broek et al. concluded that the coverage and regular testing frequency needed to achieve valuable reductions were difficult to reach. [70] The mathematical modelling studies that predicted the impact of screening interventions on Chlamydia trachomatis prevalence were much more ambitious than the results of the studies mentioned above. [81]

The RCTs about the effect of *ct* screening on PID (included in the aforementioned ECDC and Cochrane reviews) were considered of moderate quality and proved a reduced incidence compared to control groups. [46, 79] According to Low et al., the risk was reduced by around 30% compared to control clusters; however, the studies with less bias showed weaker evidence, which suggests a possible overestimation

of the protective effect of a screening test. [79] Another 2016 evidence synthesis from the United Kingdom stated that, for women aged 16–24 years, annually screened, in the best scenario, 61% of *ct*-related PID and 22% of all PID could be prevented. [41] On the other hand, the ACCEPt trial reported no impact on PID in clinical settings and a 40% reduction in hospital-based diagnosis. In both, the absolute difference was small. [80] Earlier (2013) mathematical synthesis of evidence estimated a more significant reduction of *ct*-related PID episodes with annual screening - 61% [42] – which doesn't seem to correlate with the real world. According to the 2021 USPSTF Review, screening for chlamydia was associated with reduced risk of PID. However, the outcomes were not statistically significant in most trials, and the degree of benefit was relatively minor. [4]

In addition, the 2017 Lancet article exposed that over the past two decades, there has been a decline in the hospitalisation rate for pelvic inflammatory diseases across countries with varying levels of chlamydia control activity. For example, countries like Belgium, Ireland, and Slovenia, where chlamydia testing was infrequent, saw a reduction of about 30% in hospital discharge diagnoses for PID over the last 15 years. This counterposes the idea that the decrease in PID prevalence is the causal effect of *ct* screening implemented in some places in the past years. There needs to be a careful interpretation of ecological associations like these. [9]

The practice of screening comes not only with advantages but also with unintended consequences. To understand the whole picture regarding the screening of *ct*, it is necessary to analyse which harms derive from this practice. Regarding testing acuity, there is a low rate of false-positive or false-negative findings in the most recommended tests – NAATs.[4] Still, the risk of undetected transmission has increased because of false-negative results caused by the emergence of new mutants. [10] This has happened in the past with the Swedish variant and, most recently, with the Finish variant, which evaded detection in commercial diagnostic kits. [82]

In a study aiming to analyse the changes in sexual behaviour of people tested for *ct*, the conclusion was that people with a positive test were more likely to undergo a more positive and protective behaviour, and the ones with negative tests were more likely to undertake riskier sexual behaviour - less use of condoms. [83] Receiving a positive test is also linked with labelling and anxiety. However, there is a lack of good-quality studies on this matter. [13] A sense of stigma and anxiety-related infertility can also be products of a positive STI test, *ct* included. [84]

Another possible harm derives from the premise (supported by evidence) that partial protective immunity against reinfection after genital infection in humans is gained. The arrested immunity

hypothesis supports that the detection and treatment of early infection disrupt this partial immunity and leads to individuals being more susceptible to reinfection and, consequently, sequelae. So, even though the treatment options are effective at treating disease, it leaves patients without protective immunity and does not benefit the asymptomatic majority of patients that do not get treatment. [17]

Overdiagnosis and overtreatment also need to be addressed. Overdiagnosis is determined as detecting anomalies that are either never going to cause damage, that do not progress, that do so slowly enough not to affect a person during their lifetime, or that resolve spontaneously. [85] Treatment and detection of asymptomatic *ct* infection might qualify as overdiagnosis, as they often resolve on their own without causing any symptoms or disease. Most cases of genital chlamydia infections are asymptomatic. [15] Without treatment, it typically takes about 1.36 years for the genital infection to clear up in women. [41] So, even though most cases won't develop symptoms or sequelae, people who are asymptomatic and positive for *ct* receive treatment with antibiotics, and so do their partners, mostly asymptomatic and untested, if expedited partner therapy is applied. [15] Overtreatment with antibiotics in asymptomatic individuals increases antimicrobial resistance (AMR). [15] Fortunately, there hasn't yet been detected any antimicrobial resistance to *ct*. [9, 15] However, the use of broad-spectrum antimicrobials for widespread treatment can cause AMR to emerge in other STI and non-STI pathogens. It is known that the extensive use of single-dose azithromycin to treat chlamydial infections is likely linked to the macrolide resistance seen in *M genitalium, Treponema pallidum* and *N gonorrhoeae*. [9] In MSM or other high-interconnectivity communities (with recurrent testing and treatment or prevention of STIs), overtreatment can be more problematic; the development of N. gonorrhoeae AMR has already happened in these high-connectivity groups. [86] In addition to that, the use of antibiotics disrupts the natural balance of the vaginal flora, which is proven to be important in protecting against infection and reinfection.[87] Specifically, the cervicovaginal microbiota helps protect against C. trachomatis infection by modulating host functions.[34]

9. Future perspectives

Ct infection seems to naturally prompt a solid, long-lasting immune response, which ultimately supports the concept of developing a vaccine. [88]

Vaccine design will require careful consideration of protective versus pathological host-response mechanisms in concert with an elucidation of optimal antigens and adjuvants. Research shows that a Th1 response involving CD4 T cells producing IFN-γ is crucial for creating lasting immunity that fully eliminates the infection. To develop a vaccine that can effectively trigger both arms of the immune response, it is necessary to further explore broadly neutralising antibodies and antibody-augmented cellular immunity.[89] Data shows that MOMP has promising potential as a vaccine candidate and can stimulate human antigen-specific immune responses.[90] This happens because *ct* expresses MOMP proteins at a high level, making it the most extensively studied surface antigen and vaccine antigen to date. In 2019, a vaccine candidate that incorporated MOMP proteins was trialled on humans. [24] Still, additional in-human trials are required to ensure protection against infection and establish measurable evidence of immune defences. [90] More work on discovering new pan-serovar neutralising and cost-effective candidates is crucial to provide a broadly effective vaccine. [24] Clinically, the availability of effective vaccines against C. trachomatis could significantly impact the prevention of PID.[39]

It is essential to develop consistent protocols for evaluating lower and upper genital tract microbes and inflammation and non-invasive tools to detect upper genital tract disease, such as PID. This would allow for more standardised comparisons between populations and clinical phenotypes. [9, 38] Establishing surveillance systems integrated in chlamydia management guidelines to monitor trends in PID and associated complications would be of great relevance. [9] Especially to study the proportions of PID, EP and TFI attributable to *ct*. [41] Experts agreed that research should focus on sequelae determinants instead of infection factors. [15]

Moreover, future studies on chlamydial infection and other STIs should consider the effect of the vaginal microbiota on the epithelium. It is important to have a basic understanding of the role of cervicovaginal microbiota in protecting against STIs. This knowledge can help develop new therapeutic strategies based on the microbiome to protect women from infection and improve their cervicovaginal health. [34]

Regarding the development of POC tests, they allow for the expansion of access to STI diagnostic testing, granting reduce treatment times, stopping disease transmission, and ultimately bringing benefit to public health, especially in low-income countries. [58] Thus, employing a POC test will probably be a cost-effective diagnostic approach for ct infection. [7] However, although several promising tests are in development, the available ones have low accuracy or require expensive equipment, which isn't compatible with application in developing countries. [9] Being a vital component of STI detection, particularly in low-income countries, the World Health Organisation assured to facilitate the development of POC testing.[2]

10. Conclusion

In recent years, awareness of the *ct* pathogen, disease and complications has increased. New genetic tools allowed for the discovery of *ct* proteins such as the Tarp effector and the Incs- that regulate the host cell to the benefit of the pathogen [22, 23] - and proteins like CPAF that paralyse PMNs, allowing for immune evasion. [27] An understanding of the persistent RBs was achievable, including the possible triggers that activate it [26, 32, 33] [26, 33] and the potential role of the chronic release of chlamydial hsp60 in upper genital tract inflammation, fibrosis and consequent sequelae. [26, 32] Still, there is a pressing need for investment in research to improve the understanding of chlamydia's natural history, especially the evolution to PID and the related complications. [9]

NAATs came to revolutionize the detection of ct infection. They are the most specific and sensitive tests available and should be the test of choice for screening. Nonetheless, there is still a need for more sensitive, user-friendly tests that detect new variants of *ct* and can determine its viability. The affordability of these tests is also a problem, especially in low-income countries. Point-of-care NAATs are promising but require further development.

Especially in high-income countries, the direction of chlamydia control strategies started to focus on asymptomatic detection of infection and a series of efforts was made on widespread testing and treatment. [9] Still, the current low quality of the studies on chlamydia screening's influence on infection transmission and the scarcity of studies on chlamydia-associated morbidity do not allow us to affirm that *ct* prevalence has reduced and makes it impossible to define the true impact of screening. [46, 79, 91] The NSCP - the organised screening programme implemented in England - had no significant effect on prevalence reduction. [78] The most recent trials proved no difference in prevalence between intervention and control clusters. [70, 80, 91] Moreover, both the Australian and the Dutch screening pilots evidenced the difficulty of reaching high enough levels of testing uptake to reduce chlamydia prevalence significantly. [80, 81]

The impact of screening on PID and other complications is also uncertain. Although there appears to be a reduction of PID hospital discharge compatible with the development of ct screening efforts, the prevalence reduction was global and not restricted to the countries that took on screening measures. Most studies show a generally reduced incidence of PID in intervention groups; however, the absolute difference is mostly small, with little statistical significance and more bias in the studies that prove a

more substantial relation. Although there is evidence that *ct* past infection is linked to female infertility, the risk is low (0,5-3%), and the effect of screening on lowering this risk is unknown.

The debate on the pros and cons of screening for asymptomatic *ct* infections appears to be causing controversy. The problems of overdiagnosis and overtreatment are also beginning to be considered, as well as the cost-effectiveness of widespread testing. Recent guidelines focus more on the importance of disease control and value targeted testing, intended harm reduction, and sequelae prevention. Countries like Sweden, Denmark, England and Australia, which changed their policies' aim, and the Netherlands and Ireland, could be examples to Portugal.

The ecdc guidance for chlamydia control advises countries to establish their monitorization and evaluation policies with emphasis on primary and secondary prevention strategies–including case detection and management. Anamnesis, physical exam, use of diagnostic tests, treatment, sexual partner management, sexual health promotion, follow-up, and surveillance are the main pillars. [37] In Portugal, there is an urgent need for the creation of national chlamydia management guidelines. An opportunistic testing strategy could be implemented, allowing people at higher risk of infection to get treatment and monitoring. Moreover, it is essential that the rate of notification of the disease grows to a level that translates the reality. This implies an effort from clinicians and policymakers and the promotion of sexual health nation-wise.

Nonetheless, it needs to be kept in mind that disease management ought to be the main focus, and, despite the change of paradigm, it is essential not to waste any previous efforts of getting sexual health on the agenda nor deter sexual health-seeking behaviour or compromise early detection and treatment of other STIs.

Appendix.

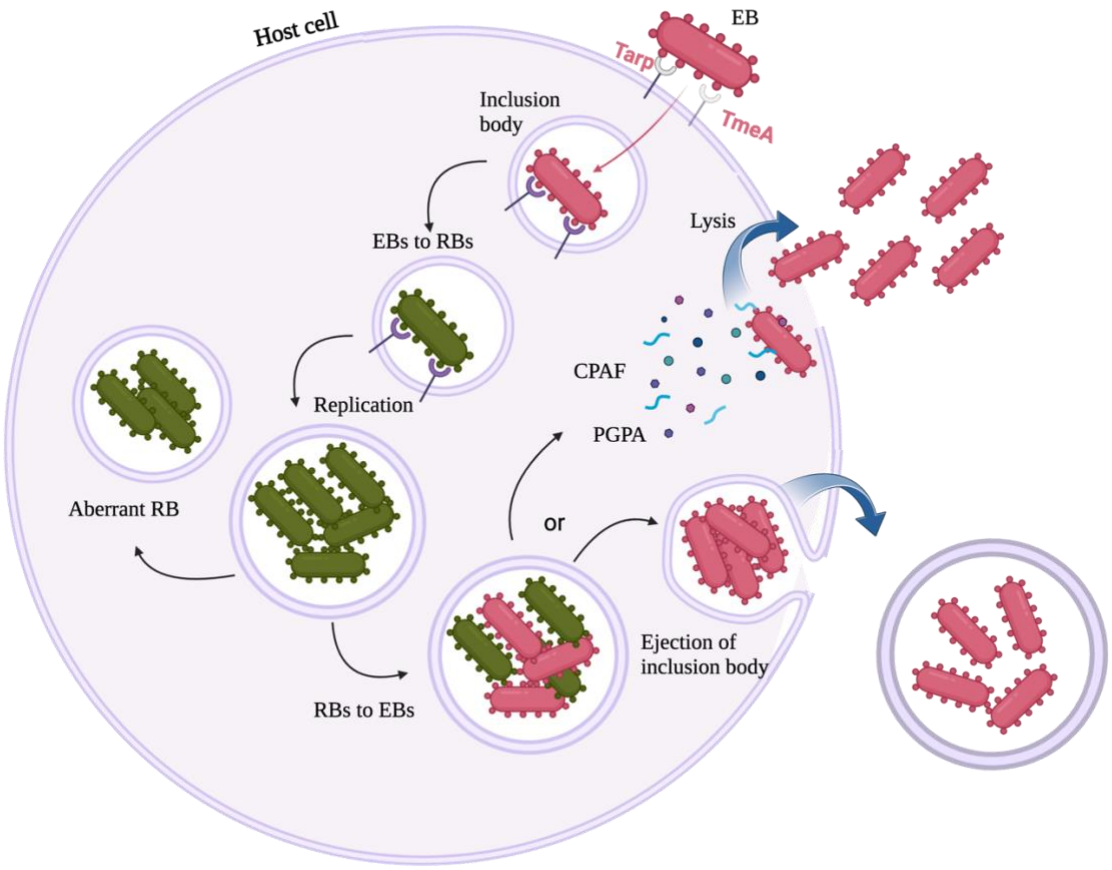

Figure 1: CT's life cycle and infection process;

*EB - Elementary Body; RB -reticulate body; Tarp- translocated actin recruiting protein; Tarp and TmeA: type III secretion system (T3S) bacterial effector proteins; CPAF: chlamydial protease-like activating factor; PGP4 - plasmid-encoded factor

Table I : Detection methods - sensitivity and specificity

Tests	Sample type	Sensitivity	Specificity	References
Culture	Endocervical Urethral	70-85% 70-85%	100% 100%	48 51
NAATs	Women: Endocervical Vaginal Men: First-void urine Urethral Meatal Rectal	 89-100% 90-100% 98-100% 99% 92% 92%	 95-100% 95-100% NI NI NI 69%	4
NAATs - POC	Genitourinary	>95%	>95%	56, 58
Antigen – POC	Genitourinary	37%–63%	≥97%	9
Serological ELISA	Genitourinary	100%	58,3%	60

* NI – no information; NAAT – nucleic acid amplification test; POC – point-of-care; ELISA - Enzyme-Linked Immunosorbent Assay

Table II : Chlamydia control activities;

Countries	Chlamydia control category	Population	Sexual partner management	Re-testing	References
US	Organized screening programme (screening programme)	Annually, women < 25 years and older in the case of a higher risk of infection, including pregnant people	Yes	Yes, 3 months after treatment	7, 13
England	Organized screening programme (screening programme)	Annually, women and other people with womb or ovaries < 25	Yes	Yes, 3 months after treatment	66, 68, 92
Australia	Opportunistic testing for selected asymptomatic individuals (opportunistic testing)	Higher risk of STIs; with known exposure/history of an STI < 12 months; member of or have partner/s from priority sub-populations	Yes	Yes, 3 months after treatment	64
Netherlands; Denmark; Sweden; Ireland	Opportunistic testing for selected asymptomatic individuals (opportunistic testing)	NI	Yes	NI	9, 69, 70, 92
Portugal; Malta; Slovakia; Slovenia;	No organized chlamydia control activities	NA	NA	NA	92

***** Column 2 categories are based on criteria from the 2012 ecdc survey [92]; NI: no information; NA: non applicable

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