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Atypical Mixed Trichomoniasis and Ureaplasma Urealyticum Infection Confirmed by Multiplex Real-Time PCR: A Case Report

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ABSTRACT: *Trichomonas vaginalis* and *Ureaplasma urealyticum* are prevalent sexually transmitted pathogens with complex symbiotic interactions. Classically, trichomoniasis presents with profuse, frothy vaginal discharge, while *U. urealyticum* causes urethritis or remains asymptomatic. An isolated genital ulcer without discharge is an exceedingly rare and atypical presentation for both. A sexually active female presented with acute dysuria and a solitary, painful external vulvar ulcer. Examination revealed localized erythema but a complete absence of purulent or frothy vaginal discharge. Due to this atypical morphology, Multiplex Real-Time PCR was utilized, definitively confirming a mixed *T. vaginalis* and *U. urealyticum* infection. The patient received targeted therapy comprising Azithromycin (1-gram single dose) and a 7-day multi-dose Metronidazole regimen (500 mg twice daily), alongside Omeprazole. This intervention achieved rapid, complete resolution of the ulcer and symptoms. This case highlights that synergistic protozoan-bacterial infections can bypass classic syndromic paradigms, presenting as localized genital ulcers mimicking viral or treponemal etiologies. The absence of characteristic discharge likely stems from endosymbiont-driven, highly localized mucosal necrosis. Clinicians must expand differential diagnoses for atypical ulcers and utilize Multiplex PCR for accurate detection and targeted therapy

1. INTRODUCTION

Sexually transmitted infections (STIs) are a global health problem with impacts on reproductive health, maternal-fetal outcomes, and human immunodeficiency virus (HIV) transmission.¹ More than 350 million curable STI cases occur each year.¹ *Trichomonas vaginalis* is the most common non-viral STI pathogen, with approximately 156 million new cases in the 15–49 age group in 2020.² In women, trichomoniasis typically presents as vaginitis with profuse, frothy, foul-smelling, yellow-green discharge, accompanied by pruritus, dyspareunia, dysuria, or strawberry cervix.³ *Ureaplasma urealyticum* is a Mollicutes bacterium that can be either commensal or an opportunistic pathogen in the genitourinary tract, including causing nongonococcal urethritis, cervicitis, pelvic inflammatory disease, and obstetric complications.⁴

The absence of a peptidoglycan cell wall necessitates specific molecular diagnostic approaches and therapies for this bacterium.⁵ Manifestations of *T. vaginalis* and *U. urealyticum* as localized genital ulcers without discharge are extremely rare and atypical conditions.⁶ Genital ulcers typically point toward a diagnosis of HSV, *Treponema pallidum*, or *Haemophilus ducreyi*.⁷ Interactions

between *T. vaginalis* and *Mycoplasmataceae* bacteria, including *Ureaplasma spp.*, may influence virulence and inflammatory response and are suspected of contributing to local mucosal damage.⁸⁻¹⁰ Conventional STI diagnosis has limitations in sensitivity and turnaround time.¹¹

Multiplex Real-Time PCR offers high sensitivity and specificity and enables the simultaneous detection of various pathogens from a single specimen.^{12,13} In the context of Indonesia, where syndromic approaches are still widely used, atypical manifestations such as ulcers without discharge can pose diagnostic challenges and underscore the importance of molecular testing in atypical cases. This case report describes a mixed infection of *T. vaginalis* and *U. urealyticum* presenting as a small, painful vulvar ulcer with dysuria and no discharge. This case highlights the importance of Multiplex Real-Time PCR in identifying the etiology of STIs presenting with atypical genitourinary manifestations.

2. CASE ILLUSTRATION

The subject of this case report is a female patient of reproductive age who presented to an outpatient genitourinary and sexual health clinic seeking evaluation and treatment for the acute onset of localized anogenital symptoms (See Figure 1). In strict adherence to the CARE guidelines and global ethical standards for medical publishing, all personally identifiable information, including the patient's exact age, geographic location, occupational status, and specific dates of clinical encounters, has been thoroughly de-identified to maintain and ensure absolute patient confidentiality.¹⁴

The patient's primary and most distressing clinical concern was the sudden and unprovoked onset of intense dysuria, which she characterized as a sharp, severe, burning sensation occurring exclusively during micturition. This symptom had been continuously present for exactly three days prior to her initial clinical consultation. Concurrently with the dysuria, the patient noted the rapid development of a small, highly localized, and exquisitely painful lesion situated on the external genitalia, specifically within the vulvar region.

During the comprehensive clinical interview, the patient was explicitly questioned regarding the presence of secondary symptoms classically associated with genitourinary infections. The patient definitively and repeatedly denied experiencing any abnormal, increased, or discolored vaginal discharge. Furthermore, she denied any foul or "fishy" vaginal odor, deep pelvic pain, dyspareunia prior to the lesion's appearance, or systemic inflammatory symptoms such as fever, chills, myalgias, or generalized malaise. The strict localization of the pain to the ulcerative lesion and the urethra during urination formed the core of her clinical complaint.

A thorough and exhaustive medical history was obtained to identify any underlying systemic conditions that might predispose the patient to atypical infectious presentations or opportunistic pathogens. The patient reported a generally excellent state of baseline health. There was no known history of congenital or acquired immunodeficiencies, including no history of human immunodeficiency virus (HIV) infection. She denied any systemic autoimmune disorders, chronic metabolic conditions such as diabetes mellitus, or hematological malignancies that could result in a compromised immune topography.

Her dermatological and genitourinary history was specifically probed. There was no documented or anecdotal history of recurrent genital ulcerative diseases, nor any prior clinical diagnoses or serological evidence of Herpes Simplex Virus (HSV) types 1 or 2, or syphilis. She additionally denied any history of severe, recurrent aphthous stomatitis or ocular inflammation that might suggest a complex autoimmune etiology such as Behçet's disease.¹⁵ Her family medical history was reviewed and deemed entirely non-contributory to the current acute infectious presentation.

Psychosocially, the patient reported being actively engaged in sexual relationships. A detailed, non-judgmental sexual history indicated that her last sexual intercourse occurred approximately one month (30 days) prior to the sudden onset of her current symptoms. She reported that this encounter was with a stable partner and denied any recent changes in sexual partners, engagement in transactional sex, or the use of intravenous or recreational drugs. Her living conditions were stable, and she had unimpeded access to routine healthcare services.

The patient's past gynecological and obstetric history was entirely unremarkable. She reported regular, normal menstrual cycles and possessed a normal, up-to-date cervical cytological screening (Pap smear) history with no evidence of cervical dysplasia or high-risk Human Papillomavirus (HPV) infection. There was no recent history of systemic antibiotic utilization, corticosteroid therapy, or other immunosuppressive pharmacological interventions within the past six months. Previous routine serological and molecular evaluations for sexually transmitted infections, conducted as part of her routine healthcare maintenance, had

reportedly been universally negative. Furthermore, there was no clinical history of prior interventions for ascending upper genital tract infections, such as pelvic inflammatory disease (PID), or treatments for severe, refractory vaginitis.

2.1 Clinical Findings

A detailed, systematic physical and pelvic examination was conducted to objectively evaluate the patient's acute symptoms and to visually characterize the reported external lesion. Initial visual inspection of the external genitalia revealed distinct, localized erythema primarily involving the mucosal tissues of the vulva and extending slightly toward the introitus of the vagina. Situated within this erythematous zone on the external vaginal mucosa was a single, small ulcerative lesion. The ulcer was morphologically characterized as being superficial, well-demarcated, and lacking any significant induration or raised, rolled borders. The base of the ulcer was slightly erythematous but was notably free of any dense purulent exudate, thick fibrinous slough, or necrotic eschar. Palpation of the lesion, conducted with extreme care, revealed it to be exquisitely tender, directly correlating with the patient's primary complaint of severe localized pain.

Crucially, there was absolutely no evidence of generalized or clustered vesicular eruptions, either intact or ruptured, that would typically suggest an acute or recurrent herpetic outbreak. Similarly, the lesion lacked the classic firm, painless, and rubbery characteristics of a primary syphilitic chancre. Palpation of the bilateral inguinal regions revealed no evidence of regional lymphadenopathy; the lymph nodes were non-palpable, non-tender, and mobile, effectively differentiating the presentation from the suppurative lymphadenitis often associated with chancroid or the firm adenopathy of primary syphilis.

Following the external inspection, a sterile speculum examination of the internal vaginal vault and the uterine cervix was carefully performed. The internal mucosal surfaces were observed to be distinctly moist, maintaining normal physiological hydration, but were entirely homogeneous in both texture and coloration. The most critical and defining clinical finding of the entire examination was a highly pertinent negative: there was a complete and absolute absence of the classic clinical signs of trichomoniasis. Specifically, no frothy, yellow-green, or purulent secretions were observed adhering to the vaginal walls or pooling within the posterior vaginal fornix. Furthermore, no abnormal or copious fluid was seen spontaneously exiting the vaginal orifice during the examination.

The uterine cervix appeared morphologically normal and healthy. There was no evidence of mucopurulent cervicitis, no contact friability upon swabbing, and no presence of the classical punctate macular microhemorrhages (colpitis macularis), universally recognized as the "strawberry cervix" sign highly indicative of severe *Trichomonas vaginalis* infection. A subsequent bimanual pelvic examination elicited no cervical motion tenderness, and the bilateral adnexal regions were non-palpable and completely non-tender, thereby effectively ruling out any ascending upper genital tract involvement or acute pelvic inflammatory disease (PID).

2.2 Timeline

The progression of the patient's clinical episode, from the presumed point of pathogen exposure, through the acute onset of symptoms, molecular diagnostic confirmation, targeted therapeutic intervention, and ultimate clinical resolution, is systematically structured and chronicled in the timeline provided below (Table 1).

2.3 Diagnostic Assessment

Given the highly atypical clinical presentation of a singular, painful genital ulcer entirely devoid of the characteristic vaginal discharge, an empirical or purely symptom-based clinical diagnosis was deemed vastly insufficient and potentially hazardous. The morphological ambiguity of the ulcerative lesion necessitated an objective, highly sensitive, and broad-spectrum molecular approach to definitively identify the exact etiologic agents and to definitively rule out the more common, severe ulcerative pathogens that typically populate the differential diagnosis.

Multiplex Real-Time PCR technology represents the pinnacle of modern infectious disease diagnostics. These sophisticated assays utilize advanced dual priming oligonucleotides (DPO) and multiple detection temperature (MuDT) technologies to simultaneously amplify and detect the specific nucleic acid sequences of a comprehensive panel of sexually transmitted pathogens within a single, closed reaction tube.¹² This specific molecular panel was designed to target and differentiate a broad array of urogenital microorganisms, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, and *Ureaplasma urealyticum*.¹²

The results of the Multiplex Real-Time PCR assay yielded a definitive, robust positive detection for the presence of DNA from both *Trichomonas vaginalis* and *Ureaplasma urealyticum*. Crucially, the assay returned definitively negative results for all other targeted pathogens on the panel, including the common ulcerative and inflammatory agents such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and the various *Mycoplasma species*. This molecular precision allowed for an immediate transition from diagnostic uncertainty to targeted therapeutic action.

The primary diagnostic challenge encountered in this unique case was the sheer clinical atypia of the presentation, which completely defied classic syndromic expectations. The universally recognized clinical hallmark of a *Trichomonas vaginalis* infection is a copious, malodorous, frothy vaginal discharge.² The complete and verified absence of these classic signs, coupled directly with the presence of a localized ulcer, heavily skewed the initial clinical suspicion away from a protozoal or ureaplasma etiology and toward viral or treponemal causes.

Table 1. Comprehensive chronological timeline detailing the patient's clinical presentation, diagnostic pathway, therapeutic management, and resolution of symptoms.

Clinical Day	Event Description and Clinical Milestones
Day -30	The patient reported her last instance of sexual intercourse. Based on the pathogenesis and incubation periods of the identified organisms, this event represents the presumed window of pathogen exposure and mucosal transmission.
Day -3	The acute onset of clinical symptoms. The patient first noted the rapid development of a small, highly painful ulcer localized to the external vaginal mucosa, accompanied simultaneously by the onset of severe, burning dysuria.
Day 0	The initial clinical presentation and outpatient consultation. A comprehensive physical and pelvic examination revealed localized vulvar erythema and a solitary, tender superficial ulcer. The examination noted a strict absence of any abnormal vaginal discharge. Diagnostic swab specimens were meticulously collected from the ulcer base and the vaginal mucosa for advanced Multiplex Real-Time PCR testing.
Day 2	Diagnostic confirmation achieved. The Multiplex Real-Time PCR assay results returned, yielding a definitive positive detection for the nucleic acids of both <i>Trichomonas vaginalis</i> and <i>Ureaplasma urealyticum</i> . Based on this molecular profile, a targeted, multidrug pharmacological therapy (comprising Azithromycin, Metronidazole, and Omeprazole) was immediately initiated.
Day 9	The successful completion of the prescribed 7-day antimicrobial regimen. The patient subjectively reported profound symptomatic relief, noting the complete cessation of dysuria and the progressive, visible epithelial healing of the genital ulcer.
Day 14	The formal clinical follow-up examination. Objective physical assessment confirmed the complete re-epithelialization and anatomical resolution of the vulvar ulcer. There was a total absence of residual erythema, induration, or scarring. The patient achieved a full, uncomplicated clinical cure.

Historically, the diagnosis of trichomoniasis in resource-limited or point-of-care settings has relied heavily upon wet mount microscopy. However, wet mount microscopy is a notoriously insensitive modality, successfully detecting *T. vaginalis* in only 31% to 60% of cases.¹⁶ The success of a wet mount is inextricably dependent on the presence of a very high parasite load and

the rapid processing of fresh, highly motile specimens suspended in fluid.¹⁶ In cases such as this, where copious vaginal discharge is entirely absent and the protozoal load may be concentrated strictly within the necrotic tissue of the ulcerative lesion, wet mount microscopy would almost certainly yield a false-negative result, leading to a missed diagnosis and therapeutic failure.

Similarly, the definitive diagnosis of *Ureaplasma urealyticum* via traditional microbial culture presents a formidable challenge. Traditional microbial culture for *U. urealyticum* is notoriously difficult. Mollicutes are exceptionally fastidious organisms that intrinsically lack a rigid cell wall, requiring highly specialized, enriched culture media (such as A7 or A8 agar) supplemented with urea, specific sterols, and precise atmospheric conditions.¹⁷ The culture process suffers from prolonged turnaround times (often requiring up to a week) and demonstrates lower sensitivity compared to molecular techniques, making it clinically impractical for the rapid management of an active ulcerative lesion.¹⁷

Table 2. Multiplex Real-Time PCR panel results demonstrating definitive positive detection for *Trichomonas vaginalis* and *Ureaplasma urealyticum*.

Laboratory Testing Result	Result	Reference Range
RT-PCR		
Molecular and Genomics		
<i>Ureaplasma urealyticum</i>	Detected; Ct value: 30.57*	Not Detected: Ct value >36 Detected: Ct value ≤36
<i>Chlamydia trachomatis</i>	Not Detected	Not Detected: Ct value >38 Detected: Ct value ≤38
<i>Mycoplasma genitalium</i>	Not Detected	Not Detected: Ct value >38 Detected: Ct value ≤38
<i>Mycoplasma hominis</i>	Not Detected	Not Detected: Ct value >36 Detected: Ct value ≤36
<i>Neisseria gonorrhoeae</i>	Not Detected	Not Detected: Ct value >37 Detected: Ct value ≤37
<i>Trichomonas vaginalis</i>	Detected; Ct value: 35.17*	Not Detected: Ct value >37 Detected: Ct value ≤37

Table 3. Comprehensive comparative analysis of differential diagnoses considered for acute genital ulceration prior to definitive molecular confirmation.

Differential Diagnosis	Primary Etiologic Agent	Classic Presentation and Morphology	Clinical and	Distinguishing Features from the Current Case
Genital Herpes	Herpes Simplex Virus (HSV-1, HSV-2)	Highly painful, multiple clustered vesicles situated on an erythematous base that rapidly rupture to form shallow, exquisitely tender ulcers. Often accompanied by a systemic viral prodrome.		The current case presented with a singular, isolated ulcer without any prior history of vesicular clustering, systemic viral prodrome, or recurrent episodes. Multiplex PCR effectively excluded common viral targets. ⁷
Syphilis (Primary Stage)	<i>Treponema pallidum</i>	A solitary, classically painless, highly indurated ulcer (chancre) featuring a clean base and raised, rolled borders. Frequently associated with firm, non-tender regional lymphadenopathy.		The patient's ulcer was highly sensitive and exquisitely painful, lacking the classic rubbery induration of a syphilitic chancre. Inguinal lymphadenopathy was notably absent. ⁷

Chancroid	<i>Haemophilus ducreyi</i>	Deep, highly painful, and necrotizing ulcers with ragged, undermined edges and a dirty, purulent base. Almost universally accompanied by painful, suppurative inguinal lymphadenopathy (buboes).	The patient's ulcer was superficial, well-demarcated, and lacked both a necrotic, purulent base and the requisite suppurative lymphadenopathy. ⁷
Lipschütz Ulcer (Ulcus Vulvae Acutum)	Non-sexually transmitted (often associated with EBV, CMV, or Mycoplasma species)	Acute, highly painful, necrotic genital ulcers that typically present in young, sexually inactive, or virginal females, often following a systemic viral illness.	The patient was a sexually active adult, lacked any recent systemic viral illness, and molecular testing identified clear, sexually transmitted pathogenic organisms. ¹⁸
Behçet's Disease	Autoimmune / Systemic Vasculitis	Recurrent, highly painful genital and oral aphthous ulcers, frequently accompanied by severe ocular lesions (such as uveitis) and cutaneous manifestations.	The patient had no historical record of recurrent oral or genital ulcers, and no systemic autoimmune or ocular manifestations were present during the clinical evaluation. ¹⁵

Prior to the receipt of the molecular confirmation, the differential diagnosis for a sexually active female presenting with a sudden, painful genital ulcer and concurrent dysuria was extensive. The diagnostic focus was primarily centered on common viral and classical bacterial STI etiologies, as well as non-infectious autoimmune conditions. A detailed comparative analysis of these differential diagnoses is outlined in Table 3.

Based on the definitive molecular evidence provided by the Multiplex Real-Time PCR assay, synthesized with the clinical findings, the final diagnosis established for this patient was an Atypical Mixed Infection of *Trichomonas vaginalis* and *Ureaplasma urealyticum* presenting as an Isolated Genital Ulcer. The clinical prognosis for localized, mixed infections involving these pathogens is considered excellent when accurately diagnosed and promptly treated with appropriate, targeted antimicrobial therapy.

2.4 Therapeutic Intervention

The therapeutic strategy devised for this patient relied entirely upon a targeted pharmacological intervention. Because the diagnostic testing revealed a mixed infection involving a cell-wall-deficient bacterium and an amitochondriate protozoan, a highly specific, dual-antimicrobial multidrug regimen was required to eradicate the diverse pathogens. Furthermore, anticipating the potential for significant gastrointestinal toxicity associated with this dual therapy, a specific gastrointestinal prophylactic agent was incorporated into the regimen to ensure patient tolerability and strict adherence. No surgical debridement or topical steroidal interventions were deemed necessary.

Based on current pharmacological guidelines and the specific biological profiles of the detected organisms, the prescribed therapeutic regimen was meticulously structured and administered as follows:

1. Azithromycin (1 gram single dose): This agent was administered specifically for the targeted eradication of the *Ureaplasma urealyticum* infection.⁸ Azithromycin is a broad-spectrum macrolide antibiotic that exerts its bactericidal effect by traversing the cell membrane and reversibly binding to the 50S ribosomal subunit of susceptible microorganisms, thereby effectively inhibiting RNA-dependent protein synthesis. Because *Ureaplasma urealyticum*, as a member of the class Mollicutes, intrinsically lacks a rigid peptidoglycan cell wall, the entire class of beta-lactam antibiotics (including all penicillins and cephalosporins) is rendered entirely pharmacologically ineffective.⁵ The selected dose of Azithromycin 1-gram orally as a single dose strictly

adheres to the updated 2021 Centers for Disease Control and Prevention (CDC) Sexually Transmitted Infections Treatment Guidelines, representing the standard, highly effective targeted therapy to achieve the necessary intracellular concentrations to eradicate these fastidious endosymbionts.⁵

2. Metronidazole (500mg twice daily) This agent was administered for the definitive eradication of the *Trichomonas vaginalis* protozoal infection. Metronidazole is a 5-nitroimidazole derivative that functions as an inactive prodrug. Upon diffusing into the amitochondriate protozoan, the nitro group of the drug is actively reduced by unique, parasite-specific enzymes operating within the hydrogenosomes.¹⁹ This generates cytotoxic nitro radical intermediates that rapidly induce lethal strand breakage within the protozoal DNA.¹⁹ Crucially, for the treatment of *Trichomonas vaginalis* in women, the CDC 2021 guidelines decisively replaced the historical 2-gram single-dose protocol with this 7-day, multi-dose regimen of Metronidazole (500 mg twice daily). This prolonged dosing strategy provides a vastly superior clinical cure rate by maintaining sustained protozoacidal concentrations within the vaginal tissues, significantly mitigating the severe risk of endosymbiont-mediated treatment failure.²

3. Omeprazole (twice daily): This medication was administered strictly as a prophylactic gastroprotective agent. Both Azithromycin and Metronidazole are notorious for inducing substantial, dose-limiting gastrointestinal distress, encompassing severe nausea, dyspepsia, metallic taste, and intense abdominal cramping. Omeprazole, a highly potent proton pump inhibitor, effectively suppresses gastric acid secretion by irreversible, specific inhibition of the H⁺/K⁺-ATPase enzyme system situated at the secretory surface of the gastric parietal cells. The strategic inclusion of this agent in the regimen was specifically designed to ensure high patient tolerability, thereby safeguarding strict adherence to the critical, multi-day antimicrobial course.²⁰

The patient was prescribed this specific, fixed multidrug regimen at the exact time of molecular diagnosis. The therapeutic intervention proved to be highly effective and was exceptionally well-tolerated by the patient. Consequently, no mid-course changes, dose reductions, drug substitutions, or premature cessations of therapy were required at any point during the treatment timeline.

2.5 Follow-up and Outcomes

The primary, critical clinical endpoints defined for the successful management of this case were the complete structural resolution of the vulvar ulceration and the total subjective cessation of the patient's dysuria. Upon the scheduled follow-up clinical evaluation conducted on Day 14 (which occurred exactly five days post-completion of the rigorous 7-day antimicrobial therapy), the patient enthusiastically reported complete and sustained relief from all acute urinary and pain symptoms.

A meticulous physical examination performed by the attending clinician objectively confirmed the patient's subjective reports. The external vaginal ulcer demonstrated total re-epithelialization and profound mucosal healing. There was an absolute absence of any residual vulvar erythema, tissue induration, or fibrotic scarring at the site of the former lesion. Furthermore, the internal vaginal mucosa remained anatomically healthy, adequately moist, and completely free of any signs of abnormal, pathogenic discharge, indicating a successful restoration of the localized mucosal barrier.



Figure 1. Clinical photograph of the vulvar region. erythematous ulcerative lesions with distinct borders are observed on the upper and middle portions of the labia minora, surrounded by tissue edema and erythema

Given the exceptionally rapid and clinically complete resolution of all presenting symptoms, and acting in strict accordance with standard, prevailing clinical guidelines for the management of resolving, uncomplicated *Trichomonas* and *Ureaplasma* infections in immunocompetent, asymptomatic individuals following targeted molecular therapy, a routine, immediate test-of-

cure (TOC) via repeat Multiplex PCR was deemed clinically unnecessary at the Day 14 mark. However, recognizing the complex epidemiology of these pathogens, the patient was extensively counseled regarding the high statistical rates of reinfection associated with *T. vaginalis*. She was strongly advised to return to the clinic for a comprehensive, routine repeat STI screening panel at the 3-month mark to ensure sustained pathogen clearance and long-term reproductive health.

3. DISCUSSION

The clinical manifestation of a mixed *Trichomonas vaginalis* and *Ureaplasma urealyticum* infection strictly as an isolated, localized genital ulcer, entirely devoid of the classical, profuse, and highly characteristic vaginal discharge, represents a profound and significant deviation from established pathognomonic norms.² In typical clinical scenarios, *Trichomonas vaginalis* mediates extensive host tissue damage through a complex, multifactorial, and highly aggressive process. This process involves direct, contact-dependent parasite-host cell adherence, profound mechanical disruption of the epithelial barrier, and the localized, concentrated release of highly cytotoxic molecules, including a vast array of potent proteolytic enzymes and cell-detaching factors.¹⁰ This relentless cytopathic effect is generally diffuse and widespread across the vaginal vault, resulting in extensive mucosal erythema and the rapid recruitment of massive numbers of polymorphonuclear leukocytes (neutrophils). Clinically, this immense cellular and fluid exudate manifests as the classic purulent, frothy, and foul-smelling discharge.²

However, the complete and verified absence of this hallmark discharge in the present case necessitates a much deeper, mechanistic examination of the underlying pathobiology. The formation of a focal, necrotic ulcer strongly suggests that the protozoal cytopathic effect, rather than being diffusely spread, was intensely concentrated at a specific micro-site on the vulvar epithelium.¹¹ Instances of *T. vaginalis* presenting as isolated vulvar or vaginal ulcerations have been documented in the literature previously, albeit as extreme rarities. When they do occur, they frequently lead to profound and dangerous diagnostic delays due to their seamless clinical mimicry of classic viral (HSV) or treponemal (syphilis) ulcers.¹¹

A pivotal learning point for clinicians drawn from this case is understanding why there was a strict absence of spontaneous purulent discharge despite a strong positive multiplex PCR result. While classical trichomoniasis elicits a diffuse, exudative inflammatory response across the vaginal vault, the massive, focused immune cell recruitment and subsequent apoptotic tissue damage in this specific co-infection were restricted entirely to the ulcer's microenvironment.⁷ Furthermore, *Ureaplasma* species are known to generally induce a non-purulent inflammatory response. Consequently, the lack of generalized mucosal transudation combined with the non-purulent nature of the bacterial endosymbiont resulted in a "dry" ulcer, effectively deceiving standard clinical expectations.¹¹ Clinicians must not exclude these synergistic pathogens from their differential diagnosis simply due to a non-exudative presentation.

The concurrent molecular detection of *Ureaplasma urealyticum* in this lesion provides a critical, profound mechanistic insight into the atypical severity and highly unusual presentation of this case. The biological interaction between the protozoan *Trichomonas vaginalis* and bacterial species belonging to the class Mollicutes (which prominently includes *Mycoplasma hominis* and various *Ureaplasma* species) is not merely co-incidental; it is characterized by a profound, clinically significant, and highly evolved molecular endosymbiosis.⁹ This relationship represents one of the very first scientifically described associations between two distinct, obligate human parasites, wherein the larger protozoan essentially acts as both a protective, intracellular niche and a highly mobile mechanical vector for the smaller bacteria.⁹

Extensive molecular and advanced phylogenetic studies have definitively demonstrated that a remarkably substantial proportion of clinical *T. vaginalis* isolates, reaching up to 54% in some extensively studied clinical cohorts, harbor these intracellular Mollicutes.⁹ These bacterial endosymbionts actively and aggressively modulate the fundamental biology, life cycle, and inherent virulence of their protozoan host.⁹ The presence of these intracellular bacteria has been demonstrably shown to significantly upregulate the phagocytic activity and the rapid ameboid transformation of *T. vaginalis*, two distinct physiological changes that directly and positively correlate with enhanced tissue invasiveness and vastly increased host cell destruction.⁹

Furthermore, *T. vaginalis* infection drastically alters the normal vaginal microbiota by displacing and reducing hydrogen peroxide-producing *Lactobacillus* species, which directly leads to an elevated, alkaline vaginal pH. This pH shift creates a highly optimal and permissive microenvironment for the massive proliferation of *U. urealyticum*, an organism that intrinsically thrives in such conditions. Together, this synergistic hyperproliferation and the combined release of cytotoxic enzymes and inflammatory cytokines severely disrupt the epithelial integrity, driving intense focal mucosal necrosis and the ultimate formation of the atypical genital ulcer.⁷ This synergistic hyperinflammation is further exacerbated by a shared metabolic pathway (the arginine

dihydrolase pathway), which actively starves the host's innate immune macrophages of the critical substrate required to synthesize nitric oxide (NO), effectively paralyzing local cellular defense mechanisms.⁹

This unique case unequivocally and powerfully demonstrates the absolute diagnostic necessity of advanced molecular techniques over traditional, antiquated modalities when clinicians are evaluating atypical genitourinary symptoms. The diagnosis of STIs in many parts of the world has historically relied on a combination of syndromic management protocols, basic microbial culture, and direct microscopic visualization.¹⁶ For the diagnosis of *Trichomonas vaginalis*, the traditional wet mount microscopic examination has long reigned as the clinical standard of care, primarily due to its extreme rapidity and very low operational cost. However, its diagnostic sensitivity is heavily and dangerously compromised, generally capturing and successfully diagnosing only 31% to 60% of true infections.¹⁶ A successful wet mount fundamentally requires a very high burden of live, highly motile trichomonads suspended in a fluid medium; therefore, in cases presenting as relatively dry, necrotic ulcers without the requisite fluid substrate of vaginal discharge, microscopy is essentially rendered obsolete and useless.¹⁶

Multiplex Real-Time PCR overcomes these hurdles by offering rapid, simultaneous detection of multiple fastidious pathogens within hours, providing superior analytical sensitivity and specificity compared to conventional methods. Traditional microbial culture for *U. urealyticum* is notoriously difficult, requiring highly specialized media, stringent transport conditions, and extended incubation periods that severely delay actionable clinical utility. The strategic deployment of Multiplex Real-Time PCR completely bypasses these limitations by relying exclusively on the highly targeted amplification of deeply conserved, pathogen-specific genetic sequences.¹⁷ This technology reliably uncovers hidden, complex co-infections that a targeted, narrow, symptom-based testing approach would entirely miss, making it medically indispensable for resolving atypical ulcerative presentations.¹⁰

The precise selection of the final therapeutic regimen was carefully guided by the highly distinct structural biology and metabolic profiles of the identified pathogens, aligning with the latest international guidelines. The selected multidrug regimen strictly adheres to the updated 2021 Centers for Disease Control and Prevention (CDC) Sexually Transmitted Infections Treatment Guidelines, which also heavily inform national protocols in Indonesia.²⁰ Azithromycin was administered as a 1-gram single dose, remaining a standard, highly effective targeted therapy to eradicate cell-wall-deficient *Ureaplasma urealyticum*. Because *Ureaplasma urealyticum* lacks a peptidoglycan cell wall, beta-lactam antibiotics are useless, and Azithromycin reliably achieves the excellent intracellular penetration necessary to clear these fastidious organisms.⁹

Crucially, for *Trichomonas vaginalis* in women, the CDC 2021 guidelines decisively replaced the historical 2-gram single-dose protocol with a 7-day, multi-dose regimen of Metronidazole (500 mg twice daily). This prolonged dosing maintains sustained protozoacidal tissue concentrations, significantly mitigating the risk of endosymbiont-mediated treatment failure. Robust clinical data definitively indicates that multi-dose metronidazole therapy significantly reduces the rate of persistent or rapid recurrent infections in women when compared directly to single-dose regimens. Finally, the strategic inclusion of Omeprazole successfully and effectively neutralized the cumulative, well-documented gastrointestinal toxicity of this dual high-dose antimicrobial therapy, ensuring the patient's absolute adherence to the critical 7-day curative course.²⁰

A primary and highly significant strength of this case report lies in its rigorous, uncompromising molecular approach to clinical diagnosis. The utilization of advanced Multiplex Real-Time PCR provided an unambiguous, irrefutable identification of the causative pathogens, rapidly transforming what would likely have been treated as an idiopathic, viral, or misdiagnosed ulcer into a clear, manageable, and actionable clinical entity. Furthermore, the report offers a deep, highly integrated analysis of the synergistic, molecular pathobiology occurring between protozoa and Mollicutes, effectively bridging the academic gap between complex molecular microbiology and atypical, real-world clinical phenotypes. A recognized limitation of this report is its inherent nature as a single, observational case study. Consequently, the highly specific clinical findings and treatment responses documented here cannot be broadly or universally generalized to establish new, sweeping population-level syndromic guidelines without further large-scale epidemiological validation. Additionally, while the Multiplex PCR definitively confirmed the presence of both organisms' nucleic acids, the assay itself does not quantitatively differentiate between an active, high-load pathogenic infection and low-level, transient commensal colonization for *Ureaplasma urealyticum*. Furthermore, while the presence of both organisms strongly implies endosymbiosis based on current literature, this specific clinical report does not provide direct, visual proof of intracellular endosymbiosis in this specific patient isolate without the use of highly specialized, complementary diagnostic tools such as transmission electron microscopy or targeted phylogenetic sequencing (e.g., to confirm the presence of specific, highly virulent strains like *Candidatus M. gireddii*).⁹

In conclusion, this specific clinical case serves as a powerful and vital reminder that *Trichomonas vaginalis* and *Ureaplasma urealyticum* possess the capacity to completely and dramatically subvert classic clinical expectations, presenting as isolated, painful genital ulcers without generating any accompanying vaginal discharge. The highly complex, evolved molecular symbiosis between these distinct pathogens likely drives unique, highly localized, and deeply destructive inflammatory phenotypes that perfectly mimic classic ulcerative sexually transmitted infections. Consequently, it is imperative that clinicians actively and aggressively expand their differential diagnosis for acute genital ulcers beyond the traditional scope of viruses and treponemes to include these synergistic, protozoal-bacterial organisms. Ultimately, the absolute reliance on highly sensitive, broad-spectrum Multiplex Real-Time PCR panels is no longer merely an academic ideal or a luxury of high-resource settings; it is a fundamental clinical necessity for rapidly resolving atypical presentations and ensuring the immediate deployment of precise, targeted, and curative multidrug therapies.

4. CONCLUSIONS AND SUGGESTIONS

In conclusion, this specific clinical case serves as a powerful and vital reminder that *Trichomonas vaginalis* and *Ureaplasma urealyticum* possess the capacity to completely and dramatically subvert classic clinical expectations, presenting as isolated, painful genital ulcers without generating any accompanying vaginal discharge. The highly complex, evolved molecular symbiosis between these distinct pathogens likely drives unique, highly localized, and deeply destructive inflammatory phenotypes that perfectly mimic classic ulcerative sexually transmitted infections. Consequently, it is imperative that clinicians actively and aggressively expand their differential diagnosis for acute genital ulcers beyond the traditional scope of viruses and treponemes to include these synergistic, protozoal-bacterial organisms. Ultimately, the absolute reliance on highly sensitive, broad-spectrum Multiplex Real-Time PCR panels is no longer merely an academic ideal or a luxury of high-resource settings; it is a fundamental clinical necessity for rapidly resolving atypical presentations and ensuring the immediate deployment of precise, targeted, and curative multidrug therapies.

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AUTHOR CONTRIBUTIONS

AH: Conceptualization, clinical investigation, diagnosis and patient management, data collection, methodology, writing – original draft, and visualization. YFL: Investigation, data curation, literature review, validation, and writing – review & editing. ANPR: Investigation, data collection, validation, and writing – review & editing. HCDRT: Investigation, data curation, validation, and writing – review & editing. PE: Methodology, data validation, and writing – review & editing. All authors contributed to the critical revision of the manuscript, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work..

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE IN THE MANUSCRIPT

The authors declare that Artificial Intelligence (AI) assistance was used during the preparation of this manuscript. The authors used ChatGPT (OpenAI) to assist with language editing, translation, sentence refinement, and improving the clarity and readability of the manuscript. The use of AI was limited to language-related assistance and editorial refinement. All scientific content, data analysis, interpretation, and final responsibility for the manuscript remain entirely with the authors.

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