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Using Wastewater-Based Epidemiology to Study Chlamydia Occurrence on a College Campus

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USING WASTEWATER-BASED EPIDEMIOLOGY TO STUDY CHLAMYDIA OCCURRENCE ON A COLLEGE CAMPUS

by

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ABSTRACT

Chlamydia is a sexually transmitted disease caused by *Chlamydia trachomatis,* commonly affecting sexually active college-aged adults. Presently, opportunistic testing, self-testing, and information campaigns are methods to screen vulnerable populations and raise awareness about chlamydia. Chlamydia remains underdiagnosed and undertested due to a lack of participation by individuals who may have been exposed to it. Wastewater-based epidemiology is a rising biomonitoring tool that detects the presence of disease- and drug-specific biomarkers in a community's wastewater. In this study, wastewater-based epidemiology was used to detect the presence of *C. trachomatis* on the University of Central Florida campus. Wastewater samples were collected from two locations on campus from January 2022 to December 2022. The samples were pasteurized and filtered. DNA was extracted from the filters and was subsequently quantified using qPCR. *C. trachomatis* was detected at both sites of the UCF campus, with peaks corresponding to periods of the academic semester at which students arrived on campus or had fewer academic responsibilities. It was concluded that wastewater-based epidemiology provided a low-cost and non-invasive tool to notify the public of potential chlamydia outbreaks and encourage testing. Exploration in wastewater-based epidemiology should continue in research of *C. trachomatis* detection.

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INTRODUCTION

Chlamydia is a sexually transmitted infection most seen in females. It is caused by the gram-negative obligate intracellular parasitic bacteria *Chlamydia trachomatis*. Chlamydia is transferred between two individuals through sexual intercourse or between an infected mother and her offspring through parturition. It is one of the most common sexually transmitted infections, with the vulnerable populations being young, sexually active women and men (Tjahyadi et al., 2022). Chlamydia typically presents asymptomatically, but it may cause symptoms such as a burning sensation during urination, vaginal discharge, vaginal bleeding after sex and/or between menstrual periods, and pelvic pain. Though chlamydia can resolve itself without treatment, it can also lead to adverse effects if left untreated in women, such as infertility, tubal scarring, ectopic pregnancy, pelvic inflammatory disease, and pregnancy complications (Baraitser et al., 2011). The current treatment for chlamydia is antibiotics (Tjahyadi et al., 2022). Medical clinics use screening programs to prevent the spread of chlamydia, detect early infections, and avoid worsening an unknown infection. However, given the asymptomatic nature of chlamydia, it is often difficult to estimate its prevalence in a community (Baraitser et al., 2011). Likewise, it is unlikely that vulnerable populations will voluntarily choose to be tested due to a lack of access to convenient testing, a lack of education about chlamydia, or a lack of privacy (Blake et al., 2003).

A community-based disease tracking and biomonitoring method that has grown in popularity in recent years is Wastewater-based Epidemiology (WBE) (Mao et al., 2021). WBE uses urine and feces samples from wastewater treatment plants or sewers to determine the presence of specific pathogens or drugs. For example, WBE has been used to track the poliovirus

(Hovi et al., 2012), heroin and other opiates (Du et al., 2017), antibiotics (Han et al., 2022), MRSA (Goldstein et al., 2012), and more recently Covid-19 (Daigle et al., 2022). WBE effectively provides an unbiased means to monitor public health and collect data from individuals unlikely to be tested for diseases while maintaining anonymity. WBE has proven helpful in preventing disease outbreaks in communities such as college campuses (Harris-Lovett et al., 2021), long-term care facilities (Davó et al., 2021), and correctional facilities (Wang et al., 2020) through early detection.

Currently, there is a gap in detecting chlamydia and preventing its spread in vulnerable populations. Voluntary screening cannot detect most chlamydia cases, given that only a minority of the vulnerable population is likely to test. Without a widespread method for detection, chlamydia can continue to circulate and remain untreated in asymptomatic individuals. To address this gap, WBE may be an ideal candidate. Previously, DNA originating from chlamydia has been detected in wastewater (Brisebois et al., 2018); thus, it is possible to use WBE methods in monitoring chlamydia in communities. Moreover, the use of WBE on college campuses, where the vulnerable population to chlamydia largely congregates, may be helpful in the prevention, screening of dormitories, and encouragement for individual testing in affected areas. WBE has already been successful in monitoring the spread of Covid-19 on college campuses (Daigle et al., 2022). Still, there is a paucity of information about its ability to track sexually transmitted infections like chlamydia.

LITERATURE REVIEW

Background of Chlamydia

Chlamydia is a sexually-transmitted disease prevalent in developed countries, such as the United States (Bosmans, 2014; Santer et al., 2000). It is caused by the gram-negative bacterium *Chlamydia trachomatis* and primarily affects the urogenital tract in both males and females (Bosmans, 2014; Tjahyadi et al., 2022). Due to its unique life cycle as an obligate intracellular parasite, *C. trachomatis* can effectively inhabit cells and promote its proliferation within a body while avoiding detection by the immune system of the host (Di Pietro et al., 2019; Moroni et al., 1996). *C. trachomatis* may also reside in the human gastrointestinal tract microbiome (Rank & Yeruva, 2014).

C. trachomatis strains can be divided into three types: trachoma, urogenital, and lymphogranuloma venereum. These types can be further divided into serovars; serovars are classifications of antigen variations within the same species of cells or viruses (Lancefield, 1933). Although they belong to the same species, cells or viruses may have diverse antigens on their surface. The trachoma type includes serovars A, B, and C. These serovars cause ocular chlamydia, or chlamydia of the eyes, non-specific eye irritation, and blindness in prolonged infections. The urogenital type includes serovars D, E, F, G, H, I, J, and K. These serovars cause genital tract infections. The lymphogranuloma venereum type includes serovars L1, L2, and L3. These serovars cause invasive anorectal or urogenital infections, common in HIV-infected men who have sexual contact with men. (Elwell et al., 2016)

Ocular chlamydia is typically transmitted from mother to child through vertical

transmission (Honkila et al., 2018). In adults, ocular chlamydia can be transmitted from hand to eye after coming into contact with infected genital secretions (Lee & Chen, 2022).

Urogenital chlamydia is spread through oral, vaginal, or anal sexual contact with an infected person or through the vaginal delivery of an infected mother to her newborn. The probability of chlamydia being transmitted during a sexual act depends on the type of sexual act, the frequency of sexual acts, and the extent of the relationship. Anal sexual contact is more likely to transmit chlamydia than vaginal sexual contact. Chlamydia is spread regardless of the infected person's symptoms or lack thereof (Tjahyadi et al., 2022). Lymphogranuloma venereum is transmitted similarly to urogenital chlamydia (White & Ison, 2008).

Chlamydia typically presents asymptomatically, with 80% of infected individuals having no symptoms throughout their infection. Asymptomatic cases do not correlate with less aggressive infections. In symptomatic cases, an infected female may have a burning sensation during urination, abnormal vaginal discharge, urinary symptoms, irregular vaginal bleeding, pelvic pain, cervicitis, abdominal pain, and dysuria. During prolonged infection, the immune response of an infected female can cause scarring and fibrosis in the fallopian tubes. Infected males can present with similar urinary discomfort. Due to the pervasive effects of chlamydia on the urogenital tract, it leaves the area vulnerable to other diseases or complications such as pelvic inflammatory disease (PID), tubal factor infertility, ectopic pregnancy, chronic pelvic pain, and human immunodeficiency virus (HIV). (Baraitser et al., 2011; Habel et al., 2016)

In Europe and the United States, 3-6% of sexually active individuals under 25 years old test positive for chlamydia. Due to a lack of participation in screening, it is difficult to accurately determine the prevalence of chlamydia (Baraitser et al., 2011; Tjahyadi et al., 2022). College students are most at risk of acquiring and spreading chlamydia due to their proclivity toward

unsafe sexual behaviors (Habel et al., 2016). Likewise, females have a 3.5-fold higher prevalence of chlamydia than males (Tjahyadi et al., 2022). This is due to the ease with which chlamydia can be established in the female urogenital tract compared to the male urogenital tract (Lewis et al., 2017). People with multiple or new sexual partners, who do not use contraceptives, or who have previously contracted an STI are also at high risk of acquiring chlamydia (Sipkin et al., 2003; Tjahyadi et al., 2022).

Chlamydia is most reliably diagnosed using a nucleic acid amplification test (NAAT). This test may be administered using cervical or vaginal swabs or urine samples. This form of testing produces results after several hours or days. Currently, rapid chlamydia tests are being developed for home use; these tests have a turnaround time between 60 minutes to 90 minutes. However, these tests have lower sensitivities than NAAT tests (Widdice et al., 2018). Chlamydia is cured with antibiotics (Tjahyadi et al., 2022).

Current Methods of Chlamydia Screening

Despite the preponderance of chlamydia in developed countries, people do not generally consent to test for it. This refusal of testing is mainly due to stigma, misinformation, and a false sense of safety. Many young people do not get tested over concern that someone will know they were tested or if they tested positive (Blake et al., 2003). This concern is rooted in the stigma that chlamydia testing is promiscuous and indicates irresponsibility and participation in risky behavior. Feeling as though one does not need to be tested reinforces the notion of goodness and purity, even if chlamydia testing is not genuinely rooted in morality (Balfe et al., 2010). Moreover, young people choose not to be tested for fear of discovering they have an STI or

immunodeficiency syndrome (Blake et al., 2003). This fear is grounded in misinformation, as many individuals do not know that chlamydia is easily treatable and not fatal (Balfe et al., 2010; Blake et al., 2003). Furthermore, many young adults perceive themselves as invulnerable to STIs like chlamydia because they trust that their partner will not infect them or because they do not frequently engage in risky behavior and are not promiscuous. The asymptomatic presentation of chlamydia also causes infected individuals to falsely believe they are not afflicted. Structural barriers such as the cost of the test, inconvenient scheduling, and long waiting times also prevent people from being tested (Balfe et al., 2010).

Given the personal nature of chlamydia, it has proven challenging to screen those in vulnerable populations. Many methods have been devised to prompt people to undergo testing. One such method is opportunistic testing which involves being tested in a clinic or doctor's office during a routine visit, regardless of the person presenting without chlamydia-like symptoms. This screening method can reduce the establishment of the infection in the body by detecting it before it progresses, thus decreasing its related morbidity and risk of other diseases (McNulty et al., 2004; Santer et al., 2000). Despite its benefits, fewer than 35% of individuals visiting a doctor's office for unrelated health concerns consent to a test (Santer et al., 2000). This phenomenon also occurs on college campuses with high populations of vulnerable individuals, with fewer than 10% of patients consenting to a test (Bosmans, 2014; Cohall et al.). This refusal of testing may likely be due to the lack of time patients are given to comprehend information about the test and decide if they are a proper candidate for it; patients are given minutes to agree to a test that must be taken at the time of their visit (Perkins et al., 2003). Likewise, opportunistic testing is unilaterally enforced; most screenings are recommended for sexually active females, leaving non-sexually active females underdiagnosed in these settings (Tjahyadi et al., 2022).

Furthermore, the lack of widespread opportunistic screening for males causes them to be underdiagnosed and portrays females as the causative agents of chlamydia, despite males also transmitting it (Perkins et al., 2003).

Self-testing was introduced to bridge the gap in opportunistic testing. Self-testing allows individuals to administer the test themselves and send their samples to a lab to determine their results. This form of screening bypasses any discomfort one would have with pelvic examination, embarrassment in agreeing to test, appointment wait times, the financial burden of clinic visits, missed work time, and travel to appointments (Rose et al., 2010). However, this method also has low participation. Those who receive self-testing kits may choose not to complete the kit or return the completed test to a lab. Self-testing necessitates individuals to identify themselves as at risk for chlamydia; this allows those in the vulnerable population to mistakenly classify themselves as safe from infection. Furthermore, where opportunistic testing is too personal, self-testing is too impersonal. People may choose not to participate in self-testing because they do not want to complete laboratory forms or send their sample through the mail without knowing who exactly is receiving it and what they may do with that information (Rose et al., 2010).

Information campaigns about chlamydia can be used in combination with opportunistic testing and self-testing to improve their efficacy. These campaigns involve improving awareness of chlamydia and how it is spread to motivate people to consent to a test. These campaigns may incentivize testing to gain groups that would not have customarily participated without perceived reward (Anderson et al., 2016). It has been shown that information campaigns do increase testing in specific populations. However, they prove to be difficult to orchestrate as they may not cater to the entirety of the target population and, as such, will not persuade everyone to be tested

(Gobin et al., 2013).

Background of Wastewater-Based Epidemiology

Wastewater-based epidemiology (WBE) is a low-cost biomonitoring tool that interprets disease- or drug-related biomarkers excreted in the urine and feces found in wastewater (Mao et al., 2021). The biomarkers are human or disease-specific and detectable, stable, and invariable in wastewater (Yang et al., 2017). This technique is useful for its near real-time response and ability to provide qualitative and quantitative information on the health of a community by relating concentrations of biomarkers to the population scale (Choi et al., 2018; Mao et al., 2021). The principal steps of WBE include collecting, pretreating and concentrating samples, extracting the RNA or DNA, and performing polymerase chain reaction (PCR) on the extracted RNA or DNA (Kabdasli & Tunay, 2021).

WBE has effectively tracked the poliovirus (Hovi et al., 2012), heroin and other opiates (Du et al., 2017), antibiotics (Han et al., 2022), MRSA (Goldstein et al., 2012), and Covid-19 (Daigle et al., 2022). In the case of Covid-19, the results obtained from WBE typically mirror the trends in new Covid-19 cases and can sometimes predict future trends in the clinical surveillance (Tiwari et al., 2022; Xiao et al., 2022). Thus, WBE can alert the public to the potential of an outbreak (Murakami et al., 2020). WBE also successfully determines if a community is free of Covid-19 (Godinez et al., 2022). However, WBE is not infallible; the results obtained can be impacted by the physical and chemical interactions within the wastewater (Tiwari et al., 2022). Likewise, non-detection of biomarkers can occur depending on when the sample is collected (Bowes et al., 2022). WBE cannot distinguish between disease biomarkers shed after the

infection has already cleared and disease biomarkers from ongoing cases (Murakami et al., 2020).

Unlike traditional clinical testing, WBE is non-invasive, and the results cannot be traced to specific individuals (Choi et al., 2018). It bypasses any stigma related to clinical testing, privacy concerns, and legal or ethical issues (Harris-Lovett et al., 2021). Additionally, it does not rely on a person's desire or ability to be tested, allowing for the detection of diseases that may not have been identified otherwise (Tiwari et al., 2022). Given that it is performed on a large scale, population data can be normalized and used to compare different communities (Choi et al., 2018).

Although WBE can be advantageous for its low cost and extensive reach, it still has numerous challenges yet to be overcome. WBE is inaccessible in developing or underdeveloped countries with no established sewage treatment plants (Goncalves et al., 2022; Saini & Deepak, 2021). Likewise, WBE requires access to a laboratory to analyze samples (Mao et al., 2021). To facilitate WBE, some sewage systems may need alterations in plumbing to improve access for wastewater sampling, prevent clogging, and enhance flow. Samplers are also at risk of being tampered with or damaged due to extreme water conditions (Harris-Lovett et al., 2021). Moreover, wastewater is an uncontrolled medium to work with due to the diversity of microorganisms and biomolecules present (Mao et al., 2021). This diversity leads to interspecies competition and may prevent the detection of some biomarkers (Harris-Lovett et al., 2021). Results obtained from WBE must be calibrated before data can be compared due to the complexity of wastewater composition. Wastewater may also contain PCR inhibitors that can affect the results and potentially lead to false positives or negatives. Wastewater samples are vulnerable to changes in weather and temperature; certain biomarkers may not survive the

transfer from the sampling site or storage if they are not kept in optimal living conditions (Mao et al., 2021).

Biomarkers are highly diluted in wastewater due to the variable composition of its environment (Jafferali et al., 2021). To obtain accurate results and improve the likelihood of detecting specific biomarkers, wastewater samples are concentrated using methods such as filtration, flocculation, ultracentrifugation, or ultrafiltration. The efficiency of a concentration method is assessed by its simplicity, rate of processing samples, amount of samples it can process, reproducibility, and cost (Saini & Deepak, 2021). Concentrating wastewater on a filter involves passing the sample through a specialized filter that can adsorb the targeted biomarkers (Kabdasli & Tunay, 2021; Lu et al., 2020). Flocculation uses a coagulating agent like skimmed milk to facilitate easier separation of biomarkers from other components of the wastewater (Kabdasli & Tunay, 2021). Ultracentrifugation is a form of centrifugation that uses higher speeds to separate the components of a sample (Daigle et al., 2022; Kabdasli & Tunay, 2021). Ultrafiltration is a form of membrane filtration that involves forcing a sample through a semipermeable membrane; it is typically coupled with centrifugation to provide the most optimal results (Jafferali et al., 2021; Kabdasli & Tunay, 2021; Saini & Deepak, 2021). Ultrafiltration is the preferred method of concentration for WBE and has been recommended by the World Health Organization as a reliable concentration technique (Kabdasli & Tunay, 2021).

During the Covid-19 pandemic, wastewater surveillance provided a convenient way of detecting the presence of Covid-19 in communities. It has been especially crucial after the creation of vaccines due to the increase in asymptomatic infections and the decrease in clinical testing (Daigle et al., 2022). WBE was implemented in numerous cities and on college campuses to alert the campus community to potential outbreaks of Covid-19 (Betancourt et al., 2021;

Harris-Lovett et al., 2021). As of July 2022, 279 college campuses have implemented WBE for tracking Covid-19 (Researchers, 2022). Given its establishment on college campuses globally and in similar communities, WBE is a promising tool for monitoring other diseases, like STIs.

Background of PCR

PCR is a molecular technique that detects genomic targets, such as DNA and RNA, within a sample and amplifies them through a polymerase chain reaction. It is used for diagnosing diseases, identifying microorganisms, and sequencing genes, rapidly and sensitively. PCR requires DNA polymerase (an enzyme that copies DNA), an extracted DNA sample, forward and reverse primers (short sequences of DNA that match a target sequence), and nucleotides (the building components of DNA). PCR involves three repeating steps of denaturation, annealing, and elongation, which are done at distinct temperatures depending on the primers and DNA involved. During denaturation, the reaction is heated to a high temperature to facilitate the separation of double-stranded DNA into two single strands of DNA. During the annealing step, the temperature is lowered, and the forward and reverse primers anneal to a specific area of the single-stranded DNA. The temperature at which this step takes place depends on the melting temperature of the primers. During the elongation step, the temperature is slightly increased to allow the DNA polymerase to elongate the single strands of DNA. These three steps are repeated 30-40 times until the targeted DNA has reached a suitable concentration for analysis. (Domingues, 2017; Shahi et al., 2018)

To quantify the DNA product for more precise analysis, qPCR (quantitative PCR) is utilized. qPCR uses the same foundational steps as PCR but uses fluorescent probes or dyes that

fluoresce at specific wavelengths and can be quantified. An example of a fluorescent probe used in qPCR is TaqMan, an oligonucleotide probe complementary to the target. It is labeled with a fluorescent reporter dye at the 5'-end and a fluorescent quencher at the 3', stopping the fluorescence after a period. During the extension step, the fluorescence created by the reporter dye on the 5'-end is measured. Standard curves are generated to quantify the fluorescence during the reaction, allowing one to determine the concentration of DNA in a sample. Melt curves are also generated to ensure that the correct targets were amplified. (Evans, 2009)

MATERIALS AND METHODS

Bacterial strains. Genomic DNA from *Chlamydia trachomatis* serovar D strain UW-3/Cx (ATCC VR-885D™) was obtained from the ATCC Global Bioresource Center (Manassas, VA) for use to assess the analytical specificity of the *C. trachomatis*-specific primers and probes. The *C. trachomatis* gene copy number was determined for each DNA extract by qPCR with a quantified *C. trachomatis* standard created from the genomic DNA.

Sample collection and pasteurization. Wastewater samples were collected during the University of Central Florida wastewater collection for Covid-19 analysis by the Beazley lab. The collection began in January 2022 and concluded in December 2022. Every step of the methods was completed using sterile techniques. Samples were allocated from the collected wastewater in two 40 mL replicates for each site, including the North and South areas of the UCF campus. The samples were pasteurized in a hot water bath of 65°C for 45 minutes to inactivate any viruses that may have been present. Samples were then stored at 4°C until they were filtered.

Sample filtration. Samples were typically filtered on the same day or within two days of collection. Filtration was done using 150 mL ThermoFisher Scientific™ Nalgene™ Sterile Analytical Filter Units (130-4020); each sample was poured into its respective filter unit while it was attached to a vacuum. The filters were removed from the unit and stored at -20°C until DNA extraction.

DNA extraction. DNA from the filters was extracted using the Qiagen DNeasy® Powerlyzer® Powersoil® Kit (12855-50) according to the manufacturer's specifications. DNA was eluted into final volumes of 100 µL of the kit non-EDTA elution buffer and then stored at -

20ºC until qPCR was completed.

qPCR assay. qPCR primers and 5'-nuclease hydrolysis TaqMan™ MGB **p**robes were purchased based on the primers developed by Stevens et. al (2010). The primers targeted *ompA* nucleotide sequences derived from *C. trachomatis* reference strains. All qPCR reactions were performed using the ThermoFisher Scientific Applied Biosystems™ StepOnePlus™ Real-Time PCR system, with each reaction comprised of a 25 µL volume containing 12.5 µL of TaqMan® Environmental Master Mix 2.0, 2 µL each primer (5 µM), 2 µL each probe (1 µM), and 4.5 µL of extracted DNA. Genomic DNA from *C. trachomatis* serovar D was used as the positive control, and molecular-grade water was used as the negative control. The PCR cycling conditions**,** primer sequence, and probe sequences are listed in Table 1.

The *C. trachomatis* group-specific multiplex PCR used one primer and two probes. The CT probe was specific to all C. trachomatis serovars and the B probe was specific to serovars B, E, D, L1, and L2 (Table 1). Serovar B is associated with ocular chlamydia, serovars E and D are associated with urogenital chlamydia, and serovars L1 and L2 are associated with lymphogranuloma venereum. The standard curves were generated using 1 gc/µL – 10000 gc/µL *C. trachomatis* serovar D genomic DNA.

qPCR data analysis. All qPCR data were analyzed using Microsoft Excel using the LINEST function to determine the concentration of *C. trachomatis* gene copies/µL of wastewater. Equation 1 was used to determine the concentration of *C. trachomatis* gene copies/mL of wastewater.

Concentration 1 * Volume 1 = Concentration 2 * Volume 2

Equation 1: The solution dilution formula

Table 1: qPCR Primer and Probe Sequences and Amplification Parameters (Sequences (5'-3')")

Primers	CT-F	CATGARTGGCAAGCAAGTTTA
	CT-R	GCAATACCGCAAGATTTTCTAG
Probes	CT	VIC- TGTTCACTCCYTACATTGGAGT-NFQ-MGB
	B	FAM-TTTCACMTCGCCAGCTCC-NFQ-MGB
PCR Conditions	1 cycle at 50ºC for 2 min, 1 cycle at 95ºC for 10 min, 45 cycles at 95ºC for 15 sec, 45 cycles at 60ºC for 45 for 10 min	

RESULTS AND DISCUSSION

Recent WBE studies have successfully detected and quantified the presence of the poliovirus (Hovi et al., 2012), MRSA (Goldstein et al., 2012), Covid-19 (Daigle et al., 2022), heroin and other opiates (Du et al., 2017), and antibiotics (Han et al., 2022). Currently, there are no studies that have attempted to detect and quantify *C. trachomatis* in wastewater. Thus, in this study, using filtration, DNA extraction, and qPCR, *C. trachomatis* was detected and quantified from wastewater collected on the North and South locations of the UCF campus.

qPCR assay results. This study adapted the qPCR protocol developed by Stevens et al., which was intended for use with clinical specimens obtained directly from patients (Stevens et al., 2010). Wastewater samples were used in place of clinical specimens. There were seven total runs of qPCR wherein an average of 6 samples were processed. The CT probe standards had an average efficiency of 87.4% and the B probe standards had an average efficiency of 84.6%. The CT probe standard curve had an R^2 range of 0.986 ± 0.011, and the B probe standard curve had an R^2 range of 0.986 ± 0.007. The average slope of the CT probe standard curve was -3.708 ± 0.120. The average slope of the B probe standard curve was -3.774 ± 0.101.

There was a total of 39 samples from each site on the UCF campus. Each duplicate of the North and South samples was run in triplicate. A sample was considered positive if at least three out of six of the replicates had a determinable concentration of *C. trachomatis*. The average concentration of *C. trachomatis* gene copies per mL wastewater is depicted in graphs 1 and 2 for each probe.

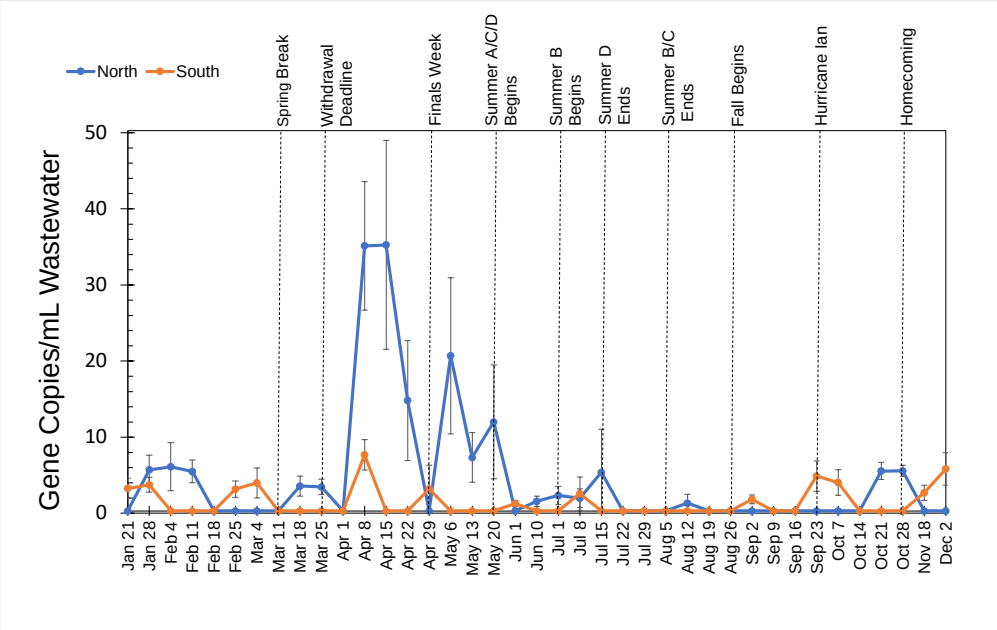

Figure 1: C. trachomatis gene copies per mL wastewater as detected by the CT probe. Samples were collected in 2022. Undetermined concentrations were reported as 0.

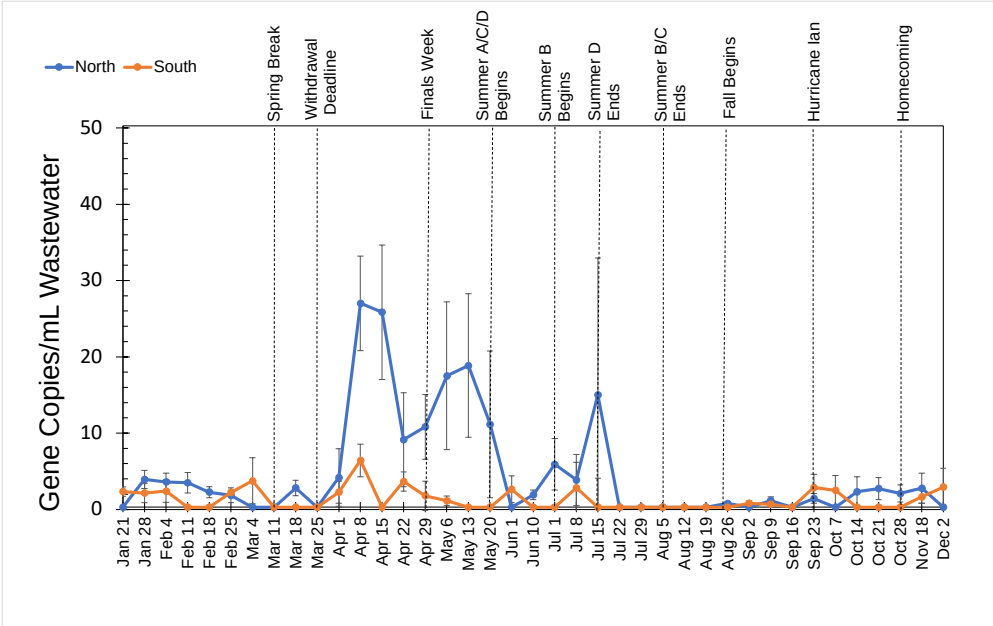

Figure 2: C. trachomatis gene copies per mL wastewater as detected by the B probe. Samples were collected in 2022. Undetermined concentrations were reported as 0.

Significance of chlamydia testing. Chlamydia is a sexually transmitted disease that can cause aggressive lifelong symptoms if left untreated (Baraitser et al., 2011; Bosmans, 2014). It typically affects sexually active, college-aged adults (Habel et al., 2016). Due to a lack of education about chlamydia and negative societal stigma, many individuals choose not to participate in testing (Balfe et al., 2010). Currently, opportunistic testing, self-testing, and information campaigns have been used to educate and screen vulnerable populations (Anderson et al., 2016; McNulty et al., 2004; Rose et al., 2010). These methods require active participation from the patient; individuals must identify themselves as needing testing. Those in the vulnerable population may fail to classify themselves as at risk due to feelings of infallibility, promiscuity, immorality, or fear (Balfe et al., 2010; Blake et al., 2003). To reinforce the current methods of screening and mitigate the effects of sexual stigma, wastewater-based epidemiology (WBE) may provide a necessary bridge.

Wastewater-based epidemiology. WBE is a cost-effective and non-invasive biomonitoring tool that detects biomarkers that can be found in feces or urine that is collected in wastewater (Mao et al., 2021). WBE can provide a rapid assessment of a community's health without giving traceable information about specific individuals (Choi et al., 2018). However, the results can be impacted by other components in the wastewater that may prevent the detection of biomarkers or contain PCR inhibitors (Harris-Lovett et al., 2021; Mao et al., 2021). The detection of biomarkers is time-sensitive and can be affected by weather conditions (Bowes et al., 2022; Mao et al., 2021). Likewise, WBE cannot differentiate between biomarkers shed during active infection or after (Murakami et al., 2020). Used in conjunction with opportunistic testing, self-testing, and information campaigns, WBE can alert communities of potential outbreaks and encourage individuals to seek testing.

Probes. The probes utilized in this study were specific to multiple serovars of chlamydia. The CT probe targeted a consensus sequence found in all serovars of chlamydia. The B probe targeted sequences found in the B, D, E, L1, and L2 serovars. The B probe detected more positive samples from both locations of the UCF campus than the CT probe. This discrepancy may have been caused by the complexity and uncontrolled nature of wastewater. Likewise, the B probe has a higher specificity for its targets, giving it a higher affinity and likelihood to contact its target sequences. Two other probes, the I probe, and the C probe, were purchased to target other serovars of chlamydia. The I probe targeted the F and G serovars. The C probe targeted the H, I, L3, A, K, J, and C serovars. A positive control that corresponded to each probe was unavailable; thus, the I and C probes could not be quantified through a standard curve.

C. trachomatis detection. The CT probe detected 18 positive samples out of the 39 (46%) samples obtained from the North location of the UCF campus and 14 positive samples (36%) from the South location. The B probe detected 24 positive samples (62%) obtained from the North location and 18 positive samples (46%) from the South location. The peaks in gene copies per mL (gc/mL) wastewater correspond with dates when students or visitors arrive on campus or have fewer academic constraints, such as after finals week or the week of the withdrawal deadline.

Confounding factors. Due to the sensitivity of wastewater and its biomarkers, there may have been complications with detecting *C. trachomatis*. Pasteurization served to inactivate any active pathogens in the wastewater. Given the high temperatures necessary for inactivation, DNA may have been destroyed in the sample, leading to a lower concentration of detectable *C. trachomatis* DNA. Although DNA is more stable than RNA, it can still be denatured by high temperatures. Additionally, for more accurate results, wastewater must be filtered as soon as

possible after collection (Mao et al., 2021). Most samples were filtered within one to two days of collection. However, the samples collected during Hurricane Ian were unable to be processed until after the UCF campus reopened. Thus, their late filtration may have led to suboptimal conditions for DNA extraction and PCR.

Future considerations. To continue this study, it will be beneficial to identify a positive control that can be used for more probes; this would allow for a broader detection of other serovars of chlamydia. Moreover, collected samples should be filtered in a timely manner. Other methods of filtration such as flocculation, ultrafiltration, and ultracentrifugation can be attempted to determine the most effective approach for concentrating wastewater. Non-thermal methods of pasteurization could also be explored to limit the amount of DNA lost due to high-temperature pasteurization (Chiozzi et al., 2022).

CONCLUSION

Chlamydia remains underdiagnosed in vulnerable populations and can cause significant long-term effects if left untreated (Baraitser et al., 2011; Tjahyadi et al., 2022). Societal stigmas and a lack of education about chlamydia greatly affect one's ability to determine the necessity of testing (Balfe et al., 2010). Current methods of screening have been unsuccessful in garnering considerable participation (Bosmans, 2014; Gobin et al., 2013; Rose et al., 2010).

Wastewater-based epidemiology is a low-cost and non-invasive tool to detect the presence of drug- and disease-related biomarkers (Mao et al., 2021). Used in combination with the current chlamydia screening methods, WBE can locate outbreaks and promote testing in affected communities.

Wastewater samples were collected from two locations on the UCF campus from January 2022 to December 2022. The samples were filtered using 150 mL ThermoFisher Scientific™ Nalgene™ Sterile Analytical Filter Units (130-4020). The DNA was extracted using the Qiagen DNeasy® Powerlyzer® Powersoil® Kit (12855-50). qPCR was run on the eluted DNA, and the results were processed using LINEST and Equation 1.

C. trachomatis was successfully detected in wastewater by both probes utilized in this study. There is potential for WBE to be used as a biomonitoring technique for the detection of chlamydia in communities with a large presence of vulnerable populations, such as college campuses. Refinement of this technique can lead to more awareness about the presence of chlamydia in a community and more encouragement to seek testing. Given that WBE provides information about which locations are affected, information campaigns and accessible testing can target those specific areas. Future work in WBE can use this study as an example of the

effectiveness of *C. trachomatis* detection in wastewater.

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