

ORIGINAL ARTICLE

**Chlamydia Trachomatis: Conjunctivitis in the Andaman and Nicobar Islands population**

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**ABSTRACT**

*Trachoma is one of the Neglected Tropical Disease caused by Chlamydia trachomatis, an intracellular bacteria which develops the follicular conjunctivitis during acute infection. Conjunctival swabs were collected during 2017-2022. Genomic DNA was extracted and qPCR and Conventional PCR for plasmid gene was performed to determine the presence of Chlamydia. During the study period, 470 conjunctival swabs were screened and a total of 7 were found to be positive for Chlamydia trachomatis from which 2 were positive for Plasmid gene. Acute Chlamydia infections mimics the common kerato-conjunctivitis symptoms and molecular epidemiology research makes it possible to identify vulnerable populations.*

**Keywords:-** Conjunctivitis, Chlamydia trachomatis, Cryptic plasmid, Covid-19, Andaman and Nicobar Islands.

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**INTRODUCTION**

Conjunctivitis, inflammation of the conjunctival mucosa, is a frequent eye disorder caused by viral, bacterial, autoimmune, toxic, and allergic factors. Trachoma is neglected tropical disease caused by CT that develops follicular inflammation of the eye [1, 2]. WHO states this public health issue blinds 1.9 million people worldwide every year and can result in irreversible blindness [3]. CT is an obligate intracellular gram-negative bacterium that can primarily affects mucosal surfaces, grows in the host cell for their metabolism [4]. The disease spreads most often through direct contact with an infected individual or indirect contact with their fluids via autoinoculation [5,6].

The genome of *Chlamydia* consists of a chromosome with 900 genes and 1MB in size and a 7.5 KB multicopy plasmid with 8 genes [7,8] that act as virulence factors. In animal studies, plasmid-free *chlamydial* strains showed reduced pathogenicity and inflammation [9]. All 19 *Chlamydia* serovars can cause human illness. Based on the site of infection, it is classified into three groups: A, B, Ba, and C. When they come into contact with genital secretions, *Chlamydia* serovars D, Da, E, F, G, Ga, H, I, Ia, J, and K cause ophthalmia neonatarium and inclusion conjunctivitis [10, 11].

India's union territory of Andaman and Nicobar Islands has 4,34,192 residents (2019) [12]. This study is to examine Ocular *Chlamydia* and to know the prevalence of CT in this archipelago. Furthermore, it is important to evaluate the effect of Covid-19 on the ocular CT infection.

**MATERIAL AND METHODS**

During August 2017 to January 2022, 470 people with keratoconjunctivitis symptoms were enrolled, and conjunctival swabs were collected in VTM. To facilitate genomic DNA extraction, the swab samples were pelleted and re-suspended in 400 µl of PBS. DNA was extracted from a 200-µl sample suspension using the Pure Link Genomic DNA Mini Kit (Invitrogen-cat no. K1820-01) and eluted with 45µl of elution buffer. A commercially available diagnostic real-time PCR kit for *Chlamydia* was used for initial screening

(HELINI *Chlamydia trachomatis* real-time PCR kit, Cat. No. 8205), and reaction conditions were according to the instructions provided with the kit.

In addition, all clinical samples were re-examined for the presence of cryptic plasmid gene using primers specific for the KL gene [13]. PCR reaction mix (25 µl) was prepared for amplification comprise of 2x PCR master mix (Cat. No. K0171), DNA template, and primers, respectively. The following cycling settings were used for the amplification: 94°C for 1 min., 64°C for 1 min., and 72°C for 1 min for 40 cycles, using an Applied Biosystems (Veriti 96) well thermal cycler (USA). Positive PCR results for *C. trachomatis* were determined when a band of size 241 bp was observed in the 2% agarose gel electrophoresis (AGE). To guarantee the purity of the isolated DNA, as an internal control, Beta-globin gene was used [14].

## RESULTS

During August 2017 to January 2022, conjunctival swab specimens from 470 individuals were collected and screened for the presence of *Chlamydia trachomatis*, to determine the prevalence of *chlamydia* associated with keratoconjunctivitis. In the first round of testing, 1.5% of patients tested positive for *Chlamydia trachomatis* (7 out of a total of n=470). Two out of 470 samples (0.4%) were tested positive for a plasmid gene unique to *Chlamydia trachomatis*. Those who had a positive *Chlamydia* test displayed symptoms within a week. Female (57%) patients were shown to have a higher prevalence of *Chlamydia* infection than male patients (42.9%). The average age of confirmed cases were 26 years (IQR-interquartile range, Q1-Q3). Approximately (57.1%) of confirmed cases of associated keratoconjunctivitis included only one eye. Eye redness (100%), itching (71.4%), ocular pain (71.4%) and swollen eyelids (57.1%) were the most often reported symptoms (Table 1).

**Table 1:- Distribution of symptoms among *C. trachomatis* -infected keratoconjunctivitis patients.**

No. of Positive Cases	No. of Patients (N=7)
Male	3
Female	4
Unilateral	4
Bilateral	3
Symptoms	
Redness of Eye	7
Eye Irritation/Itching	5
Eye pain	5
Swelling of eyelid	4
Swelling of Conjunctiva	3
Increased Tearing	3
Blurry vision	3
Photophobia	2
Follicles	2
SPK	2
Lid Edema	1

## DISCUSSION

*Chlamydia* that cause disease in humans come from a thriving and distinct bacterial family [15]. Since most *chlamydial* infections are asymptomatic, they often go misdiagnosed and untreated, resulting in persistent inflammation. Similarly, during this study patients presenting with typical keratoconjunctivitis symptoms were also found to have *Chlamydia* infection, irrespective of the associated symptoms. Recurrent infections are prevalent, and acquiring significant ophthalmic (irreversible blindness) or urogenital (infertility) sequelae increases with many infections [16].

The plasmid *chlamydial* was originally considered as the "*cryptic plasmid of Chlamydia*" due to its lack of visibility for a long time [17]. Earlier study with wild-type and plasmid-free *C. trachomatis* strains in the urogenital tract of mouse showed that the plasmid regulates infectivity [18]. The infection caused by the *C. trachomatis* bearing plasmid was responsible for a global epidemic with increased severity [19, 20]. In this study, two out of seven were found to be carrying the plasmid. It is a well-documented occurrence that CT can be detected in urogenital tract specimens despite the absence of any sexual contact due to autoinoculation of *C. trachomatis* from the ocular infection to the urogenital tract region [21].

Another study showed that plasmids have diverged from the common bacterial ancestor of all species. High level of conserved genome across the genus *Chlamydia*, suggested that there is considerable

evolutionary selection in favor of *chlamydia species* preserving their plasmids. [22] The absence of the plasmid gene in the respiratory strain, however, makes this a real possibility. Additionally, findings of this study revealed that the prevalence of ocular *Chlamydia* dropped dramatically during and after Covid-19, with no positive cases being found at all (from 2017-2019 all 7 cases were detected, whereas during 2020-2022 none of the case found positive). That could be attributable to both good hand hygiene and non-pharmaceutical interventions among the population.

## CONCLUSION

The early stages of *C. trachomatis* infections mimics the common keratoconjunctivitis symptoms, molecular epidemiology makes it possible to identify this among vulnerable populations and societal structures. Apart from this, the non-pharmaceutical measures during Covid-19 played a vital role in reducing the transmission of *C. trachomatis* infection. Therefore, the crucial information gathered from this study can be used to create screening programs, focused preventative strategies, and therapeutic measures to decline the infection burden in future.

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## ETHICAL APPROVAL

This study was approved by the Institutional Human Ethical Committee, Jawaharlal institute of post-graduate medical education and research (JIPMER), Pondicherry for the project entitled "A multi-centric hospital based study on epidemiology of keratoconjunctivitis in India" (JIP/IEC/20 17 /0279).

## INFORMED CONSENT

Informed consent was obtained from each individual enrolled in this study. A written informed consent form was obtained before the sample collection.

## AUTHOR CONTRIBUTIONS

Conceptualization, NM, NB; methodology, NB; data curation, NB; writing–original draft preparation, NB; writing–review and editing, AV, and NB.

## DISCLOSURE STATEMENT

No conflict of interest was reported by the authors.

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**Abbreviations:-** CT- Chlamydia trachomatis, PCR- Polymerase Chain Reaction, SPK- Superficial Punctate Keratitis, DNA- Deoxyribonucleic acid, VTM- Viral transport medium, WHO- World Health Organisation.

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