Chapter 7

Summary
7. SUMMARY

7.1 GENITAL CHLAMYDIAL INFECTION IN THE APPARENTLY HEALTHY POPULATION OF TAMIL NADU

The epidemiology of genital chlamydial infection in the apparently healthy population of Tamil Nadu was studied by taking a representative sample size from 90 randomly selected clusters in three different districts, Tanjore, Ramnad and Dindigul based on the 'Probability Proportional to Size' (PPS) cluster survey technique. The selected study population consisted of 1849 subjects including 1066 females and 783 males of sexually active age (15-45 yrs). Both serology and molecular methods were used to study the prevalence of genital chlamydial infection in them.

Using *Chlamydia trachomatis* IgM ELISA, the overall seroprevalence of genital chlamydial infection was 2.4% (95% CI 1.6%-3.2%) (45/1849).

Employing PCR in urine samples as the methodology, the rate of active genital chlamydial infection as evidenced by the presence of CT-DNA was found to be 1.1% (95% CI 0.5%-1.7%) (16/1444).
Seropositivity was significantly high in females (3.3%) compared to that in males (1.3%) \((P<0.05)\). In females, the positivity rates with respect to the age groups differed significantly \((P=0.0001)\), while no significant differences were observed in the case of males \((P<0.05)\). In both gender, younger age groups appeared to have higher positivity rate. No positivity was observed in 41-45 yrs age group. Overall, the differences in the positivity rates between age groups were statistically significant \((P=0.0000)\).

The PCR results showed similar prevalence in males (1.2%) and females (1.1%). In both gender, 31-35 yrs age group had a higher positivity rate, while no positivity was seen in the age groups 15-20 yrs and 36-40 yrs. Overall, the differences in the positivity rates between the age groups showed statistical significance \((P<0.05)\), eventhough these differences were not significant when analysed separately for males and females.

The analysis of district wise positivity revealed a higher seroprevalence of 3.7% (21/570) in Tanjore compared to 1.5% (10/646) in Ramnad and 2.2% (14/633) in Dindigul.

Urine PCR analysis showed a higher positivity for CT-DNA in Dindigul 1.5% (8/534) compared to Ramnad 0.9% (4/470) and Tanjore 0.9% (4/440).
By taking IgM antibody and chlamydial DNA positivity, an overall chlamydial infection positivity of 3% (95% CI 2.2%-3.8%) was observed in the general population of Tamil Nadu.

No symptoms were observed in majority of the study population. Discharge (30.2%) was the major symptom observed among the study subjects and also when analysed against positivity. Discharge was seen in nearly half (48.9%) of the IgM ELISA positive cases and in 25% of the urine PCR positive cases. It was observed that majority of the PCR positive cases (68.8%) did not have any symptom.

The decoded results of the quality control study revealed an absolute agreement with the laboratory testing performance and between PCR and LCR methods adopted.

A poor correlation was observed between the serology and PCR. Out of 1443 subjects tested by both PCR and IgM ELISA, only 5 (0.3%) were positive for both the tests while PCR additionally picked up 11 (0.8%) positives from the IgM negative group.

Risk factor variables like young age (<30 yrs), premarital sex, extramarital sex, CSW visit, non-use of condoms, multiple sexual partners, homosexuality and infected spouse were analysed. None of these risk factors showed significant
correlation with ELISA positivity as well as PCR positivity in this study population of apparently healthy adults.

7.2 GENITAL CHLAMYDIAL INFECTION IN SYMPTOMATIC STD PATIENTS (n=143).

- By using conventional and molecular markers, the overall prevalence of proven *C. trachomatis* infection in this study population of symptomatic STD patients was 30.8% (95% CI 23.5%-39.1%).

- A higher prevalence rate was observed in men (34.9%) than women (27.5%). Younger age groups had higher positivity rate.

7.2.1 Clinical and Microbiological correlations

- Among the 143 cases enrolled, there were 58 (40.5%) urethritis cases, 53 (37.1%) cervicitis cases, 22 (15.4%) PID cases, 5 (3.4%) cases of epididymitis and 5 (3.4%) cases of infertility.

- Confirmed *C. trachomatis* positivity was observed in 36.2% (21/58) of the urethritis cases; 30.2% (16/53) of the cervicitis cases; 18.2% (4/22) of the PID cases; 1 out of the 5 epididymitis cases and 2 out of the 5 infertility cases.
Among the genitourinary symptoms observed in the study subjects, discharge (66.4%) and dysuria (49.7%) were found to be the major symptoms. Other symptoms observed were inflammation (23.8%), pain (16.8%), itching (16.1%), and rashes (7.0%). Among the PID cases, lower abdominal pain was seen in 86.3% and chronic pelvic pain was observed in 63.6%.

In the proven cases of genital chlamydial infection, dysuria (61.4%) and discharge (59.1%) were found to be the predominant symptoms. When these two symptoms were analysed separately and in combination with the positive cases, 36.4% had only dysuria, 34.1% of the cases had only discharge, and 25% cases experienced both the symptoms. However, these correlations were not statistically significant. Other symptoms showed poor correlation with C. trachomatis positivity.

Risk factor analysis included variables like young age (defined as <30 yrs), premarital sex, extra marital sex, visit to CSW (males), multipartner sex, past history of STDs, non-use of condoms (males), use of oral contraceptives (OCs) and intrauterine devices (IUD) in women.

C. trachomatis infection was seen to be associated with risk factors like premarital sex, multiple numbers of sexual partners and the use of oral contraceptives (OC) in women. Other risk factors did not show significant correlation with Chlamydia positivity.
7.3 EVALUATION OF CONVENTIONAL AND MOLECULAR MARKERS FOR THE DIAGNOSIS OF GENITAL CHLAMYDIAL INFECTION.

7.3.1 Isolation in Cell culture

- *C. trachomatis* was isolated from 27 (18.9%) out of the total 143 cases by cell culture using McCoy cell line. Culture positivity was 17.5% (11/63) and 20% (16/80) in males and females respectively with no significant difference in the isolation rates.

- Higher isolation rate was observed in younger age groups.

- *C. trachomatis* isolation was seen in 19% (11/58) of the urethritis cases, 20.8% (11/53) of the cervicitis cases, 13.6% (3/22) of the PID cases, 1 out of the 5 epididymitis cases and 1 out of the 5 infertility cases.

7.3.2 Antigen Detection by DFA.

- DFA detected *C. trachomatis* specific antigen in 35 (24.5%) cases and showed good correlation with isolation in cell culture.

- Compared to cell culture, antigen detection by DFA picked up 9 additional positive cases (4 from urethritis, 3 from cervicitis, 1 from PID and 1 from infertility cases), but missed one culture positive cervicitis case.
7.3.3 Use of serological markers

- The analysis of *C. trachomatis* seropositivity in the patient group (n=143) and in a control group of apparently healthy adults (n=50) revealed that the prevalence of anti-*C. trachomatis* antibodies (IgM, IgA and IgG) was significantly high in the patient group compared to the control group (*P*<0.05).

- *C. trachomatis* seropositivity by IgM ELISA in the patient group was 22.4% (32/143). IgA positivity was seen in 28.7% (41/143) of the cases. A high prevalence of IgG antibodies was seen in 58.7% (84/143) of the patient population.

- Among the 27 culture positive cases, IgM antibody positivity was seen in 10 (37%) cases, while IgA and IgG positivity was seen in 11 (40.7%) and 18 (66.7%) cases respectively.

- Anti-CT IgG titer estimation in single serum specimen by the commercial IPAzyme immunoperoxidase assay has shown that high levels of IgG antibodies (1:128) were seen in female upper genital tract disease groups, PID and infertility. This suggests the utility of IgG titer estimation in single serum specimen to identify active/chronic/recurrent chlamydial infection in these disease groups.
The serological marker pattern vs. clinical patient groups revealed the following:

- IgM positivity alone was observed in 15 cases (10.5%) suggesting that these may be of primary infections with *C. trachomatis*. IgA was seen with IgG and was observed in all the clinical categories, the percentage of positivity being relatively high in the PID cases (31.8%).

- Seropositivity by any one of the markers (IgM or IgA or IgG) was observed in 98 (68.7%) cases. Notably, 19 (86.4%) out of the 22 PID cases and 3 of the 5 infertility cases were serologically positive by at least one marker.

- 36 out of 44 (81.8%) proven cases were positive by any one of the serological markers, which suggests that serology would be helpful if all the markers (IgM, IgA and IgG) are simultaneously analysed.

### 7.3.4 PCR assays for *C. trachomatis*

The diagnostic efficacy of different PCR assays in genital swab specimens and urine specimens of men and women was analysed. While the in-house PCR methods (plasmid and MOMP based) were used to test both swab as well as urine, the commercial Amplicor PCR (plasmid-based) was applied in urine specimens only. The following observations were made:
• The in-house plasmid PCR detected *C. trachomatis* DNA in the genital swab specimens of 46 (32.2%) cases; 38.1% (24/63) in males and 27.5% (22/80) in females.

• Compared to culture, plasmid PCR additionally detected 19 cases. The nested MOMP PCR showed positivity in 44 out the 46 plasmid PCR positive cases, but showed negative results in 2 male urethral specimens.

• Both plasmid and MOMP PCR assays were significantly more sensitive than culture, when performed in genital swab specimens.

• The plasmid, MOMP and the Amplicor assays detected *C. trachomatis* DNA in the urine samples of 36 (25.2%), 33 (23.1%) and 32 (22.4%) cases respectively. The assays differed in the detection rates in female urine samples as well.

• The Amplicor internal control detection revealed the presence of PCR inhibitory substances in 6.3% (9/143) of the urine specimens, the rate of inhibition being higher in female urine samples than male urine samples (8.8% vs. 3.2%). Urine specimens from 3 culture positive cases missed by Amplicor PCR were PCR inhibitory. None of the swab specimens were PCR inhibitory.

• By using the multiplex Amplicor PCR, *N. gonorrhoeae* was positive in 26 (18.6%) of the total cases. 11(7.7%) cases were positive for both *C. trachomatis* and *N. gonorrhoeae*. 25% (11/44) of the proven *C. trachomatis* infected cases had
concomitant gonococcal infection. A high rate of *C. trachomatis* positivity (42.3%) was observed in gonococcus positive cases.

- *C. trachomatis* positivity by different diagnostic methods vs. clinical groups showed that PCR methods improved the diagnostic sensitivity when compared to culture and antigen detection in almost all clinical groups especially in lower genital tract complications, urethritis and cervicitis. Serologic tests identified more number of cases from the upper genital tract disease groups compared to the other direct tests.

- Taking culture alone as gold standard,

  - Antigen detection by DFA technique showed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 96.3%, 92.2%, 74.3% and 99.1% respectively.

  - Serological markers showed poor performance values.

  - The in-house plasmid and MOMP PCR assays in swabs as well as urine were 100% sensitive (as all the culture positive cases were picked up as positive), but showed reduced specificities (as additional positives were considered as false positives).
7.3.5. **Evaluation of diagnostic systems using an expanded gold standard system**

An expanded gold standard was established by the combination of multiple tests including cell culture, DFA and in-house PCR assays. A patient was considered 'truly infected' if positive by culture or if positive by any two of the other tests. Thus there were 44 confirmed positives out of the total 143 cases. Based on this expanded standard of positivity, performance values were determined for each of the tests used for *C. trachomatis* diagnosis.

- The sensitivity of culture was found to be 61.4% in this study, which clearly shows that it is an inadequate standard for the evaluation of diagnostic tests for *C. trachomatis*.

- Antigen detection using DFA showed a sensitivity, specificity, PPV and NPV of 79.5%, 100%, 100%, and 91.7% respectively.

- The serological markers have shown poor sensitivities, specificities and predictive values. IgM (ELISA): sensitivity 31.8%; specificity 81.8%; PPV 43.8%; NPV 73%. IgA (immunoperoxidase assay): sensitivity 40.9%; specificity 76.8%; PPV 43.9% and NPV 74.5%. IgG (immunoperoxidase assay): sensitivity 68.2%; specificity 45.5%; PPV 35.7% and NPV 76.3%.
- The in-house plasmid PCR in genital swabs showed a sensitivity, specificity, PPV and NPV of 100%, 98%, 95.7% and 100% respectively. The corresponding values in urine were 81.8%, 100%, 100% and 92.5%.

- The in-house nested MOMP PCR in genital swab was 100% sensitive and 100% specific. The predictive values were also absolute. In urine, the MOMP PCR showed a sensitivity, specificity, PPV and NPV of 75%, 100%, 100% and 90% respectively. Corresponding values for the commercial Amplicor urine-PCR were 72.7%, 100%, 100% and 89.2%.

- The overall analysis revealed that PCR is the most sensitive technique for the diagnosis of genital chlamydial infections when performed in genital swab specimens from symptomatic patients.

### 7.4 THE ASSOCIATION BETWEEN CT INFECTION AND HIV INFECTION.

#### 7.4.1 HIV status in C.trachomatis positive cases

- HIV seropositivity was 16.8% (24/143) in the symptomatic STD population studied. Positivity was significantly high in males compared to females (27% vs. 8.6%; \( P<0.05 \)).
• CT and HIV co-infection was observed to be 8.4% in the study population.

• *C. trachomatis* positivity was significantly higher in the HIV positive cases than in the HIV negative cases (12/24 (50%) vs. 32/119 (26.9%); *P*<0.05).

• The study revealed that HIV positivity rate was significantly high among the *C. trachomatis* positive cases (*P*<0.05).

7.4.2 *C. trachomatis* positivity in HIV proven cases

• In this study, *C. trachomatis* positivity was observed in 37.1% (13/35) of the HIV seropositive women, while positivity was seen in only 3 of 25 (12%) controls (HIV seronegative women), which was statistically significant (*P*<0.05).