Chlamydia trachomatis

Insights on genetics, molecular epidemiology, diagnostics and treatment, and host-pathogen interactions

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Chapter 11

General Discussion
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*Chlamydia trachomatis* remains the most common bacterial sexually transmitted infection (STI), in spite of huge public health efforts, such as treatment, partner notification, and counselling to reduce its prevalence [1,2]. The majority of these infections remain mostly asymptomatic (up to 80%) and therefore unnoticed, constituting a large reservoir for continued sexual transmission of *C. trachomatis* [3]. Moreover, if not properly treated, infection with *C. trachomatis* may result in significant morbidity including epididymitis and pelvic inflammatory disease, leading to infertility in women and possibly also in men [2,4,5].

In this thesis, genetic, epidemiological, and in-vitro studies were combined to gain a more comprehensive understanding of *C. trachomatis* infections. The performed studies were described in four parts. Part 1 focussed on the genetics of *C. trachomatis* infections, part 2 on the molecular epidemiology of *C. trachomatis* infections, part 3 on diagnostics and effective treatment of *C. trachomatis* infections and part 4 on *C. trachomatis* host-pathogen interactions.

**Genetics of Chlamydia trachomatis infections**

Various host genetics have been associated with disease severity of *C. trachomatis* infection [6-16]. However, successful infection, disease severity and continuous transmission cannot be solely attributed to host genetics, since *C. trachomatis* strains must also successfully evolve in order to survive within the human host [13,15-17]. Therefore, it is important to understand the genetic diversity of *C. trachomatis* and to identify different strains circulating within a population.

Several methods can be used to investigate the genetic variation of *C. trachomatis* strains. Whole genome sequencing (WGS) achieves maximum resolution to investigate all genetic variation present within *C. trachomatis* strains. However, as described in the introduction (Chapter 1) of this thesis, it remains technically challenging to perform WGS directly on clinical specimens, mainly due to the complex intracellular lifecycle and the fact that WGS of *C. trachomatis* to date requires DNA quantities only obtained after cultivation. Since many molecular diagnostics often include sample collection tubes containing lysis buffer, the use of cell culture is prohibited [18]. Moreover, the *C. trachomatis* genome is highly conserved (>98% similarity), and therefore genetic variation is often restricted to specific polymorphic genes [13,16,19]. Since WGS is not (yet) feasible, other
genetic typing methods, such as MLST, are still needed to better understand the genetic variation of *C. trachomatis* and discriminate between strains that circulate within a population.

In Chapter 2, we compared two multilocus sequence typing (MLST) schemes that can be used to assess the genetic diversity of *C. trachomatis*. We found that no distinct transmission of *C. trachomatis* could be observed in MSM and heterosexuals using an MLST scheme based on 7 housekeeping genes (MLST-7). In contrast, a high-resolution MLST scheme based on 6 highly polymorphic genes (hr-MLST) did show sufficient discrimination of strains allowing epidemiological analysis. Moreover, we demonstrated that typing using the hr-MLST-6 scheme is able to identify genetically related clusters of *C. trachomatis* strains within each of the clusters that were also identified by using the MLST-7 scheme.

The original MLST principle aims to index genetic diversity using conserved housekeeping genes [20-22]. However, housekeeping genes are presumed to be under neutral or nearly neutral selection pressure making them stable over time within a species [20]. The use of a typing method that includes housekeeping genes is therefore very suitable to answer evolutionary questions within a genus and to investigate the genetic differences in *C. trachomatis* strains over a longer time-frame. The hr-MLST scheme was designed to be highly discriminating using non-housekeeping genes that are under immune pressure or have variable repeat regions [23,24]. The genetic variation in these highly polymorphic genes allow the identification of detailed genetic differences between *C. trachomatis* strains in a short time-frame of only a few years. The use of MLST schemes to investigate genetic variation among different *C. trachomatis* strains has also resulted in some discussion. A study by Harris et al. was the first to study a large set (52) full genome sequences and revealed the whole genome phylogeny of *C. trachomatis* [19]. They subsequently reported that multilocus sequencing techniques that are based on housekeeping loci show greater congruency with whole genome phylogeny [19].

In Chapter 3, we demonstrated that phylogenetic analysis of the hr-MLST targets is also in agreement with whole genome phylogeny. Although any scheme based on a small number of loci lacks resolution compared to whole genome sequencing, hr-MLST may still be used to facilitate phylogenetic studies. Moreover, MLST is a relatively fast method that can be performed on direct patient samples. Recently, a very comprehensive analysis has been performed including 563 *C. trachomatis*
General Discussion

genomes to reveal the evolutionary history of C. trachomatis strains [25]. This study demonstrated that the history of C. trachomatis species comprises two phases, namely deep variation and contemporary mixing. Whole genome phylogenetic analysis of these 563 genomes revealed four deeply divergent lineages (two urogenital, one ocular and one LGV lineage) that are in agreement with the phylogenetic clusters that were previously identified by others [19,25]. Since the whole genome phylogeny is in agreement with hr-MLST phylogeny, phylogenetic analysis of the hr-MLST targets likely provide a robust estimate of the true whole genome phylogeny. One should however keep in mind that variation that occurs in the remaining genes due to recombination will be missed, potentially resulting in a confounded tree topology [19]. The genetic typing method of choice should therefore be determined on a case-by-case basis, depending on the research question and resolution that is required, which is indeed performed in the Netherlands.

Besides facilitating phylogenetic studies, adequate tools for genetic typing are also important to understand the population structure of C. trachomatis infections. Typing is especially helpful since genetic variation within C. trachomatis strains may result in biological advantages, such as increased virulence, tissue tropism, antibiotic or diagnostic resistance or accelerated proliferation of strains. An example of this is the hypervirulent L2C strain, which was identified to be a recombination of a genovar L2 and a genovar D strain. This was suggested to be hypervirulent in terms of clinical signs and symptoms, as it produced severe hemorrhagic proctitis [26]. Moreover, genetic variation can eventually lead to the emergence of strains that are not detected by current diagnostics as was the case for the new Swedish variant. This new variant harboured a 377 bp deletion in the pgp7 plasmid gene that prevented detection of infections using plasmid based (commercial) PCR diagnostics that targeted the pgp7 gene [27,28]. These studies indicate the need to actively monitor genetic variation among different C. trachomatis strains.

Since its development in 2007, hr-MLST has been performed on many clinical specimens, including urogenital [23,24,29-36], LGV [33,37], and trachoma [38] samples. Genetic typing information from all specimens included in these studies is stored in a public database (http://mlstdb.bmc.uu.se/). In Chapter 3, we studied 415 unique sequence types (STs) obtained from 2089 specimens present in the public database at that time (2014) [39]. The specimens were generated from 13
studies and represented 16 countries on different continents. Today it constitutes by far the largest genetic typing dataset for *Chlamydia trachomatis* strains. Analysis of this dataset revealed that 8 STs are predominant in all countries. Of these 8 STs, four were mostly present among men who have sex with men (MSM) and 4 were mostly present among heterosexuals. Although these predominating STs might suggest that these strains have biological advantages, *in silico* analysis of 12 publicly available full genomes did not reveal a specific (set of) gene(s) that could be linked to transmission efficiency of certain STs. Transmission efficiency might of course also be host related, or could perhaps even be the result of more efficient host-pathogen interactions due to niche specific (e.g. tissue tropism) adaption of *C. trachomatis* strains [13-17]. In any case, genetic analyses of the pathogen alone are insufficient to reveal why some STs prevail in space and time.

More refined population-based WGS studies may help to better understand the genetic population structure of *C. trachomatis* and its entire genetic variation. These studies are becoming more feasible since new improved methods that allow *C. trachomatis* whole genome sequencing (WGS) on direct (non-cultured) clinical specimens are rapidly being developed [40-43]. One of these new methods involves the use of MOMP specific antibodies that are attached to magnetic beads. These beads can be used to separate *C. trachomatis* infected cells from the remaining native clinical samples [40,41]. However, this method cannot be performed on those clinical samples collected for test platforms that utilize lysis buffer, since these disrupt the MOMP structure preventing any antibody binding. Another culture-independent WGS assay uses PCR enrichment technology to amplify a 100 kb region of the *C. trachomatis* genome with 1.1–1.3 kb overlapping amplicons resulting in enrichment of the entire *C. trachomatis* genome [18]. The advantage of this method is that it can also be used on clinical samples that are collected in lysis buffer. Moreover, by estimating the number of SNPs and comparing it to a database of known SNPs from existing genome sequences, it can help to identify mixed infections [18]. To deal with the growing collection of full genomes, new promising tools such as the bacterial isolate genomics database (BIGSdb) were developed to catalogue bacterial diversity and to rapidly identify bacterial species, virulence factors, and outbreaks [22,44,45].

In Chapter 4, we used BIGSdb and applied a gene-by-gene approach to study the relationship between the *C. trachomatis* genome, plasmid types and infection
pathology. Various studies have implicated that *C. trachomatis* plasmids function as an important virulence factor contributing to infectivity and pathogenicity, but limited data is available on its genetic diversity and whether distinct plasmid types are associated with different tissue tropism and pathologies. In Chapter 4, we analysed 157 isolates available in the Chlamydiales pubMLST database (http://pubMLST.org/chlamydiales/). Analysis of the core genome of these isolates revealed four phylogenetic clusters that are in concordance with the whole genome phylogeny as described by Harris et al [19]. Analysis of the plasmid sequences revealed six plasmid clusters that showed a strong association with their *C. trachomatis* core genome and their plasmid sequences suggesting co-evolution of *C. trachomatis* plasmids and their chromosome [19,46]. These core genome and plasmid clusters were in turn linked to *ompA* genovars and disease phenotype showing exceptional horizontal genetic exchange of three urogenital isolates that obtained ocular plasmids.

The *pgp3* and *pgp4* genes are specific plasmid genes that have been associated with chlamydial virulence [47-50]. In Chapter 4, we also showed that of all plasmid genes, *pgp3* is the most polymorphic gene. The *pgp3* gene is secreted into the host cell cytosol and more variation could be observed among the *pgp3* gene due to immune selection [46]. In contrast, *pgp4* encodes a transcriptional regulator acting on *pgp3* and chromosomal genes and was identified as the most conserved gene. This suggests that *pgp4* might be an indispensable factor for chlamydial virulence and infection, although previous studies have demonstrated that *pgp4* is dispensable for *in vitro* growth of *C. trachomatis* [50,51]. Therefore, it is more likely that variation of the *pgp4* gene is limited due to its function (i.e. interaction with a specific nucleotide sequence and RNA polymerase). Although we were able to define the population structure, future research should apply the gene-by-gene approach on a well-defined population with known epidemiological and clinical data, as this will help to improve our understanding of chlamydial transmission and disease.

**Molecular epidemiology of Chlamydia trachomatis infection**

Besides only looking at genetic variation to understand chlamydia diversity, this information can also be linked to epidemiological and clinical data to facilitate molecular epidemiological studies [52]. Most *C. trachomatis* infections occur within sexual transmission networks, and its study is complicated by the disclosure of the
number and nature of sexual partners of each index patient [53]. Genetic typing methods can help to better understand how these strains are transmitted within networks and to detect specific modes of transmission and populations at risk, without the need for possibly biased information from the patient [24,35,54].

Previous studies found conflicting results regarding associations between specific urogenital *C. trachomatis* strain types and ethnicity or symptomatology using only 
ompA based genotyping, what could be explained by a lack of resolution [55-61]. In Chapter 5, we used hr-MLST, to assess the relation between urogenital *C. trachomatis* strain types and ethnicity or symptoms in a young screening population. We found no association between strain type and self-reported symptoms. However, we did find an association between urogenital *C. trachomatis* strain types and ethnicity, as some strains were over-represented in participants of non-Dutch ethnicity, of whom the majority were from Surinamese/Antillean ethnicity, whereas other strains were over-represented by participants with Dutch ethnicity. This association with ethnicity likely reflects differences in socio-economic status, sexual risk behaviour, and relative scarcity of mixing of sexual partners between various ethnic groups [31,61-64]. Several previous studies reported that high-risk sexual behaviour is more common among people with Antillean and Surinamese ethnicity, as they more often have multiple partners in the preceding 6 months, have their first sexual experience at a younger age, and are less likely to use a condom [31,61,64]. However, it has also been reported that this higher prevalence is likely not due to increased risk behaviour. In stead, it is suggested that the higher *C. trachomatis* prevalence found among Surinamese/Antilleans results from lower health-seeking behaviour or lower access to healthcare, as the higher prevalence reflects their lower educational level and neighbourhood, which are two markers of lower socio-economic status [63].

The lack of an association between strain types and self-reported symptoms is in concordance with previous studies [32,34]. This could be the result of using hr-MLST as this method is limited by the small fraction of the genome (6 genes) that is included. WGS, also covering the virulence associated extrachromosomal plasmid, might provide more insight into the possible association between *C. trachomatis* strain types or genes and urogenital symptoms. However, it is also possible that symptoms are associated with host immune factors, e.g. activation by previous and re-occurring infections [65-67]. Therefore, possibly a relation between the
General Discussion

genomes of *C. trachomatis* strains and reported symptoms does not exist. Instead, the interaction between *C. trachomatis* infections and their subsequent effects on the human host should be carefully monitored and examined to identify any gene or set of genes that might contribute to a more severe course of disease.

Although molecular epidemiological data of *C. trachomatis* infections are sufficiently available for European countries, these data are still lacking for South Africa. In Chapter 6, we used hr-MLST to investigate the molecular epidemiology of urogenital *C. trachomatis* strains circulating among women in the Mopani District, South Africa, and compared those to strains circulating among women in Amsterdam, the Netherlands. We observed heterogeneous clusters with strains shared between Mopani district and Amsterdam, but also novel STs and a cluster that were unique to Mopani district. In addition, we observed two identical STs that were present in both geographical locations. The occurrence of identical STs in these geographically distant locations is likely explained by the genomic stability of some *C. trachomatis* strains over a long period of time, since these STs were also identified to predominate in heterosexual populations, as was discussed in Chapter 3. Similar findings were also reported for strains circulating in Nanjing, China, in comparison to Amsterdam [29], and for strains circulating in Sfax, Tunisia, in comparison to Amsterdam [68].

**Diagnostics and effective treatment of *Chlamydia trachomatis* infections.**

Concerns have been raised over potential *C. trachomatis* treatment failure and persistent infections [69-72]. Therefore, a test of cure (TOC) is based on a second nucleic acid amplification test (NAAT) test performed after treatment is recommended in some situations [73,74]. Although modern NAAT diagnostics are highly sensitive and specific, they fail to differentiate between viable bacteria and non-viable molecular remnants [73,74,76]. Recently, a study demonstrated that NAAT used as TOC intermittently detected *C. trachomatis* nucleic acids (DNA and RNA) in up to 42%, and up to 51 days after treatment [75]. Likely these NAAT results were partly false positive, and based on nucleic acid remnants after successfully cleared infections. In contrast to NAAT, cell culture remains the most specific method available to discriminate a persistent viable *C. trachomatis* infection from a cleared infection with nucleic acid remnants and may therefore help to interpret the value of a positive NAAT after treatment. Recently a viability PCR was developed which relies on the use of membrane impermeable DNA intercalating dyes as a
pre-treatment step before conducting a molecular assay [76]. A disadvantage of this method is that it also detects dead chlamydial cells that still contain an intact cell membrane. Moreover, like cell culture the use of this viability PCR still requires special logistics as this assay cannot be performed on most present day clinical samples since these often utilize lysis buffer in sample collection tubes, which destroys the *C. trachomatis* membrane and also disintegrates all other molecular structures.

NAAT has replaced culture as the standard method for *C. trachomatis* diagnostics because of its superiority in specificity and sensitivity [74]. *C. trachomatis* culture to date is only sporadically performed and only a few laboratories still have the means, expertise and logistics for it. In Chapter 7, we used cell culture to assess the use of a DNA and RNA based NAAT platform (resp. Cobas 4800 CT/NG and Aptima combo) as TOC and their capacity to differentiate truly persisting infections after treatment from successfully treated infections with nucleic acid remnants causing false positive NAAT results. We observed that DNA and RNA-positive results at 7, 21 and 49 days post treatment cannot be confirmed by a positive culture suggesting that these observations might be due to nucleic acid remnants. However, it cannot be excluded that chlamydia persisted in a metabolically inert yet viable form after treatment. *C. trachomatis* gastrointestinal infections have been demonstrated in humans in the absence of clinical disease[77-79]. This could also explain urogenital re-infections or persisting infections among women, via autoinoculation from the rectal site [77,80]. Many women acquire rectal chlamydia infection without anal sex, and therefore autoinoculation could be a potential explanation for the high co-occurrence of anorectal and urogenital infections that are often reported [81]. Therefore, it is well possible that these *C. trachomatis* infections constitute a reservoir for persisting infections.

Current guidelines recommend that patients should be advised to abstain from sexual contact for seven days after treatment [74,82-84]. However, in a minority of three patients that were included in our study in Chapter 7, viable *C. trachomatis* was found in cultures 7 days after treatment. These findings suggest that abstinence from sexual contact for 7 days after treatment may not always be sufficient to prevent ongoing transmission. However, culture positive results in these three persons could also be explained by *C. trachomatis* reticulate bodies that have reversibly transitioned from their normal developmental cycle into a
state of persistence [85,86], which has also been described in the introduction of this thesis (Chapter 1). During this persistent stage, Chlamydia inclusions present as morphologically enlarged and non-dividing RBs, which cannot differentiate into EBs preventing completion of the developmental cycle [85]. This persistent state is often induced by exposure to antibiotics or the result of nutrient deficiency and can be reversed by removal of the particular inducer [86]. Therefore, it could be that these RBs persisted 7 days after treatment, and induced a new infection after the antibiotic had left the body.

Besides diagnostics and treatment of C. trachomatis infections among heterosexuals, there is also a need to evaluate the current diagnostic practice of C. trachomatis infections among MSM. In the current LGV epidemic among MSM, LGV-inducing strains predominantly cause ano-rectal infections [87,88]. The male urethra is likely the most common factor in the transmission of LGV-inducing strains among MSM. However, most guidelines do not recommend routine testing for urethral LGV [89-91], raising the question whether many urethral LGV-infections remain underdiagnosed and subsequently contribute to the on-going LGV epidemic. In Chapter 8, we observed an LGV positivity rate in male urine samples of only 0.06% (7/12,174 positivity). This was 15 times lower compared to the ano-rectal LGV infections (0.9%, 109/12,174) in the same time period. This discrepancy in positivity suggests that other modes of transmission might drive the current LGV epidemic. Moreover, the discrepancy cannot be explained by tissue tropism of C. trachomatis strains with a preference for anorectal tissue circulating among MSM [92-94]. The lack of knowledge on the pathogenicity and transmission of LGV infections indicates the need for further research to clarify the discrepancy found in the rate of ano-rectal and urethral LGV infections.

**Chlamydia trachomatis host-pathogen interaction**

The use of molecular epidemiology has helped to reveal several host factors that affect C. trachomatis infection. For instance, several polymorphisms within cytokines, interleukins and HLA DQ have been associated with the risk of C. trachomatis induced tubal factor infertility and disease severity [6-12,77]. Moreover, the development of an active C. trachomatis infection has recently been associated with cervicovaginal microbiota of the human host that is dominated by Lactobacillus iners or by diverse anaerobic bacteria instead of by Lactobacillus crispatus [95]. In addition, previous studies have suggested that there is directional
evolution of *C. trachomatis* toward niche-specific adaptation contributing to a more effective host pathogen interaction [13-17]. Some positively selected genes and codons were identified that were hypothetically involved in specialisation towards different human cell types (e.g. columnar epithelial cells of ocular or genital mucosae and mononuclear phagocytes) [15]. These studies indicate the need to better understand *C. trachomatis* host-pathogen interactions as this knowledge can help to develop better diagnostic, treatment and prevention strategies to reduce *C. trachomatis* infections.

Several studies demonstrated distinct transmission networks among MSM and in heterosexuals [24,33]. However, in most studies samples from MSM were primarily obtained from the ano-rectal tract whereas samples from heterosexuals were obtained from the urogenital tract. Therefore, this difference in transmission networks could be the result of *C. trachomatis* strains adapting to specific niches and developing tissue tropism due to a preference and specialisation to infect specific human anatomic tissue [13-17]. In Chapter 9, we investigated whether the occurrence of these distinct transmission networks in MSM and heterosexuals can be explained by tissue tropism. We compared *C. trachomatis* strains found in anorectal samples among MSM and women, and we compared *C. trachomatis* strains found in concurrent anorectal, urogenital and/or, pharyngeal infections in women. The results of our study showed that ano-rectal *C. trachomatis* infections in women were caused by different strains than ano-rectal infections in MSM, suggesting that tissue tropism is not the most likely explanation for the observed separate transmission networks. These findings suggest an absence of sexual mixing between the groups. Moreover, we have observed that MSM associated strains formed less diverse and more clonal clusters, whereas heterosexual strains are more diverse and heterogeneous. This could also be explained by the differences in sexual host behaviour with separate sexual contacts. MSM more often mix with sexual partners that differ in age, ethnicity, nationality and lifestyle which eventually leads to a large international transmission network [96]. In contrast, the heterogeneous clusters among heterosexuals indicate the existence of local transmission networks. Finally, we observed that *C. trachomatis* strains do not preferentially infect urogenital, ano-rectal, or pharyngeal tissue, as we did not observe differences in strains causing concurrent urogenital, rectal and/or pharyngeal infections in women. This suggests that *C. trachomatis* pathogens that reside in specific different niches within the human body do not specialise towards these niches.
Genetics and molecular epidemiology alone are not sufficient to better understand *C. trachomatis* infections and their effect on the human host in terms of symptomology, biological advantages and prevalence, mainly because most effects following *C. trachomatis* infections are likely the result of host-pathogen interaction. Molecular epidemiology offers some insight in the interaction between chlamydia genetics and the human host, but these studies have their limitations since they lack information on invasion, infection and tissue damage caused by *C. trachomatis* in patients. In fact, many of these mechanisms behind invasion, infection and tissue damage caused by *C. trachomatis* still need to be elucidated and this remains difficult due to the absence of appropriate research models and tools. To overcome these limitations, there is a need for novel tools to combine the study of genetics and molecular epidemiology with *in-vitro* culture models that accurately reflect *C. trachomatis* infections as they occur in the human host.

In Chapter 10, we developed a 3D organotypic reconstructed human urethra (RhU), constructed from primary urethral keratinocytes and fibroblasts derived from human donor tissues that could be used to study *C. trachomatis* infection. Primary urethra cells were used to reconstruct epithelium on a fibroblast populated collagen-fibrin hydrogel. RhU and native urethral tissue were characterized and compared by immunohistochemical staining for proliferation and epithelial differentiation to demonstrate that RhUs closely resemble native urethral donor tissue, which they did. The next step was to expose these RhUs to genetically defined *C. trachomatis* strains. After exposure for 1, 3, 7 and 10 days using invasive (genovar L2b) and non-invasive (genovar D) *C. trachomatis* strains, differences in growth and differences in infection capacity were observed. Only the invasive (genovar L2b) strain was able to form inclusions bodies within the epithelial layer of the RhU, indicating an active and replicating chlamydial infection. The absence of inclusion bodies for RhUs exposed to non-invasive (genovar D) *C. trachomatis* strains was likely the result of the length of the exposure times that were used. There are some differences in exposure of RhUs compared to standard 2D cell culture, for instance lower volumes of cultivated *C. trachomatis* bacteria were used for exposure of 3D RhUs and these were also distributed over a larger surface. Moreover, standard cell culture is completed by centrifuge-assisted inoculation [73], which is not possible for RhUs and this likely slowed down the infection process. Therefore, prolonged exposure times could result in an active non-invasive *C. trachomatis* infection and formation of inclusion bodies.
There have also been recent advances in the development of organoid models [97]. These models are constructed by providing and maintaining the correct signaling to adult stem cells of the human mucosa, which gives rise to differentiated organoids containing ciliated and secretory cells [97]. The use of organotypic models or organoids might help to better understand chlamydial infections. These models are indispensable for chlamydia research as they help to study the interaction between *C. trachomatis* and the human host and allow to accurately assess and follow *C. trachomatis* infections over time. These models can therefore help to study chlamydia genetics in relation to infection of the human host, possibly providing more insight in why certain strains are predominating in space and time as was described in Chapter 3. Moreover, these models can help to translate WGS data with the occurrence of tissue damage and potential symptoms providing more insight into chlamydial virulence. Lastly, these models can provide new opportunities to study the effect of antibiotic treatment on *C. trachomatis* infections.

**Concluding remarks**

Although huge public health efforts, such as treatment, partner notification, and counselling have not been able to reduce *C. trachomatis* prevalence, these are still of critical importance to prevent sequelae that may arise due to *C. trachomatis* infection. This failure to reduce *C. trachomatis* prevalence indicates the need for more basic research to gain a better understanding of *chlamydia*. Over the last decades much progress has been made in the diagnostics and treatment of *C. trachomatis* infections. Moreover, new research tools have been developed and used to investigate chlamydial genetics, molecular epidemiology, diagnostics and treatment, and these will be helpful to increase our knowledge and understanding of *C. trachomatis* infections today. However, previous research and the results of studies in this thesis have also addressed knowledge gaps that cannot be resolved by separately investigating chlamydial genetics, molecular epidemiology, diagnostics or treatment. We need an integrated model to get a full picture of the nature of *C. trachomatis* infections, including their host-pathogen interactions. Therefore, future research should combine the use of research methods to study *C. trachomatis* infections in relation to host-pathogen interactions. The use of novel culture models such as 3D tissue engineered anatomical equivalents, and organoids are a promising tool to conduct these studies. Although organoids that are constructed by providing appropriate signaling to stem cells to stimulate these cells to differentiate into specific tissue are perhaps the brighter future for chlamydial research.
General Discussion

References


8. Murillo LS, Land JA, Pleijster J, Bruggeman CA, Pena AS, Morre SA: Interleukin-1B (IL-1B) and interleukin-1 receptor antagonist (IL-1RN) gene polymorphisms are not associated with tubal pathology and Chlamydia trachomatis-related tubal factor subfertility. Hum Reprod 2003, 18: 2309-2314.


81. van Liere GA, Hoebe CJ, Wolffs PF, Dukers-Muijters NH: High co-occurrence of anorectal chlamydia with urogenital chlamydia in women visiting an STI clinic revealed by routine universal testing in an observational study; a recommendation towards a better anorectal chlamydia control in women. BMC Infect Dis 2014, 14: 274.


