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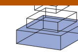

Genomic stability of genotyping markers in *Chlamydia trachomatis*

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A commentary on

Genotyping markers used for multi locus VNTR analysis with ompA (MLVA-ompA) and multi sequence typing retain stability in *Chlamydia trachomatis*
by Labiran, C., Clarke, I. N., Cutcliffe, L. T., Wang, Y., Skilton, R. J., Persson, K., Bjartling, C., Herrmann, B., Christerson, L., and Marsh, P. (2012). Front. Cell. Infect. Microbiol. 2:68. doi: 10.3389/fcimb.2012.00068

Chlamydia trachomatis is an obligate intracellular human pathogen that infects columnar epithelial cells of ocular or genital mucosae, and mononuclear phagocytes. More than 50 fully sequenced genomes are publically available (Harris et al., 2012), and despite low polymorphic character when compared with most bacteria, these data allow a better understanding of *C. trachomatis* chromosomal dynamics. Indeed, it revealed not only the chromosome regions more prone to genetic mutations, but also that recombination is highly chromosome dispersed, although the frequency and relative weight of recombination and mutation events undoubtedly posits *C. trachomatis* as a low recombining bacterium (Joseph et al., 2011; Ferreira et al., 2012).

In order to understand the epidemiology and pathogenesis of the highly prevalent chlamydial diseases, the classification of clinical isolates has been a priority of researchers for decades. However, the traditional typing system, which is based on the polymorphism of the gene (*ompA*) encoding the major outer membrane protein (Yuan et al., 1989) has been jeopardized in the last years as it does not group the isolated strains according to their cell-appetence or virulence. Thus, with the progressive release of sequenced genomes, several typing systems have been developed (Klint et al., 2007; Pannekoek et al., 2008; Pedersen et al., 2008; Dean et al., 2009; Bom et al., 2011), although none of them is consensual due to the dissimilar genetic characteristics or discriminatory power of the loci enrolled in each of those.

Although it is assumed that the typing schemes should ideally ensure that the chosen loci are stable over the course of evolution, this issue is of difficult assessment. Labiran et al. (2012) have now presented a study where they have assessed the stability of some regions of the *C. trachomatis* genome by using innovate approaches. They have used the genetic markers from the described typing schemes that provide the highest Simpson index of diversity: the multi-sequence typing (MST) system described by Klint et al. (2007) and the multi locus variable number tandem repeat (VNTR) analysis (MLVA-*ompA*) described by Pedersen et al. (2008). Labiran et al. (2012) have initially evaluated the stability of those genetic markers through the first stages of adaptation of *C. trachomatis* to cell culture. This is interesting as the first attempt to study mutational patterns over adaptation to cell culture had been performed back in 1998 (Stothard et al., 1998), by studying solely the stability of *ompA* over 20 *in vitro* passages. Now, by analyzing three VNTR besides *ompA*, the authors undoubtedly increase the chances of observing genetic alterations, as these loci possess a high discriminatory power. Furthermore, as the repeated sequences of VNTR loci are generated as a consequence of an increased error rate during DNA replication at these regions, they are hypothetically more prone to additional variation. In this first approach, the authors found no alterations in those genomic regions by studying seven clinical isolates over eight passages. The initial inoculations were properly done from primary swabs in order to avoid previous contact of the strains with the *in vitro* system. Interestingly, the eight passages represent about 16 days in culture aiming to reflect the *in vivo* estimated period for appearance of symptoms for *C. trachomatis* infection of both genitalia and conjunctivae (Black, 1997). There is no doubt that there is a radical change of environmental conditions when transiting from the *in vivo* to the *in vitro* system, mostly because the latter lacks immune system, competing flora, pH alterations, hormonal fluctuations, etc. Nevertheless, although the parallelism between these two scenarios remains speculative for these reasons, the impossibility to perform this evaluation *in vivo* and under controlled conditions makes this an interesting approach.

In a second stage, the authors went even further by studying the "long-term" genome stability under influence of *in vitro* environmental conditions. To achieve this, they used the MLVA-*ompA* and also the five-locus MST system to monitor the stability of the enrolled loci throughout 72 passages in tissue culture. The coverage and the runtime of this approach make it an unprecedented evaluation of genome stability. In this study Labiran et al. (2012) used the so called "Swedish new variant," a *C. trachomatis* isolate that, besides the higher number of pseudogenes than most sequenced genital isolates (Jeffrey et al., 2010), shows both a deletion and duplication within the plasmid, which are genomic changes that had never been described before (Unemo et al., 2010). These kind of genomic alterations are infrequent as *C. trachomatis* presents a nearly identical core and pan genome and a DNA sequence similarity of >98% (Harris et al., 2012). So, this isolate could hypothetically be considered as genomically unstable, making this a valuable strain to test stability over a long time *in vitro* passage. Similarly to the previous approach, no genetic changes were observed throughout the long-term *in vitro* maintenance, suggesting that the MLVA-*ompA* and MST systems are, on a genetic stability basis, highly accurate for both short-term (such as partner tracing) and long-term molecular epidemiology.

Moreover, the results from the Labiran et al. (2012) study indirectly bring to light a major controversy in the field, which is the uncertainty of whether the prototype

strains that have been used worldwide leading to thousands of papers, accurately represent current circulating clinical strains. In fact, the former are laboratory adapted for decades but at this stage one cannot know the extent of genomic alterations that may occur due to laboratory adaptation. On one hand, the results from Labiran et al. (2012) point to specific genomic stability, which, together with the *C. trachomatis* transmission bottlenecks (Andersson and Hughes, 1996) and the high doubling time of 2.5–4 h (Borges et al., 2010), disfavors genetic variability. On the other hand, it is known that some loci are prone to generate multi-alleles, such as the putative virulence factor CT135 (Sturdevant et al., 2010), and that *Chlamydia* undergoes adaptive changes when subjected to specific environmental alterations (Kutlin et al., 2005; Suchland et al., 2005). Ultimately, unless the entire genome is analyzed (at the stage of the strain isolation and after long-term *in vitro* passage), the controversy will certainly remain.

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