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Commentary

Call for consensus in *Chlamydia trachomatis* nomenclature: moving from biovars, serovars, and serotypes to genovariants and genotypes

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The International Committee on Systematics of Prokaryotes (ICSP) Subcommittee on the Taxonomy of Chlamydiae (STC) was founded in 2010 to solve major taxonomy and nomenclature issues regarding members of the order Chlamydiales. The ICSP-STC clarifies how species and Candidatus species should be reported. When based on genomic data, the genome sequence (at least 80% complete) should reveal the presence of core genes [1], with the taxonomically informative genes defined by Pillonel et al. [2] as the preferred core subset. These include genes (a) encoding DnaA, SucA, Hyp325, and FabI that, with similarity cut-offs of 70%, 64%, 57%, and 78 %, respectively, facilitate classification of isolates at the genus level; (b) encoding RpoN, FtsK, PepF, and Adk that, with similarity cut-offs of 96%, 98%, 96%, 95%, and 95 %, respectively, facilitate classification of isolates at the species level; and (c) encoding 16S rRNA and 23S rRNA that, with similarity cut-offs of 92.5% and 91 %, respectively, facilitate classification of isolates at the family level [2].

Another important nomenclature issue that was recently identified by the members of the ICSP-STC was the large diversity of terms historically applied to refer to a given chlamydial type (i.e. variants, biovar, serovar, genovar, serotype, genotype, subtype, subspecies, genomovariant) among all *Chlamydia* spp. With respect to *C. trachomatis*, typing is essential to better understand strain emergence and identify populations at risk and to unravel

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transmission networks. Typing also contributes to improving screening and prevention interventions [3]. Thus, it seems important to attempt to improve type-and subtype-level nomenclature, particularly as new variants of *C. trachomatis* are appearing at increased frequency. The proposed nomenclature presented in this article reports the conclusions of the ICSP-STC.

The first *C. trachomatis* typing method was based on characterization of the major outer membrane protein (MOMP) using specific monoclonal antibodies for various serologic immunoassays [4]. Different serovars (designated by single capital letters A–L) could be identified, substituted later with several serovariants (designated by capital and lowercase letter/number combinations). Based on MOMP serotyping, these serovars/variants could be grouped into three serogroups: the B serogroup (serovars B, Ba, D, E, L1, and L2), the intermediate serogroup (serovars F, G, K, and L3), and the C serogroup (serovars A, C, H, I, and J) [5]. This grouping has been very important clinically because serovars have been shown to correspond to different clinical manifestations. Thus, serovars/variants A, B, Ba, and C are the etiologic agents of trachoma, the world's leading cause of infectious blindness. Serovars D through K are sexually transmitted pathogens that cause urethritis, epididymitis, cervicitis, and pelvic inflammatory disease. Serovars L1 through L3 cause lymphogranuloma venereum, an invasive ulcerative sexually transmitted infection associated with marked inflammation and locoregional lymphadenopathy. Despite its clinical importance, serotyping (e.g. using the micro-immunofluorescence method developed by San-ping Wang [6]) was historically difficult and rarely used because it required labour-intensive cell culture, as well as highly experienced investigators.

The advent of new molecular techniques offers direct and more accurate alternatives for typing *C. trachomatis* strains. Moreover, whereas serologic tests require cultured strains, molecular techniques can be applied to DNA extracted directly from clinical samples. Results from genotyping based on *ompA* (encoding MOMP, also referred to as the OmpA protein) are highly concordant with those of traditional OmpA protein-based serotyping [7]. Strains identified and based on the *ompA* gene have been designated either as serovars or genovars [8,9].

However, a major disadvantage of *ompA* or OmpA-based typing of *C. trachomatis* strains is that this gene is frequently subject to recombination between strains, as revealed by comparison of whole-genome sequencing (WGS) data with the reference genome [10]. Thus, *ompA* strain typing can be misleading, as was recently reported in lymphogranuloma venereum case reports from Hungary and Slovenia [11]. In these reports, based on *ompA* sequence data only, isolates from two patients with lymphogranuloma venereum were initially misclassified as hypervirulent L2c strains that were earlier described by Somboona et al. [12]. This original L2c strain exhibited a recombination encompassing a large genomic portion from a serovar D strain and the *ompA* sequence of a standard serovar L2 strain. The *ompA* sequence of the Hungarian strain was identical to L2 (AM884176), as well as L2c (NC_015744); yet it was identified as L2c rather than L2. The authors later corrected this in an addendum [13]. A similar initial misidentification occurred in a Slovenian paper that was published earlier [14].

Although WGS can identify recombined strains, a current disadvantage is that it is not performed routinely on clinical (noncultured) specimens and is currently impractical and too expensive for large-scale population studies. Indeed, WGS still requires relatively large quantities of DNA from isolates of *C. trachomatis,* which most often will require cultivation. As an alternative to WGS, several multilocus typing systems that can be applied directly on noncultured samples have been developed successfully. These approaches, including multilocus variable tandem repeat analysis (MLVA) and multilocus sequence typing (MLST), have been validated for *C. trachomatis* and have provided insight into the epidemiology and transmission of different strains [15]. The MLVA typing method is based on the difference in tandem repeats between strains and combines *ompA*-typing with analyses of three highly variable genomic targets: CT1291, CT1299, and CT1335. In contrast to MLVA, MLST is based on the sequence variation in several genomic loci, and three MLST schemes have been described for *C. trachomatis* [15]. The discriminatory power of these multilocus typing methods is much higher than using *ompA* only for genotyping [3]. An open database containing molecular data of chlamydia strains can be found at https://pubmlst.org/organisms/chlamydiales-spp.

Although typing based on serologic assays has been largely abandoned, publications using molecular *C. trachomatis* strain identification methods have continued to use terms such as serovar and serotype to distinguish various strains [16,17]. However, the terms serovar, serotype, and serovariant are not appropriate when molecular DNA-based approaches are used.

Biovar is another term often misused in the current *Chlamydia* literature [18]. Yet, strictly speaking, the term *biovar* is reserved for a variant prokaryotic strain that differs physiologically and/or biochemically from other strains in a particular species (http://medical-dictionary.thefreedictionary.com/biovar; https://www.dictionary.com/browse/biovar). Because neither physiological nor biochemical assays are used in *C. trachomatis* strain identification, in our opinion the use of the term biovar should thus be discontinued. A third term used to refer to *C. trachomatis* strains is *genovar* [14]. However, this term was previously coined using an algorithm for the detection and visualization of copy number-variable regions in DNA sequencing data to enable the manual exclusion of erroneous signals (http://genovar.sourceforge.net) [19].

To eliminate confusion and inconsistencies, the ICSP-STC now makes the following recommendations for strain classification and identification at the subspecies level:

1. The term *genotype* should be used specifically to refer to strains identified based on a molecular approach; and
2. The term *serotype* should be used only for strains that have been characterized by serotyping only. Thus, isolates that have been characterized previously by serotyping, but are now characterized by molecular approaches, should be classified by genotype.

Moreover, because *C. trachomatis* exhibits frequent genomic recombination [10], typing techniques based on a single gene sequence (or parts thereof) or a few loci may be misrepresentative of the correct underlying genomic background. WGS and detailed phylogenetic analyses are required to fully understand the relationship of a given isolate in the wider network and epidemiologic context.

The ISCP-STC therefore makes the additional recommendations:

3. Authors, reviewers, and editors must ensure that manuscripts that are submitted for publication and contain sequencing data are clear as to which genotyping method has been used in strain identification.
4. Before publication, authors should thoroughly compare their *ompA* sequences against a database of known genotypes, and reviewers and editors should require authors to do so before accepting any manuscript containing sequencing data for publication.
5. *Chlamydia* researchers are encouraged to rigorously identify (or refer to) the method used for identification in any study that involves a given strain(s)/isolate(s).

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Table 1
International Committee on Systematics of Prokaryotes Subcommittee recommendation on the taxonomy of *Chlamydia* nomenclature

Method	Nomenclature to define strain	Nomenclature to refer to group of strains associated with given clinical characteristic (i.e. biovar)
OmpA serotyping (using mono- and/or polyclonal antibodies)	Serotype	Serovar
Molecular typing (e.g. multilocus sequence typing, multilocus variable tandem repeat analysis, restriction fragment length polymorphism, sequencing (part of) the *ompA* gene)	Genotype	Genovar: if identical to any of the major 19 serovars originally typed by *omp*A sequence Genovariant: if not identical to the major 19 serovars originally typed by *omp*A sequence
Whole-genome sequencing (≥80% completeness)	Genomotype	Genomovar

6. The ICSP-STC proposes to use the term *genotype* when describing strains that have been identified by molecular sequencing typing methods and are identical to the major 19 serovars originally typed by *ompA* sequence.
7. The term *genovariant* should be used to refer to a group of genotypes that exhibit similarities and are not identical to the major 19 serovars originally typed by *ompA* sequence (Table 1).
8. Finally, the ICSP-STC recommends using the term *genomovar* to designate a strain that has been characterized using a whole genome-based approach, even if previously typed using OmpA serotyping (Table 1).

We hope that our proposed re-evaluation of the subspecies-level *Chlamydia* nomenclature will provide clarity and, as such, will help not only the *Chlamydia* research community, but also infectious diseases specialists, epidemiologists, veterinarians, and clinical microbiologists, to improve communication and prevent confusion. In turn, this will enhance patient care, animal health, and the management of outbreaks.

Transparency declaration

The authors declare that they have no conflicts of interest.

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