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Meeting report

Polymicrobial infections and biofilms in women's health Gahro Expert Group Meeting Report

1. In the beginning …

The conference took place between the 11th and 14th of May 2017 in the medieval village of Gahro, near Berlin. It was organized by the Molecular Genetics Laboratory for Polymicrobial Infections and Biofilms, Charité, Universitätsmedizin Berlin. Scientists representing 11 European centers involved in research on the vaginal microbiome came together to exchange their opinion over present and future frontiers of research.

2. Is bacterial vaginosis lost in translation?

A key objective of this meeting was to address the multidisciplinary recent developments highlighting the role of multi-species biofilms in the etiology of bacterial vaginosis (BV). Throughout the meeting, a recurrent question was asked: When we describe BV, are we talking about the same thing? In other words, are there different types of BV? Or are we using the term BV for a different set of etiological conditions with similar symptoms? Per Goran Larsson (Department of Obstetrics and Gynecology, Linköping University Hospital, 581 85 Linköping, Sweden) argued that BV research is now on the rise, but despite the hundreds of yearly publications, there are still a lot of controversial studies that are not helping to unravel BV etiology.

Traditionally, vaginal dysbiosis includes *Candida vaginitis* [1] Trichomonas vaginitis [2] and BV [3], but as Gilbert Donders (Department of Obstetrics & Gynecology Antwerp University, Antwerp, Belgium) points out, other significant dysbioses are being neglected and can account for significant consequences for women's health. Donders argued that aerobic vaginitis (AV) or BV mixed flora could be more predictive of preterm labor than BV [4]. A major problem with differentiation between BV and other dysbioses is the lack of proper diagnostic methods, since neither the classical Nugent score nor the Amsel criteria provide very reliable results (sensitivity and specificity often below 90%) [5], nor do they add understanding to the pathogenesis of disease, which is purely descriptive.

Furthermore, Mario Vaneechoutte pointed out the possibility of the existence of different triggers for development of BV, with the possibility of spontaneous and transient physiological BV [6] and a sexually transmittable, persistent BV [7], by means of fragments of biofilms consisting of *Gardnerella* and other species [8].

The confusion of attitudes is probably unavoidable as long as everybody understands something different about BV. Working with clearly defined entities like *Gardnerellosis* (defined as *Gardnerella* conditioned polymicrobial biofilm disease) would be more helpful. FISH is highly efficient in the direct visualization of polymicrobial BV biofilms and their components, but requires skilled staff, is not available everywhere and is probably over-used for clinical purposes. The commercially available multiplex qPCR tests based on estimation of the ratio between concentrations of *Lactobacillus* spp DNA and *Gardnerella vaginalis and Atopobium vaginae* are quick, reliable, adequate and meet clinical needs. Other diagnostic methods are in progress. Simon Cameron (Imperial College London, Charing Cross Hospital, London, UK) presented data on in situ ionization mass spectrometry, which offers a scope of new possibilities to visualize and investigate metabolomics of microbiota directly in human tissues.

3. Microbiome diversity and diversity of bacterial species

Novel deep sequencing studies have improved our understanding of the vaginal microbiome [9,10]. However, these studies fail to provide functional or mechanistic evidence to support a direct cause–effect link [11]. In fact, as pointed out by Nuno Cerca, among the few direct functional studies addressing virulent traits of BV-associated bacteria, often *G. vaginalis* stands out as the most promising virulent candidate [12,13]. Elena Spasibova (D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology Mendeleevskaya line, 3, St. Petersburg, Russia) presented data on the diversity of vaginal lactobacilli in healthy women and pointed out that the composition of the vaginal biotope is an indicator of a woman's reproductive health, with significant differences between the lactobacilli population usually found in healthy and BV women [14].

A closely related issue is whether BV exists without *G. vaginalis* and whether health can co-exist with *G. vaginalis*. Most of the sequence_based investigations demonstrate that

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Gardnerella is obligatorily present in BV. However, depending on the definition criteria of dysbiosis and diagnostic methods used, some investigators claim the possibility of *Gardnerella*_negative BV [10,15]. On the other hand, its co-occurrence in health does not necessarily indicate the inoffensiveness and regular distribution within a normal population, but more likely the requirement for additional factors for *Gardnerella* to express pathogenicity. *G. vaginalis* is a common component of the vaginal microbiota. It is much more abundant in women with bacterial vaginosis [16]. The phenotypic heterogeneity of *G. vaginalis* is well known, and several biotyping and genotyping methods have been developed [17,18]. Recent genomic analysis has revealed four *G. vaginalis* genome groups, with great differences between each other [19].

Kira Shalepo (D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology Mendeleevskaya line, 3, St. Petersburg, Russia), in collaboration with Alexander Guschin's group, followed up on the discussion by presenting detailed data on the diversity and prevalence of the different *G. vaginalis* genotypes in healthy and BV women. Since *G. vaginalis* is known to establish synergistic relationships with other bacterial species, Guschin proposed that different *G. vaginalis* genotypes could interact synergistically and be responsible for the development of BV.

4. The polymicrobial biofilm hypothesis

An important milestone in BV research was the discovery that the different species involved in BV were associated in a structured polymicrobial biofilm, dominated by *G. vaginalis* and often including *A. vaginae* and several lactobacilli spp. [20]. Alexander Swidsinski argued that to understand polymicrobial cultures, we have to investigate them as a structure-functional unity. Swidsinski proposed that BV biofilms contain core organisms, which are necessary for propagation, highly specialized for this task, and disabled for autonomic growth outside of the consolidated polymicrobial biofilm. He argued that *Gardnerella*-driven biofilms may be a transitional state and the missing link in evolution from isolated living prokaryotes to completely interdependent polymicrobial communities, giving rise to eukaryotes. It is unclear which participants in the polymicrobial BV biofilm belong to the essential core of the biofilm, which are individual symbionts or accidental beneficiaries. Besides various *Gardnerella* genotypes, the most interesting participants seem to be *A. vaginae*, *Lactobacillus iners* and *Mycoplasma*.

5. Taking home a message

BV is not just amorphous dysbiosis, but rather, a number of different diseases which still have to be precisely defined based on pathogenic rather than descriptive criteria and using proper diagnostic methods. New sequencing and functional data seem to suggest that *Gardnerella* is not uniform.

The future may reveal that some of the known genotypes are, in fact, distinct species. Furthermore, it is now evident that lactobacilli are not totally beneficial. Some may be involved in pathogenesis of dysbiosis and adverse pregnancy outcome. This seems to be evident, at least in the case of *L. iners*. Strong data indicate that *L. iners* and AV might be even more strongly associated with preterm birth than BV.

Conflict of interest

The authors have no conflict of interest to declare.

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