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Gardnerella vaginalis diversity and ecology in relation to vaginal symptoms

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Abstract

Gardnerella vaginalis was first described in 1953, and subsequently identified as the causative agent of a cluster of vaginal symptoms currently known as vaginosis. Research has so far failed to confirm whether and by which mechanism *G. vaginalis* initiates vaginosis, with, consequently, poor diagnostics and treatment outcomes. Recent molecular analyses of protein-coding genes demonstrate that the taxon *G. vaginalis* consists of at least four distinct species. This development may represent a critical turning point in clarifying ecological interactions and virulence factors contributing to symptoms and/or sequelae of vaginosis.

Keywords: *Gardnerella vaginalis*; Phylogeny; Virulence; Microbial ecology; Microbiome; Bacterial vaginosis

1. Introduction

"*Gardnerella vaginalis*" was first described in the early 1950s, following a jump in the number of publications concerning sexually transmitted infections and vaginitis after the Second World War (Fig. 1). Gardner was not the first to observe the Gram-variable vaginal bacillus eventually named after him (despite his disapproval), but he was the first to suggest it as the causative agent of what had previously been known as "non-specific vaginitis", in the seminal paper of the field [1]. The first paper to use the term "vaginosis", in 1964, was referring to cysts of non-microbiological origin (but coincidentally mentions Gardner by name) [2]. The term "vaginosis" did not re-appear until 1981, when it was used, with the qualifier "bacterial", to signify an overgrowth of *G. vaginalis* and other anaerobes, not characterized by typical inflammatory changes generally implied by the suffix '*−itis*' [3]. The utility of this clinical designation, also referred to as "cytolytic vaginosis", has recently been questioned, and yet another qualifier has been suggested ("polymicrobial vaginosis") [4]. Clearly, the sizeable accumulation of clinical and microbiological observations, since Catlin's review [5], has yet to result in a coherent division between ubiquitous commensals of the genital tract and pathogens, resulting in either vaginal symptoms or in symptomless states that can nevertheless compromise sexual and reproductive health.

G. vaginalis is found in most women with vaginosis and in many or most women without vaginosis, especially in higher-resolution datasets [6]. These studies also confirm that *G. vaginalis* is present at higher concentrations and forms typically different ecological partnerships when women are experiencing vaginosis, or in women more likely to be affected by HIV, STI or pre-term birth. Several conceptual and technical advances have re-defined the modern understanding of *G. vaginalis* in relation to vaginosis, including: 1) massive expansion of readily available molecular biology techniques and reagents, ranging from multi-target quantitative PCR to systems biology/omics via whole genome high-throughput sequencing and mass spectrometry techniques, 2) increasingly refined culture-based strategies to describe potentially virulent or protective properties of bacterial strains in vaginal secretions, 3) microscopic analysis of the arrangement of

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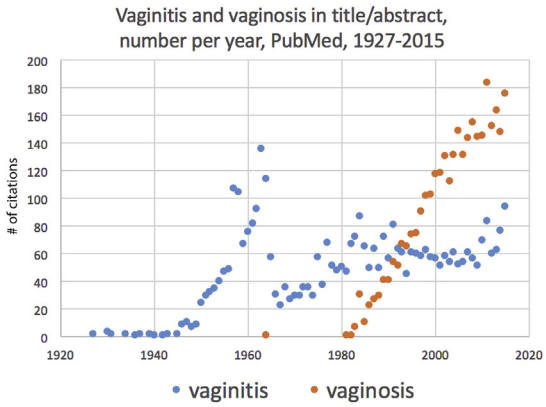

Fig. 1. Left: Use of the terms "vaginitis" and "vaginosis" in PubMed articles since 1927, indicating year of publication for articles by Gardner and Dukes, linking "*G. vaginalis*" to "vaginosis". Note that the first use of vaginosis (1964) does not concern vaginal microbiology. Right: H.L. Gardner at the First International Conference on Vaginosis – Non-specific Vaginitis, Kristiansand, Norway, April 16–17, 1982. He provided the introduction to the proceedings [79] and, unfortunately, was also the subject of the leading obituary.

bacterial cells in mucosal strata including adherent polymicrobial biofilm, and 4) increased characterization of mucosal innate and acquired immune effectors in response to specific virulence factors, microbes or microbial combinations. Freed from an exclusive reliance on culture, molecular microbiologists have discovered previously unrecognized microbial diversity within the vaginal microbiome and within *G. vaginalis*, suggesting potentially significant associations between *G. vaginalis* and other microbial species, as recently reviewed [6]. Despite this advance, enhanced culture techniques are still required in order to test hypotheses about microbial functions and interactions. Additionally, both culture and target-based molecular studies inherently underemphasize the physical arrangement of cells of different species in vaginal mucosal layers, with subsequent analyses necessarily based on description of co-occurrence of *G. vaginalis* and other microbial species in proportional terms. In contrast, microscopic techniques ranging from wet mount and Gram stain to the most advanced confocal microscopy with phylogenetically-targeted fluorophores provide more or less detailed information about bacterial diversity, but are essential to understand physical arrangement of bacterial and human cells *in vivo*. Although *G. vaginalis* and/or polymicrobial biofilm has been recognized as a factor in vaginosis for decades as "clue cells", microscopy has recently provided more insight into the phylogenetic diversity and physical structure of *G. vaginalis* biofilms intimately associated with the vaginal mucosa [7,8], as well as of intracellular *G. vaginalis* [9].

The original case for fulfillment of Koch's postulates linking the cause of vaginosis with *G. vaginalis*, made by Gardner and Dukes (1955), continues to be defended and derided, even in current literature [10,11], but its specific role in the natural history of specific vaginal symptoms and/or immune impairment leading to silent reproductive health risks remains elusive [12]. Since the clinical category "vaginosis" is poorly descriptive, with little agreement in the literature as to its etiology and natural course, and no cure in sight, our goal is to review the state of knowledge regarding the phylogenetic diversity, microbial associations and clinical significance of *G. vaginalis*, the Actinobacterium originally described as the cause of this enigmatic syndrome.

2. Phylogenetics of protein-coding genes reveals *G. vaginalis* diversity

Phenotypic heterogeneity within *G. vaginalis* has been recognized since the small, pleomorphic, rod-shaped organism was first identified and observed to give variable results in Gram staining. Based on current understanding of the cell wall structure and biochemical properties of *G. vaginalis*, it is considered a Gram-positive bacterium [13]. Efforts to identify phenotypic traits shared universally by *G. vaginalis*, which would be clinically useful in order to distinguish it from other catalase-negative coryneforms, resulted in a rather short list including beta-hemolysis on human blood agar, negative catalase reaction, hippurate hydrolysis and lack of growth on nutrient agar or in the presence of 2% (w/v) sodium chloride [14,15]. Proposals have been made for disambiguating *G. vaginalis* based on phenotypic properties ("biotyping") [16,17] or targeted genotyping methods such as amplified ribosomal DNA restriction analysis (ARDRA) [18]. However, there has been little success in reconciling the genotypic and phenotypic characteristics with each other, or in identifying patterns of association of any genotype or phenotype with demographic or clinical characteristics. Reports of correspondence between specific biotypes and clinical status are variable, with some authors reporting significant associations between particular biotypes and vaginosis symptoms [19–23]. Observations of ARDRA genotypes and their association with biotype or specific virulence factors are similarly variable [23–26]. While these approaches for classification are somewhat useful for examining cultured isolates, the requirement for culture means that they cannot be readily applied to addressing questions of the role of *G. vaginalis* in the context of the

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