for the first time in syphilis research, we describe a transformation protocol that successfully replaced the *tpaA* (tp0009) pseudogene in the SS14 *T. pallidum* strain with a kanamycin resistance (*kanR*) cassette.

Principal findings A suicide vector was constructed using the pUC57 plasmid backbone. In the vector, the *kanR* gene was cloned downstream of the *tp0574* gene promoter. The *tp0574*prom-*kanR* cassette was then placed between two 1-Kbp homology arms identical to the sequences upstream and downstream of the *tpaA* pseudogene. To induce homologous recombination of the arms into the *T. pallidum* chromosome, with resulting integration of the *kanR* cassette, in vitro-cultured SS14 strain spirochetes were exposed to the engineered vector resuspended in a transformation buffer and let recover for 24 hours before adding kanamycin-containing selective media. Integration of the *kanR* cassette was demonstrated by qualitative PCR, droplet digital PCR (ddPCR) and whole genome sequencing (WGS) of transformant treponemes propagated in vitro and in vivo. ddPCR analysis of RNA and mass spectrometry confirmed expression of the *kanR* message and protein in treponemes propagated in vitro. Moreover, *tpaA* knockout (*tpAko*-SS14) treponemes grew in kanamycin concentrations that were 64 times higher than the MIC for the wild-type SS14 (wt-SS14) strain and in infected rabbits treated with kanamycin.

Conclusion We demonstrated that genetic manipulation of *T. pallidum* is attainable. This discovery will allow the application of functional genomics to study syphilis pathogenesis and improve syphilis vaccine development.

001.8

CONTEMPORARY SYPHILIS IS CHARACTERISED BY RAPID GLOBAL SPREAD OF PANDEMIC TREPONEMA PALLIDUM LINEAGES

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Background Syphilis is an important sexually transmitted infection caused by the bacterium *Treponema pallidum* subspecies *pallidum*. The last two decades have seen syphilis incidence rise in many high-income countries, yet the evolutionary and epidemiological relationships that underpin this are poorly understood, as is the global *T. pallidum* population structure.

Methods We assembled a geographically and temporally diverse collection of clinical and laboratory samples, performing direct sequencing on the majority, and combining these with 133 publicly available sequences to compile a dataset comprising 726 *T. pallidum* genomes. We analysed the resulting genomes using detailed phylogenetic analysis and clustering.

Results We show that syphilis globally can be described by only two deeply branching lineages, Nichols and SS14. We show that both of these lineages can be found circulating concurrently in 12 of the 23 countries sampled. To provide further phylodynamic resolution we subdivided *Treponema*

pallidum subspecies *pallidum* into 17 distinct sublineages. Importantly, like SS14, we provide evidence that two Nichols sublineages have expanded clonally across 9 countries contemporaneously with SS14. Moreover, pairwise genome analysis showed that recent isolates circulating in 14 different countries were genetically identical in their core genome to those from other countries, suggesting frequent exchange through international transmission pathways. This contrasts with the majority of samples collected prior to 1983, which are phylogenetically distinct from these more recently isolated sublineages. Bayesian temporal analysis provided evidence of a population bottleneck and decline occurring during the late 1990s, followed by a rapid population expansion a decade later. This was driven by the dominant *T. pallidum* sublineages circulating today, many of which are resistant to macrolides.

Conclusion Combined we show that the population of contemporary syphilis in high-income countries has undergone a recent and rapid global expansion. This dataset will provide a framework for future characterisation and epidemiological investigation of syphilis populations.

Vaginal microbiota

002.1

CERVICOVAGINAL MICROBIOTA SPECIES DISTINCTLY MODULATE THE IMMUNOMETABOLIC MICROENVIRONMENT IN A HUMAN THREE-DIMENSIONAL CERVICAL MODEL

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Background Bacterial vaginosis-associated bacteria (BVAB) have been linked to gynecologic and obstetric sequelae, including an increased risk of STI acquisition and pre-term birth. However, there is a fundamental gap that exists in understanding the function of these microorganisms in the local microenvironment that contribute to disease. Hence, our objective was to identify immunometabolic signatures of cervicovaginal microbiota species in the context of cervical epithelium that can relate to clinical findings.

Methods Human three-dimensional (3D) cervical epithelial cell models were infected under anaerobic conditions with *Gardnerella vaginalis*, *Prevotella bivia*, *Atopobium vaginae*, *Sneathia amnii*, a polymicrobial community of BVAB, or health-associated *Lactobacillus crispatus*. Cell culture supernatants were collected 24 h post infection and analyzed using multiplex cytometric bead arrays and ultrahigh-performance liquid chromatography-mass spectroscopy.

Results *Lactobacillus* and BVAB effectively colonized the surface and crevices of human 3D cervical model visualized by scanning electron microscopy. Immunoproteomics analysis (28 targets) revealed that *A. vaginae*, *S. amnii* and polymicrobial community exert the greatest proinflammatory potentials, whereas *G. vaginalis* and *P. bivia* mostly altered epithelial barrier targets. *S. amnii* also induced proteins related to cellular stress and angiogenesis. The metabolomics analysis yielded 418 known metabolites. Random Forest analysis of metabolic profiles highlighted excellent prediction (93.75%) of infections. Furthermore, *A. vaginae*, *S. amnii* and the polymicrobial community profiles clustered

separately from *G. vaginalis*, *P. bivia*, *L. crispatus* and controls. BVAB induced production of biogenic amines in a species-specific manner. *A. vaginae* and *S. amnii* impacted arginine/citrulline metabolism, leading to pro-inflammatory signaling, and induced production of oxidative stress-related metabolites. In contrast, *G. vaginalis* and *P. bivia* altered epithelial barrier through mucin and collagen degradation and potential ammonia production.

Conclusions We demonstrated the utility of our 3D model to recapitulate the cervicovaginal microenvironment and identified unique and species-specific mechanisms by which BVAB contribute to pathophysiological changes favorable for STIs.

002.2 THE IMPACT OF CONCURRENT PARTNER TREATMENT FOR BACTERIAL VAGINOSIS ON THE GENITAL MICROBIOTA OF HETEROSEXUAL COUPLES: A PILOT STUDY

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Background Approximately 40% of women receiving first-line antibiotics for bacterial vaginosis (BV) experience recurrence within 3-months. Reinfection from an untreated regular sexual partner (RSP) likely contributes to BV-recurrence. We investigated the impact of concurrent partner treatment for BV on the genital microbiota of heterosexual couples.

Methods Women with symptomatic BV (≥ 3 Amsel criteria and Nugent Score[NS]=4–10) were recruited between March 2018–March 2019 with their RSP. Couples received oral metronidazole 400mg BID 7-days, and men additionally applied 2% clindamycin cream topically to penile skin BID for 7-days. Participants self-collected genital samples (women: vaginal; men: cutaneous penile, first-void urine [representing the urethra]) pre-treatment (day-0), post-treatment (day-8) and then 4-weekly until endpoint (week-12 or BV-recurrence). We characterised the genital microbiota using 16S-rRNA gene sequencing. Differential abundance testing was performed using ALDEX2. Changes in microbiota composition post-treatment were assessed using ANOSIM.

Results Data from 29 couples were analysed. Women had a high prevalence of risk factors for BV-recurrence; 79% had a history of BV, 83% had an uncircumcised RSP, all reported condomless sex during the study and 38% had an intrauterine device. Only 4/27 (15%) women completing clinical follow-up experienced BV-recurrence (≥ 3 Amsel criteria, NS=4–10). BV-associated bacteria, including *Atopobium*, *Prevotella* and *Dialister*, significantly decreased in abundance at all three genital sites immediately post-treatment (false discovery rate (FDR)-corrected p-value<0.05). BV-associated bacteria remained decreased at endpoint at all sites, but following FDR-correction this was only significant for the vaginal site. Overall,

partner treatment significantly and immediately changed the composition of the female and male genital microbiota (AON-ISM; p-value=0.001).

Conclusion In a cohort of women at high risk for BV-recurrence, only 15% experienced recurrence within 3-months of concurrent partner treatment. Treatment reduced the abundance of BV-associated bacteria and altered the genital microbiota composition of both partners; although only changes at the vaginal site remained significant at endpoint.

002.3 RECLASSIFICATION OF ATOPOBIUM VAGINAE AS THREE NOVEL FANNYHESSEA SPECIES: IMPLICATIONS FOR UNDERSTANDING THEIR ROLE IN BACTERIAL VAGINOSIS

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Background *Atopobium vaginae* is a prevalent vaginal bacterium associated with bacterial vaginosis (BV). Molecular studies targeting its 16S rRNA gene have shown *A. vaginae* is a useful diagnostic indicator of BV and important secondary colonizer of the vaginal biofilm. Both *A. vaginae* and the vagina's primary biofilm-forming species, *Gardnerella vaginalis*, exhibit extreme genomic heterogeneity. This led to *Gardnerella*'s recent split into approximately nine genomospecies indistinguishable by their 16S genes. The objective of this work was to ascertain whether similar subdivisions should be made for *A. vaginae*.

Methods We performed comparative genomics on publically available *A. vaginae* whole genome sequences. We also genotyped 20 *A. vaginae* isolates from women participating in a BV treatment study and phenotypically characterized select strains to identify their biochemical properties (API/MIC tests) and virulence potential.

Results Three genomospecies were defined by cross-species nucleic acid identity <74%; each shares $\geq 99\%$ 16S rRNA gene identity and belongs to the new genus *Fannyhessea*. Two species previously cultured and sequenced are *Fannyhessea vaginae* (CCUG 38953T, representing 85% of cultured isolates) and *Fannyhessea massiliense* (Marseille-P4126T, PB189–14T). These species differ in alkaline and acid phosphatase activity, as well as leucine arylamidase activity. Novel *Fannyhessea* species type 2 was distinguished by its inability to ferment sucrose or tagatose and its sensitivity to metronidazole. Colony morphology, Gram stain, and lack of detectable catalase, indole, sialidase, proteinase, and hyaluronidase activities were similar for all three species. Functional genomics revealed *F. vaginae* uniquely possesses sialic acid response machinery and several adhesion proteins.

Conclusions Genomic and functional differences support redefining *A. vaginae* as three novel *Fannyhessea* species. More work is needed to re-examine its role in BV in the absence of confounding by the other species, but our findings suggest *F. vaginae* may contribute to treatment failure and interact specifically with the *Gardnerella* biofilm and host epithelium.