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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10.1136/sextrans-2021-sti.55

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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10.1136/sextrans-2021-sti.56

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