heteroresistant to fosfomycin. Of the isolates found to be susceptible by standard testing, 1(0.3%) and 9(3.6%) were heteroresistant to nitrofurantoin and trimethoprim-sulfamethoxazole by PAP, respectively. DISCUSSION/SIGNIFICANCE OF IMPACT: Despite low rates of HR to nitrofurantoin and trimethoprim-sulfamethoxazole (0.84%, 4.8%), HR to fosfomycin was more frequent (7.6%). Given that susceptibility is not generally performed for fosfomycin, this could have implications for including fosfomycin as a first-line treatment for *E. coli* UTIs.

## Assessing genetic diversity of the Pfs25 vaccine candidate: Implications for malaria transmission-blocking vaccine in Africa

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OBJECTIVES/GOALS: Transmission-blocking vaccines hold promise for malaria elimination by reducing community transmission. But a major challenge that limits the development of efficacious vaccines is the vast parasite's genetic diversity. This work aims to assess the genetic diversity of the Pfs25 vaccine candidate in complex infections across African countries. METHODS/STUDY POPULATION: We employed next-generation amplicon deep sequencing to identify nonsynonymous single nucleotide polymorphisms (SNPs) in 194 Plasmodium falciparum samples from four endemic African countries: Senegal, Tanzania, Ghana, and Burkina Faso. The individuals aged between 1 and 74 years, but most of them ranged from 1 to 19 years, and all presented symptomatic P. falciparum infection. The genome amplicon sequencing was analyzed using Geneious software and P. falciparum 3D7 as a reference. The SPNs were called with a minimum coverage of 500bp, and for this work, we used a very sensitive threshold of 1% variant frequency to determine the frequency of SNPs. The identified SNPs were threaded to the crystal structure of the Pfs25 protein, which allowed us to predict the impact of the novel SNP in the protein or antibody binding. RESULTS/ANTICIPATED RESULTS: We identified 26 SNPs including 24 novel variants, and assessed their population prevalence and variant frequency in complex infections. Notably, five variants were detected in multiple samples (L63V, V143I, S39G, L63P, and E59G), while the remaining 21 were rare variants found in individual samples. Analysis of countryspecific prevalence showed varying proportions of mutant alleles, with Ghana exhibiting the highest prevalence (44.6%), followed by Tanzania (12%), Senegal (11.8%), and Burkina Faso (2.7%). Moreover, we categorized SNPs based on their frequency, identifying dominant variants (>25%), and rare variants (DISCUSSION/ SIGNIFICANCE OF IMPACT: We identified additional SNPs in the Pfs25 gene beyond those previously reported. However, the majority of these newly discovered display low variant frequency and population prevalence. Further research exploring the functional implications of these variations will be important to elucidate their role in malaria transmission.

## Disparities in healthcare discrimination among sexual minority groups: Insights from the NIH All of Us Program Chen Zhang, Caitlin Deisbach, Wonkyung Chang, Yang Yu and

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OBJECTIVES/GOALS: Discriminatory experiences within healthcare settings significantly hinder equitable health access for sexual minority groups (SMPs) in the USA. These discriminatory experiences can manifest in various forms (e.g., refusal of care). We aimed to explore different types of discrimination encountered by SMPs in the healthcare settings. METHODS/STUDY POPULATION: This study utilized secondary data from the NIH All of Us Research Program. For this analysis, we selected cohorts self-identifying as gay (n = 9,454), bisexual (n = 15,284), lesbian (n = 5,267), and straight (n = 349,748), enabling robust comparisons across SMPs and straight individuals. We employed analysis of variance and Chisquare analyses to assess group differences in healthcare discrimination, using key indicators from the Discrimination in Medical Settings Scale. These indicators captured experiences such as being treated with less respect or courtesy and feeling ignored by healthcare providers, providing a comprehensive view of discriminatory encounters in healthcare settings for SMPs. RESULTS/ ANTICIPATED RESULTS: Our analyses revealed that bisexual individuals reported the highest levels of healthcare discrimination (mean = 3.64, SD = 2.45), followed by lesbians (mean = 3.37, SD = 2.47), other SMPs (mean = 3.36, SD = 2.53), gay (mean = 2.69, SD = 2.47), and straight participants (mean = 2.60, SD = 2.42). Among the seven discrimination indicators, the most reported experience was feeling like a doctor or nurse was not listening, with 76.8% of bisexual participants, 72.3% of lesbians, 68.8% of other SMPs, and 56.9% of gay participants reporting this experience. This was followed by reports of being treated with less respect and being treated with less courtesy in healthcare settings. These findings highlight the pervasive nature of healthcare discrimination among particularly bisexual individuals. DISCUSSION/ SIGNIFICANCE OF IMPACT: SMPs experience higher levels of