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Sulfamethoxazole Susceptibility of *Mycobacterium tuberculosis* Isolates from HIV-Infected Ugandan Adults with Tuberculosis Taking Trimethoprim-Sulfamethoxazole Prophylaxis

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Additional drugs are needed for the treatment of multidrug-resistant tuberculosis (TB). Sulfamethoxazole has been shown to have *in vitro* activity against *Mycobacterium tuberculosis*; however, there is concern about resistance given the widespread use of trimethoprim-sulfamethoxazole prophylaxis among HIV-infected patients in sub-Saharan Africa. Thirty-eight of 40 *Mycobacterium tuberculosis* isolates (95%) from pretreatment sputum samples from Ugandan adults with pulmonary TB, including HIV-infected patients taking trimethoprim-sulfamethoxazole prophylaxis, were susceptible with MICs of ≤38.4 μg/ml.

Alternative drugs are urgently needed to treat multidrug-resistant (MDR) tuberculosis (TB). Given the difficulties of new drug development, repurposing currently licensed antibiotics is practical and efficient. Trimethoprim-sulfamethoxazole (SXT) is a fixed-dose drug combination used worldwide as treatment and prophylaxis for multiple infections. Sulfamethoxazole (SMX) is in the sulfonamide class of antibiotics, which were explored as an anti-TB treatment in the mid-20th century with early studies showing potential value for the treatment of pulmonary and miliary TB (1–5). More recently, Forgacs et al. reported defervescence of a patient with pulmonary TB who was initially treated with SXT alone and also demonstrated *in vitro* susceptibility to SXT in 43 of 44 *Mycobacterium tuberculosis* isolates (6). These drug susceptibility results were independently confirmed in laboratory strains (7, 8) and in patient isolates demonstrating SMX to be the active agent with MICs within achievable serum levels (9, 10). In addition, Alsaad and colleagues reported the use of SXT as part of a combination regimen used to treat 10 patients with MDR-TB in the Netherlands (11). They also reported *M. tuberculosis* susceptibility to SXT in 17 of 18 patients with TB-HIV coinfection; however, only 1 was taking SXT prior to TB diagnosis (12). Given the development of drug resistance when active TB is treated with a single drug, there is concern for resistance to SMX among TB-HIV-coinfected patients taking SXT prophylaxis. To address this concern, we performed drug susceptibility testing (DST) on *M. tuberculosis* isolates obtained from pretreatment sputum specimens of HIV-infected patients taking SXT prophylaxis at the time of diagnosis of active TB.

Sputum isolates used for this study were collected from patients enrolled in the Kawempe Community Health Study (KCHS), a prospective cohort of adults with newly diagnosed pulmonary TB and their household contacts (13, 14). Kawempe is located in Kampala, Uganda, which is a high-TB-burden country with an annual incidence of approximately 166 TB cases per 100,000 population (15). The isolates included in this study were a convenience sample collected between November 2007 and July 2012, prior to the initiation of anti-TB treatment. Patients underwent HIV testing and self-reported concomitant medications were recorded at baseline. In addition, isolates were excluded if they were resistant to isoniazid, rifampin, pyrazinamide, or ethambutol.

Susceptibility testing was adapted from the Bactec MGIT 960 SIRE kit instructions (Becton Dickinson, Sparks, MD) and the CLSI approved standard M24-A2 (16). Briefly, pure *M. tuberculosis* colonies grown on solid media (Löwenstein-Jensen slants or Middlebrook 7H10 plates; Becton-Dickinson) were inoculated into 7-ml MGIT tubes supplemented with oleic acid-albumin-dextrose-catalase (OADC) and polymyxin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) antibiotics, incubated in the MGIT 960 until positive, diluted based on time since positive per SIRE instructions, and then 500 μl was used to inoculate each of 8 MGIT tubes. Drugs were prepared from powder (Sigma-Aldrich, St. Louis, MO) dissolved in dimethyl sulfoxide (DMSO). Further dilution in DMSO was carried out to obtain the drug concentrations in a standardized 100-μl volume. The no-drug control was 100 μl DMSO. Eight MGIT 7-ml tubes were inoculated for each isolate: undiluted no-drug control; rifampin, 1 μg/ml control; and SMX concentrations of 2.4, 4.8, 9.6, 19.2, 38.4, and 76.8 μg/ml. An additional growth control tube was prepared using 500 μl of a 1:100 dilution from the inoculum used in the other tubes. Growth was measured in the MGIT instrument in drug-containing tubes in relation to the growth control tube to determine drug susceptibility or nonsusceptibility. Quality control strains for SMX and rifampin were *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922, which were done on each testing day, according to CLSI methods (17).

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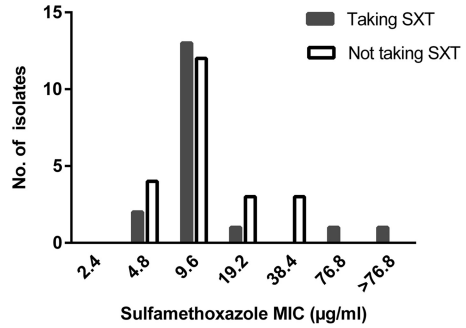

FIG 1 Distribution of sulfamethoxazole MICs among pretreatment *M. tuberculosis* isolates obtained from patients taking or not taking trimethoprim-sulfamethoxazole (SXT) at the time of TB diagnosis.

Pretreatment sputum *M. tuberculosis* isolates from 40 adults with pulmonary TB were included in this study. Median age was 27 years (interquartile range [IQR], 23 to 38 years) and median body mass index (BMI) was 20 (IQR, 18 to 22). Eighteen isolates were from patients who reported taking SXT prophylaxis, and all were HIV seropositive, with a median CD4 count of 340 cells/μl (IQR, 103 to 558 cells/μl). All 18 patients reported taking SXT at a dose of 800 mg sulfamethoxazole and 160 mg trimethoprim once daily. The start date of SXT prophylaxis was recorded in 15 of the 18 patients, and the median duration of SXT prophylaxis prior to TB diagnosis was 22 weeks with a range of 1 to 181 weeks. The remaining 22 *M. tuberculosis* isolates were from patients who did not report taking SXT; all were HIV negative. Thirty-eight of 40 isolates (95%) were susceptible to SMX with an MIC of ≤38.4 μg/ml (Fig. 1). Two of 18 isolates (11%), collected from HIV-positive patients taking SXT at the time of their TB diagnosis, had higher MICs (76.8 μg/ml and >76.8 μg/ml). The relative proportions of isolates susceptible to SMX comparing isolates from HIV-positive patients taking SXT prophylaxis and HIV-negative patients not taking SXT prophylaxis at the time of TB diagnosis did not differ when using MIC cutoffs of ≤9.6 μg/ml, ≤19.2 μg/ml, or ≤38.4 μg/ml ($P = 0.48, 0.36, 0.20$, respectively, Fisher's exact test).

In this study, of pretreatment sputum *M. tuberculosis* isolates collected from Ugandan patients with pulmonary TB, we found that 38 of 40 isolates (95%) had MICs of ≤38.4 μg/ml and were susceptible to SMX. Among HIV-positive patients taking SXT at the time of their TB diagnosis, 16 of 18 isolates (89%) had MICs of ≤38.4, while all 22 isolates from HIV-negative patients not taking SXT had MICs of ≤38.4 μg/ml. While this difference was not statistically significant, it does raise the possibility that there may be an increased prevalence of SMX resistance among TB-HIV-coinfected patients taking SXT prophylaxis. The small sample size of our study limits our ability to assess adequately a low level of resistance but does support the finding that SMX resistance is not frequent. Another limitation of our study is that medication use was self-reported, and we could not verify that patients were actually taking SXT. Despite these limitations, our findings add to a growing body of research demonstrating the *in vitro* activity of SMX against *M. tuberculosis* and provide evidence that this activity is maintained even in populations taking regular SXT prophylaxis. A limited number of second-line drugs are available for the treatment of MDR-TB, and many have intolerable side effects. SXT is approved for use around the world and is readily available, inexpensive, and well-tolerated. Current *in vitro* and clinical data support additional evaluation of SMX for the treatment of TB. A phase 2 early bactericidal activity trial of SMX is a reasonable next step (18).

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