

Mycobacterium tuberculosis and Sulfamethoxazole Susceptibility

We noted with interest the paper by Forgacs et al. (2) reporting on a case of tuberculosis with an apparent response to treatment with trimethoprim-sulfamethoxazole (TMP-SMX) and the subsequent *in vitro* studies indicating that the majority of *Mycobacterium tuberculosis* strains may be susceptible to TMP-SMX.

As the Forgacs paper implied that it is the sulfonamide component of TMP-SMX that has anti-*M. tuberculosis* activity, we sought to further test this hypothesis by testing 12 sequential patient *M. tuberculosis* isolates against SMX alone in a Mycobacterium Growth Indicator Tube (MGIT; Becton Dickinson [B-D]) broth dilution drug susceptibility test (DST) system.

SMX concentrations were chosen to match those used by Forgacs et al. and those found in commercially available broth microdilution systems for DSTs of nontuberculous mycobacteria. Briefly, suspensions of *M. tuberculosis* grown on solid media were prepared according to the B-D MGIT 960 SIRE antimicrobial susceptibility testing kit instructions, with the exception that the initial suspensions were made in Ringer's solution rather than Middlebrook 7H9 broth. A working solution of 60.8 mg of SMX (Fluka brand, supplied by Sigma-Aldrich Australia [batch number 048K1024]) dissolved in 10 ml distilled water was prepared and then diluted to give the final concentrations in MGIT tubes shown in Table 1. Growth of *M. tuberculosis* in both drug-containing and control tubes was automatically monitored in an MGIT 960 instrument.

Isolates used in the study were sourced from eight female and four male patients ranging in age from 1 to 86 years. Nine isolates were from respiratory specimens and three from extrapulmonary specimens. All isolates tested susceptible to first-line antituberculous drugs.

The MICs of the 12 isolates are shown in Table 1. All appear susceptible to SMX at a concentration ≤ 38 $\mu\text{g/ml}$ in this system.

This brief study indicates that SMX alone does indeed appear to have at least bacteriostatic activity against *M. tuberculosis* in this DST system. The MICs are within readily achievable serum levels of SMX (3) and generally within the TMP-SMX breakpoints quoted for a wide range of vegetative bacteria (1). SMX and TMP-SMX are widely available and cheap, have favorable pharmacokinetic and tissue penetration properties (3), and are generally well tolerated. Their use as potential second-line antituberculous agents is

an exciting prospect and deserves further *in vitro* and *in vivo* study.

REFERENCES

1. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement, vol. M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA.
2. **Forgacs, P., N. L. Wengenack, L. Hall, S. K. Zimmerman, M. L. Silverman, and G. D. Roberts.** 2009. Tuberculosis and trimethoprim-sulfamethoxazole. *Antimicrob. Agents Chemother.* **53**:4789–4793.
3. **Kucers, A., S. Crowe, M. Grayson, and J. Hoy.** 1997. The use of antibiotics: a clinical review of antibacterial, antifungal, and antiviral drugs, 5th ed. Butterworth-Heinemann, Oxford, United Kingdom.

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Authors' Reply

We thank Leslie et al. for performing additional testing to look into the susceptibility of *M. tuberculosis* to sulfonamides using a technique different from the two used in our study (3). They describe the results of testing a moderate number ($n = 12$) of consecutive clinical isolates of *M. tuberculosis* for susceptibility to SMX using the MGIT system for broth dilution susceptibility.

All 12 isolates of *M. tuberculosis* were found to be susceptible to ≤ 38 $\mu\text{g/ml}$ SMX in this system. These results with SMX are similar to our findings on the susceptibility of *M. tuberculosis* to TMP-SMX, with the exception that 11/44 (25%) of our isolates were susceptible at 1- to 2-fold lower SMX concentrations when tested in combination with TMP (2.4 and 4.8 $\mu\text{g/ml}$) (3). It is not clear whether this is a significant difference and, if so, whether it is due to the use of a different methodology or to the synergy of TMP with SMX.

Leslie et al. feel that the sulfa moiety is likely the active part of the antituberculous effect of TMP-SMX. Wallace et al., however, found that the addition of TMP decreased 2- to 4-fold the SMX MIC of approximately one-third of *M. fortuitum* isolates (4).

Synergy of the combination of TMP and SMX has been observed in 88% of aerobic bacteria; the occurrence of synergy is not infrequent, even in bacteria resistant to one or both of the components of TMP-SMX, and may be of a sufficient extent to bring the MIC of SMX and/or TMP into the susceptible range (1). Testing for synergy between TMP and SMX has not, to our knowledge, been performed with *M. tuberculosis* isolates.

It is also not known whether using the combination of TMP-

TABLE 1. Number of *M. tuberculosis* isolates^a inhibited by SMX at the indicated concentrations

SMX concn ($\mu\text{g/ml}$)	4.75	9.5	19	38	76	152
No. of isolates susceptible	1	5	5	1	0	0

^a A total of 12 isolates were tested.

SMX in *M. tuberculosis* infections helps to prevent the development of resistance and adds bactericidal activity, as in aerobic bacteria (1).

We are pleased that our *in vitro* susceptibility findings have been independently supported by Leslie et al. We agree with their conclusion that further study of the effects of TMP-SMX on *M. tuberculosis* is warranted. In an era of multiple and extended antituberculous drug resistance, there is an urgent need for additional second-line antituberculous drugs. There is extensive data, prior to 1955, on the effect of older, more toxic sulfonamides and sulfones *in vitro* on *M. tuberculosis*, as well as *in vivo* in experimental animals and humans with tuberculosis; this includes a randomized, non-controlled human study of sulfapyridine monotherapy showing frequent sputum conversion and clinical efficacy (2). We feel, therefore, that the evaluation of TMP-SMX as an antituberculous agent should not follow the pathway used for a completely new drug found through the drug discovery process.

REFERENCES

1. **Bushby, S. R.** 1969. Combined antibacterial action *in vitro* of trimethoprim and sulfonamides. The *in vitro* nature of synergy. *Postgrad. Med. J.* **45**(Suppl.): 10-18.
2. **Ellman, P.** 1941. Investigation in the value of sulfapyridine in the treatment of pulmonary tuberculosis. *Tubercle* **22**:296-302.
3. **Forgacs, P., N. L. Wengenack, L. Hall, S. K. Zimmerman, M. L. Silverman, and G. D. Roberts.** 2009. Tuberculosis and trimethoprim-sulfamethoxazole. *Antimicrob. Agents Chemother.* **53**:4789-4793.
4. **Wallace, R. J., Jr., K. Wiss, M. B. Bushby, and D. C. Hollowell.** 1982. *In vitro* activity of trimethoprim and sulfamethoxazole against the nontuberculous mycobacteria. *Rev. Infect. Dis.* **4**:326-331.

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