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*J. Clin. Microbiol.* 2012, 50(12):4067. DOI:  
10.1128/JCM.02195-12.  
Published Ahead of Print 10 October 2012.

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# Is *Streptococcus pyogenes* Resistant or Susceptible to Trimethoprim-Sulfamethoxazole?

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*Streptococcus pyogenes* is commonly believed to be resistant to trimethoprim-sulfamethoxazole (SXT), resulting in reservations about using SXT for skin and soft tissue infections (STTI) where *S. pyogenes* is involved. *S. pyogenes*' *in vitro* susceptibility to SXT depends on the medium's thymidine content. Thymidine allows *S. pyogenes* to bypass the sulfur-mediated inhibition of folate metabolism and, historically, has resulted in apparently reduced susceptibility of *S. pyogenes* to sulfur antibacterials. The low thymidine concentration in Mueller-Hinton agar (MHA) is now regulated. We explored *S. pyogenes* susceptibility to SXT on various media. Using two sets of 100 clinical *S. pyogenes* isolates, we tested for susceptibility using SXT Etests on MHA containing defibrinated horse blood and 20 mg/liter  $\beta$ -NAD (MHF), MHA with sheep blood (MHS), MHA alone, MHA with horse blood (MHBA), and MHA with lysed horse blood (MHLHBA). European Committee on Antibacterial Susceptibility Testing (EUCAST) breakpoints defined susceptibility (MIC,  $\leq 1$  mg/liter) and resistance (MIC,  $> 2$  mg/liter). In study 1, 99% of *S. pyogenes* isolates were susceptible to SXT on MHA, MHBA, and MHLHBA, with geometric mean MICs of 0.04, 0.04, and 0.05 mg/liter, respectively. In study 2, all 100 *S. pyogenes* isolates were susceptible to SXT on MHF, MHS, MHA, and MHLHBA with geometric mean MICs of 0.07, 0.16, 0.07, and 0.09 mg/liter, respectively. This study confirms the *in vitro* susceptibility of *S. pyogenes* to SXT, providing support for the use of SXT for STTIs. A clinical trial using SXT for impetigo is ongoing.

*Streptococcus pyogenes* was one of the first bacterial infections to be treated with sulfur antibacterials in the 1930s (16) and proved to be clinically effective in the treatment and prophylaxis of *S. pyogenes* infections (10, 16, 23, 29). However, when sulfadiazine, an early short-acting sulfur antibacterial, was used in mass prophylaxis programs to prevent *S. pyogenes* tonsillitis and acute rheumatic fever (ARF) in military recruits in the 1940s, the clinical efficacy of this antibacterial was limited due to the presumed development of resistance (13, 15, 27, 31, 42) among some strains. Initial antibacterial susceptibility testing (AST) of *S. pyogenes* to sulfur antibacterials using a broth dilution method demonstrated that some strains were resistant (25, 53); however, AST was in its infancy and no standardized reference methods existed at that time. This early experience resulted in the belief that trimethoprim-sulfamethoxazole (SXT) is ineffective against *S. pyogenes*, and its use has been discouraged in clinical practice for decades (35).

Subsequent antibacterial susceptibility experiments showed apparently reduced susceptibility of *S. pyogenes* (and other bacteria) (6, 40) to the sulfur antibacterials due to antagonism of the inhibition of folate metabolism. Harper and Cawston discovered an inhibitory substance in 1945, eventually identified as thymidine, which was interfering with the ability of sulfur antibacterials to kill the organism (25). Because the activity of SXT is determined by the antibacterial's ability to deprive an organism of folate coenzymes (7), there is a direct relationship between the thymidine levels in culture media and SXT resistance (11). High thymidine content in agar provides an exogenous substrate which can be used by an organism to maintain folate metabolism and hence appear resistant to SXT. In early studies, most culture media contained sufficient thymidine to antagonize the inhibitory effects of

sulfur drugs and hence produced resistant results when this class of antibacterials was tested (6).

Notably, lysed horse blood was found to contain the enzyme thymidine phosphorylase, which neutralized thymidine (46) and overcame this effect (21, 25). No other mammalian blood contains thymidine phosphorylase (21). However, the addition of lysed horse blood was not recommended for AST, despite several authors (5, 6, 21, 54) advising supplementation with lysed horse blood for any medium used to test sulfur antibacterial susceptibility if the thymidine concentration was above 0.03  $\mu$ g/ml (5) (below which inhibition does not occur). In this context, the notion that *S. pyogenes* was resistant to sulfur antibacterials perpetuated.

SXT was introduced in 1968 (3, 28, 43) and has since become one of the most widely used antibacterials in the world. However, recommendations against the use of sulfur antibacterials, including SXT, for *S. pyogenes* infections continue in the belief that the organism is intrinsically resistant (33, 47, 51). Two studies (33, 51) have reported *S. pyogenes* uniformly resistant to SXT, but this was prior to thymidine content standardization in Mueller-Hinton agar (MHA) and was on agar containing sheep blood. There have also been reported clinical failures in the use of this agent in eradicating *S. pyogenes* from nasopharyngeal carriage (30). However, other centers have demonstrated full *in vitro* susceptibility of *S.*

Received 19 August 2012 Returned for modification 10 September 2012

Accepted 4 October 2012

Published ahead of print 10 October 2012

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doi:10.1128/JCM.02195-12

*pyogenes* to SXT (14, 22, 36, 54). Since 2006, when the thymidine content of MHA became strictly regulated by the Clinical and Laboratory Standards Institute (CLSI) to maintain a low level of thymidine and hence avoid inhibition (M6-A2 protocol) (9), it has no longer been necessary to add lysed horse blood to the medium for AST. However, current methods do use agar supplemented with mammalian blood.

Given the prevailing view that caution should be exercised in using SXT for infections involving *S. pyogenes* and the paucity of clinical data of SXT efficacy against *S. pyogenes*, we sought to confirm or disprove the notion that *S. pyogenes* is resistant to SXT *in vitro* on various antibacterial susceptibility testing media. Coinfection of *S. pyogenes* with *Staphylococcus aureus* in skin and soft tissue infections (SSTI) and the rising prevalence of methicillin-resistant *S. aureus* (MRSA) provide added stimulus to explore the utility of SXT in the treatment of these infections.

## MATERIALS AND METHODS

**Swab collection and identification of *S. pyogenes*.** Skin, throat, or nose swabs were collected using a rayon tipped cotton swab (Copan, Interpath Services, Melbourne, Australia). In study 1, swabs were plated on horse blood agar (HBA) (Oxoid, Basingstoke, United Kingdom) and HBA containing colistin and nalidixic acid (HBA + CNA) (Oxoid) within 48 h of collection. In study 2, swabs were stored in skim milk-tryptone-glucose-glycerol broth (STGGB) at  $-70^{\circ}\text{C}$  prior to plating on the above-described media. Incubation was at  $37^{\circ}\text{C}$  for 16 h in 5%  $\text{CO}_2$ .  $\beta$ -Hemolytic colonies were identified morphologically, and confirmation of *S. pyogenes* was with the Lancefield streptococcal grouping test for group A (Oxoid). Isolates were stored in glycerol at  $-70^{\circ}\text{C}$  until subsequent replating for AST.

**Selection of isolates. (i) Study 1.** We began by exploring the issue with a preliminary study of 100 skin and throat isolates of *S. pyogenes* collected from 3 remote Australian Aboriginal communities between 2003 and 2005 during surveillance studies (39). We used an SXT Etest strip (bioMérieux, France) on 3 different agars: MHA, MHA supplemented with horse blood (MHBA), and MHA supplemented with lysed horse blood (MHLHBA) (Oxoid). Interpretation was based on European Committee on Antibacterial Susceptibility Testing (EUCAST) breakpoints (20) released in 2010.

**(ii) Study 2.** The Skin Sore Trial is a randomized, controlled trial (RCT) comparing benzathine penicillin G (BPG) treatment of impetigo (the standard of care) with oral SXT. The first 100 *S. pyogenes* isolates from skin and nasal swabs from participants in the Skin Sore Trial were used in study 2. The participants were 3 months to 13 years old and were from 4 remote Aboriginal communities of the Top End of the Northern Territory, Australia, recruited in 2010. Swabs were collected from the anterior nares and at least 2 purulent or crusted sores from all children. Swabs were collected from skin sores on day 0 (pretreatment), day 2 (midtreatment), and day 7 (completion of treatment). Consent for participation and collection of specimens was obtained from the guardian or parent of each participant. The study was approved by the Northern Territory Top End Human Research Ethics Committee (HREC 09/08) and has been registered with the Australian and New Zealand Clinical Trials Registry (ACTRN 12609000858291).

**Antibacterial susceptibility testing methods and agar used.** The two internationally accredited, standardized methods for AST (Table 1) are the CLSI (8) and EUCAST (19) methods. CLSI does not provide reference breakpoints for *S. pyogenes* susceptibility to SXT and recommends the use of Mueller-Hinton agar (MHA) supplemented with 5% sheep blood for testing the susceptibility of *S. pyogenes* to other antibacterials. In contrast, EUCAST has released breakpoints for the disk diffusion method and MIC on appropriate media. The medium recommended for *Streptococcus* groups A, B, C, and G is MHA supplemented with 5% defibrinated horse blood + 20 mg/liter  $\beta$ -NAD. This agar is commonly referred to as MHF (<http://www.eucast.org>, accessed 25 July 2012).

TABLE 1 Antibiotic susceptibility testing methods and agar used

Method	Study	Agar used	Abbreviation
CLSI (8)	2	Mueller-Hinton agar supplemented with 5% sheep blood	MHS
EUCAST (19)	2	Mueller-Hinton agar supplemented with 5% defibrinated horse blood + $\beta$ -NAD	MHF
Experimental	1 and 2	Mueller-Hinton agar	MHA
Experimental	1 and 2	Mueller-Hinton agar containing 5% lysed horse blood	MHLHBA
Experimental	1	Mueller-Hinton agar containing horse blood	MHBA

Two experimental agars were also used for susceptibility testing. MHA is routinely used in a number of other antibacterial susceptibility tests not involving  $\beta$ -hemolytic streptococci. MHLHBA was used to explore the hypothesis based on historical literature that the lysing of horse blood releases thymidine phosphorylase which breaks thymidine down to thymine.

Using the EUCAST breakpoints (20), with an MIC of  $\leq 1$  mg/liter as sensitive and an MIC of  $> 2$  mg/liter as resistant, and an SXT Etest to perform antibacterial susceptibility, these studies compared the various media that have been recommended for AST. The Etest interpretation is based on the trimethoprim component of the trimethoprim-sulfamethoxazole combination, in a ratio of 1:19. The EUCAST methodology using the Etest was chosen due to the availability of published breakpoints; however, due to the widespread use of CLSI, the medium upon which this organism is tested was also used and results obtained were referenced to the EUCAST breakpoints.

**Antibacterial susceptibility testing.** Single colonies were isolated from frozen stocks following overnight incubation on HBA in 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ . Susceptibility testing for SXT was performed using a 0.5 McFarland suspension to create a confluent lawn inoculum and then applying an SXT Etest as per the manufacturer's instructions. Plates were read by 2 readers following incubation in 5%  $\text{CO}_2$  at  $35^{\circ}\text{C}$  for 16 to 20 h. The MIC was recorded where the inhibition ellipse intersected the scale. Where a difference in results of more than 2 gradations was noted between the 2 readers, a repeat test was performed with a fresh subculture of *S. pyogenes*. As SXT is a bacteriostatic antibacterial, this mode of action can alter the appearance of an MIC endpoint, resulting in hazy zones. Where haze was present, both the 80% and 100% points of ellipse intersection were recorded. A penicillin, erythromycin, and clindamycin disk diffusion test according to CLSI guidelines (8) was conducted concurrently on all 100 strains in study 2.

**Quality control.** In study 2, for every 20 *S. pyogenes* clinical isolates, a control strain of *S. pyogenes* (ATCC 19615) was also tested on the 4 agars and found to be susceptible. Quality control of the SXT Etests was performed throughout the study using *Escherichia coli* (ATCC 25922) on MHA and *Streptococcus pneumoniae* (ATCC 49619) on MHS. The reference ranges for each organism were achieved, namely, 0.064 to 0.25  $\mu\text{g}/\text{ml}$  for *E. coli* and 0.125 to 1  $\mu\text{g}/\text{ml}$  for *S. pneumoniae*.

STATA version 12.0 (STATAcorp, College Station, TX) was used to determine the geometric means. The data were logarithmically transformed to a normal distribution, and paired *t* tests were used to determine the difference between agars for studies 1 and 2.

TABLE 2 SXT Etest susceptibility results for study 1

Medium	% of isolates		Geometric mean MIC (mg/liter)	SD (log)
	Susceptible (MIC ≤ 1 mg/liter)	Resistant (MIC > 2 mg/liter)		
MHBA	100	0	0.04	1.52
MHA	99	1	0.04	2.2
MHLHBA	100	0	0.05	1.78

**RESULTS**

**Study 1.** On MHBA and MHLHBA, *S. pyogenes* isolates from the skin (*n* = 36) and throat (*n* = 64) were uniformly susceptible to SXT (Table 2). Ninety-nine isolates tested on MHA were susceptible to SXT (Table 2). The single isolate which appeared resistant on MHA was susceptible on both MHBA and MHLHBA.

There was no statistically significant difference between the geometric mean MIC measurements on MHA and MHBA. However, isolates tested on both MHA and MHBA had lower MICs than isolates tested on MHLHBA (Table 3).

**Study 2.** One hundred isolates of *S. pyogenes* from 43 children were included in this analysis. *S. pyogenes* isolates utilized were from sores (*n* = 98) and the anterior nares (*n* = 2). The majority of isolates (76%) were from day 0 (before antibacterial treatment); 19% were from day 2, and 5% were from day 7. Sixty-four of the swabs from which *S. pyogenes* was identified also cultured *S. aureus*. Of these, 14% were methicillin-resistant *S. aureus* (MRSA) and 86% were methicillin-susceptible *S. aureus* (MSSA).

All 100 isolates of *S. pyogenes* were susceptible to SXT on all agars by both readers (Table 4). Interrater reliability was excellent, with 96% of all MIC readings within ± 1 MIC gradation. In view of this, all analyses were done on results from reader 1. All 100 *S. pyogenes* isolates were also susceptible to penicillin, erythromycin, and clindamycin.

The geometric means were similar for MHA, MHBA, and MHLHBA (Table 4). MHS had a higher geometric mean MIC than the other media. This was statistically significant, with isolates tested on MHS having higher geometric mean MICs than the same isolates tested on all other agars (Table 5). Despite the higher MICs, all isolates tested on MHS were still susceptible to SXT. There was no difference in MIC between isolates tested on MHA and those tested on MHF. As in study 1, isolates tested on MHA had lower MICs than the same isolates tested on MHLHBA. This was also found for isolates tested on MHF (Table 5).

Ongoing surveillance with *in vitro* susceptibility testing is needed to monitor for changes in rates of SXT resistance with increased use of SXT. To date, we have tested 910 *S. pyogenes* isolates cultured from impetigo and anterior nares of children

TABLE 3 Difference in MIC in study 1<sup>a</sup>

Medium	Difference in MIC <sup>b</sup>	
	MHA	MHLHBA
MHBA	MHBA lower by 7% (-9% to 20%)	MHBA lower by 25%* (13% to 35%)
MHA		MHA lower by 20%* (3% to 33%)

<sup>a</sup> Pairwise comparisons of geometric mean MICs of various media.

<sup>b</sup> 95% confidence level in parentheses. \*, *P* < 0.05.

TABLE 4 SXT Etest susceptibility results for study 2

Medium	% of susceptible isolates (MIC ≤ 1 mg/liter)	Geometric mean MIC (mg/liter)	SD (log)
MHF	100	0.07	2.05
MHS	100	0.16	1.79
MHA	100	0.07	2.03
MHLHBA	100	0.09	2.3

randomized in the Skin Sore Trial on MHF using an SXT Etest according to EUCAST guidelines. Only 8 (0.9%) have been found to be resistant, with MICs of >2 mg/liter (unpublished data). These results are consistent with those reported from the EUCAST group.

**DISCUSSION**

Although SXT is no longer commonly recommended for treatment of respiratory tract infections, it remains one of the most widely used and cheapest antibacterials in the world and is an important option for treatment of SSTI, where *S. pyogenes* and *S. aureus* are often copathogens (4, 12, 24, 34). In the era of rising MRSA prevalence, antibacterials that are active against both bacteria are highly valued.

Impetigo is a significant therapeutic problem in remote communities in the Northern Territory of Australia (37, 49, 50), with community-associated MRSA having become highly prevalent in this region (50). Impetigo is also an endemic problem in many less-developed countries (41, 45), and MRSA is likely to be on the rise in these contexts also (50). In patients with MRSA and *S. pyogenes* coinfection, finding a single oral agent that is effective, affordable, and easy to use would be a significant advance. Penicillins and cephalosporins are no longer an option for MRSA treatment. In the Northern Territory context, clindamycin is not an option, with up to 22% of MRSA isolates resistant (49), aside from its poor palatability in young children and the difficulties in maintaining adherence to a thrice-daily regimen. Tetracyclines are not recommended in children under 8 years of age (17), and linezolid is currently too expensive for the empirical treatment of such a common childhood condition. SXT, which is cheap, widely available, and well tolerated and requires only twice-daily dosing, is a potential single agent for treatment of both MRSA and *S. pyogenes* infections. Several studies have con-

TABLE 5 Difference in MIC in study 2<sup>a</sup>

Medium	Difference in MIC <sup>b</sup>		
	MHF	MHA	MHLHBA
MHS	MHS higher by 128%* (107% to 150%)	MHS higher by 132%* (113% to 153%)	MHS higher by 92%* (70% to 116%)
MHF		MHF higher by 2% (-12% to 7%)	MHF lower by 16%* (3% to 27%)
MHA			MHA lower by 17%* (6% to 27%)

<sup>a</sup> Pairwise comparisons of geometric mean MICs of various media.

<sup>b</sup> 95% confidence level in parentheses. \*, *P* < 0.05.

TABLE 6 Summary of published results of *S. pyogenes in vitro* susceptibility to SXT<sup>a</sup>

Publication	Yr	Country	Method and medium used	Findings	Resistance
Yourassowsky et al. (54)	1974	Belgium	AST determined by the agar dilution method on Wellcotest agar supplemented with 5% lysed horse blood	59/59 strains of <i>S. pyogenes</i> susceptible to TMP, SMZ, and SXT	0%
Finland et al. (22)	1976	United States	Plate dilution method on a modified thymidine-deficient Mueller-Hinton medium containing 5% laked blood	35/35 strains of <i>S. pyogenes</i> susceptible to SXT; 32/35 strains of <i>S. pyogenes</i> susceptible to SMZ alone	0%
Darrell et al. (14)	1968	United Kingdom	MIC determined by the plate dilution method on diagnostic sensitivity agar containing 5% lysed horse blood	14/14 strains of <i>S. pyogenes</i> susceptible to TMP with MIC $\leq$ 1 $\mu$ g/ml	0%
Liebowitz et al. (36)	2003	South Africa	MICs determined by the broth microdilution method according to NCCLS guidelines	66/66 <i>S. pyogenes</i> strains susceptible to SXT	0%
Hartman and Hoes (26)	1949	United States	Wilson's method (53, 53a), a semi-solid medium with low sulfonamide antagonist content	94/96 strains of <i>S. pyogenes</i> susceptible to sulfadiazine	1.9%
Schultz and Frank (44)	1958	United States	Wilson's method (53, 53a), a semi-solid medium with low sulfonamide antagonist content	84/86 strains of <i>S. pyogenes</i> susceptible to sulfur antibiotics	2.3%
Eliopoulos and Wennersten (18)	1997	United States	MICs determined by agar dilution methods on Mueller-Hinton II agar + 5% lysed horse blood or with thymidine phosphorylase at 0.2 IU/ml added	58/60 <i>S. pyogenes</i> susceptible to SXT	3.3%
Berger-Rabinowitz and Davies (2)	1970	Israel	Wilson's method (53, 53a), a semi-solid medium with low sulfonamide antagonist content	849/890 <i>S. pyogenes</i> strains susceptible to sulfadiazine	4.6%
Dhanda et al. (15a)	2011	India	Kirby-Bauer disk-diffusion test as per CLSI guidelines; agar not specified	24/26 <i>S. pyogenes</i> strains susceptible to SXT	6.7%
Bushby (6)	1973	United States	Disk diffusion using TMP/SMZ disks 1.25/23.75 $\mu$ g on various media	699/757 <i>S. pyogenes</i> strains susceptible to SXT	7.7%
Lakshmy et al. (34a)	2011	India	Kirby-Bauer disk-diffusion test as per CLSI guidelines. Agar not specified	95/119 <i>S. pyogenes</i> strains susceptible to SXT	21.8%
Dumre et al. (16a)	2009	Nepal	Kirby-Bauer disk-diffusion test as per CLSI guidelines; agar not specified	11/38 <i>S. pyogenes</i> strains susceptible to SXT	71%
Traub and Leonhard (51)	1997	Germany	Agar disk diffusion using NCCLS criteria on sheep blood MHA	0/63 strains of <i>S. pyogenes</i> susceptible to SXT	100%
Kaplan et al. (33)	1999	United States	Etest performed on MHA containing 5% sheep blood	0/169 strains of <i>S. pyogenes</i> susceptible to SXT with MIC $\geq$ 32 $\mu$ g/ml	100%

<sup>a</sup> TMP, trimethoprim, SMZ, sulfamethoxazole.

firmed the ongoing susceptibility of *S. aureus* to SXT in this region of Australia (38, 49).

The breakpoints utilized for this study were defined by EUCAST using data collated from a wide range of sources on more than 2,500 isolates of *S. pyogenes* tested for susceptibility to SXT using a variety of methods (32). Of the 2,596 tests reported from multiple sources, geographical areas, and time periods, 2,559 were susceptible to SXT of  $\leq$ 1 mg/liter and 23 isolates were resistant (0.9%) (European Committee on Antimicrobial Susceptibility Testing, data from the EUCAST MIC distribution website, <http://mic.eucast.org>, last accessed 18 September 2012). On disk diffusion testing for *S. pyogenes*

using SXT disks in a ratio of 1:19, a zone size of  $\geq$ 18 mm indicates susceptibility and  $<$ 15 mm indicates resistance. Using these breakpoints, a total of 358 tests were performed, with 5 confirmed resistant strains (1.4%) (<http://mic.eucast.org>, last accessed 18 September 2012). This can be contrasted with results found in U.S.-based literature using the CLSI methods, where AST for *S. pyogenes* is performed on agar supplemented with defibrinated sheep blood and SXT is not routinely tested, as *S. pyogenes* strains are considered universally resistant (51). Defibrinated sheep blood is utilized, as the hemolytic reactions of  $\beta$ -hemolytic streptococci on blood agar containing sheep blood are deemed "true" (1).

The *in vitro* results reported in the current study confirming the susceptibility of *S. pyogenes* to SXT suggest that treatment of SSTI with SXT is worth considering. Our current RCT to assess the noninferiority of SXT to the standard treatment with benzathine penicillin G (BPG) for impetigo will provide the necessary clinical evidence to inform guidelines. It is based on a pilot study of 13 participants which indicated that both BPG and SXT were efficacious in healing impetigo (48). There is one study published comparing these agents for *S. pyogenes* infection in tonsillitis, which reported a 70% treatment efficacy for SXT compared to 88% for penicillin, a non-statistically significant difference (52).

The infrequent reports of susceptibility of *S. pyogenes* to SXT demonstrate resistance rates ranging from 0% to 100% depending on which medium and testing conditions are used (Table 6). Although the variation in results may relate to the particular strains included or the local prescribing patterns of SXT, it is most likely related to the methodology of testing. All of the studies reporting high resistance rates either used media known to have high concentrations of thymidine or did not provide details of the medium used. As standardization to ensure a low thymidine concentration of Mueller-Hinton medium was introduced only in 2006, it is likely that, unless low-thymidine media were specified (2, 14, 18, 22, 26, 44, 54), studies in publications prior to this may not have controlled for thymidine content.

Alongside this, *S. pyogenes* has remained 100% susceptible *in vitro* to penicillin. Hence there has been no pressing need to understand SXT susceptibility as an alternative antibacterial in the public health approach to treatment of *S. pyogenes* infections. However, this is changing in the context of rising MRSA rates for SSTI.

Our results show that testing of *S. pyogenes* for susceptibility to SXT on MHS gives a higher MIC than all of the other agars, although the organism remains in the susceptible range. This could possibly be due to the availability of thymidine or other inhibitory substances in this medium. However, thymidine concentrations of the various media utilized were not assessed. Alternatively, the absence of an enzyme to reduce the inhibition in sheep blood compared to horse blood may be the explanation.

The original paper describing the identification of the Harper-Cawston factor (25) as thymidine (21) reports an interesting observation that our study has partially demonstrated. Only lysed horse blood contains thymidine phosphorylase to convert thymidine to thymine and hence overcome the inhibition of folate metabolism that occurs in the presence of thymidine. No other mammalian blood contains this enzyme, which is a possible reason for the higher MICs reported on MHS than on those agars containing horse blood. In the original paper, the presence of thymidine at concentrations of 1.6 µg/ml was sufficient to completely prevent inhibition by the drugs. The inclusion of lysed horse blood restored the inhibition. This has also been shown by Coll et al. (11). However, the MIC of *S. pyogenes* isolates tested on MHLHBA was higher than those of isolates tested on MHF (study 2), MHA (studies 1 and 2), or MHBA (study 1), which suggests other factors at play.

A limitation of this study is the reliance upon a single method for susceptibility testing, the Etest, which is a commercially derived method. Further work using broth or agar dilution methods would add to our understanding of the susceptibility of *S. pyogenes* to SXT. As shown in Table 6, when these additional methods have been assessed, the susceptibility of *S. pyogenes* ranges from 0%

(54) to 3.3% (18) resistant, similarly low resistances to those reported in this study.

Reading MICs for SXT can be challenging due to haze. In particular, only faint growth of *S. pyogenes* was achieved on MHA (due to the absence of blood), and this made reading endpoints more difficult. MHLHBA and MHF had problems similar to those of MHA with respect to haze. Despite this in study 2, the MICs were reproducible between readers, with a high level of interrater reliability within 1 MIC gradation.

**Conclusions.** The widespread belief that SXT is ineffective for *S. pyogenes* infections because of inherent antimicrobial resistance is a fallacy due to technical limitations in laboratory methodology: namely, the use of media containing high concentrations of thymidine, which inhibits the action of sulfur antibacterials. When media containing low concentrations of thymidine and/or high concentrations of the enzyme thymidine phosphorylase are used, resistance rates are low in most cases, although this must be monitored over time and may vary with local epidemiology and antibacterial prescribing patterns. This study provides justification to proceed to clinical trials of SXT for *S. pyogenes* infections. Corroboration with clinical trial data may convince clinicians that SXT can safely and appropriately be used for infections involving *S. pyogenes*. The Skin Sore Trial will answer the clinical applicability of this current *in vitro* study. In the era of rising MRSA prevalence, more clinical trials of SXT for treatment of SSTI, where *S. pyogenes* and *S. aureus* are frequently copathogens, are needed.

#### ACKNOWLEDGMENTS

The work of the research assistants in study 1 and the Skin Sore Trial team (Irene O'Meara, Jane Nelson, Tammy Fernandes, Melita McKinnon, Di- anne Halliday, Colleen Mitchell, Valerie Coomber, and Christine Francis) in recruiting participants for study 2 has made this research possible. Study scientists who carried out this work include R.A.L., P.W., and Vanya Hampton. Mark Chatfield provided assistance with the statistical analysis. We also acknowledge all of the participants and their families who have participated in the Skin Sore Trial and Rheumatic Heart Disease studies.

This work was supported through a National Health and Medical Research Council (NHMRC) Project Grant (545234) on which A.C.B., S.Y.C.T., M.I.M., B.J.C., and J.R.C. are all investigators. A.C.B. is the recipient of a NHMRC scholarship for Ph.D. research (605845) as well as an Australian Academy of Sciences Douglas and Lola Douglas scholarship. S.Y.C.T. is the recipient of a NHMRC Early Career Fellowship (605829).

We have no conflicts of interest to declare.

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