Susceptibility of Anaerobic Bacteria to Sulfamethoxazole/Trimethoprim and Routine Susceptibility Testing

JÜRGEN WÜST¹* AND TRACY D. WILKINS²

Institute for Medical Microbiology, University of Zurich, CH-8028 Zurich, Switzerland,¹ and Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060²

Received for publication 5 July 1978

The minimal inhibitory concentrations (MICs) of sulfamethoxazole and trimethoprim against 144 strains of obligately anaerobic bacteria were determined on Diagnostic Sensitivity Test agar (Oxoid) or in prereduced Diagnostic Sensitivity Test broth, both supplemented with sodium pyruvate (1 mg/ml), hemin (5 µg/ml), and vitamin K₁ (1 µg/ml). Fifty-eight percent of the strains were susceptible to sulfamethoxazole alone (MIC ≤ 16 µg/ml), only 12% were susceptible to trimethoprim alone (MIC ≤ 1 µg/ml), and 85% were susceptible to the combination of sulfamethoxazole plus trimethoprim (MIC ≤ 16 µg/ml) at a ratio of 19:1. All 45 strains of the *Bacteroides fragilis* group were susceptible to the combination. Synergy of the combination was often observed by a checkerboard MIC determination of 123 strains, usually most markedly when the ratio of the two components was near 1:1. However, there was also synergism at the ratio of sulfamethoxazole to trimethoprim of 16:1 in 61 (53.5%) of the 114 strains that could be evaluated for synergistic activity. When the strains were tested by the broth-disk test proposed by Wilkins and Thiel, modified by using prereduced Diagnostic Sensitivity Test broth instead of brain heart infusion broth and by using a smaller inoculum, there was over 90% correlation with the MICs. Poor results were found when the broth-disk tests were performed in brain heart infusion broth. There was very poor correlation between inhibition zone diameters by an agar diffusion method and MICs.

In the United States the combination of sulfamethoxazole (SMZ) and trimethoprim (TMP) is approved only for the treatment of urinary tract and *Pneumocystis carinii* infections, but in many European countries these drugs are used for numerous types of infections. These include, among others, infections of the respiratory tract, septicemia, typhoid fever, otitis, and gonorrhea (1, 11, 16). However, it is not recommended for the treatment of anaerobic infections, and there is relatively little known about the activity of SMZ and TMP on anaerobes.

There are very limited reports on clinical efficacy of cotrimoxazole, the combination of SMZ and TMP, for anaerobic infections. Okubadejo (21) mentioned that five patients with *Bacteroides fragilis* infections responded well to cotrimoxazole treatment. Hanson and Woods (13) treated two patients with *Bacteroides* infections successfully with sulfadimidine and TMP; however, because they added either cloxacillin or cephalothin to the regimen, an exact judgment is difficult. On the other hand, Darrell et al. (6) noted that a patient with a *Bacteroides* septi-

cemia and a liver abscess did not improve when treated with sulfisoxazole plus TMP and that two patients with *Escherichia coli* bacteremia complicating septic abortion developed *Bacteroides* bacteremia when the original organism was eliminated by this therapy.

There are a few reports on in vitro susceptibility. Rosenblatt and Stewart (24) found little activity of SMZ and TMP against anaerobes. In contrast to this, other workers have reported, in general, good susceptibility of *B. fragilis* to SMZ alone and to cotrimoxazole, whereas most strains were resistant to TMP alone (7, 21-23, 25). The in vitro data have been obtained by agar diffusion methods or by determination of minimal inhibitory concentrations (MICs), a procedure that is not suitable for routine susceptibility testing in most clinical laboratories unless large numbers of strains have to be tested each day. The purpose of this study was to determine the susceptibility of a variety of anaerobic bacteria to SMZ and TMP by checkerboard MIC determinations and to investigate the reliability of susceptibility testing by a modification of the

broth-disk method proposed by Wilkins and Thiel (33) and by an agar diffusion method.

MATERIALS AND METHODS

Bacterial strains. All 144 strains were obtained from the Virginia Polytechnic Institute Anaerobe Laboratory culture collection. They had all been isolated from humans. They were identified according to the criteria outlined by Holdeman et al. (14). The strains comprised the following: 21 *B. fragilis*, 24 other strains of the *B. fragilis* group (5) (seven *B. distasonis*, four *B. ovatus*, four *B. thetaiotaomicron*, five *B. uniformis*, four *B. vulgatus*, two *B. asaccharolyticus*, six *B. bivius*, one *B. capillosus*, one *B. corrodens*, three *B. disiens*, two *B. melaninogenicus* subsp. *intermedius*, one *B. melaninogenicus* subsp. *melaninogenicus*, two *B. oralis*, two *B. putredinis*, one *B. ruminicola* subsp. *brevis*, two *B. splachnicus*; four *Fusobacterium mortiferum*, two *F. necrophorum*, six *F. nucleatum*, one *F. varium*; two *Peptococcus asaccharolyticus*, three *P. magnus*, two *P. prevotii*; five *Peptostreptococcus anaerobius*; one *Streptococcus constellatus*, four *S. intermedius*, one *S. morbillorum*; four *Veillonella parvula*; one *Actinomyces israelii*, one *A. naeslundii*, one *A. odontolyticus*, one *A. viscosus*; one *Clostridium bifermentans*, one *C. butyricum*, two *C. clostridiforme*, one *C. difficile*, one *C. innocuum*, one *C. limosum*, one *C. novyi* A, seven *C. perfringens*, five *C. ramosum*, two *C. septicum*, one *C. sordellii*, three *C. sporogenes*, one *C. tertium*, one *C. tetani*; one *Eubacterium alacalyticum*, three *E. lentum*, one *E. limosum*, one *E. moniliforme*; two *Propionibacterium acnes*, one *P. avidum*. The cultures were maintained in chopped meat broth (14) at room temperature.

Media. Throughout the experiments, prereduced, anaerobically sterilized media were used. These were prepared by the methods described by Holdeman et al. (14). The basic media were supplemented brain heart infusion (BHI-S) broth, chopped meat carbohydrate (CMC) broth, and Diagnostic Sensitivity Test (DST) broth (Oxoid) supplemented with vitamin K₁ (1 µg/ml), hemin (5 µg/ml), and sodium pyruvate (1 mg/ml). The media evaluated for agar dilution MIC determinations were: BHI agar (Difco) supplemented with 0.5% yeast extract (Difco), DST agar (Oxoid), Isosensitest agar (Oxoid), Mueller-Hinton agar (BBL), and Wilkins-Chalgren agar (32). All agar media contained vitamin K₁ (1 µg/ml), hemin (5 µg/ml), and sodium pyruvate (1 mg/ml) (32). In part of the experiments, thymidine phosphorylase (0.1 IU/ml; supplied by S. R. M. Bushby, Burroughs Wellcome Co., Research Triangle Park, N.C.) was added to counteract the antagonistic effect of thymidine in the media (10). No blood was added because we wanted to use media defined as much as possible (32).

Antimicrobial agents. SMZ and TMP were supplied by Hoffmann-La Roche, Inc., Nutley, N.J. SMZ was dissolved in 0.05 N NaOH, and TMP was dissolved in 0.02 N lactic acid, both to a final concentration of 5.12 mg/ml. These stock solutions were kept at -70°C.

MIC determinations. Plates were poured aerobically on the day of the experiment to complete a checkerboard series of twofold dilutions, with SMZ ranging from 128 to 0.25 µg/ml and TMP ranging from

64 to 0.125 µg/ml. Plates were also prepared in duplicate with the antimicrobial agents incorporated singly for determination of individual MICs and with SMZ and TMP (SMZ/TMP), in a ratio of 19:1, the ratio usually obtained in serum. Before use, the plates were dried at 37°C for 30 min.

The inoculum was prepared anaerobically by diluting an overnight culture in CMC broth to half the turbidity of a McFarland no. 1 turbidity standard and making a 1:100 dilution thereof in BHI-S broth. The agar plates were inoculated aerobically with a Steers replicator (27), resulting in a final inoculum of approximately 5×10^8 bacteria per spot. Appropriate anaerobic and aerobic control plates without antimicrobial agents were also inoculated (29). The plates were incubated in GasPak (BBL) jars at 37°C for 42 to 44 h. After incubation, the MIC was determined as the lowest concentration of the antimicrobial agents showing no growth, a barely visible haze, or one discrete colony (9). One strain of *B. fragilis* (ATCC 25285) was included in each experiment to determine the reproducibility of the method. MICs for this strain were always in a fourfold range. The MICs were plotted graphically on semilog paper, and the plots were read for 50 and 80% efficacy.

For evaluation of synergistic activity of the two components, the fractional inhibitory concentration (FIC) index was calculated as follows (8, 23): FIC index = (MIC of SMZ in combination/MIC of SMZ alone) + (MIC of TMP in combination/MIC of TMP alone). Any value of the FIC index ≤ 0.7 was considered to indicate synergy (18). Results were not interpreted when the MIC for either drug was at the lowest or next to lowest concentration tested for that drug.

MIC determinations of 19 strains that did not grow sufficiently on the plates and for two swarming clostridia were performed in DST broth. For these strains, only the combination of SMZ and TMP at the ratio of 19:1 was tested. The tubes contained 5 ml of DST broth with a twofold dilution series of SMZ/TMP. They were inoculated with 0.05 ml of a 1:100 dilution of half the turbidity of a McFarland no. 1 turbidity standard in DST broth, resulting in a final inoculum of approximately 10^4 bacteria per ml. Results were read after 42 to 44 h of incubation at 37°C. No difference between agar and broth dilutions was detected with four strains tested by both methods in parallel.

Results did not usually differ by more than one or, in some instances, two dilutions. In the case of larger discrepancies, tests were repeated. When the data were evaluated, the higher value was used when tests differed by not more than two dilutions.

Broth-disk method. The broth-disk method described by Wilkins and Thiel (33) was followed with the exception that in the preparation of the inoculum an overnight culture in CMC broth was first diluted approximately 1:100 by adding one drop to 5 ml of BHI-S broth. This was done because there was too much background turbidity with susceptible strains when the original CMC culture was used as the inoculum, presumably because the organisms were able to multiply to produce visible growth before the drugs became inhibitory (26). One drop of the diluted inoculum was added to 5 ml each of BHI-S broth, BHI-S broth containing 0.1 IU of thymidine phosphorylase per ml, and DST broth. We added four paper disks

(Difco) containing a combination of 23.75 µg of SMZ and 1.25 µg of TMP to each tube, resulting in a final concentration of 20 µg of SMZ/TMP per ml. Control cultures in BHI-S broth and DST broth without antimicrobial agents were inoculated in parallel. Incubation was at 37°C for 18 to 24 h. Susceptibility was defined as either absence of growth or less than 50% the turbidity of the corresponding control culture (3, 33).

Agar diffusion test. The agar diffusion test proposed by Sutter et al. (29) was followed, with the exception that DST broth and agar (both supplemented with sodium pyruvate [1 mg/ml], hemin [5 µg/ml], and vitamin K₁ [1 µg/ml]) were used instead of brucella broth and agar. Disks containing 25 µg of SMZ/TMP were added to the inoculated plates. With each day's experiments, *B. fragilis* ATCC 25285 and *B. vulgatus* WAL 1887 (29) were included as controls.

RESULTS

In preliminary experiments, the MICs of TMP against 17 strains of six different species were similar in all five media tested, probably because by addition of thymidine phosphorylase the thymidine in the media had been converted to the far less antagonistic thymine (4, 10). The MICs of SMZ were much higher in supplemented BHI and Wilkins-Chalgren media, probably due to the yeast extract contained in these two media. Growth of many anaerobes was poor on Isosensitest agar as compared with the other media. We finally chose DST agar for further work as some workers have made objections about Mueller-Hinton agar because of batch-to-batch variation (9, 12, 34). This medium also has been used by others in investigating the effect of SMZ/TMP on anaerobes (21-23, 25), although with the addition of lysed horse blood which may affect the antimicrobial activity or improve growth. Thymidine phosphorylase had no effect on MICs in DST agar and was therefore omitted.

Table 1 shows the concentrations of SMZ,

TMP, and SMZ/TMP required for inhibition of 50 and 80% of the strains, respectively. Only 58% of the strains were inhibited by 16 µg of SMZ per ml, and only 12% were inhibited by 1 µg of TMP per ml. The levels of SMZ in serum usually reached are 16 to 32 µg and those of TMP are 0.8 to 1.6 µg (17). As shown in Table 1, as well as in the concentrations required for inhibition of increasing percentages of strains shown in Table 2, the combination SMZ/TMP was most effective, with 85% of the strains inhibited by 16 µg/ml. Of particular interest is the finding that all 45 strains of the *B. fragilis* group were susceptible to such levels. Moderately resistant to SMZ/TMP (MIC = 32 µg/ml) were *B. bivius* (1 of 6 strains tested), *B. corrodens* (1/1), *B. melaninogenicus* subsp. *intermedius* (1/2), *B. putredinis* (1/2), *F. mortiferum* (1/4), *P. asaccharolyticus* (1/2), *E. alactolyticum* (1/1), and *C. clostridiiforme* (2/2). Resistant to SMZ/TMP (MIC ≥ 128 µg/ml) were *B. disiens* (1/3), *B. melaninogenicus* subsp. *intermedius* (1/2), *C. innocuum* (1/1), *C. novyi* A (1/1), *C. tetani* (1/1), *C. sporogenes* (3/3), *E. lentum* (2/3), *P. magnus* (2/3), and *P. prevotii* (1/2).

In general, MIC determinations were easy to read with the low inoculum used. When the 100-fold-denser inoculum recommended by Sutter et al. (29) for MIC tests with other antibiotics was used (equivalent to half the turbidity of a McFarland no. 1 turbidity standard), it was often very difficult to determine end points. With most strains, there was a haze of growth even at concentrations much higher than the MICs. This was probably due to an extended growth phase before the antimicrobial agents became effective, as reviewed and described by Seydel et al. (26).

The only problems encountered in MIC determinations were with three out of seven strains of *C. perfringens* and all four strains of *F. mor-*

TABLE 1. Activities of SMZ, TMP, and 19:1 combinations of these agents against various anaerobic bacteria

Organisms	No. of strains	µg/ml required to inhibit growth of:					
		50% of test strains			80% of test strains		
		SMZ	TMP	SMZ + TMP	SMZ	TMP	SMZ + TMP
<i>B. fragilis</i>	21	2.4	11.0	2.09 + 0.11	5.3	19.5	3.32 + 0.18
<i>B. fragilis</i> group (other)	24	8.8	12.5	1.33 + 0.07	28	34	2.85 + 0.15
<i>Bacteroides</i> species (other)	15	53	20.0	6.65 + 0.35	128	64	17.10 + 0.90
<i>Fusobacterium</i> spp.	5	11.0	51	6.65 + 0.35	32	90	11.88 + 0.63
<i>Peptococcus</i> and <i>Peptostreptococcus</i> spp.	18	32	0.3	1.9 + 0.1	>128	>128	23.28 + 1.23
<i>Clostridium</i> species	26	32	32	7.6 + 0.4	>128	>128	76 + 4
Nonspore-forming gram-positive rods	10	4.0	16.0	2.37 + 0.13	>128	>128	19.95 + 1.05
Total	119	7.5	22.0	2.56 + 0.14	>128	128	11.88 + 0.63

tiferum. These did not show a distinct end point; growth diminished only slowly with increasing concentrations. For the *F. mortiferum* strains, we had to determine MICs in DST broth, in which a sudden distinct drop in turbidity to about 5 to 10% of the one in the control tube could be observed.

Table 3 shows the number of strains that were synergistically influenced by different combinations of SMZ and TMP. Synergism was most marked when the ratio of the two compounds was near 1:1. The number of strains showing effects of synergism was lower at an SMZ/TMP ratio of 4:1 and even lower at an SMZ/TMP ratio of 16:1, a ratio near the one obtained in blood (19:1). FIC indexes were usually lowest with an SMZ/TMP ratio of 1:1. Drug antagonism was never observed.

Table 4 compares the results obtained with the broth-disk test in different media and MICs obtained by the dilution methods. Poor correlation was found with BHI-S broth (61.8% agreement for BHI-S and 70.8% for BHI-S broth with

0.1 IU of thymidine phosphorylase per ml). This was probably due to the yeast extract. Good correlation (92.4%) was found with DST broth. Four strains classified as susceptible by the broth-disk test were slightly resistant, with MICs of 32 $\mu\text{g/ml}$. Of the seven strains with a false-resistant reading, the MIC for five was 16 $\mu\text{g/ml}$ and that for two was 8 $\mu\text{g/ml}$. When the tests were repeated, there were only four false-resistant readings, with strains having an MIC of 16 $\mu\text{g/ml}$. If the inherent error in dilution methods of plus or minus one dilution was considered, there were only 2 (1.4%) false reactions out of 144 observed in DST broth, but still 38 (26.3%) in BHI-S broth and 28 (19.4%) in BHI-S broth with thymidine phosphorylase.

In general, the results of the broth-disk method were easy to read. Often, there was slightly less growth in DST broth than in BHI-S broth, but that did not pose any problems. The tubes either showed good growth or were clear. With a few strains, there was some turbidity in the drug-containing tubes (up to approxi-

TABLE 2. Activity of SMZ/TMP (19:1) against anaerobic bacteria

Organisms	No. of strains	MIC ($\mu\text{g/ml}$) for following % of strains:			
		50	80	90	100
<i>B. fragilis</i>	21	2.2	3.5	4.0	8.0
<i>B. fragilis</i> group (other)	24	1.4	3.0	5.0	16.0
<i>Bacteroides</i> spp. (other)	23	7.2	17.5	30	>256
<i>Fusobacterium</i> spp.	13	6.6	13.5	15.5	32
<i>Peptococcus</i> and <i>Peptostreptococcus</i> spp.	18	2.0	24.5	128	>256
<i>V. parvula</i>	4		2.4	3.5	4.0
<i>Clostridium</i> spp.	28	8.0	85	>256	>256
Nonspore-forming gram-positive rods	13	2.5	20.0	>256	>256
Total	144	2.8	12.5	32	>256

TABLE 3. Synergistic activity of SMZ and TMP

Organisms	No. of strains evaluated	SMZ/TMP (1:1)		SMZ/TMP (4:1)		SMZ/TMP (16:1)	
		No. showing synergism	FIC index (mean)	No. showing synergism	FIC index (mean)	No. showing synergism	FIC index (mean)
<i>B. fragilis</i>	21	21	0.29	13	0.47	5	0.40
<i>B. fragilis</i> group (other)	23	23	0.14	21	0.16	20	0.19
<i>Bacteroides</i> species (other)	15	13	0.22	13	0.21	12	0.23
<i>Fusobacterium</i> species	9	5	0.27	5	0.29	5	0.32
<i>Peptococcus</i> and <i>Peptostreptococcus</i> species	8	1	0.13	1	0.27	1	0.27
<i>V. parvula</i>	4	4	0.24	3	0.21	2	0.28
<i>C. perfringens</i>	7	6	0.25	5	0.29	4	0.45
<i>Clostridium</i> species (other)	18	13	0.34	12	0.38	10	0.39
<i>Propionibacterium</i> species	2	2	0.39	2	0.39	2	0.39
<i>Eubacterium</i> species	4	0		0		0	
<i>Actinomyces</i> species	3	1	0.52	2	0.52	0	

TABLE 4. Comparison of results obtained with the broth-disk and the agar MIC dilution methods

Broth medium	Resistant strains ^a		Susceptible strains ^a		Overall agreement between the two methods (%)
	No.	No. of false-susceptible readings ^b	No.	No. of false-resistant readings ^b	
DST	21	4(0) ^c	123	7 (2) ^c	92.4 (98.6) ^c
BHI-S	21	0	123	55 (38)	61.8 (73.7)
BHI-S and TP ^d	21	0	123	42 (28)	70.8 (80.6)

^a According to MIC values.^b In broth-disk test.^c Numbers within parentheses indicate false readings or overall agreement, respectively, when strains with MICs one dilution step above or below the break point were excluded.^d TP, 0.1 IU of thymidine phosphorylase per ml.

mately 20% of the control tubes), but it was easy to ascertain a marked difference from the grown control tube. A total of 124 results could be interpreted after 18 to 24 h of incubation. Twenty strains required incubation for another 24 h. These strains comprised mainly *B. oralis*, *B. melaninogenicus*, *B. capillosus*, *B. putredinis*, *F. nucleatum*, eubacteria, actinomycetes, and *P. asaccharolyticus*. The only problem encountered with DST broth was that it was easily oxidized. The problem of oxidized media was eased when the SMZ/TMP disks were stored under CO₂ overnight before they were added to the DST broth.

The agar diffusion method that we tried gave very poor results. Only 76 (52.8%) of the 144 strains produced confluent growth and distinct inhibition zones; these strains consisted mainly of the *B. fragilis* group and the clostridia. The 68 strains that did not grow well enough to be tested consisted mainly of *Bacteroides* species other than the *B. fragilis* group, fusobacteria, actinomycetes, eubacteria, propionibacteria, and some of the cocci. There was extensive variation in diameters for a given MIC. When control strains were tested 10 times on different days, *B. fragilis* ATCC 25285 showed a range of diameters from 27 to 36 mm, with a standard deviation of 3.4 mm. *B. vulgatus* WAL 1887 gave diameters from 29 to 39 mm, with a standard deviation of 4.0 mm.

DISCUSSION

Phillips and Warren (23) have shown that most *B. fragilis* strains in their series were susceptible to SMZ and SMZ/TMP. The main factor that led to the conclusion by Rosenblatt and Stewart (24) that the majority of anaerobes are resistant to sulfonamides appears to have been the higher inoculum that they used and other differences in methods as discussed in detail by Phillips and Warren (23). The inoculum used in the present study corresponds to the one recommended by Ericsson and Sherris

(9) and also used by Phillips and Warren.

We found that most strains of a random selection of anaerobic bacteria were susceptible to SMZ/TMP and that there was often synergy. SMZ alone was less active than the combination, and TMP alone showed little activity. The relative resistance of anaerobic bacteria to TMP is probably due to a lower susceptibility of the dihydrofolate reductases from anaerobes than those from facultatively anaerobic and aerobic bacteria. Inhibition of dihydrofolate reductases from *B. fragilis* and *C. perfringens* has been shown to require 100 to 1,000 times more TMP than the enzymes isolated from *E. coli* and *Staphylococcus aureus* (30).

Synergy was most marked when the ratio of the two components was near 1:1. However, there was also synergism at an SMZ/TMP ratio of 16:1 in 61 (53.5%) of the 114 strains that could be evaluated for synergistic activity. The free amounts of SMZ and TMP in the blood after reaching equilibrium with a standard dose of 800 mg of SMZ and 160 mg of TMP twice per day are 16 to 32 µg/ml for SMZ and 0.8 to 1.6 µg/ml for TMP (17). The level of TMP cannot be increased significantly without side effects (31). However, the ratio of SMZ to TMP is often lower in body fluids than in blood (E. Böhni, Hoffmann-La Roche & Co. AG, Basel, Switzerland, personal communication). Therefore, in theory, synergy against anaerobes would occur more often in tissue and body fluids than in blood. However, levels of the two drugs reached in anaerobic abscesses have not been determined.

Because routine MIC determinations are not feasible for most clinical laboratories, an easier approach for susceptibility testing is needed. We found that a modification of the broth-disk test by Wilkins and Thiel (33) gave reliable results in testing the susceptibility of anaerobic bacteria to SMZ/TMP. A level of 20 µg of SMZ/TMP per ml was chosen to differentiate between susceptible and resistant organisms because such a

concentration is usually reached in serum. The main drawback for the broth-disk test is its need for prerduced media and for opening the tubes only under a stream of oxygen-free CO₂.

In contrast to the modified broth-disk method, very poor correlation was found between zone diameters in the agar diffusion test and MICs. When two control strains were tested several times, the standard deviation in zone size was more than double the standard deviation allowed by Sutter et al. (29) with their method. In addition, almost half of the strains could not be tested due to insufficient growth or indistinct inhibition zones. Therefore, the agar diffusion test used cannot be recommended for testing susceptibility of anaerobic bacteria to SMZ/TMP.

The *in vitro* results of activity of SMZ/TMP against anaerobic bacteria are promising. SMZ/TMP might represent an alternative to current regimens for therapy of infections due to anaerobic bacteria, especially when mixed with facultatively anaerobic bacteria susceptible to it.

These results and those obtained by other workers (7, 21-23, 25) are contradictory to the observation by Näff (20) that cotrimoxazole has no effect on the anaerobic bowel flora. However, he did not identify the anaerobic bacteria as to species, and his cultural counts of 4.3 to 15.5% of the microscopic counts are much lower than the 35% obtained by Attebery et al. (2) or 63 and 93% found in two studies at the VPI Anaerobe Laboratory (15, 19) with improved methods. It is possible that an originally susceptible population has been replaced by resistant strains under cotrimoxazole treatment. Such a shift was observed by Sutter and Finegold (28) with clindamycin; whereas total anaerobic counts were little or not influenced, the *B. fragilis* group, lactobacilli, and bifidobacteria were replaced by clostridia and eubacteria. Therefore, Näff's study has to be interpreted with caution.

Potential usefulness of the combination SMZ/TMP has to be evaluated further in animal studies and clinical trials.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM 14604 from the National Institute of General Medical Sciences and a grant from the Swiss National Science Foundation.

LITERATURE CITED

- Anderson, R. J. (ed.). 1975. Combination chemotherapy of infectious diseases. *Can. Med. Assoc. J.* 112(Suppl.):1S-100S.
- Attebery, H. R., V. L. Sutter, and S. M. Finegold. 1974. Normal human intestinal flora, p. 81-97. In A. Balows, R. M. DeHaan, V. R. Dowell, Jr., and L. B. Guze (ed.), *Anaerobic bacteria: role in disease*. Charles C Thomas, Publisher, Springfield, Ill.
- Blazevic, D. J. 1975. Evaluation of the modified broth-disk method for determining antibiotic susceptibilities of anaerobic bacteria. *Antimicrob. Agents Chemother.* 7:721-723.
- Bushby, S. R. M. 1973. Trimethoprim-sulfamethoxazole: *in vitro* microbiological aspects. *J. Infect. Dis.* 128(Suppl.):S442-S462.
- Cato, E. P., and J. L. Johnson. 1976. Reinstatement of species rank for *Bacteroides fragilis*, *B. ovatus*, *B. distasonis*, *B. thetaiotaomicron*, and *B. vulgatus*: designation of neotype strains for *Bacteroides fragilis* (Veillon and Zuber) Castellani and Chalmers and *Bacteroides thetaiotaomicron* (Distaso) Castellani and Chalmers. *Int. J. Syst. Bacteriol.* 26:230-237.
- Darrell, J. H., L. P. Garrod, and P. M. Waterworth. 1968. Trimethoprim: laboratory and clinical studies. *J. Clin. Pathol.* 21:202-209.
- Dornbusch, K., C. -E. Nord, and T. Wadström. 1974. Biochemical characterization and *in vitro* determination of antibiotic susceptibility of clinical isolates of *Bacteroides fragilis*. *Scand. J. Infect. Dis.* 6:253-258.
- Elion, G. B., S. Singer, and H. H. Hitchins. 1954. Antagonists of nucleic acid derivatives. *J. Biol. Chem.* 208:477-488.
- Ericsson, H. M., and J. C. Sherris. 1971. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol. Microbiol. Scand.* 217(Suppl.):1-90.
- Ferone, R., S. R. M. Bushby, J. J. Burchall, W. D. Moore, and D. Smith. 1975. Identification of Harper-Cawston factor as thymidine phosphorylase and removal of substances interfering with susceptibility testing to sulfonamides and diaminopyrimidines. *Antimicrob. Agents Chemother.* 7:91-98.
- Finland, M., and E. H. Kass (ed.). 1973. Trimethoprim-sulfamethoxazole. The University of Chicago Press, Chicago.
- Garrod, L. P., and P. M. Waterworth. 1971. A study of antibiotic sensitivity testing with proposals for simple uniform methods. *J. Clin. Pathol.* 24:779-789.
- Hanson, G. C., and R. L. Woods. 1975. Intravenous trimethoprim/sulfamidine in the treatment of *Bacteroides septicaemia*. *Postgrad. Med. J.* 51:105-106.
- Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.). 1977. *Anaerobe laboratory manual*, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
- Holdeman, L. V., I. J. Good, and W. E. C. Moore. 1976. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl. Environ. Microbiol.* 31:359-375.
- Kallings, L. O., and J. -E. Johansson (ed.). 1976. Sulfamethoxazole-trimethoprim. *Scand. J. Infect. Dis.* 8(Suppl.):1-106.
- Kayser, F. H., and J. Wüst. 1973. Determination of bacterial resistance to trimethoprim/sulfamethoxazole using the single disk diffusion method. *Chemotherapy (Basel)* 18:162-168.
- Kerry, D. W., J. M. T. Hamilton-Miller, and W. Brumfitt. 1975. Trimethoprim and rifampicin: *in vitro* activities separately and in combination. *J. Antimicrob. Chemother.* 1:417-427.
- Moore, W. E. C., and L. V. Holdeman. 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl. Microbiol.* 27:961-979.
- Näff, H. 1971. Ueber die Veränderungen der normalen Darmflora des Menschen durch Bactrim^R. *Pathol. Microbiol.* 37:1-22.
- Okubadejo, O. A. 1974. Susceptibility of *Bacteroides fragilis* to cotrimoxazole. *Lancet* i:1061.
- Okubadejo, O. A., P. J. Green, and D. J. H. Payne. 1973. *Bacteroides* in the blood. *Lancet* i:147.
- Phillips, I., and C. Warren. 1976. Activity of sulfame-

- thoxazole and trimethoprim against *Bacteroides fragilis*. Antimicrob. Agents Chemother. 9:736-740.
24. Rosenblatt, J. E., and P. R. Stewart. 1974. Lack of activity of sulfamethoxazole and trimethoprim against anaerobic bacteria. Antimicrob. Agents Chemother. 6:93-97.
25. Schoutens, E., M. Labbé, and E. Yourassowsky. 1973. Sépticémies à *Bacteroides fragilis*. Incidence et sensibilité des souches aux antibiotiques. Pathol. Biol. 21:349-353.
26. Seydel, J. K., E. Wempe, G. H. Miller, and L. Miller. 1973. Quantification of the antibacterial action of trimethoprim alone and in combination with sulfonamides by bacterial growth kinetics. J. Infect. Dis. 128(Suppl.):S463-S469.
27. Steers, E., E. L. Foltz, B. S. Graves, and J. Riders. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. (Washington, D.C.) 9:307-311.
28. Sutter, V. L., and S. M. Finegold. 1974. The effect of antimicrobial agents on human fecal flora: studies with cephalixin, cyclacillin and clindamycin, p. 229-240. In F. A. Skinner and J. G. Skarr (ed.), The normal microbial flora of man. Academic Press Inc., London.
29. Sutter, V. L., V. L. Vargo, and S. M. Finegold. 1975. Wadsworth anaerobic bacteriology manual, 2nd ed. Anaerobic Bacteriology Laboratory, Wadsworth Hospital Center, and Department of Medicine, University of California at Los Angeles, Los Angeles.
30. Then, R. L., and P. Angehrn. 1978. Low trimethoprim susceptibility of anaerobic bacteria based on insensitive dihydrofolate reductases, p. 662-666. In W. Siegenthaler and R. Lüthy (ed.), Current chemotherapy. Proceedings of the 10th International Congress of Chemotherapy, Zurich, 1977, vol. 1. American Society for Microbiology, Washington, D.C.
31. Whitman, E. R. 1969. Effects in man of prolonged administration of trimethoprim and sulfasoxazole. Postgrad. Med. J. Suppl. 45:46-51.
32. Wilkins, T. D., and S. Chalgren. 1976. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. 10:926-928.
33. Wilkins, T. D., and T. Thiel. 1973. Modified broth-disk method for testing the antibiotic susceptibility of anaerobic bacteria. Antimicrob. Agents Chemother. 3:350-356.
34. Yourassowsky, E., M. P. Vanderlinden, and E. Schoutens. 1974. Sensitivity of *Streptococcus pyogenes* to sulfamethoxazole, trimethoprim and cotrimoxazole. J. Clin. Pathol. 27:897-901.