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Effect of Trimethoprim and Trimethoprim-Sulfamethoxazole on Development of Drug-Resistant Vaginal and Fecal Floras

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Trimethoprim-sulfamethoxazole (TMP-SMX) or trimethoprim (TMP) alone was given on a random double-blind basis to 26 young women to treat urinary tract infections. Fecal and introital aerobic bacterial floras were identified at 1, 7, 14, and 42 days to analyze changes in these floras or development of resistance to TMP or TMP-SMX. Neither TMP alone nor the TMP-SMX combination administered for 2 weeks selected a resistant fecal or introital flora. In the few individuals who had strains resistant to TMP or TMP-SMX before initiation of therapy, these organisms did not persist once therapy began. Both programs effectively cleared the introitus and rectal areas of *Enterobacteriaceae*. Concentrations of TMP adequate to inhibit the majority of *Escherichia coli* strains causing urinary tract infections were found in the vaginal secretions.

Trimethoprim in combination with sulfamethoxazole (TMP-SMX) has proved to be an effective treatment of urinary tract infections (5, 12). Increasing clinical experience with the combination preparation has led to an appreciation of its wide spectrum of activity. The majority of *Escherichia coli*, *Proteus*, and *Klebsiella* strains, as well as many *Enterobacter* and *Serratia* strains, are susceptible to TMP (6). Because of the broad spectrum of activity of the combination against *Enterobacteriaceae*, the question of alteration of intestinal flora in patients treated with the agents has arisen. Suppression of intestinal *Enterobacteriaceae* by the administration of TMP-SMX has been reported in both animals and humans (3, 10, 15).

In the United States, where TMP is available only in combination with SMX, primary resistance to the TMP component has remained low despite heavy and increasing clinical use for a variety of infectious processes (9, 18). It has been thought that the combination of TMP with SMX delays or prevents the emergence of resistance among the *Enterobacteriaceae* and that the use of TMP alone in simple infections would foster selection of greater numbers of TMP-resistant organisms, thus diminishing the use of a very valuable chemotherapeutic agent (8, 12). In Europe, where TMP is available alone, primary TMP-resistant strains among members of the *Enterobacteriaceae* especially have been more commonly encountered than they have been in the United States (9).

We undertook a study of the effect that treatment of urinary tract infections with TMP alone or with TMP-SMX in combination would have on the prevalence of TMP-resistant *Enterobacteriaceae* in the flora of the vaginal introitus and bowel.

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MATERIALS AND METHODS

Subjects. A total of 30 female patients who came to the Health Service of Columbia University and The Columbia-Presbyterian Medical Center, New York, N.Y., with uncomplicated urinary tract infections were enrolled in this study. All patients, after being informed of the goals and risks of the study, signed informed consents in accordance with the guidelines of the Committee on Human Investigation of the institution. After enrollment each patient was assigned prospectively, in a double-blind fashion, to receive either TMP (100 mg) plus placebo twice daily for 14 days or to receive TMP-SMX (160 mg + 800 mg) plus placebo twice daily for 14 days. Placebo and active tablets were identical. Since each patient took one of each tablet, blindedness was assured.

Any patient with a history of allergy to sulfonamides was excluded. Patients were clinically free of underlying or chronic diseases, had normal renal function (normal creatinine and blood urea nitrogen levels), and were not pregnant. No patient had a history of chronic exposure to antibiotics or had taken any medication (except birth control pills) for the 6 months before enrollment. Each had a clinical diagnosis of acute uncomplicated urinary tract infection which clinically fitted the pattern expected of cystitis, and all had had at least one prior episode of simple cystitis. Methods of birth control ranged from none to oral contraceptives. All subjects were sexually active.

Patients ranged in age from 22 to 55 years with a mean age of 27.4 years and median age of 25 years. Of the 30 enrolled patients, 26 completed all of the protocol requirements and underwent complete evalua-

tion. Four patients failed to complete follow-up visits and were excluded on that basis. There were 12 patients in the TMP-only group and 14 patients in the TMP-SMX combination group.

Sample protocol. At the initial visit, cultures of urine, the vaginal introitus, and the anal canal were obtained. These samples were repeated at visits after 1 week of therapy, at the close of therapy, and finally at 4 weeks after completion of treatment.

Blood levels of antimicrobial agents were obtained approximately 6 h after a dose on day 7 of therapy. Samples of blood were allowed to clot, and the serum was extracted by centrifugation and stored at −20°C until assay.

On day 7 of therapy, vaginal secretions were obtained. By using a standard vaginal speculum with water lubrication, the cervix, vault, and vagina were irrigated with 50 ml of sterile water via a sterile syringe and a no. 16 French catheter. The washings were collected in a sterile basin by aspiration and lyophilized. The dried, powdered, concentrated residue was stored at −20°C until it was suspended in 5 ml of normal saline and assayed for SMX and TMP content.

Bacteriological methods. Urine cultures were obtained as "clean-catch" specimens. All isolates were identified by an Analytab Products, Inc. or Roche enterotube system, and antibacterial susceptibility was determined by the standard Kirby-Bauer disk diffusion technique (2).

Introital and rectal floras were sampled with Culturette sterile swabs (Scientific Products Div.). After sampling, swabs were immediately placed in sterile test tubes containing 2 ml of normal saline and blended in a Vortex mixer for 60 s. This suspension was then serially diluted in 10^{-2} steps to a final dilution of 10^{-10}. A sample of 0.1 ml of each dilution was plated onto MacConkey agar and incubated for 24 h. The plate with the most uniform colony distribution was selected, and five colonies were selected at random and identified by the Enterotube system (Roche Diagnostics). This profile on each sample provided the background floras present. At the same time, each of the original dilutions was streaked on Mueller-Hinton agar plates—one plate containing 4 μg of TMP and 0.05 IU of thymidine phosphorylase per ml and another plate containing 1 μg of TMP and 20 μg of SMX plus 0.5 IU of thymidine phosphorylase per ml. These selective media plates were incubated for 48 h at 35°C, and organisms were subcultured to MacConkey agar and identified by the Enterotube system (Roche). Resistance to TMP, TMP-SMX, or SMX or to organisms which grew on the plates containing TMP or TMP-SMX was confirmed by retesting on another set of plates for growth and lack of zones of inhibition to disks containing the antibiotics. Resistance transfer was performed by standard techniques with *E. coli* K-12 strain W 1485 (11).

RESULTS

Infecting organisms. In 23 of the 26 patients (89%), *E. coli* was the etiological agent in their urinary tract infections. One patient each was infected with either *Enterobacter aerogenes* or *E. cloacae*, and one patient was infected with both *E. coli* and *E. cloacae*. All pretreated isolates from urine were susceptible to TMP and to SMX, except for three *E. coli* which were resistant to SMX.

Therapeutic results. The results of therapy were uniformly good. Both the TMP and TMP-SMX groups had no antibiotic-associated failures, and follow-up urines remained sterile, with the exception of a single patient who redeveloped an *E. coli* urinary tract infection 2 weeks after completion of treatment with TMP. Table 1 lists the results of therapy and the incidence of side effects.

Three patients developed asymptomatic bacteriuria during and after the study. One patient exhibited *E. coli* on follow-up urine cultures and responded to long-term suppression after the study was completed. Two additional patients developed bacteriuria after the completion of the study—one with *E. coli* and the other with *Klebsiella pneumoniae*. These isolates were susceptible to both agents. These patients had the same strain in their vaginal introitus at the time the bacteriuria was noted.

Medication side effects were confined to the TMP-SMX group; these consisted of mild skin rashes occurring on days 13 and 14 of therapy in two patients. Therapy was completed in both cases. There were no side effects in the TMP group.

Initial gram-negative aerobic rectal flora included predominantly *E. coli* and *Enterobacter* and occasional *Proteus* species, as well as *Aci-*

TABLE 1. *Outcome of therapy of urinary tract infections with TMP or TMP-SMX*

Treatment with:	No. excluded	Determination[a]			
		Clinical and bacteriological cure at end of therapy	Recurrence		Untoward effects
			Before week 6	After week 6	
TMP-SMX	2/16	14/14	0/14	1/14	2/14
TMP	2/14	12/12	1/12[b]	1/12[c]	0/12

[a] Number of patients exhibiting effects per total number.
[b] *E. coli* was susceptible to all agents and located also in the vagina.
[c] *K. pneumoniae* was susceptible to all agents located also in the vagina.

netobacter and *Klebsiella* species. Table 2 summarizes the number of isolates and their susceptibility patterns. Five isolates from each patient were selected from the MacConkey plates. Five isolates would have been selected from the plates which contained TMP or TMP-SMX. In some cases no resistant strains were found. In some instances, strains which grew on the TMP or TMP-SMX plate were shown not to be resistant, which explains the number of organisms. Pretherapy TMP-resistant isolates were present in all groups of organisms: *E. coli*, *Enterobacter*, *Proteus*, *Acinetobacter*, and *Klebsiella*. These organisms were not restricted to only one or two patients but were found in most of the patients. TMP-SMX resistance was also noted but not as frequently. There were 16 TMP-resistant isolates and 4 SMX-resistant isolates obtained from the 26 patients. Most of the isolates resistant to either TMP or SMX were susceptible to the TMP-SMX combination.

Despite the presence of initially resistant isolates after 1 and 2 weeks of therapy in either the TMP- or the TMP-SMX-treated patients, the isolates disappeared from the rectal flora. Only a few strains of *Klebsiella* and *E. coli* susceptible to inhibition by both agents could be isolated. These were all obtained from TMP-SMX patients. The rectal flora during this period consisted of enterococci, anaerobic bacteria, and, in a few patients, *Candida albicans*.

By week 6, 4 weeks after therapy, *Enterobacteriaceae* were uniformly reestablished in the fecal flora, but the strains had not returned to pretreatment levels in all cases (Table 2). Isolates were uniformly susceptible to the antimicrobial agents. Only one TMP-resistant *E. coli* strain could be isolated from all 26 patients. Resistance to TMP could not be transferred to a recipient *E. coli*. Floras were, as before, largely *E. coli*, with a few *Klebsiella*, *Enterobacter*, and *Citrobacter* isolates present. The initially resistant isolates did not overgrow or even persist. During the 6-week period they were no longer evident.

The floras of the vaginal introitus exhibited a similar pattern (Table 3). The initial bacterial isolates were predominantly *E. coli* with some *Enterobacter*, *Klebsiella*, *Serratia*, and *Citrobacter*. A number of these isolates were resistant to TMP and TMP-SMX before initiation of therapy. By weeks 1 and 2 of therapy the introital cultures were cleared of *Enterobacteriaceae*. At week 1, two patients receiving TMP, but none on the combination, had positive introital cultures. At week 2, one patient on combination therapy, but none receiving TMP, had introital bacteria. The numbers of isolates obtained during this period represented only one or two colonies on a plate streaked with undiluted material and may possibly represent random contamination during sampling. Nevertheless, introital samples were less uniformly sterile than were rectal cultures during this period.

At week 6, 4 weeks after therapy, *Enterobacteriaceae* strains were found in a number of introital samples. With the exception of one *E. cloacae* which was resistant to TMP, isolates were susceptible to both TMP and SMX.

Initially *Enterobacteriaceae* isolates were cultured from the vaginal introitus of 22 of 26 patients (85%). In each case the urinary pathogen was the same as that found in the introitus. Table 4 outlines the patients with positive introital cultures. At 6 weeks, in five patients in each treatment group, *Enterobacteriaceae* strains

TABLE 2. *Rectal isolates*[a]

Strain	Pretherapy				TMP patients		
	No. susceptible	No. resistant to:			TMP patients		TMP-SMX patients (no. susceptible)[c]
		TMP	SMX	TMP-SMX	No. susc.	No. resist. to TMP[b]	
E. coli	133	8	2	1	47	1	67
E. aerogenes	5	1	0	1	0	0	2
E. agglomerans	0	1	0	0	0	0	0
E. cloacae	1	0	0	0	0	0	6
E. hafniae	2	0	0	0	0	0	0
P. mirabilis	1	0	0	0	0	0	0
P. rettgeri	1	2	0	0	0	0	0
A. lwoffi	1	0	0	0	0	0	0
A. anitratus	0	2	2	2	0	0	0
K. pneumoniae	0	2	0	1	4	0	3
S. marcescens	0	0	0	0	0	0	0
C. diversus	0	0	0	0	0	0	2

[a] Total, 26 Patients. No resistant isolates were obtained while patients were on therapy.
[b] There were no isolates resistant to SMX or TMP-SMX.
[b] There were no isolates resistant to TMP, SMX, or TMP-SMX.

TABLE 3. *Introital isolates*[a]

Organism	Pretherapy				4-wk posttherapy		
	No. susceptible	No. resistant to:			TMP patients (no. susceptible)[b]	TMP-SMX patients	
		TMP	SMX	TMP-SMX		No. susceptible	No. resistant to TMP[c]
E. coli	94	4	0	3	11	20	0
E. agglomerans	2	1	0	0	0	1	0
E. aerogenes	0	0	0	0	1	0	0
E. cloacae	3	0	0	0	0	3	1
E. hafniae	4	0	0	0	0	0	0
A. anitratus	0	0	0	0	0	0	0
K. pneumoniae	6	1	0	0	4	2	0
S. marcescens	1	1	0	0	0	0	0
C. freundii	0	1	0	0	0	1	0

[a] Total, 26 patients.
[b] There were no isolates resistant to TMP, SMX, or TMP-SMX.
[c] There were no isolates resistant to SMX or TMP-SMX.

TABLE 4. *Presence of bacteria in introital cultures of patients with urinary infection*

Drug	Initial sample	Week 1	Week 2	Week 6
TMP	9/12[a] (75%)	2/12 (17%)	0.12 (0%)	5/12 (42%)
TMP-SMX	13/14 (93%)	0/14 (0%)	1/14 (7%)	5/14 (36%)

[a] Number of patients with positive cultures/number tested.

were isolated from the introitus. One patient in each group had a subsequent urinary tract infection in the next few weeks.

Introital floras of normal women. To aid in assessing the significance of *Enterobacteriaceae* cultures from the vaginal introitus, an additional 17 normal females of the same age and sexual activity as the subjects in the study, but with no history of urinary tract infection and no antibiotic therapy for the previous 6 months, had introital samples taken according to the previously described technique. Table 5 outlines this population. Of the 17 individuals sampled, in 11 completely sterile introital cultures were taken, in two subjects *Acinetobacter lwoffi* was isolated, and in three *P. aeruginosa* was isolated. *E. coli* was isolated in only one patient. Greater than 10^5 organisms in the introital flora was found in only two patients. The one subject discovered to have asymptomatic *E. coli* bacteriuria had a negative introital culture.

Serum and vaginal antimicrobial levels. Serum samples for TMP or TMP-SMX were obtained from 22 patients (Table 6). Measurable levels were found in all but one patient. Vaginal washings showed a wide range of drug levels in both agents, although they were taken at the same time as the serum samples. However, the concentrations of TMP (0.9 μg/ml), particularly in the TMP-SMX group, were adequate to inhibit the majority of the *E. coli* isolates; on the other hand, the SMX concentrations were inadequate to inhibit most of the *E. coli* isolates.

DISCUSSION

This study was an attempt to determine whether brief use (2 weeks) of TMP as a single agent to treat urinary tract infections was more likely to result in the selection of an intestinal or vaginal introital flora resistant to TMP than would occur when TMP was combined with SMX. Resistance to TMP was first noted in London in 1971, and by 1972 strains of bacteria resistant to TMP were found in a number of London hospitals (7). It has been suggested that strains of bacteria carrying TMP resistance factors are sporadically distributed in nature but are present at a low frequency in the normal intestinal floras of the community. Strains of bacteria resistant to TMP on the basis of a mechanism other than that of a plasmid are more common than are plasmid-carrying strains (8). It has been suggested that the use of TMP and SMX as a synergistic combination will decrease the likelihood that resistance to TMP will increase (12).

The patient population of this study is one which normally develops lower urinary tract infections. *E. coli* accounted for 89% of the infecting isolates, and the majority of patients had the same organism as part of their introital floras (16). The results of this study support those of previous reports showing that there is a high degree of carriage of *E. coli* in the introitus in females before and during urinary tract infections; on the other hand, women who infrequently develop urinary tract infections do not

TABLE 5. *Introital flora of normal females*

Sample	Negative		Positive									
					E. coli		*A. lwoffi*		*P. aeruginosa*[a]		*K. pneumoniae*	
	No. of cultures	%	No. of cultures	%	No. of cultures	No. of organisms	No. of cultures	No. of organisms	No. of cultures	No. of organisms	No. of cultures	No. of organisms
Introital cultures	11	65	6	35	1	$>10^5$	2	$<10^5$	3	$2 < 10^5$,	1	$>10^5$
Urine cultures[b]	16	94	1	6	1	(6%)	0		0	$1 > 10^5$	0	

[a] One subject had *Pseudomonas* and *Klebsiella*.
[b] Total number of cultures, 17.

TABLE 6. *Serum and vaginal levels of TMP and SMX*

Group	Drug	Serum concn (μg/ml)		Vaginal concn (μg/ml)	
		Range	Mean	Range	Mean
TMP	TMP	1.6–3.3	2.5	<0.5–1	0.3
TMP-SMX	TMP	0.6–6.4	3.5	<0.5–2.6	0.9
	SMX	4–29	17.6	<0.5–1.5	0.5

have *E. coli* in their periurethral flòras (4, 16).

Although organisms resistant to TMP or the combination of TMP-SMX were isolated from the stool before therapy, these organisms did not persist as a part of the fecal or introital flora. TMP alone or the TMP-SMX combination was equally effective at clearing bacteria from the intestine. Furthermore, the fecal floras returned to normal within a month of completing the therapy. These results are in agreement with those of Toivanen et al. (17) and Sietzen and Knothe (13) who gave TMP or TMP-SMX to healthy persons, both males and females, for 4 weeks and did not find that resistant species developed in the group which received TMP alone.

TMP used alone was no less effective in the treatment of urinary tract infections than was the combination. Brumfitt and Pursell (5) noted a similar result. In our study TMP alone was equally effective as TMP-SMX in eradicating the introital carriage of bacteria which contributes to recurrent urinary infection. This disappearance would appear to be due to the presence of TMP in vaginal secretions (14). The concentration of sulfonamide in vaginal secretions was significantly less than the concentration of TMP in all patients. Why the concentrations of TMP in the vaginal secretions of the TMP-SMX group were greater than those of the group which received TMP alone is not known but is probably due to the 160-mg dose of TMP-SMX compared with the 100-mg of the TMP alone. In both groups concentrations of TMP in vaginal secretions were above a concentration which would inhibit 85% of *E. coli* found in urinary tract infections (1).

There were no untoward effects in the group that received TMP alone, whereas two patients in the group that received TMP-SMX developed a rash. The number of patients is too few however to evaluate this difference.

This study suggests that brief therapy with TMP alone in young, non-hospitalized women will not result in the selection of TMP-resistant bacteria. The results cannot be extrapolated to the use of TMP alone in other populations, such as hospitalized patients, or to those who would receive the drug for long periods, i.e., for 6 weeks to 6 months. The studies of Toivanen et al. (17) were performed on normal volunteers for 3 weeks and may not be applicable to the individual with recurrent urinary tract infections who had previously received a variety of antimicrobial agents. Further studies such as this are needed before one can safely recommend that TMP be used as a single agent. It would be unfortunate to compromise the effectiveness of TMP by selecting a drug-resistant population of bacteria or population of bacteria intrinsically resistant to TMP.

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