

Emergence of Trimethoprim Resistance in Relation to Drug Consumption in a Finnish Hospital from 1971 through 1984

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Emergence of trimethoprim resistance among urinary tract *Escherichia coli* strains, isolated mostly from long-term patients in the Turku City Hospital, Turku, Finland, was studied from 1971 through 1984. Emergence of resistance to trimethoprim was associated with changes in the consumption of both trimethoprim-sulfamethoxazole and trimethoprim, with occurrence of high-level trimethoprim resistance and sequences homologous to trimethoprim resistance transposon Tn7. Since 1971, resistance of *E. coli* to trimethoprim-sulfamethoxazole increased from 8 to between 32 and 35% in 1983 and 1984; resistance to sulfamethoxazole varied from 39 to between 40 and 44%. The frequency of DNA sequence homology with our Tn7 probe among trimethoprim-resistant *E. coli* strains was 42% from 1980 to 1981 and 64% in 1983 ($P < 0.005$). Fourteen years after the introduction of trimethoprim therapy in this hospital, resistance has reached the level of resistance to sulfonamide.

Since 1969, trimethoprim has been widely used alone or in combination with sulfonamides in the treatment and prophylaxis of urinary tract infections (12-14, 16). The development of trimethoprim resistance, which is often transposon mediated, has been a problem. Transposons Tn7 and Tn402 are the best known trimethoprim resistance transposons (1, 5, 21). The former also mediates resistance to streptomycin (1).

The incidence of trimethoprim resistance in outpatients has reached a plateau in the Turku area of Finland, but resistance is still slowly increasing in the Helsinki area (P. Huovinen, O.-V. Renkonen, L. Pulkkinen, R. Sunila, P. Grönroos, M.-L. Klossner, S. Virtanen, and P. Toivanen, J. Antimicrob. Chemother., in press). The level of trimethoprim resistance in *Escherichia coli* strains isolated from urine samples in the Turku area was 10% from 1981 through 1984. In the Helsinki area the corresponding resistance increased from 2.9 to 11.1%. In the United Kingdom, trimethoprim resistance has also increased slowly (2, 22). In hospitals for geriatric patients, development of trimethoprim resistance differed from that in other hospitals or in outpatients (6). In the Turku City Hospital, a hospital for geriatric patients, trimethoprim resistance in *E. coli* strains isolated from urine samples was 21%. The corresponding levels in the Turku University Central Hospital and the Kuopio University Central Hospital, which are hospitals for acutely ill patients, were only 4.1 and 6.2%, respectively (10). The increased resistance was due principally to colonization of indwelling catheters by *E. coli* strains; trimethoprim resistance among *E. coli* isolates collected from patients with and without indwelling catheters was 38 and 13.4%, respectively (9). The present study describes the relationship between the development of trimethoprim resistance, consumption of trimethoprim-sulfamethoxazole and trimethoprim in the Turku City Hospital from 1971 through 1984, and prevalence of *E. coli* isolates with DNA sequences homologous to transposon Tn7 from 1980 through 1981 and in 1983.

MATERIALS AND METHODS

Five thousand *E. coli* isolates from urine specimens collected in the Turku City Hospital from 1971 through 1984 were studied; 200 consecutive strains were studied each year (except in 1974) from January to February and July to August. *E. coli* isolates from patients with indwelling catheters were also included; 40 to 50% of strains studied were from catheter samples (9). Only one specimen from each patient was included. Over 80% of isolates were from patients aged ≥ 65 years (9). Strains were identified by routine methods, including the API 20E procedure (Analytab Products, Plainview, N.Y.) (15, 17). Resistance to trimethoprim, trimethoprim-sulfamethoxazole, and sulfamethoxazole was tested by the disk diffusion method (7). Determinations of susceptibility to trimethoprim-sulfamethoxazole and sulfamethoxazole were carried out during the entire study from 1971 through 1984, and determination of trimethoprim resistance alone was carried out since 1978. A semiconfluent inoculum was used (7). Paper disks containing 1.25 μg of trimethoprim, 250 μg of sulfasomidine or sulfamethoxazole, and 1.25 ± 23.75 μg of trimethoprim-sulfamethoxazole were applied to PDM-ASM agar (AB Biodisk, Solna, Sweden). Strains were classified as resistant when inhibition zones were smaller than 15 mm in diameter. Strains with inhibition zones between 11 and 19 mm constituted a small minority (8.3% for sulfamethoxazole, 2.5% for trimethoprim-sulfamethoxazole, and 1.8% for trimethoprim) during the whole study. The agreement between the disk diffusion and the plate dilution methods was found to be acceptable for testing the trimethoprim susceptibility of *E. coli* strains (9). MIC determinations involved the use of a plate dilution method with doubling dilutions (0.0625 to 1024 $\mu\text{g}/\text{ml}$) of trimethoprim lactate (Burroughs Wellcome, Helsinki, Finland) (10). The resistance breakpoint was ≥ 8 μg of trimethoprim per ml (10).

Occurrence of DNA sequences homologous to our probe for transposon Tn7 (a *Bam*HI fragment of ColE1::Tn7) was determined by DNA colony hybridization (18). This DNA

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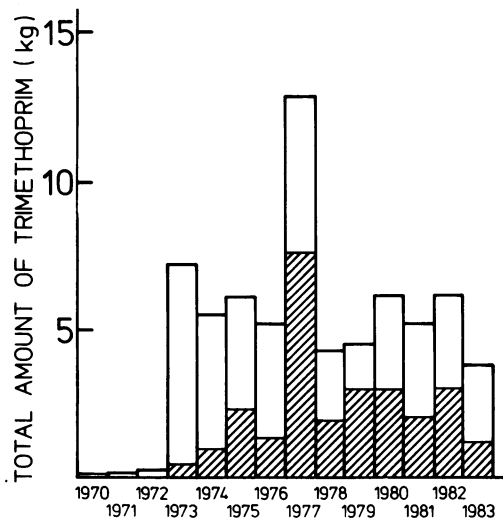


FIG. 1. Annual consumption of trimethoprim-sulfamethoxazole (□) and trimethoprim (▨) in the Turku City Hospital.

probe does not contain genes for trimethoprim or streptomycin resistance. However, all strains showing DNA homology with this probe appeared highly resistant to trimethoprim (MIC, >1,000 µg/ml) (18).

Consumption of trimethoprim and trimethoprim-sulfamethoxazole were estimated from the amounts purchased by the hospital (drugs were used only for inpatients). The total consumption of trimethoprim included that of trimethoprim alone and that of trimethoprim in combination.

RESULTS

Trimethoprim-sulfamethoxazole was first used in Turku City Hospital in 1970 (Fig. 1). From 1970 through 1972, only minor amounts of this drug were used. In 1973 consumption increased markedly, and use of pure trimethoprim contributed 5.3% of the total amount of 7.6 kg of trimethoprim consumed. In 1977, use of pure trimethoprim increased drastically, bringing the total amount of trimethoprim consumed to almost 14 kg; pure trimethoprim constituted 59% of the total amount. In 1978, the amounts were even again, and in 1983 the total trimethoprim consumption was only 3.9 kg (Fig. 1).

Resistance to trimethoprim-sulfamethoxazole has clearly increased since 1971 (Fig. 2). The basic level of resistance to trimethoprim-sulfamethoxazole from 1971 through 1973 was 5 to 10% among urinary *E. coli* isolates studied. From 1975 through 1978 resistance increased slightly, to 8 to 17%; in 1979 a clear increase in resistance to 25% occurred, and a plateau of 18 to 25% was reached which continued until 1982. Moreover, from 1983 through 1984 resistance increased again; a level of 32 to 35% was reached. Susceptibility testing for trimethoprim alone began in 1978, and 10% of *E. coli* isolates were determined to be resistant. However, in 1979 a level of 28.5 to 3.15% already existed, and this level continued until 1982. From 1983 through 1984 an increase in the level of trimethoprim resistance occurred again, reaching 34 to 40%.

Sulfonamide resistance among *E. coli* strains varied between 23 and 59% during the whole study. Only a minor decrease in resistance was found from 1977 through 1978

(Fig. 2). From 1983 through 1984, the level of sulfonamide resistance was 40 to 44%.

DNA homology of *E. coli* strains with the Tn7 probe was determined from 1980 to 1981 and again in 1983 (18). From 1980 to 1981, 42% of trimethoprim-resistant *E. coli* isolates (MIC ≥ 8 µg/ml) showed DNA homology with the probe (Table 1). Among all members of the family *Enterobacteriaceae*, the frequency of sequences homologous with the probe was 47.2%. In 1983, 64% of trimethoprim-resistant *E. coli* strains carried these Tn7 DNA sequences. The corresponding figure for all members of the family *Enterobacteriaceae* was 56.1%.

In 1970 patients stayed in the Turku City Hospital for an average of 33 days, and in the geriatrics department the duration was 57 days; the corresponding times, in 1975, 1980, and 1984 were 36 and 73 days, 37 and 73 days, and 40 and 85 days, respectively.

DISCUSSION

Opinions on the relationship of trimethoprim usage and emergence of resistance have varied (2, 16, 20). Results presented in this paper support the findings that emergence of trimethoprim resistance is linked to changes in the consumption of both trimethoprim-sulfamethoxazole and pure trimethoprim.

Trimethoprim resistance in relation to the consumption of trimethoprim in hospitals has previously been reported by Lacey (16). In the hospitals of the King's Lynn Health District, King's Lynn, England, use of trimethoprim alone began in 1980. In 1982, trimethoprim resistance was still at the original level; however, the follow-up time of 2 years was probably too short for detection of increased resistance. Grüneberg followed development of trimethoprim resistance in London (8). In 1971, he found that 96.8% of *E. coli* isolates were fully susceptible to trimethoprim; the corresponding figure in 1982 was 85.7%. However, drug consumption was not tabulated. Datta and co-workers also found a clear increase in trimethoprim resistance between 1970 and 1980, from 5.6 to 15.4%, among members of the family *Enterobacteriaceae* studied in a London hospital (4, 19). Towner and Wise followed the development of trimethoprim resistance in inpatients in the Nottingham area; the same trend was seen: trimethoprim resistance of *E. coli* isolates increased from 1.9 to 9.3% from 1978 through 1983 (22).

Trimethoprim resistance of *E. coli* strains isolated from urine samples in the Turku City Hospital reported in our earlier studies (9–11) is in agreement with the present results. In 1981 and 1982 we found that 22% of *E. coli* strains were trimethoprim resistant (9). Of these strains, 43% were collected from patients with indwelling urinary tract catheters; trimethoprim resistance among these strains was 38%. Among all members of the family *Enterobacteriaceae*, trimethoprim resistance occurred in 35.4% (11).

The first increase of trimethoprim resistance in the Turku City Hospital occurred after wider use of trimethoprim-sulfamethoxazole (Fig. 1 and 2). However, after the drastic increase of consumption of trimethoprim alone in 1977, trimethoprim resistance increased, reaching a level of 28 to 32% (Fig. 1 and 2); from 1977 through 1978, no remarkable changes occurred in the consumption of trimethoprim-sulfamethoxazole. After 1977, total consumption of trimethoprim as well as consumption of pure trimethoprim gradually decreased. Despite this, trimethoprim resistance has increased; in 1983 trimethoprim resistance reached a level of 34 to 37%. In 1984, 14 years after introduction of trimethoprim, trimethoprim resistance had reached approximately

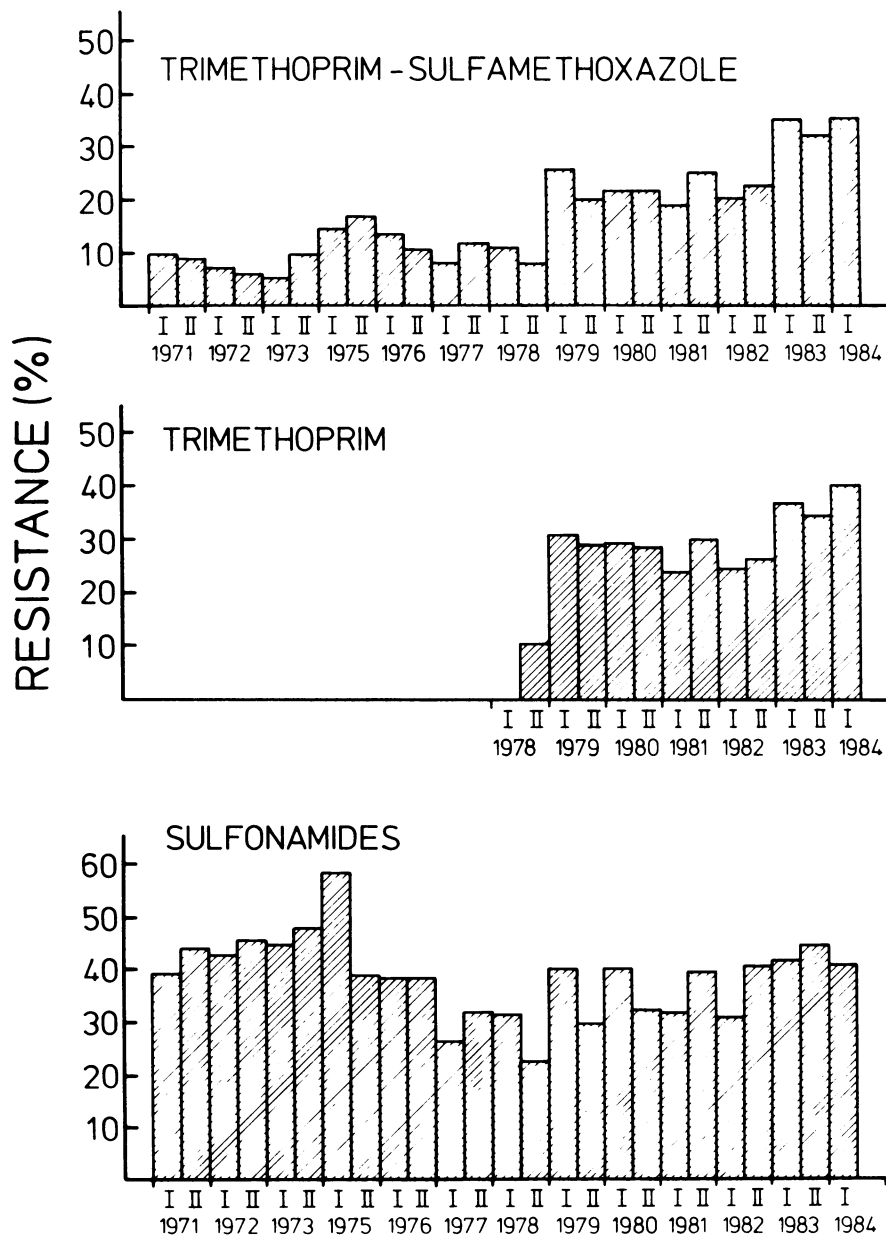


FIG. 2. Development of trimethoprim-sulfamethoxazole, trimethoprim, and sulfonamide resistance of *E. coli* strains isolated from urine in the Turku City Hospital from 1971 through 1984. Symbols: I, January and February; II, July and August.

TABLE 1. Trimethoprim-resistant (MIC \geq 8 μ g/ml) members of the family *Enterobacteriaceae* which showed DNA homology with the transposon Tn7 probe from 1980 to 1981 and in 1983 in the Turku City Hospital (18)

Organism	No. of trimethoprim-resistant strains studied		% positive in DNA hybridization with Tn7 probe	
	1980 to 1981	1983	1980 to 1981	1983
<i>E. coli</i>	76	126	42.0 ^a	64.3 ^a
<i>Proteus mirabilis</i>	69	67	75.4	56.7
<i>Klebsiella</i> spp.	31	17	12.9	23.5
<i>Providencia stuartii</i>	8	19	0	15.8
<i>Citrobacter freundii</i>	7	8	71.4	87.5
Other <i>Enterobacteriaceae</i>	8	2	16.7	50.0
Total	199	239	47.2 ^b	56.1 ^b

^a Difference between the two numbers marked is significant ($P < 0.005$; binomial t test).

^b $P < 0.10$.

the level of sulfonamide resistance, which, in this hospital, is about 40% among *E. coli* strains isolated from urine samples. On the basis of these findings, it appears likely that the development of resistance is related to the total use of trimethoprim, in combination or alone.

Increase in trimethoprim resistance in 1983 and 1984 might be explained by a transposon Tn7-mediated "hospital infection." Prevalence of sequences homologous to Tn7 was studied in 1980 and 1981, when homology among trimethoprim-resistant *E. coli* isolates and the Tn7 probe occurred in 42%, and homology among members of the family *Enterobacteriaceae* occurred in 47.2% of isolates (Table 1). In 1983, the corresponding numbers were 64.2% for *E. coli* and 56.1% for all *Enterobacteriaceae* ($P < 0.005$ and $P < 0.10$; Table 1). Increase in the occurrence of homologous sequences to Tn7 occurred in 1983 and 1984 without any notable increase in the use of trimethoprim. Possibly only a low level of trimethoprim consumption can build selection pressure enough to promote the spread of transposon-mediated resistance.

Of the 54 trimethoprim-resistant *E. coli* strains (MIC $>1,000$ $\mu\text{g/ml}$) collected in 1980 and 1981, 32 had DNA sequences homologous to our Tn7 probe. From these strains only seven transferred their resistance into the recipient *E. coli* strain (P. Huovinen, M.D. thesis, University of Turku, Turku, Finland). This might reflect the spread of chromosomally located Tn7 in this particular hospital. In addition, 15 of the 22 *E. coli* isolates without any DNA homology to our Tn7 probe transferred their resistance into the recipient strain. According to our preliminary studies, these isolates include several strains which have DNA homology with the trimethoprim resistance transposon Tn402 (11). In addition, some of the trimethoprim-resistant strains showed homology to both Tn7 and Tn402 probes. Similar finding were previously reported by Burchall et al. (3). It is apparent that further studies are needed to evaluate the role of transposon Tn402 in the spread of trimethoprim resistance in this hospital.

In conclusion, although the patients in this hospital are geriatric, and long-term stay and use of urinary tract catheters are common, our findings indicate that emergence of trimethoprim resistance is linked with changes in the consumption of both trimethoprim-sulfamethoxazole and pure trimethoprim.

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