The distribution of the DHFR genes in trimethoprim-resistant urinary tract isolates from Taiwan

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SUMMARY

Between July 1987 and June 1989, 1054 urinary isolates of enterobacteria from Kaohsiung, Taiwan were studied for their trimethoprim resistance. Trimethoprim resistance was defined as MIC greater than 4 μg/ml and high-level resistance by MIC greater than 1000 μg/ml. The incidence of trimethoprim resistance increased from 33·6 % in 1987 to 42·1 % in 1989. Among the resistant strains studied, 90 % were resistant to high levels of trimethoprim. An increase in the proportion of resistant strains (33·9–46·3 %) exhibiting high-level non-transferable trimethoprim resistance was noted. The distribution of the dihydrofolate reductase (DHFR) genes by colony hybridization in 374 trimethoprim-resistant isolates revealed the presence of type I and type V DHFR genes in most of these isolates (45·4 % and 10·4 % respectively). Type I was predominant in *Escherichia coli* whereas type V was frequently seen in *Enterobacter* spp. None showed homology with the type II and type III DHFR probe DNA. In addition, transposon Tn7 was present in 7·8 % of 374 trimethoprim-resistant enterobacteria.

INTRODUCTION

Trimethoprim, either alone or in combination with sulphonamides, has been used to treat infections of the urinary tract for several years. Following its widespread use, bacterial pathogens resistant to trimethoprim have emerged as a significant clinical problem [1–2]. The most important mechanism of high level trimethoprim resistance in bacteria is production of plasmid-encoded trimethoprim-resistant dihydrofolate reductase (DHFR) [3]. Several plasmid-encoded DHFRs or their genes have been already characterized [4, 5]. Type I DHFR found in many countries, is the most important mediator of trimethoprim resistance in enterobacteria, and is determined by transposon Tn7 [6, 7]. Isolates with type II DHFR have also been reported in Europe and America [8]. Type III DHFR occurs less commonly and mediates only moderate level of resistance to trimethoprim [9]. Type V DHFR has been found predominantly in Sri Lanka [10].

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The trimethoprim-resistant rate of urinary isolates in Kaohsiung, Taiwan was about 32% between 1982 and 1983 [11]. Because transposons are believed to be involved in the spread of antibiotic resistance among different plasmids in Gram-negative bacteria [12], we surveyed the prevalence of trimethoprim-resistant transposon in urinary isolates in the same area from 1985 to 1986. Fifty-two per cent of trimethoprim-resistant isolates showed a positive reaction when a Tn7 DNA probe was used [13].

As changing trends in susceptibility to trimethoprim in recent years need to be monitored at the molecular level, the present study has undertaken to survey trimethoprim resistance from 1987 to 1989. Using DNA hybridization, we detected and discriminated the type of DHFR genes responsible for the spread of trimethoprim resistance in various clinical bacterial isolates with high-level trimethoprim resistance. We also present data to show the prevalence of transposon in clinical isolates.

MATERIALS AND METHODS

Bacterial strains

Isolates of enterobacteria from urinary tract infections were collected from Kaohsiung, Taiwan; these included *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp. Minimal inhibitory concentration (MIC) were determined for these strains by using a multiple-inoculator replicator to inoculate 10^4–10^5 c.f.u. onto Mueller–Hinton agar (Difco Laboratories, Detroit, Michigan, USA) containing various concentrations of trimethoprim (Sigma Chemical, St Louis, Mo, USA). Resistance was defined as growth on plates containing greater than $4 \mu g/ml$ trimethoprim. Other plasmids which were not from our isolates are listed in Table 1.

Conjugation, plasmid DNA extraction, gel electrophoresis and southern transfer to membrane filters

Conjugation experiments were performed by mixing donor and recipient cells and incubating overnight at 37 °C and 28 °C without agitation [14]. Donors were from our clinical isolates. *E. coli* K12 F^- lac$^-$ nal^r (14R, 519) obtained from Dr B. Rowe (Central Public Health Laboratory, London, England), which is devoid of extra-chromosomal DNA was used as the recipient. The transconjugants were selected from agar plates containing appropriate antibiotics. The plasmid content of transconjugants was extracted by the method of Kado and Liu [15] with subsequent electrophoresis in 0·7% agarose gels (Bethesda Research Laboratories, Gaithersburg, USA). Gels were stained with ethidium bromide and photographed using ultraviolet transilluminator. The gels were then partially depurinated in 0·25 N-HCl and denatured in 0·5 N-NaOH. DNA was then transferred to nitrocellulose filters (Schleicher and Schuell, Dassel, N.H., USA) by the technique of Southern [16].

Description and isolation of DNA fragments and nick translation

The sources of probes used in this study are listed in Table 1. The probe for the type I DHFR gene was the 500 bp *Hpa* I restriction fragment from plasmid pFE872, whereas the DNA probe used to detect the type II DHFR gene consisted

Table 1. *Sources of plasmids*

Plasmid	Relevant characteristics	Source and reference
pFE872	Tp Ap Tc (type I DHFR)	Lynn Elwell [23]
pFE1975	Tp Ap (type II DHFR)	Lynn Elwell [23]
pFE1242	Tp Ap (type III DHFR)	Lynn Elwell [23]
pLK022	Tp Ap (type V DHFR)	Ola Sköld [32]
ColE1::Tn7	Tp Sm Sp	Lynn Elwell [23]
R483	Sp TP (type I DHFR)	VM Hughes
R388	Su Tp (type II DHFR)	VM Hughes
pFE364	Ap Tp (type II DHFR)	Lynn Elwell [23]
pUB307	Km Tc	PM Bennett

Tp, trimethoprim; Ap, ampicillin; Tc, tetracycline; Sm, streptomycin; Sp, spectinomycin; Su, sulfamethoxazole; Km, kanamycin.

of the 284 bp *Bam*H I–*Eco*R I restriction fragment derived from plasmid pFE1975. Plasmid pFE1242 that contains the type III DHFR structural gene on an 855 bp *Eco*R I–*Hin*d III fragment was the source of type III DHFR gene. For the type V DHFR gene, a 480 bp *Kpn* I–*Bam*H I fragment of pLK022 plasmid was used. The probe for tnsC consisted of the 1 kb *Bam*H I fragment from ColE1::Tn7, which does not include the trimethoprim resistance gene [17], was used to detect Tn7 in bacterial strains by colony hybridization.

The plasmid DNA described above was purified by a modification of the alkaline extraction method [18]. Plasmid DNA was digested with the appropriate restriction enzyme combinations (New England Biolabs Inc., Beverly, USA) and the fragments were separated electrophoretically on 1% low melting-point agarose (Bethesda Research Laboratories, Gaithersburg, USA). The specific fragments were cut out and DNAs were eluted by Tris-saturated phenol extraction [16].

The fragments were radiolabelled by using [α-^{32}P]dCTP (50 μCi) (New England Nuclear, USA) and DNase plus DNA polymerase I (Boehringer-Mannheim GmbH, Mannheim, Germany) under the conditions as described by Maniatis and colleagues [16]. Before hybridization, the probes were denatured by heating at 100 °C for 10 min.

Colony hybridization, southern hybridization and autoradiography

The colony hybridization technique [16] was used with slight modification. Bacterial colonies were lysed on nitrocellulose filters and the released DNA was immobilized on filters. Nitrocellulose filters from southern transfer and colony hybridization were pre-hybridized followed by hybridization with nick-translated ^{32}P-labelled DNA probes for 20 h at 65 °C. Autoradiography of filters was performed with Kodak X-Omat AR film together with intensifying screens at −70 °C.

RESULTS

Characterization of trimethoprim-resistant strains

A total of 1054 urinary isolates of *E. coli*, *Enterobacter* spp., and *Klebsiella* spp. was collected between July 1987 and June 1989. Table 2 shows the number of isolates resistant to $\geqslant 4$ μg/ml trimethoprim in these 3 years. An increase from 34

Table 2. *Proportion of enterobacteria resistant to* $\geqslant 4\ \mu g/ml$ *trimethoprim*

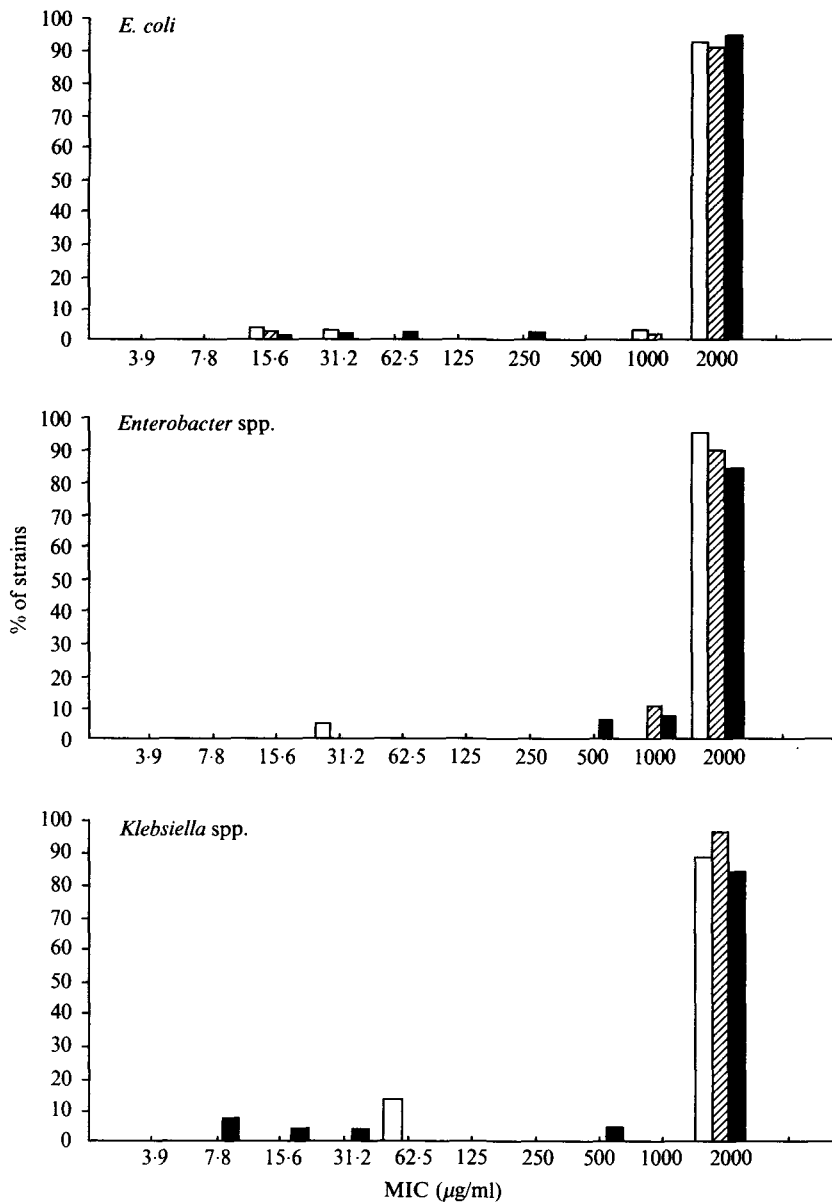| Species	Number of strains tested	Number (%) of resistant isolates in each year		
		1987	1988	1989
E. coli	824	49 (28·5%)	101 (27·9%)	119 (41·0%)
Enterobacter spp.	85	20 (57·1%)	10 (55·5%)	14 (43·8%)
Klebsiella spp.	145	8 (42·1%)	25 (41·7%)	30 (45·5%)
Total	1054	77 (34%)	136 (30·9%)	163 (42·0%)

Fig. 1. Percentages of isolates of *E. coli*, *Enterobacter* spp., and *Klebsiella* spp. resistant to trimethoprim as expressed in MIC. □, 1987; ▨, 1988; ■, 1989.

Table 3. *Transferability of trimethoprim resistance in trimethoprim-resistant (Tp^r) isolates*

Species	1987		1988		1989	
	tra$^+$	tra$^-$	tra$^+$	tra$^-$	tra$^+$	tra$^-$
E. coli	30	16	65	26	67	41
Enterobacter spp.	14	3	9	11	10	14
Klebsiella spp.	3	5	10	13	8	18
Total	47(66·1%)		84(62·6%)		85(53·7%)	

tra$^+$, transferable; tra$^-$, non-transferable.
Numbers in parentheses indicate percent of transferable isolates in each year.

to 42% in the overall incidence of resistance to this concentration of trimethoprim was noted. However, the prevalence of trimethoprim resistance among clinical isolates of enterobacteria varied widely between species. The resistant rates in *Enterobacter* spp. and *Klebsiella* spp. did not change appreciably, but the percentage of trimethoprim resistance of *E. coli* increased significantly (from 28·5% in 1987 to 41% in 1989).

Degrees of resistance

The degrees of resistance to trimethoprim in each of the three groups of bacteria are shown in Fig. 1. There was a significant number of isolates resistant to $\geqslant 1000~\mu g/ml$ trimethoprim. In these groups 95·8% (257 of 268) of *E. coli*, 95·4% (42 of 44) of *Enterobacter* spp. and 88·8% (56 of 62) of *Klebsiella* spp. exhibited high-level resistance.

Transfer of resistance

Trimethoprim resistance isolates were tested for their ability to transfer resistance to a plasmid-free recipient strain of *E. coli* K12 in the conjugation experiment. Table 3 gives the numbers of isolates capable of transferring resistance. From 1987 to 1989 the proportion of resistant isolates owing their resistance to a transferable plasmid decreased from 66·1 to 53·7%. Further study by using the southern hybridization revealed that the transferable resistance was, in fact, plasmid-mediated (Fig. 2). However, the incidence of transferable resistance were greater among *E. coli* than in the other species.

Detection of specific DHFR structural genes

In order to determine the type of DHFR genes in these 374 trimethoprim-resistant isolates, colony hybridization with probes representing types I, II, III and V DHFR, were used. In addition, *E. coli* strains containing the following plasmids were used as controls: ColE1::Tn7 (type I DHFR), pFE364 (2·6 kb R67 insert, type IIa DHFR), p1975 (284 bp R67 insert, type IIa DHFR), p1242 (type III DHFR), pLK022 (type V DHFR), R388 (type IIb DHFR) R483 (type I DHFR). The probes for the type I, II, III and V DHFR hybridized only with their own positive control strains; no other cross-hybridizations were detected. The results are presented in Table 4. Among the isolates tested, 169 isolates (45·4%)
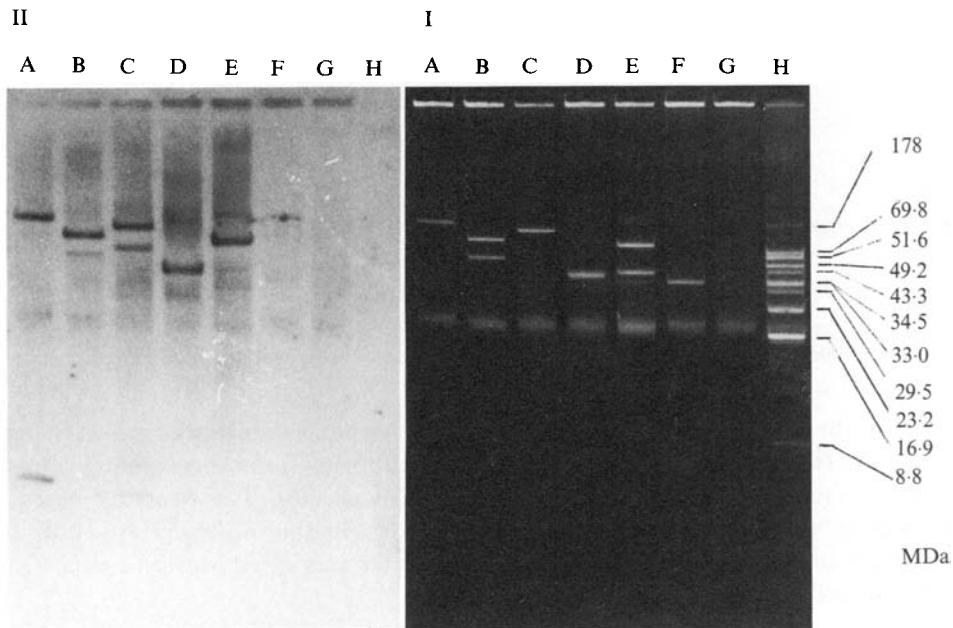
Fig. 2. Agarose gel electrophoresis (I) and southern hybridization (II) of plasmid DNA obtained from transconjugants. (A–E), transconjugants of clinical strains; (F) pUB307 (negative control); (G) *E. coli* K12 (14R, 519), recipient of conjugation experiment; (H) *Erwinia stewartii* sw2 (MW marker).

Table 4. *Distribution of types I, II, III and V DHFR genes, as inferred from colony hybridization experiments*

Fragments used as probes

Percentage of enteric isolates reacting with probes

DHFR genes	500 bp DNA fragment of pFE872	284 bp DNA fragment of pFE1975	855 bp DNA fragment of pFE1242	480 bp DNA fragment of pLK022
type I	45·4%	—	—	—
type II	—	0%	—	—
type III	—	—	0%	---
type V	—	—	—	10·4%

hybridized with the type I DHFR probe. Thirty-nine isolates (10·4%) hybridized with the type V DHFR probe. No positive hybridization was found either with the probes specific for the type II or type III DHFR.

The distribution of the DHFR genes differs in each group of bacteria are shown in Table 5. In *E. coli*, type I was dominant (62·2%) (Fig. 3). The percentage of type I DHFR was significantly smaller in *Enterobacter* spp. and *Klebsiella* spp. than in *E. coli* during this study period, whereas type V frequently occurred in *Enterobacter* spp. (56·8%). In order to determine whether a trimethoprim-resistant gene existed as a functional transposable element in these clinical isolates, we used a *Bam*H I fragment of Tn7 as a probe to detect the prevalence of Tn7. Of the 374 isolates studied, 7·8% were positive in colony hybridization. In these studies. about 10% (27 of 267) of *E. coli* and 3·2% (2 of 63) of *Klebsiella* spp. were found

Table 5. *Distribution of type I and type V dihydrofolate reductase (DHFR) genes in bacteria with trimethoprim resistance*

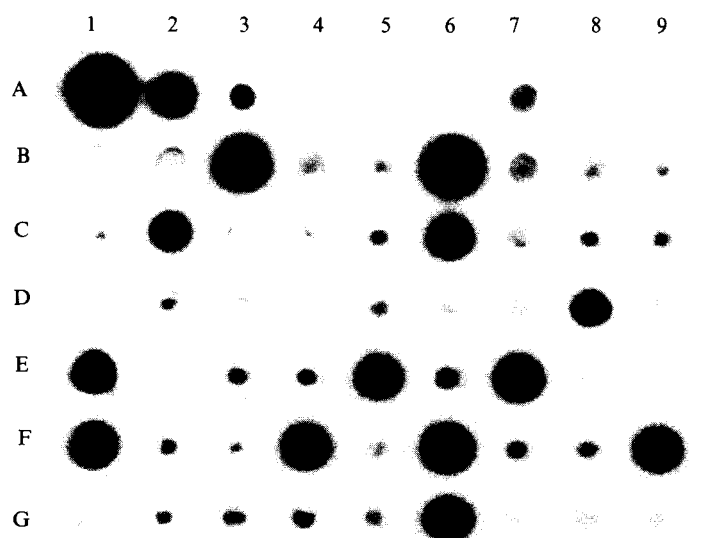| Organism	Total no. of strains	No. of strains with indicated DHFR type	
		I	V
Escherichia coli	267	166 (62·2%)	10 (3·7%)
Enterobacter spp.	44	0	25 (56·8%)
Klebsiella spp.	63	3 (4·8%)	4 (6·3%)

Fig. 3. Colony hybridization of 57 *E. coli* strains with nick-translated *Hpa* I fragment of pFE872 used as a probe. Hybridization and autoradiography were performed as described in the text. As positive controls, *E. coli* C600 (pFE872) (A1), *E. coli* C600 (ColE1::Tn7) (A2), *E. coli* J53 (R483) (A3) were used. The negative controls were pLKO22 (type V DHFR) (A4), pFE1975 (type II DHFR) (A5), and pFE1242 (type III DHFR) (A6).

Table 6. *Distribution of Tn7 in bacteria with trimethoprim resistance. (A 1 kb BamH I fragment of plasmid colE1::Tn7 was used as a probe)*

Organism	Total no. of strains	No. of Tn7-like transposons
Escherichia coli	267	27 (10·0%)
Enterobacter spp.	44	0
Klebsiella spp.	63	2 (3·2%)
Total	374	29 (7·8%)

to carry this transposable element (Table 6). However, none was found in *Enterobacter* spp.

DISCUSSION

The great consumption of trimethoprim as an anti-bacterial drug is the cause of rising resistance among pathogenic bacteria [1, 2]. Resistance to trimethoprim has been demonstrated in many bacterial species [19]. Reports from Italy [20] and

Finland [21] indicated an overall level of resistance to trimethoprim ranging from 20 to 40%. However, resistance rates of 5–8% have been reported from the UK, Denmark and the US [8, 22]. The incidence of trimethoprim-resistant bacteria is especially great in developing countries, e.g. 44% in Chile and 40% in Thailand [23]. In Kaohsiung Hospital, Taiwan, the trimethoprim resistance remained at approximately 32% from 1983 to 1987. However, the resistance rate increased from 33·6% in 1987 to 42·1% in 1989. Control of trimethoprim and other antibiotics used in various countries may be the cause of this variable resistance. There was also some evidence to demonstrate that ampicillin, which is widely used in clinical settings, may select bacteria harbouring plasmids encoding joint resistance to ampicillin and trimethoprim [4]. Therefore the combination of widespread use of trimethoprim, related antibiotics and even unrelated antibiotics may explain the emergence and spread of trimethoprim resistance in different species.

The increasing frequency of drug-resistant bacteria is clearly related to the ability to transfer resistance among bacteria [12]. The high level of trimethoprim resistance ($\geqslant 1000$ μg/ml) is mostly associated with the presence of plasmids or transposons [4]. The first trimethoprim-resistant plasmid was described in England in 1972 [24]. Subsequently, plasmid-mediated trimethoprim resistance has become widely disseminated, according to reports from the UK [25], France, Italy [20], Finland, Canada and Bangladesh [26, 27].

Decreased transferability of high-level trimethoprim resistant genes among pathogenic bacteria was observed recently, even though the incidence of high-level resistance remained high. We found that the rate of the transferable trimethoprim resistance was 66% in 1987 but decreased to 53·7% in 1989. In recent years, the increasing prevalence of nontransferable resistance to large concentrations of trimethoprim has been reported [28]. These findings indicate that resistance may be carried on non-transferable plasmids or be mediated by a transposable element inserted into the bacterial chromosome [29]. The mobilization experiment will be tested to characterize the location of the DHFR gene in our present study.

We screened 374 naturally occurring bacterial isolates with high-level trimethoprim resistance for the presence of either type I, type II, type III or type V DHFR genes. Of the 169 isolates that hybridized with the type I DHFR probe, only 17·2% hybridized with the Tn7 probe. These results indicate that type I DHFR gene existed not only on its acceptor site in Tn7 but also can occur in genetic structures other than Tn7. For this reason the trimethoprim-resistant trait in general is moveable. An integron which contains the *sulI* gene has been shown to harbour a site-specific recombination system for the integration of various antibiotic resistance genes [30]. In addition, the integron was distributed by integration into transposons belonging to the Tn21 family [31]. In our hands (unpublished data) the location of the *dhfrI* gene previously found only in Tn7, was observed to occur at other specific sites on a Tn21-like structure [32]. This result may indicate a fairly ubiquitous spread of *dhfrI* outside Tn7 in our survey.

We noticed no type III DHFR in our isolates. This result is plausible as type III DHFR mediates only a moderate level of resistance to trimethoprim. Thus, it was not seen in this high proportion (> 90%) of highly-resistant isolates. The type

V DHFR gene that also confers a high level of trimethoprim resistance and that shows 75 % nucleotide homology with the type I DHFR gene, has been reported to be dominant in enterobacteria only in Sri Lanka and the UK. In this study, 10·4 % of the trimethoprim-resistant strains hybridized with the type V DHFR probes that we used.

The genetic location of the *dhfrI* gene changed (from 52 to 7·8 % in Tn7) during 1985–9. One possibility was the combination of trimethoprim and sulphonamides to treat urinary tract infection, which might have promoted the recombination of dhfr and sulI genes [30]. The combination relationship between dhfr gene from our clinical isolates and integron will be reported in the future.

REFERENCES

1. Richmond M. Antibiotic resistance and the evolution of bacteria. Nature 1983; **302**: 657.
2. Huovinen P, Pulkkinen L, Helin HL, Makila M, Toivanen P. Emergence of trimethoprim resistance in relation to drug consumption in a Finnish hospital from 1971 through 1984. Antimicrob Agents Chemother 1986; **29**: 73–6.
3. Amyes SGB, Smith JT. R-factor trimethoprim resistance mechanism: An insusceptible target site. Biochem Biophys Res Commun 1974; **58**: 412–8.
4. Amyes SGB, Towner KJ. Trimethoprim resistance; epidemiology and molecular aspects. J Med Microbiol 1990; **31**: 1–19.
5. Jansson C, Sköld O. Appearance of a new trimethoprim resistance gene, dhfrIX, in *Escherichia coli* from Swine. Antimicrob Agents Chemother 1991; **35**: 1891–9.
6. Steen R, Sköld O. Plasmid-borne or chromosomally mediated resistance by Tn7 is the most common response to ubiquitous use of trimethoprim. Antimicrob Agents Chemother 1985; **27**: 933–7.
7. Pulkkinen L, Huovinen P, Vuorio E, Toivanen P. Characterization of trimethoprim resistance by use of probes specific for transposon Tn7. Antimicrob Agents Chemother 1984; **26**: 82–6.
8. Mayer KH, Fling ME, Hopkins JD, O'Brien TF. Trimethoprim resistance in multiple genera of Enterobacteriaceae at a U.S. hospital: spread of the type II dihydrofolate reductase gene by a single plasmid. J Infect Dis 1985; **151**: 783–9.
9. Fling ME, Kopf J, Richards C. Characterization of plasmid pAZ1 and the type III dihydrofolate reductase gene. Plasmid 1988; **19**: 30–3.
10. Sundström, L, Vinayagamoorthy T, Sköld O. Novel type of plasmid-borne resistance to trimethoprim. Antimicrob Agents Chemother 1987; **31**: 60–6.
11. Chang LL, Chang SF, Chow TY. R factor in urinary tract infection. J Formosan Med Assoc 1984; **83**: 268–77.
12. Datta N, Richards H. Trimethoprim-resistant bacteria in hospital and in the community: spread of plasmids and transposons. In: Levy SB, Clowes RC, Koenig RL, eds. Molecular biology: pathogenicity and ecology of bacterial plasmids. New York: 1981: 21–30.
13. Chang SF, Chang LL, Chow TY, Wu WJ, Chang JC. Prevalence of transposons encoding kanamycin, ampicillin and trimethoprim resistance in isolates from urinary tract infections detected by using DNA probes. Kaohsiung J Med Sci 1992; **8**: 141–7.
14. Lin SR, Chang SF. Drug resistance and plasmid profile of shigellae in Taiwan. Epidemiol Infect 1991; **108**: 87–97.
15. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981; **145**: 1365–73.
16. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.

17. Gosti-Testu F, Norris V, Brevet J. Restriction map of Tn7. Plasmid 1983; **10**: 96–9.
18. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 1979; **7**: 1513–23.
19. Goldstein FW, Papadopoulou B, Acar JF. The changing pattern of trimethoprim resistance in Paris, with a review of worldwide experience. Rev Infect Dis 1986; **8**: 725–37.
20. Romero E, Perduca M. Compatibility groups of R-factors for trimethoprim resistance isolated in Italy. J Antimicrob Chemother 1977; **3**: 35–8.
21. Huovinen P, Pulkkinen L, Toivanen P. Transferable trimethoprim resistance in three Finnish hospitals. J Antimicrob Chemother 1983; **12**: 249–56.
22. Brumfitt W, Hamilton-Miller JMT, Wood A. Evidence for a slowing in trimethoprim resistance during 1981 – a comparison with earlier years. J Antimicrob Chemother 1983; **11**: 503–9.
23. Murray BE, Alvarado T, Kim KH, et al. Increasing resistance to trimethoprim-sulfamethoxazole among isolates of *Escherichia coli* in developing countries. J Infect Dis 1985; **152**: 1107–13.
24. Fleming MP, Datta N, Grüneberg RN. Trimethoprim resistance determined by R factors. Br Med J 1972; **1**: 726–8.
25. Jobanputra RS, Datta N. Trimethoprim R factors in enterobacteria from clinical specimens. J Med Microbiol 1974; **7**: 169–77.
26. Bannatyne RM, Toma S, Cheung R, Hu G. Resistance to trimethoprim and other antibiotics in Ontario *Shigellae*. Lancet 1980; i: 425–6.
27. Threlfall EJ, Rowe B, Huq I. Plasmid-encoded multiple antibiotic resistance in *Vibrio Cholerae* El Tor from Bangladesh. Lancet 1980; i: 1247–8.
28. Kraft CA, Timbury MC, Platt DJ. Distribution and genetic location of Tn7 in trimethoprim-resistant *Escherichia coli*. J Med Microbiol 1986; **22**: 125–31.
29. Heikkila E, Sundström L, Skurnik M, Huovinen P. Analysis of genetic localization of the type I trimethoprim resistance gene from *Escherichia coli* isolated in Finland. Antimicrob Agents Chemother 1991; **35**: 1562–9.
30. Radstrom P, Swedberg G, Sköld O. Genetic analyses of sulfonamide resistance and its dissemination in gram-negative bacteria illustrate new aspects of R plasmid evolution. Antimicrob Agents Chemother 1991; **35**: 1840–8.
31. Sundström L, Radstrom P, Swedberg G, Sköld O. Site specific recombination promotes linkage between trimethoprim and sulfonamide resistance genes. Sequence characterization of *dhfrV* and *sulI* and a recombination active locus of Tn21. MGG 1988; **213**: 191–201.
32. Sundström L, Sköld O. The dhfrI trimethoprim resistance gene of Tn7 can be found at specific sites in other genetic surroundings. Antimicrob Agents Chemother 1990; **34**: 642–50.