J. Hyg., Camb. (1985), 95, 391–395
Printed in Great Britain

Transferable resistance to trimethoprim in enteric pathogens isolated in Kuwait

By T. D. CHUGH

Faculty of Medicine, University of Kuwait, P.O. Box 24923, *Kuwait*

(*Received* 26 *February* 1985; *accepted* 17 *April* 1985)

SUMMARY

Trimethoprim resistance was seen in 14·8% of 500 strains of salmonella, 43·1% of 153 strains of shigella and 59% of 27 strains of *Escherichia coli* isolated from stools of patients with diarrhoea. Strains with a high level of trimethoprim resistance (MIC of $\geqslant 512\ \mu g$) were subjected to conjugal transfer. Trimethoprim resistance was plasmid-mediated in all of 42 strains of shigella and 12 strains of *E. coli* examined. However, 2 of the 47 strains of salmonella could not transfer their trimethoprim resistance either directly or by mobilization with the transfer factors *X* and *Δ* both at 37 and 25 °C overnight incubation.

INTRODUCTION

Trimethoprim, alone or in combination with sulphamethoxazole, has been used in clinical practice since 1969. Transferable resistance to trimethoprim was first observed in 1971 and since then has been reported in enteric pathogens in several countries (Rowe & Threlfall, 1981). Towner *et al.* (1980) examined clinical isolates of enterobacteria for resistance to trimethoprim in two 6-month surveys during 1978 and 1979. The incidence showed a slight decrease but the proportion of strains with transferable trimethoprim resistance almost trebled during the second survey period. In previous publications we reported plasmid-encoded drug resistance in salmonella and shigella isolated from human patients, sea water and clams (Chugh, 1982; Chugh & Bishbishi, 1983; Chugh *et al.* 1984; Chugh & Kadri, 1984). This communication is an account of plasmid-mediated trimethoprim resistance in the enteric pathogens isolated from clinical cases in Kuwait.

MATERIALS AND METHODS

Bacterial strains

A total of 680 strains of enteric pathogens (500 salmonella, 153 shigella and 27 enteropathogenic *Escherichia coli*) were examined. These had been isolated from stools or rectal swabs from the same group of epidemiologically unrelated patients suffering from acute diarrhoea during the period 1980–3. Isolates were identified biochemically (API–20E) and serologically using commercial antisera.

Table 1. *Minimum inhibitory concentration of enteric pathogens to trimethoprim*

Enteric pathogens	No. examined	No. resistant*	MIC (μg/ml) 0·5	1	2	4	8	16	32	64	128	256	512
Salmonella sp.	500	74 (14·8%)	345	57	14	11	1	4	4	4	7	8	47
S. typhimurium	223	59	113	43	3	5	—	—	—	2	6	7	44
S. wien	35	1	30	1	1	2	—	—	—	—	—	—	1
S. bovismorbificans	30	2	26	1	1	—	—	—	1	—	—	—	1
S. typhi	17	—	16	1	—	—	—	—	—	—	—	—	—
Others	195	12	158	11	9	4	1	4	3	2	1	1	1
Shigella sp.	153	66 (43·1%)	71	6	—	4	3	6	4	8	2	4	42
Sh. dysenteriae	7	3	4	—	—	—	—	—	—	2	—	1	—
Sh. flexneri	99	39	48	6	3	3	—	3	1	—	2	3	30
Sh. boydii	18	11	6	—	—	1	—	2	—	3	—	—	6
Sh. sonnei	29	13	13	—	—	—	3	1	3	3	—	—	6
E. coli	27	16 (59·2%)	9	1	1	—	—	—	1	3	—	—	12
Total	680	156	423	64	18	15	4	10	9	15	9	2	101

* Strains with MIC against trimethoprim more than 8 μg/ml were considered resistant.

Antibiotic susceptibility

This was determined by disk diffusion on DST agar (Oxoid CM 261) with *E. coli* (NCTC 10418) as the sensitive control. The antimicrobials used and the disk load of each drug were: ampicillin (A) 30 μg, chloramphenicol (C) 30 μg, gentamicin (G) 10 μg, kanamycin (K) 30 μg, streptomycin (S) 10 μg, sulphonamide (Su) 300 μg, tetracycline (T) 30 μg, trimethoprim (Tm) 1·25 μg and nalidixic acid (Nx) 30 μg. Minimal inhibitory concentrations (MIC) were determined by plate dilution technique by incorporating trimethoprim lactate in twofold serial dilutions to get a final concentration of 0·5–512 μg/ml. Plates were inoculated with bacterial suspension (10^3 c.f.u. per spot) by a multipoint inoculator. The lowest concentration of the drug that prevented visible growth after overnight incubation at 37 °C was recorded as MIC. Isolates with MIC against trimethoprim more than 8 μg per ml were considered as resistant.

Resistance transfer

All strains with MIC ⩾512 μg/ml were examined for the ability to transfer trimethoprim resistance to recipient *E. coli* K12F$^-$Nx^r at 37 and 25 °C. The transconjugants were selected by inoculating 0·1 ml of the overnight mixture of equal volumes of donor and recipient cultures on to lactose/neutral-red/peptone agar each containing 40 μg/ml of nalidixic acid and 8 μg/ml of trimethoprim lactate. Mobilization of non-self transferable resistance was attempted in triparental crosses with transfer factors *X* and *Δ* (Anderson & Threlfall, 1974). The transfer frequency was determined as the ratio of total number of transconjugants to the number of recipients.

https://doi.org/10.1017/S0022172400062811 Published online by Cambridge University Press

Table 2. *Transfer of resistance to trimethoprim in enteric pathogens*

	No. tested	Resistance transfer: Autotransferable	Resistance transfer: Non-autotransferable	Total with transferable trimethoprim resistance
Salmonella sp.	47	38 (80·8%)	7 (14·9%)	45 (95·7%)
Shigella sp.	42	27 (64·3%)	15 (35·7%)	42 (100·0%)
E. coli	12	11 (91·7%)	1 (8·3%)	12 (100·0%)
Total	101	76 (75·2%)	23 (22·8%)	99 (98·0%)

RESULTS

The results are shown in Tables 1 and 2. None of the resistant strains were thymidine-dependent as they could grow both on lysed blood agar and routine primary isolation media.

Salmonella sp.

Resistance to trimethoprim (TmR) was seen in 74 of the 500 isolates (14·8%) of salmonella examined. It was more prevalent in *S. typhimurium* (59 of the 223 strains, 26·5%) than in other serotypes (15 of 277 strains, 5·4%). None of the 17 isolates of *S. typhi* examined was resistant to trimethoprim. The level of resistance (MIC) was higher in *S. typhimurium* (44 of the 59 TmR strains have MIC of $\geqslant 512$ μg/ml, 74·6%) than in other *Salmonella* sp. (3 of 15 strains have MIC of $\geqslant 512$ μg/ml, 20·0%). All the 74 isolates of salmonella resistant to trimethoprim were multidrug-resistant (resistant to $\geqslant 3$ drugs) and 20 of these (27%) were resistant to all the eight drugs used (ACGKSSuTTm). Resistance to trimethoprim was transferable in 45 of the 47 (95·7%) strains examined, being directly transferable in 38 and mobilizable with transfer factors (*X* and *Δ*) in another 7 strains. In 2 strains of *S. typhimurium* the transfer of resistance was not detected, either directly or by mobilization with transfer factors both at 37 and 25 °C.

Shigella sp.

Trimethoprim resistance was observed in 66 of the 153 (43%) clinical isolates of shigella examined. Resistance was equally distributed in the four subgroups. There were two isolates of *Sh. dysenteriae* 1 in the present series. One of these was sensitive to trimethoprim while the second isolate with the R-type ACGKSSuTTm (MIC, 256 μg/ml) could transfer all the R determinants directly. Forty-two of the 66 resistant strains (64%) had an MIC of 512 μg/ml or more and, with one exception, all were multi-resistant. All the 42 strains examined could transfer their trimethoprim resistance to recipient *E. coli*, directly in 27 and by mobilization with transfer factors (*X* and *Δ*) in 15.

E. coli

Sixteen of the 27 (59%) clinical isolates of enteropathogenic *E. coli* obtained from cases of diarrhoea were resistant to trimethoprim. The majority of the resistant strains (75%) had an MIC of $\geqslant 512$ μg/ml and all except one were multi-resistant.

All the 12 strains examined could transfer their resistance to recipient *E. coli* directly in 11 and by mobilization with transfer factors in the 1 strain.

The frequency of transfer of trimethoprim resistance in the various enteric pathogens varied from $3{\cdot}4 \times 10^{-4}$ to $4{\cdot}1 \times 10^{-8}$. In one strain of *S. typhimurium* the transfer was higher ($3{\cdot}6 \times 10^{-4}$) at 25 °C than at 37 °C ($5{\cdot}2 \times 10^{-7}$), the plasmid being thermosensitive (ts).

DISCUSSION

Trimethoprim resistance in salmonella from the Middle East was first reported by Anderson *et al.* (1977) and was observed in *S. typhimurium* isolated in Israel (9 strains in 1975 and 3 strains isolated during 1976). The resistance in all the strains was determined by a non-autotransferable plasmid (F_1 me). They also described one isolate from Iran with the resistance pattern of ACGKSSuTTm but the resistance to trimethoprim was not transferable. Subsequently, Rowe *et al.* (1980) reported the spread of a clone of *S. typhimurium* in Saudi Arabia and India. The majority of strains were resistant to eight drugs (ACGKSSuTTm) and in all strains the resistance was plasmid-determined. Threlfall *et al.* (1983) reported the incidence of plasmid-encoded trimethoprim resistance in salmonellae in Britain. Resistance to trimethoprim was transferable in one of three resistant strains of *S. typhi*, in all the 1448 strains of *S. typhimurium* and in 48 of 67 strains of other salmonella serotypes that were examined. Similar reports have been made from India (Rangnekar, Banker & Jhala, 1983; Walia *et al.* 1981), New Zealand (Anderson, 1980) and Italy (Falbo *et al.* 1982). The present studies show that high-level resistance to trimethoprim (MIC $\geqslant 512$ μg/ml) in the clinical isolates of enteric pathogens is common and is invariably plasmid-mediated except in the two strains of *S. typhimurium* where it could not be transferred directly or by mobilization at 37 and 25 °C. Both were of the R-type ACGKSSuTTm and the MIC was more than 512 μg/ml. The lack of transfer of resistance in the two isolates could be due to location of the determinant gene on a chromosome (Towner *et al.* 1978) or on a transposon. Datta & Richards (1981) reported transposons (Tn7, Tn79, Tn80) that conferred resistance to trimethoprim alone or linked to streptomycin in some enteric bacteria. However, the possibility of the presence of a plasmid in our two strains mobilizable by transfer factors other than *X* and *Δ* cannot be ruled out. Towner *et al.* (1980) reported an increase in the resistant isolates of enterobacteria that failed to transfer their high-level trimethoprim resistance (23 % in 1979 compared to 4·7 % in 1978).

Resistance of shigella to trimethoprim is reported to be high in Spain (96 %) and Korea (71 %) and conjugally transferable, suggesting that resistance is mediated by R-plasmids (Lopez-Brea *et al.* 1983; Chun *et al.* 1984). In a study of shigella subgroups A, B and C isolated in England and Wales during 1974–83 Gross *et al.* (1984) found strains resistant to trimethoprim in significant numbers for the first time in 1979 (1·3 %), which increased to 9·9 % in 1982 and 16·8 % during the first half of 1983. It was transferable in 29 of 55 strains examined (53 %), directly in 28 and by mobilization in 1 strain.

The high incidence of plasmid-coded resistance to trimethoprim among our strains of salmonella, shigella and *E. coli* strains may be a result of widespread use of antibiotics including cotrimoxazole in Kuwait or, alternatively, due to the large

circulation of immigrants from many Asian countries where trimethoprim strains are endemic.

This project (MI 002) was funded by the University of Kuwait.

REFERENCES

ANDERSON, D. M. (1980). Plasmid studies of *Salmonella typhimurium* phage type 179 resistance to ampicillin, tetracycline, sulphonamides and trimethoprim. *Journal of Hygiene* **85**, 293–300.

ANDERSON, E. S. & THRELFALL, E. J. (1984). The characterization of plasmids in the Enterobacteria. *Journal of Hygiene* **72**, 471–487.

ANDERSON, E. S., THRELFALL, E. J., CARR, J. M., MCCONNELL, M. M. & SMITH, H. R. (1977). Clonal distribution of resistance plasmid-carrying *Salmonella typhimurium*, mainly in the Middle East. *Journal of Hygiene* **79**, 425–448.

CHUGH, T. D. (1982). Plasmid-linked drug resistance in *S. typhimurium* in Kuwait. *Antonie van Leeuwenhoek* **48**, 401–405.

CHUGH, T. D. & BISHBISHI, E. (1983). Trimethoprim R-plasmids in clinical isolates of salmonella in Kuwait. *Proceedings of 13th International Congress of Chemotherapy*, Vienna.

CHUGH, T. D. & KADRI, M. H. (1984). Detection of R-plasmids in *Salmonella* isolated from clams and marine waters of Kuwait. *Water, Air and Soil Pollution*. (In the Press.)

CHUGH, T. D., SUHEIR, A., MAHBOOB, A. G., NEIL, L. & BISHBISHI, E. (1984). Plasmid mediated drug resistance of Shigellae in Kuwait. *Antonie van Leeuwenhoek*. (In the Press.)

CHUN, D., CHO, D. K., SEOL, S. Y., SUH, M. H. & LEE, Y. C. (1984). R-Plasmids conferring multiple drug resistance from *Shigella* isolated in Korea. *Journal of Hygiene* **92**, 153–160.

DATTA, N. & RICHARDS, H. (1981). *Salmonella typhi* in vivo acquires resistance to both chloramphenicol and cotrimoxazole. *Lancet* i, 1181–1183.

FALBO, V., CAPRIOLI, A., NONDELLA, F., CACACE, M. L., LUZI, S. & GRECO, D. (1982). Antimicrobial resistance among salmonella isolates from hospitals in Rome. *Journal of Hygiene* **88**, 275–284.

GROSS, R. J., ROWE, B., CHEASTY, T. & THOMAS, L. V. (1984). Increase in drug resistance among *Sh. dysenteriae*, *Sh. flexneri* and *Sh. boydii*. *British Medical Journal* **283**, 575–576.

LOPEZ-BREA, M., CALLADO, L., VINCENTE, F. & PEREZ-DIAZ, J. C. (1983). Increasing antimicrobial resistance of *Sh. sonnei*. *Journal of Antimicrobial Chemotherapy* **11**, 598–9.

RANGNEKAR, V. M., BANKER, D. D. & JHALA, H. I. (1983). Antimicrobial resistance and incompatibility groups of R-plasmids in *S. typhimurium*. *Antimicrobial Agents and Chemotherapy* **23**, 54–58.

ROWE, B. & THRELFALL, E. J. (1981). Multiple antimicrobial drug resistance in enteric pathogens. *Journal of Antimicrobial Chemotherapy* **7**, 1–3.

ROWE, B., FROST, J. A., THRELFALL, E. J. & WARD, L. R. (1980). Spread of a multiresistant clone of *Salmonella typhimurium* phage type 66/122 in South East Asia and the Middle East. *Lancet* i, 1070–1071.

THRELFALL, E. J., FROST, J. A., KING, H. C. & ROWE, B. (1983). Plasmid-encoded trimethoprim resistance in salmonellas isolated in Britain between 1970 and 1981. *Journal of Hygiene* **90**, 55–60.

TOWNER, K. J., PEARSON, N. J., CATTELL, W. R. & O'GRADY, F. (1978). Chromosomal resistance to trimethoprim. *Lancet* i, 1371–1372.

TOWNER, K. J., PEARSON, H. J., PINN, P. A. & O'GRADY, F. (1980). Increasing importance of plasmid-mediated trimethoprim resistance in enterobacteria: two six-month surveys. *British Medical Journal* i, 517–519.

WALIA, S. K., GUPTA, P. N., ARORA, D. R. & CHUGH, T. D. (1981). Trimethoprim-sulphamethoxazole resistance conferred by R-plasmids in clinical isolates of *Salmonella typhimurium*. *Indian Journal of Medical Research* **74**, 631–634.