Epidem. Inf. (1988), **100**, 73–81
Printed in Great Britain

Occurrence of multiple antibiotic resistance and R plasmids in Enterobacteriaceae isolated from children in the Sudan

By P. SHEARS†, G. SULIMAN* and C. A. HART†

†*University Department of Medical Microbiology, Royal Liverpool Hospital, Liverpool and* **Children's Emergency Hospital, Khartoum, Sudan*

(*Accepted 21 September 1987*)

SUMMARY

The prevalence of resistance to six commonly-used antimicrobial agents in faecal coliforms from children in Khartoum, Sudan was studied. A relatively high prevalence of resistance was found, ranging from 96% of children with isolates resistant to ampicillin to 70% of children with isolates resistant to chloramphenicol. Seventy-seven percent of children had isolates with high-level resistance to trimethoprim (MIC > 1000 μg/ml). Twenty-nine different resistance patterns were found. Thirty-nine percent of the children had isolates resistant to all six antibiotics studied, and 80% of children had isolates resistant to at least four. Transfer of resistance to each of the antimicrobials, in varying combinations, was demonstrated, but did not occur for all resistance patterns. Plasmid analysis showed plasmids ranging from 160 MDa to 2·8 MDa and isolates contained from one to five plasmids of different sizes. There were no consistent relationships between resistance pattern and plasmid profile, but multiple resistance transfer was mediated commonly by plasmids with a molecular weight of 62 MDa.

The high prevalence of potentially transferable antibiotic resistance in gut commensals of children in the Sudan may be of importance in the management of enteric and other infections requiring antimicrobial treatment.

INTRODUCTION

Resistance to commonly prescribed antimicrobial agents is an increasing problem in many developing countries (Burke & Levy, 1985) and has been associated with outbreaks of infection due to multiply resistant enteric pathogens with significant morbidity and mortality (Mata *et al.* 1970; Frost *et al.* 1978; Pal, 1984; Farrar, 1985). Extensive and inappropriate use of antimicrobials in the treatment of childhood gastroenteritis is common in many developing countries (Tomson & Sterky, 1986; Chetley, 1987), and numerous studies have suggested this may result in the development of resistance in commensal gut bacteria (Levy *et al.* 1985; Murray *et al.* 1985), and in enteric pathogens (Shahid *et al.* 1985). There are only limited data available for much of African on either the prevalence or genetic basis of antibiotic resistance, and the need for effective surveillance studies has been emphasized (O'Brien, 1986). Previous studies in the Sudan have noted antibiotic resistance in both commensal gut bacteria (Shears *et al.* 1987), and in

enteric pathogens (Hassan, 1985). No information is currently available on the genetic basis of antibiotic resistance in the Sudan. We have undertaken a study to determine the prevalence of antibiotic resistance in children in Khartoum, and to investigate the genetic basis of resistance and resistance transfer.

Patients and methods

The Children's Emergency Hospital in Khartoum is the principal paediatric outpatient and inpatient hospital for Khartoum and Omdurman, the adjacent principal cities of Sudan. Over a 2-week period, stool samples were collected from 87 children, 60 of whom were outpatients and 27 inpatients. For each child, the following data were collected: name, age, sex, residence, current illness, duration of current illness, and antimicrobial therapy in the previous 7 days. The 60 outpatient children had all presented with diarrhoea, and, generally, were the first 10 children presenting with diarrhoea on each of 6 days. These children were not known to have been in hospital for at least the previous 2 weeks. The 27 inpatients included children with acute medical problems, principally lower respiratory tract infections, and children with chronic illnesses including diabetes, rheumatic fever and anaemia. The inpatients had been in hospital for periods from 2–21 days.

Isolation of bacteria

Each swab was plated onto MacConkey media, and onto direct sensitivity agar plates each containing one of the following six antibiotics: ampicillin (10 μg/ml), tetracycline (10 μg/ml), sulphonamide (50 μg/ml), trimethoprim (5 μg/ml), streptomycin (25 μg/ml) and chloramphenicol (10 μg/ml). Plates were cultured aerobically overnight at 37 °C. From the antibiotic-containing plates, oxidase negative, Gram negative bacilli were replated onto MacConkey agar. Isolates were identified biochemically using the API 10 system (API Products, Basingstoke, UK).

Antibiotic sensitivity testing

Isolates from the antibiotic-containing plates were tested for sensitivity to each of the antibiotics using a disk diffusion method (Stokes & Waterworth, 1972). For 58 children, the isolates for a given child from each of the different antibiotic-containing plates were found to have the same sensitivity pattern, and one representative isolate was used in each case for further studies. For 25 children, two different isolates were obtained, resulting in a total of 108 isolates. Minimum inhibitory concentrations (MIC's) of each of the antibiotics were determined for each isolate using the agar incorporation method and a multi-point inoculator. Eight concentrations of each antibiotic were used, for trimethoprim 0·25–32 μg/ml and 1000 μg/ml, and for the rest, 1–128 μg/ml.

Conjugation studies

Conjugation experiments according to the method of Ackerman & Obbink (1979) were performed on 40 of the isolates. The nalidixic acid-resistant *Escherichia coli* K.12 (strain J62-1) was used as the recipient. Both donors and recipients were grown to logarithmic phase, and conjugation allowed to take place for 16 h at both 30 and 37 °C. For selective media direct sensitivity test agar containing

Table 1. *No.* (%) *of children with isolates resistant to the individual antibiotics*

	Ap*	Tc	Su	Tm	Sm	C
Outpatients ($n = 56$)	54 (96)	50 (89)	53 (95)	41 (73)	49 (87)	39 (70)
Inpatients ($n = 27$)	26 (96)	26 (96)	22 (81)	23 (85)	21 (78)	21 (78)
Total ($n = 83$)	80 (96)	76 (92)	75 (90)	64 (77)	70 (84)	60 (72)

* Ap, ampicillin MIC > 32 μg/ml; Tc, tetracycline MIC > 12 μg/ml; Su, sulphonamide MIC > 128 μg/ml; Tm, trimethoprim MIC > 16 μg/ml; Sm, streptomycin MIC > 16 μg/ml; C, chloramphenicol MIC > 25 μg/ml.

30 μg/ml nalidixic acid, plus an antibiotic to which the donor was resistant, was used. Resistance pattern of transconjugants was determined by the disk diffusion method. No attempt was made in this study to mobilize resistances that were non-conjugative.

Plasmid studies

Plasmid DNA was extracted by the method of Kado & Liu (1981) and electrophoresed in a 0·7% agarose gel at 80 volts for 4 h, using a horizontal electrophoresis system (H5-BRL, Bethesda Research Laboratories). Gels were stained with ethidium bromide (5 μg/ml) for 30 min and photographed with Polaroid 665 film. Plasmid profiles were determined for 82 isolates, including 19 donors and their respective transconjugants. The following plasmid standards were used as controls: R1/19 (62 MDa), R6k (25 MDa), PIV51 (5·2 MDa).

RESULTS

In the outpatient children, 22 were less than 1 year of age, 33 aged 1–5 years and 5 were over 5 years. In the hospital group, 5 were less than 1 year of age, 11 aged 1–5 years and 11 were over five. Information for the outpatient children on recent antibiotic usage was obtained from the mothers, and was probably only reliable where mothers could provide an old prescription or antibiotic container. On this basis, 6 of the children had had ampicillin, and 4 had had cotrimoxazole prior to coming to the outpatients. Of the inpatients, 10 were currently being treated with ampicillin, 8 with penicillin, 1 with cotrimoxazole and 1 with chloramphenicol. Because of the uncertainty of antibiotic prior usage in the outpatient children, we have not attempted to analyse the results in relation to antibiotic usage in this study.

Coliform bacteria were grown from specimens from 83 of the 87 children. Of the 108 isolates obtained, 101 were *E. coli*, the remaining 7 being *Klebsiella*, *Citrobacter* and *Enterobacter* spp.

Table 1 shows the number and percentage of children with isolates individually resistant to the different antimicrobials. There was no significant difference in antibiotic resistance (χ^2, 0·50 > 0·10) between the outpatient and hospital group for each of the six antimicrobials tested, and in the remainder of the study, the two groups are considered together. All of the isolates resistant to trimethoprim were shown to have high-level trimethoprim resistance (MIC > 1000 μg/ml). Twenty-nine different resistance patterns were found. Thirty-two (39%) of the children

Table 2. *Principal antibiotic resistance patterns**

Resistance pattern	No. (%) of children
Ap Tc Su Tm Sm C	32 (39)
Ap Tc Su Tm Sm	13 (16)
Ap Tc Su Sm C	7 (8)
Ap Tc Su Sm	4 (5)
Ap Su Tm Sm	3 (4)
Ap Tc C	4 (5)

* Ap, ampicillin; Tc, tetracycline; Su, sulphonamide; Tm, trimethoprim; Sm, streptomycin; C, chloramphenicol.

Table 3. *Plasmid profiles and resistance patterns of donors and transconjugants*

Donor		Transconjugant	
Resistance pattern	Plasmid profile	Plasmid(s) transferred	Resistance transferred
Ap Tc Su Tm Sm C	(4·6, 62)	(62)	Ap Tc Su Sm C
Ap Tc Su Tm Sm C	(4·1, 4·6, 5·8, 37, 62)	(62)	Ap Su Tm Sm
Ap Tc Su Tm Sm C	(4·0, 4·3, 32, 62, 110)	(32)	Tc Su Tm
Ap Tc Su Tm Sm C	(33, 62)	(62)	Tc C
Ap Tc Su Tm Sm C	(90)	(90)	Tm Sm
Ap Tc Su Tm Sm	(62)	(62)	Ap Tc Su Tm Sm
Ap Tc Su Tm Sm	(62)	(62)	Ap Tc Su Tm Sm
Ap Tc Su Tm Sm	(62)	(62)	Ap Su Tm Sm
Ap Tc Su Tm Sm	(62)	(62)	Ap Su Tm Sm
Ap Tc Su Tm Sm	(40, 62, 90)	(40, 62)	Ap Su Tm Sm
Ap Tc Su Tm Sm	(42, 62, 110)	(62)	Ap Tc Sm
Ap Tc Su Tm Sm	(4, 62, 110)	(62)	Tm
Ap Su Tm Sm	(4·7, 62)	(62)	Ap Su Tm Sm
Ap Su Tm Sm	(37, 62)	(37, 62)	Ap Su Tm Sm
Ap Su Tm Sm C	(62)	(62)	Ap C
Ap Tc Su Tm C	(62)	(62)	Tm
Ap Tc Su Tm	(62)	(62)	Ap Tc Su Tm
Ap Su Tm	(62)	(62)	Ap Tm
Ap Su Tm	(25)	(25)	Ap Su Tm

had isolates resistant to all 6 antimicrobials, and 66 children (80%) had isolates resistant to at least 4 antimicrobials. The most commonly occurring resistance patterns are shown in Table 2. The resistance patterns for the isolates other than *E. coli* were similar to those of *E. coli* isolates.

Of 40 isolates tested, 19 were able to transfer all or part of their resistance pattern. All transferred at 37 °C and 8 also at 30 °C, the same resistance being transferred at the lower temperature. The patterns of transfer are shown in Table 3, and are discussed below in relation to plasmid transfer. Agarose gel electrophoresis of 63 isolates, including isolates showing each of the 29 resistance patterns, showed 45 different plasmid profiles. Plasmid sizes ranged from 160 MDa to 2·8 MDa. Forty isolates contained more than one plasmid, but no isolate contained more than five plasmids. There were no consistent relationships between plasmid profile and antibiotic resistance pattern. However, in most cases,

Table 4. *Plasmid profiles (size in MDa) associated with different resistance patterns*

Resistance pattern	No. of isolates tested	Plasmid profile (MDa) and frequency [ ] if > 1.
Ap Su Tm Sm C	11	(100), (3·0, 5·5, 100), (90), (3·2, 62, 88), (110, 62, 32, 4·3, 4·0), (62) [2], (33, 62), (4·6, 62), (3·2, 62) (62, 37), (62, 37, 5·8, 4·6, 4·1)
Ap Tc Su Tm Sm	9	(4, 62, 110), (40, 62, 90), (62, 90), (62) [4], (3·3, 33), (42, 62, 110)
Ap Tc Su Sm C	4	(3·0, 48, 120), (3·0, 3·6, 120), (62), (4·0)
Ap Tc Su Sm	4	(3·8, 88), (2·6, 62), (3·2, 62), (3·6, 4·1, 62, 48)
Ap Su Tm Sm	3	(62), (37, 62), (4·6, 62)
Ap Tc C	3	(88), (3·2, 62), (2·8, 3·2, 62)
Ap Su Tm Sm C	1	(62)
Ap Tc Su C	3	(3·3, 3·7, 4·1, 62), (2·4, 3·1, 62), (46)
Ap Tc Su Tm C	1	(62)
Ap Tc Tm Sm C	1	(4·5, 40, 62)
Ap Tc Su Tm	2	(62, (3·1, 46, 53)
Ap Tm Sm C	1	(4·5, 48)
Ap Su Tm	2	(62, (25)
Tc Su Tm	2	(3·0, 4·1), [2]
Tc Su Sm	2	(2·5, 3·4, 120), (3·1, 3·4, 46)
Ap Tc	1	(3·5, 62, 88)
Ap Tc Tm Sm	1	(3·5, 62)
Ap Tc Tm C	1	(2·4, 120)
Ap Su Tm C	1	(88)
Ap Su Sm C	1	(4·6, 160)
Tc Su Tm Sm	1	(3·8, 4·1, 62)
Tc Su Sm C	1	(4·7, 54, 74)
Ap Su Sm	1	(3·8, 46, 62)
Ap Su	1	(3·7, 25, 62, 160)
Ap C	1	(120)
Su Sm	1	(3·3, 3·8)
Tm Sm	1	(3·8, 4·1, 62)
Ap	1	(88)
Tc	1	(2·7)

isolates with the same resistance pattern had at least one plasmid of a similar size in common. Plasmid profiles and associated resistance patterns are given in Table 4. A 62 MDa plasmid was present, either alone or in combination with plasmids of other sizes, in 18 (62%) of the different resistance patterns. In three resistance patterns (TcSuTm, SuSm, Tc), only small (< 5 MDa) plasmids were present.

Plasmid analysis of donors and transconjugants are shown in Table 3 and Figure 1. All 16 donors with a 62 MDa plasmid co-transferred this plasmid. No attempt was made in this study to determine homogeneity of the 62 MDa plasmids by compatibility grouping or restriction endonuclease analysis. Eight of these donors contained at least one other plasmid in association with a 62 MDa plasmid, but in only two cases was an additional plasmid co-transferred. Resistance transfer in association with a 62 MDa plasmid varied from all resistance determinants (Ap Tc Su Tm Sm C) to trimethoprim (Tm) alone. In five cases

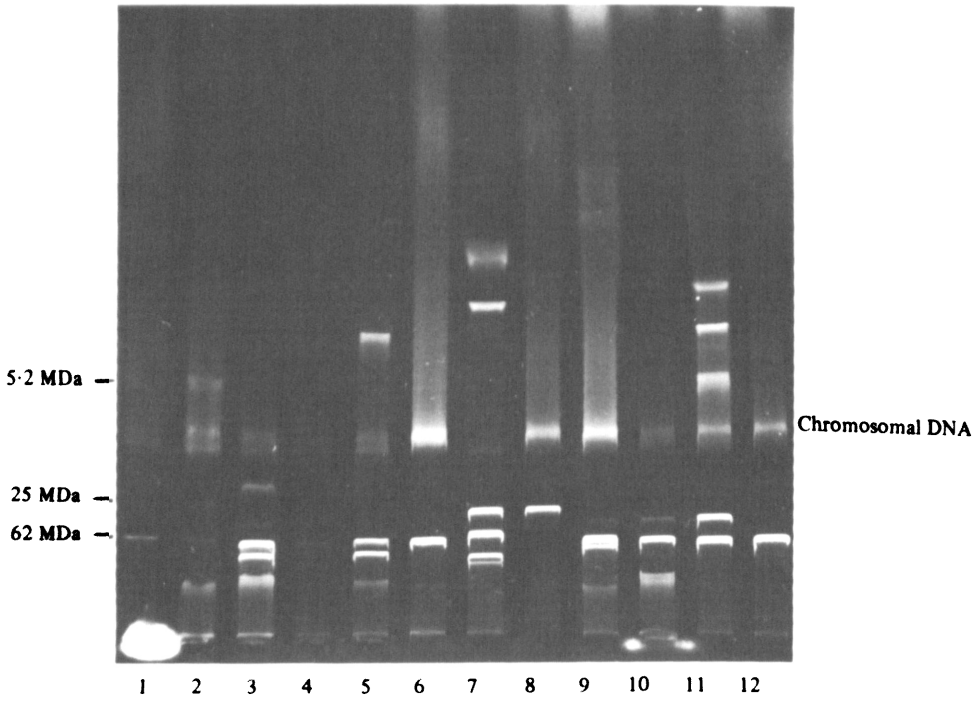

Fig. 1. Agarose gel electrophoresis of plasmid DNA extracted from donors and transconjugants. Lanes: 3(Ap Tc Su Tm Sm); 4, transconjugant of 3(Ap Tm Sm); 5(Ap Tc Su Tm Sm); 6, transconjugant of 5(Tm); 7(Ap Tc Su Tm Sm C); 8, transconjugant of 7(Tc Su Tm); 9(Ap Su Tm Sm); 10, transconjugant of 9(Ap Su Tm Sm), 11(Ap Tc Su Tm Sm C); 12, transconjugant of 11(Ap Su Tm Sm). Plasmids of known molecular weight are contained in lanes 1; R1/19 (62 MDa): 2; R1/19, R6k, PLV, 5·1 (62 MDa, 25 MDa, 5·2 MDa).

where a 62 MDa plasmid was the only plasmid present in the donor, only part of the resistance phenotype was transferred with the plasmid. In the three conjugations not involving a 62 MDa plasmid, the plasmids transferred were 90, 32 and 25 MDa. Trimethoprim (Tm) transfer occurred most frequently, occurring in 17 (90%) of the 19 conjugations where Tm resistance was present in the donor. Tm transfer was associated with plasmids ranging in size from 25 to 90 MDa, but was most commonly associated with a 62 MDa plasmid (15 of the 17 transfers). Tetracycline (6 of 15 possible conjugations) and chloramphenicol (3 of 8 possible conjugations) were the least frequently transferred. Streptomycin resistance was consistently co-transferred with trimethoprim (in all 11 cases of streptomycin transfer), and was associated with transfer of a 62 MDa plasmid in 10 of the 11 transfers.

DISCUSSION

A remarkably high prevalence of resistance to commonly used antimicrobial agents was present in this group of children, ranging from 96% with resistance to ampicillin to 72% with resistance to chloramphenicol. In addition to resistance to individual antimicrobials, most children harboured multiply resistant isolates.

Thirty-two children (39%) had isolates resistant to all 6 antimicrobials, and 66 children (80%) had isolates resistant to at least 4 of the antimicrobials. These findings are in agreement with those of other workers in developing countries (Levy *et al.* 1985; Koh, 1986), and with our earlier preliminary studies in the Sudan (Shears *et al.* 1987; Shears, Suliman & Hart, 1987). The prevalence of trimethoprim resistance was considerably greater than that seen in a comparable age-group of children in UK (McDowell *et al.* 1987) where trimethoprim-resistant isolates were found in only 14% of children. We found no significant difference between the prevalence of resistance to antimicrobials in the 'community' group (outpatients) and the 'hospital' group (inpatients). Because of the small numbers involved, we do not feel it appropriate to interpret the results from these two groups further, but do feel that the prevalence of resistance found is a true indication of the prevalence of resistance in the community. The limited data on antibiotic usage in the community in this study do not allow conclusions to be drawn between antibiotic use and resistance prevalence in this population, and we are planning a longer-term study to obtain data on antibiotic use.

Our conjugation studies demonstrated that some of the resistance determinants are potentially transferable, though successful conjugation occurred in less than 50% of cases. If transfer to other members of the enterobacteriaceae is possible, these commensal bacteria may act as a reservoir of multiple resistance. Plasmid analysis of the isolates showed a wide range of plasmid sizes present, but there was no consistent relationship between plasmid profile and antimicrobial resistance pattern. Other workers have found similar variation in plasmid content in relation to resistance pattern in Enterobacteriaceae isolates from developing countries. Young *et al.* (1986) found 58 different plasmid types associated with trimethoprim resistance in isolates from south India. Murray *et al.* (1985) found plasmid sizes ranging from 42 to 80 MDa associated with trimethoprim resistance in Chilean isolates. In our study, plasmids of molecular weight 62 MDa occurred most frequently, and were associated with different resistance patterns. Investigation by restriction endonuclease analysis is planned to determine whether the 62 MDa plasmids are homogeneous. Conjugation studies showed that 62 MDa plasmids were important in the transfer of resistance to each of the antimicrobials under consideration. While additional work is required to characterize further the resistance determinants that may be located on plasmids of this molecular weight, the co-transfer of streptomycin and trimethoprim in all cases of streptomycin transfer suggests the presence of transposon Tn7 (Young *et al.* 1986).

Several factors may be important in promoting antibiotic resistance in gut bacteria in developing countries. Considerable attention has been paid to the indiscriminate use of antibiotics (Murray *et al.* 1985; Koh, 1986; Burke & Levy, 1985). This is particularly the case in the management of diarrhoeal diseases despite W.H.O. recommendations on rehydration therapy alone (Moshaddeque, Glass & Khan, 1982). A recent survey (Chetley, 1987) has shown that 65% of antidiarrhoeal medicines available in developing countries contain antimicrobials. An increasing prevalence of resistance after introduction of specific antibiotics has been shown by Shahid *et al.* (1985) in Bangladesh. In addition to poor antibiotic usage, circulation of resistant bacteria in environments with limited sanitation facilities may be of importance (Al-Jebouri & Al-Meshhadani, 1985). Further

studies are required in the Sudan to determine the impact of antibiotic usage and environmental spread in the development and dissemination of resistant isolates, and to investigate in more detail the genetic determinants of transferable resistance.

The clinical and laboratory staff of the Children's Emergency Hospital, Khartoum participated with interest and enthusiasm in this study. The study has been supported by the Wellcome Trust, the University of Liverpool Research Development Fund, and a Medical Research Council Equipment Grant.

REFERENCES

ACKERMAN, V. P. & OBBINK, D. J. (1979). Screening for transferable antibiotic resistance in the clinical laboratory. *Journal of Antimicrobial Chemotherapy* **5**, 167–172.

AL-JABOURI, M. M. & AL-MESHHADANI, N. S. (1985). A note on antibiotic-resistant *Escherichia coli* in adult man, raw sewage and sewage-polluted River Tigris in Mosul (Iraq). *Journal of Applied Bacteriology* **59**, 513–518.

BURKE, J. P. & LEVY, S. B. (1985). Summary report on worldwide antibiotic resistance: international task forces on antibiotic use. *Reviews of Infectious Diseases* **7**, 560–564.

CHETLEY, A. (1987). Antibiotics – the wrong drugs for diarrhoea, pp. 22. Netherlands: Health Action International.

FARRAR, W. E. (1985). Antibiotic resistance in developing countries. *Journal of Infectious Diseases* **152**, 1103–1106.

FROST, J. A., VANDEPITTE, J., ROWE, B. & THRELFALL, E. J. (1978). Plasmid characterisation in the investigation of an epidemic caused by multiply resistant *Shigella dysenteriae* type 1 in central Africa. *Lancet* ii, 1074–1076.

HASSAN, S. H. (1985). Sensitivity of salmonella and shigella to antibiotics and chemotherapeutic agents in the Sudan. *Journal of Tropical Medicine and Hygiene* **88**, 243–248.

KADO, C. I. & LIU, S. T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *Journal of Bacteriology* **145**, 1365–1373.

KOH, C. L. (1986). Antibiotic resistance and conjugative R plasmids in clinical isolates of Enterobacteriaceae in Peninsular Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 158–161.

LEVY, S. B., HEDGES, R. W., SULLIVAN, F., MEDEIROS, A. A. & SOSROSEPUTRO, H. (1985). Multiple antibiotic resistance plasmids in Enterobacteriaceae isolated from diarrhoeal specimens of hospitalised children in Indonesia. *Journal of Antimicrobial Chemotherapy* **16**, 7–16.

MATA, L. J., GANGAROSA, E. J., CACERES, A., PERERA, D. R. & MEJICANOS, M. L. (1970). Epidemic shiga bacillus dysentery in Central America. *Journal of Infectious Diseases* **122**, 170–180.

MCDOWELL, H. P., SHEARS, P., HART, C. A. & MARTIN, J. (1987). Acute lymphoblastic leukaemia: trimethoprim-resistant organism during treatment. *Archives of Disease in Childhood* **62**, 573–575.

MOSHADDEQUE, H. M., GLASS, R. I. & KHAN, M. R. (1982). Antibiotic use in a rural community in Bangladesh. *International Journal of Epidemiology* **11**, 402–405.

MURRAY, B. E., ALVARODO, T., KIM, H. H. & VORACHIT, M. (1985). Increasing resistance to trimethoprim – sulfamethoxazole among isolates of *Escherichia coli* in developing countries. *Journal of Infectious Diseases* **152**, 1107–1113.

O'BRIEN, J. F. (1986). Resistance to antibiotics at medical centres in different parts of the world. *Journal of Antimicrobial Chemotherapy* **18**, Suppl. C, 243–253.

PAL, S. C. (1984). Epidemic baciliary dysentery in West Bengal, India. *Lancet* i, 1962.

SHAHID, N. S., RAHMAN, M. M., HAIDER, K., BANU, H. & RAHMAN, N. (1985). Changing pattern of resistant Shiga bacillus in Bangladesh. *Journal of Infectious Diseases* **152**, 1114–1119.

SHEARS, P., HART, C. A., BROADHEAD, R. L. & COULTER, J. B. S. (1987). A note on antibiotic resistance in *E. coli* isolated from children with diarrhoea in the Sudan. *Annals of Tropical Paediatrics* **7**, 38–41.

Sheears, P., Suliman, G. & Hart, C. A. (1987). A preliminary investigation of antibiotic resistance in Enterobacteriaceae isolated from children with diarrhoea from four developing countries. *Annals of Tropical Medicine and Parasitology*. In the press.

Stokes, E. J. & Waterworth, P. M. (1972). Association of Clinical Pathologists Broadsheet No. 55.

Tomson, G. & Sterky, G. (1986). Self-prescribing by way of pharmacies in three Asian developing countries. *Lancet* ii, 620–622.

WHO Expert Committee on Biological Standardisation (1977). Technical report·series 610, pp. 98–128. Geneva: World Health Organisation.

Young, H. K., Jesudason, M. V., Koshi, G. & Amyes, S. G. B. (1986). Trimethoprim resistance among urinary pathogens in South India. *Journal of Antimicrobial Chemotherapy* **17**, 615–621.