TRIMETHOPRIM R FACTORS IN ENTEROBACTERIA FROM CLINICAL SPECIMENS

R. S. JOBANPUTRA* AND NAOMI DATTA

Department of Bacteriology, Royal Postgraduate Medical School, London W12 0HS

R FACTORS conferring a high level of resistance to trimethoprim were first detected in London in 1971 (Fleming, Datta and Grüneberg, 1972). During the winter of 1971–72 trimethoprim-resistant enterobacteria were quite frequently isolated from clinical material in the UK (Grüneberg, 1971; Lacey *et al.*, 1972; Datta and Hedges, 1972*b*) but trimethoprim R factors were detected only in material from hospitals in a limited area of London. They were all *fi*⁻, belonged to the same compatibility group, W, and conferred the same pattern of resistance, to trimethoprim and sulphonamides, but occurred in various bacterial hosts; thus it appeared that we were observing the spread of a single transmissible plasmid (Datta and Hedges, 1972*b*).

In 1972, *Escherichia coli* strains carrying trimethoprim R factors were isolated from calves that had been fed high doses of a trimethoprim-sulphonamide preparation for experimental purposes (Fleming, 1973). The R factors were different from those previously identified. They did not belong to compatibility group W, and the resistance pattern they conferred was to trimethoprim and streptomycin, but not to sulphonamides (Hedges, Datta and Fleming, 1972).

In this paper we report the results of a survey of trimethoprim-resistant enterobacteria performed during the winter of 1972–73. The purpose was to show whether the W trimethoprim-resistance plasmid had spread further, whether the trimethoprim R factor found in calves had appeared in clinical isolates from man, and whether trimethoprim-resistance genes had been acquired by plasmids of further compatibility groups.

MATERIALS AND METHODS

Trimethoprim-resistant bacteria

These had been isolated in diagnostic laboratories in five London hospitals, and in Glasgow, Belfast, Cardiff and Birmingham. They were identified, their trimethoprim resistance was measured and their capacity to synthesise thymine determined. They were tested for transfer of trimethoprim resistance to *E. coli* K12. The bacteria tested are listed in table I. At Hammersmith Hospital, isolates of *Klebsiella* from infected urine from in-patients were monitored over a 3-month period and the proportion of trimethoprim-resistant organisms calculated.

Identification of the bacteria was by methods employed in the diagnostic laboratory at Hammersmith Hospital (Darrell, 1967) and by reference to the tables of Cowan and Steel (1970).

Received 8 June 1973; accepted 22 June 1973.

* Present address: Department of Microbiology, Northwick Park Hospital, Harrow, HA1 3UJ.

Minimal inhibitory concentrations (MIC). For the purpose of this survey, strains were grouped according to their inhibition by 10-fold differences in trimethoprim concentration. Cultures were diluted to give well-separated colonies on drug-free medium and were seeded on to plates of Oxoid DST Agar (CM267) containing 4% lysed horse blood and 1, 10, 100 or 1000 μg trimethoprim per ml medium. The MIC was taken as the concentration that prevented visible growth. Sensitive *E. coli* K12 was inhibited by 1 μg per ml.

Thymine dependence. Trimethoprim has been used in the laboratory to select mutant bacteria unable to synthesise thymine (Stacey and Simson, 1965; Andrew, 1973). Some strains of naturally occurring bacteria, from patients treated with trimethoprim, have also been found to be thymine-requiring. Such bacteria are unable to grow on media containing lysed horse blood (Barker, Healing and Hutchison, 1972; Okubadejo and Maskell, 1973).

Any trimethoprim-resistant strains in this study that failed to grow on Oxoid DST Agar with 4% lysed horse blood were tested for thymine requirement on minimal salts agar (Clowes and Hayes, 1968) supplemented with glucose, with or without thymine.

Trimethoprim-resistance transfer

Strains of Escherichia coli K12 used in these experiments were J62-2 (rifampicin resistant, non-lactose-fermenting; Datta and Hedges, 1972b), and J53 and HfrC, both lactose fermenting (Clowes and Hayes, 1968).

Bacteriophages used as indicators for sex pili were MS2 (Davis, Strauss and Sinsheimer, 1961) for F pili, If1 (Meynell and Lawn, 1968) for I pili, and PRR1 (Olsen and Shipley, 1973; R. H. Olsen, personal communication) for pili determined by P plasmids.

Strains under test were mixed with *E. coli* K12, sub-strain no. J62-2 in broth culture in a proportion of one part donor to 10 of the K12 recipient. Cultures were incubated overnight at 37°C and plated on minimal salts agar (Clowes and Hayes, 1968) with glucose as carbon source, the amino acids (proline, histidine and tryptophan) required for growth of the recipient strain, trimethoprim 5 μg per ml, and rifampicin, 50 μg per ml. Neither donor nor recipient would grow on this medium. The colonies that developed were *E. coli* K12 to which plasmids (R factors) conferring resistance to trimethoprim had been transferred in the mixed broth culture. This was confirmed after purification on MacConkey Agar (Oxoid CM7b) (i) by identification of *E. coli* K12 J62-2 by its biochemical properties, including amino-acid requirements, and (ii) by showing that trimethoprim resistance was transmissible from the first K12 recipient to another sub-strain of *E. coli* K12, no. J53.

Classification of R factors

1. *By fi character.* Transmissible plasmids, including R factors, are divisible into two classes, fi^+ (fertility inhibition⁺) and fi^- according to their effect on the F (fertility) factor (Watanabe *et al.*, 1964). To demonstrate this character, we transferred trimethoprim-resistance factors to strain HfrC and tested for lysis by F-specific phage MS2, spotting undiluted phage preparation on to nutrient-agar plates inoculated with strain HfrC R^+ . Lysis indicated that pilus production by the F factor of strain HfrC was uninhibited, i.e., that the R factor was fi^- . Lack of lysis indicated that the R factor was fi^+ .

2. *By production of sex pili.* Some fi^- R factors determine I pili, which act as receptors for phage If1. I pili were detected by demonstrating an increase in titre of phage If1 when a known quantity of the phage was incubated with a bacterial culture (Lawn *et al.*, 1967; Datta *et al.*, 1971). Phage PRR1 specifically lyses bacteria carrying P plasmids (Olsen and Shipley, 1973). *E. coli* K12 carrying trimethoprim R factors were tested by spotting an undiluted preparation of phage PRR1 (titre 10^{11} plaque-forming-units per ml) on bacterial lawns on nutrient-agar plates.

3. *By compatibility.* Plasmids that are alike are incompatible, i.e., cannot co-exist stably in a single bacterial host (Watanabe *et al.*, 1964; Meynell, Meynell and Datta, 1968). On this basis, R factors can be classified by ability to co-exist in *E. coli* K12 (Datta and Hedges, 1971; Chabbert *et al.*, 1972; Coetzee, Datta and Hedges, 1972; Grindley, Grindley and Anderson, 1973). Trimethoprim-resistance factors identified in this study were tested for

compatibility with a set of standard *fi*⁻ R factors of various compatibility groups (listed in table I) in the following manner. Trimethoprim R factors were transferred from strain J62-2 to strain J53 cultures that carried resident R factors. The mixed broth cultures were incubated for 1 hour and plated on minimal-salts-agar supplemented with methionine and proline, 20 µg per ml of each (required for growth of J53), and trimethoprim, 10 µg per ml. The donor cells would not grow on this medium because of the lack of histidine and tryptophan. The colonies that appeared were clones of strain J53 that had received the trimethoprim R factor. From each such cross, 10 colonies were purified by streaking on Mac-

TABLE I
Standard fi⁻ R factors of known compatibility groups

Factor	Compatibility group	Resistance* determinants	Reference
R144	I _z	TK	Meynell, Meynell and Datta (1968)
R483	I _β	STp	Hedges, Datta and Fleming (1972); Datta and Hedges, 1973
R621a	I _γ	T	Hedges and Datta (1973)
JR66a	I _ω	SK	Datta and Hedges (1973)
N3	N	STSu	Datta and Hedges (1971)
RP4	P	ATK	Datta <i>et al.</i> (1971)
S-a	W	SCKSu	Hedges and Datta (1971)
R57b-1	C	CSu	Datta and Hedges (1972c)
RA1	A	TSu	Hedges and Datta (1971)
R446b	M	ST	Hedges <i>et al.</i> (1973)
R391	J	K	Coetzee, Datta and Hedges (1972)
R6K	X	AS	Hedges <i>et al.</i> (1973)
R387	K	SC	Hedges and Datta (1971)

* A = Ampicillin (and carbenicillin); S = streptomycin; T = tetracycline; C = chloramphenicol; K = kanamycin; Su = sulphonamides; Tp = trimethoprim.

Conkey agar containing 4% lysed horse blood and trimethoprim, 10 µg per ml. This medium did not permit growth of trimethoprim-sensitive bacteria; on it lactose-fermenting colonies were easily distinguished from non-lactose fermenting ones, despite the lysed blood, if the plates were held up to the light. Ten purified clones of each cross were tested on double ditch plates for the presence of resistance markers of transferred and resident plasmids. One ditch contained trimethoprim with lysed blood and the opposite ditch contained an appropriate antibiotic.

Where the introduction of a trimethoprim R factor eliminated a resident R factor, the test was made also in the opposite direction, the standard R factor being transferred to strain J62-2 with a resident trimethoprim R factor. Mutual elimination was taken as evidence that the two plasmids belonged to the same compatibility group.

RESULTS

Trimethoprim-resistant strains

The number of strains tested, and their identification, is shown in table II, their origin and degree of trimethoprim-resistance in table III. In all, 11 cultures were resistant to trimethoprim at levels of >1000 µg per ml medium. Ten of these transferred resistance to *E. coli* K12 (see below); the exception was a strain of *Proteus rettgeri* from Cardiff.

TABLE II

Number and identification of naturally occurring trimethoprim-resistant bacteria

Identification	Number tested
<i>Escherichia coli</i>	21
<i>Klebsiella aerogenes</i>	38
<i>Klebsiella</i> sp.*	13
<i>Proteus mirabilis</i>	5
<i>P. morganii</i>	1
<i>P. rettgeri</i>	2
<i>Providencia</i> sp.	4
<i>Enterobacter</i> sp.	8
<i>Acinetobacter</i> sp.	3
Any of above	95

* MR+, VP+.

TABLE III

Origin of the strains and levels of trimethoprim resistance

Source	Number of strains				
	examined	with MIC (μ g per ml) in the range			
		1-10	10-100	100-1000	>1000
Hammersmith Hospital, London	33	12	10	4	7
St Thomas's Hospital, London	7	...	4	2	1
St Bartholomew's Hospital, London	6	2	3	1	...
The Hospital for Sick Children, London	3	...	1	1	1
West Middlesex Hospital, Isleworth, London	4	...	3	...	1
Dudley Road Hospital, Birmingham	10	7	1	2	...
Stobhill General Hospital, Glasgow	10	6	4	...	...
Glasgow Royal Infirmary, Glasgow	5	3	2	...	...
Belfast City Hospital, Belfast	5	2	2	1	...
The Welsh National School of Medicine, Cardiff	12	4	4	3	1
Any of above	95	36	34	14	11

Proportion of urinary isolates of Klebsiella that were resistant to trimethoprim

During a 3-month period, klebsiella strains were isolated in significant numbers from the urine of 83 hospital patients in Hammersmith Hospital. Of these, 61 strains were sensitive and 22 resistant to trimethoprim when tested with a 1.25- μ g disk (Oxoid) by the Stokes method (1968). Thus the proportion of trimethoprim resistant *Klebsiella* spp. was 26%. The levels of trimethoprim resistance were as follows: five strains had MICs of >1000 μ g per ml; two

strains, 100–1000 μg per ml; nine strains, 10–100 μg per ml; six strains, 1–10 μg per ml. All the five strains with a MIC of >1000 μg per ml carried the W plasmid (see below). None of the other strains was shown to carry a trimethoprim R factor. Thus the proportion of urinary klebsiella isolates with the R factor was about 6%.

Thymine requirement

Of the 95 trimethoprim-resistant strains examined, only one—*E. coli* no. HH32—failed to grow on lysed blood agar. This strain did not grow on minimal-salts-agar unless thymine (2.5 μg per ml) was added. When tested

TABLE IV
Characters of trimethoprim R factors

Identification	Host strain*		Resistance transferred†	R-factor no.	Compatibility group
	Source‡	Number of isolates			
<i>Klebsiella</i> sp.	H.H.	6	Su,Tp	...§	W
<i>Pr. morganii</i>	W.M.H.	1	Su,Tp	...	W
<i>E. coli</i>	G.O.S.	1	Su,Tp	...	W
<i>E. coli</i>	H.H.	1	S,Tp	R721	Iδ
<i>K. aerogenes</i>	S.T.	1	Tp	R751	P

* All were resistant to >1000 μg per ml of trimethoprim.

† H.H. = Hammersmith Hospital; G.O.S. = Hospital for Sick Children; W.M.H. = West Middlesex Hospital; S.T. = St Thomas's Hospital.

‡ Su = Sulphonamide; Tp = trimethoprim; S = streptomycin.

§ R-factor numbers were not allotted to the W plasmids, which all appeared identical with factor R388 previously described (Datta and Hedges, 1972b).

with a 1.25- μg trimethoprim disk on minimal salts medium with added thymine, no. HH32 was resistant and the control *E. coli* was sensitive.

Transfer of trimethoprim resistance

Trimethoprim R factors detected in this study, as in previous studies, all caused resistance to >1000 μg per ml of this substance. Three distinguishable trimethoprim R factors were identified (table IV).

W plasmids. These conferred resistance to trimethoprim and sulphonamides, were apparently identical with those previously described, and were present in strains isolated at Hammersmith Hospital and the West Middlesex Hospital, but not in strains from any of the other hospitals listed in table III. At Hammersmith Hospital, the W plasmid was identified in cultures isolated from six in-patients, the five urinary isolates mentioned above, and one from sputum. The patients were not all in the same ward blocks. In every case the W plasmid was carried by klebsiella strains with the same biochemical characteristics (MR+, VP+) as previously reported as the most common

host for this plasmid at University College Hospital and the Whittington Hospital (Datta and Hedges, 1972*b*). This same combination of bacterial host and W plasmid was also present in the St Pancras branch of University College Hospital (R. N. Grüneberg, personal communication). Characteristic MR+, VP+ klebsiella strains carrying W plasmids isolated at University College Hospital, the Whittington Hospital and Hammersmith Hospital all belonged to capsular type 9 (M. W. Casewell, personal communication).

Apparently identical W plasmids were detected also in *P. morganii* from a single patient at the West Middlesex Hospital, and in *E. coli* (serotype O4) isolated from urine and faeces of a patient attending the Hospital for Sick Children as an outpatient and being treated with co-trimoxazole. The R factor was not detected among in-patients at the Hospital for Sick Children.

I δ plasmid. An R factor (R721) which conferred resistance to trimethoprim and streptomycin was present in *E. coli* isolated from a urine specimen of an out-patient at Hammersmith Hospital, who was on a long-term course of co-trimoxazole. The factor R721 closely resembled the trimethoprim-streptomycin R factors previously identified in calves (Hedges *et al.*, 1972) not only in its resistance pattern but also in being *fi*⁻ and determining I pili (Meynell *et al.*, 1968). However, it was distinguishable from the calf R factor, R483, in its compatibility properties. Factor R483 (group I β) was incompatible with R factor JR66a, which determines resistance to streptomycin and kanamycin and the synthesis of I pili (Datta and Hedges, 1973), whereas factor R721 was compatible with factor JR66a. Factor R721 was compatible with *fi*⁻ plasmids of all defined groups, and therefore belongs to a new group, designated I δ to indicate that it is a member of the I-pilus-determining complex (Hedges and Datta, 1973).

P plasmid. Another R factor identified in only a single bacterial isolate was present in a strain of *K. aerogenes* (capsular type 37) isolated from a peritoneal-dialysis catheter from a patient at St Thomas's Hospital, London. This R factor, R751, determined resistance to trimethoprim only. It was *fi*⁻ and incompatible with factor RP4, an R factor from *Pseudomonas aeruginosa*, which is the prototype of compatibility group P (Datta *et al.*, 1971). *E. coli* K12(R751) was sensitive to the P-specific phage PRR1.

DISCUSSION

Among 95 strains of trimethoprim-resistant Gram-negative bacilli, isolated from various parts of the UK, only 10 transferred trimethoprim resistance to *E. coli* K12. The MIC of trimethoprim for the remainder of the strains was with one exception lower than for those whose resistance was determined by R factors. The mechanism of their resistance was not further investigated; it had presumably resulted from mutation. Only one of the 95 strains was thymine-requiring (Barker *et al.*, 1972; Okubadejo and Maskell, 1973), but this low proportion may not reflect the true frequency of thymine-requiring trimethoprim-resistant bacteria in human infections. As pointed out by Okubadejo and Maskell (1973), such strains may go unrecognised in diagnostic

laboratories because of their inability to grow on the media normally employed for testing sensitivity to sulphonamides and trimethoprim.

Among the cultures whose resistance was determined by R factors, three different trimethoprim-resistance plasmids were identified. The R factor of compatibility-group W, previously identified in London, was found in two London hospitals (Hammersmith and West Middlesex) where it had not been seen a year earlier. However, it was not found in cultures from two other London hospitals, St Bartholomew's and St Thomas's, nor in cultures from Belfast, Glasgow, Birmingham or Cardiff. Trimethoprim R factors have not been observed in Bristol (Lewis and Lacey, 1973) or in Dublin (Moorhouse and Farrell, 1973).

Trimethoprim resistance was determined by R factors of two other compatibility groups besides group W, but each of these was identified only in one single bacterial isolate. Factor R721, which belongs to group I δ , was very similar to the trimethoprim-streptomycin R factor R483, which occurred in *E. coli* in calves. Factors R721 and R483 both determine I pili. I-pilus-determining plasmids constitute a compatibility complex (Hedges and Datta, 1973) and factors R721 and R483 are probably closely related phylogenetically. They may both be variants of a single trimethoprim-resistance plasmid; alternatively, genes determining trimethoprim resistance may have been acquired by plasmids of the I β and I δ compatibility groups as separate events.

The appearance of a trimethoprim-resistance factor of compatibility-group P is of interest because this group has a wide host range among bacteria (Datta and Hedges, 1972; Olsen and Shipley, 1973). P-group R factors have been identified in bacteria of several genera, including *Ps. aeruginosa*, in infected patients in the Birmingham Accident Hospital (Roe and Lowbury, 1972). The P plasmids in that hospital conferred resistance to a number of antibiotics, including carbenicillin, but not to trimethoprim. They were eradicated from the hospital by a careful antibiotic policy (Lowbury, Babb and Roe, 1972). There has been no evidence of spread of the P plasmid, R751, at St Thomas's Hospital; no further bacterial strains with high-level trimethoprim resistance have appeared there (Dr I. Phillips, personal communication).

From our results it appears that *Klebsiella* sp., type K9, carrying the W trimethoprim-sulphonamide resistance factor is established as an "opportunistic" pathogen in several London hospitals. Although there are many studies of the spread of such organisms within hospitals, little is known of factors that determine their presence in particular environments or their spread between hospitals.

In addition, bacteria carrying trimethoprim R factors appear to be distributed sporadically. This suggests that trimethoprim R factors are present in the great pool of potential plasmid hosts, the normal intestinal flora of the community, but that their frequency is not high. That we did not detect any in bacteria isolated in centres other than London was probably only because our sample was too small. Given that they are present in the community and that trimethoprim (as co-trimoxazole) is widely used, the frequency of trimethoprim R factors was low.

SUMMARY

Among 95 strains of trimethoprim-resistant Gram-negative bacteria isolated from clinical material in various centres in the UK, one was a thymine-requiring variant of *Escherichia coli* and 10 carried transmissible trimethoprim R factors. The trimethoprim R factors belonged to three different compatibility groups. All gave rise to high levels of trimethoprim resistance. Eight belonged to group W, conferred resistance to trimethoprim and sulphonamides and appeared to be identical with one another and with those found in some London hospitals a year earlier. One belonged to a new group, I δ , conferred resistance to trimethoprim and streptomycin and determined I pili. One belonged to group P and conferred resistance to trimethoprim alone. The W plasmid, carried by a particular klebsiella strain of capsular type 9, appeared to be established in several hospitals in London, but it also occurred sporadically and in other bacterial hosts. The other trimethoprim R factors were identified only in single bacterial strains.

We are grateful to the following who provided trimethoprim-resistant cultures for this study; Professor J. A. Dudgeon, Dr R. A. W. Girdwood, Dr J. W. Harding, Dr I. Phillips, Dr Elizabeth J. Shaw, Dr W. Shepherd, Dr J. S. Stevenson, Dr M. Sussman, Dr J. D. Williams; and also to Dr M. W. Casewell, St Thomas's Hospital, for information about the serotypes of the R+ klebsiella strains and to Dr R. H. Olsen, University of Michigan Medical School, for phage PRR1. N. D. is in receipt of a grant from the Medical Research Council.

REFERENCES

- ANDREW, M. H. E. 1973. Use of trimethoprim to obtain thymine-requiring mutants of *Streptococcus faecalis*. *J. gen. Microbiol.*, **74**, 195.
- BARKER, J., HEALING, D. AND HUTCHISON, J. G. P. 1972. Characteristics of some cotrimoxazole-resistant Enterobacteriaceae from infected patients. *J. clin. Path.*, **25**, 1086.
- CHABBERT, Y.-A., SCAVIZZI, M. R., WITCITZ, G. R., GERBAUD, G. R. AND BOUANCHAUD, D. H. 1972. Incompatibility groups and the classification of *fi*⁻ resistance factors. *J. Bact.*, **112**, 666.
- CLOWES, R. C. AND HAYES, W. 1968. Experiments on microbial genetics, *Oxford and Edinburgh*.
- COETZEE, J. N., DATTA, N. AND HEDGES, R. W. 1972. R factors from *Proteus rettgeri*. *J. gen. Microbiol.*, **72**, 543.
- COWAN, S. T. AND STEEL, K. J. 1970. Manual for the identification of medical bacteria, *London*.
- DARRELL, J. H. 1967. In Progress in medical laboratory technique, vol. 4, edited by F. J. Baker, *London*, p. 2.
- DATTA, N. AND HEDGES, R. W. 1971. Compatibility groups among *fi*⁻ R factors. *Nature, Lond.*, **234**, 222.
- DATTA, N. AND HEDGES, R. W. 1972a. Host ranges of R factors. *J. gen. Microbiol.*, **70**, 453.
- DATTA, N. AND HEDGES, R. W. 1972b. Trimethoprim resistance conferred by W plasmids in Enterobacteriaceae. *J. gen. Microbiol.*, **72**, 349.
- DATTA, N. AND HEDGES, R. W. 1972c. R factors identified in Paris, some conferring gentamicin-resistance, constitute a new compatibility group. *Ann. Inst. Pasteur, Paris*, **123**, 849.
- DATTA, N. AND HEDGES, R. W. 1973. An I-pilus determining R factor with anomalous compatibility properties, mobilizing a gentamicin-resistance plasmid. *J. gen. Microbiol.*, **77**, 11.

- DATTA, N., HEDGES, R. W., SHAW, E. J., SYKES, R. B. AND RICHMOND, M. H. 1971. Properties of an R factor from *Pseudomonas aeruginosa*. *J. Bact.*, **108**, 1244.
- DAVIS, J. E., STRAUSS, J. H. AND SINSHEIMER, R. L. 1961. Bacteriophage MS2: another RNA phage. *Science (N.Y.)*, **134**, 1427.
- FLEMING, M. P. 1973. Trimethoprim-resistance and its transferability in *Escherichia coli* isolated from calves treated with trimethoprim-sulphadiazine: a two year study. *J. Hyg., Camb.*, **71**, 669.
- FLEMING, M. P., DATTA, N. AND GRÜNEBERG, R. N. 1972. Trimethoprim resistance determined by R factors. *Br. med. J.*, **1**, 726.
- GRINDLEY, N. D. F., GRINDLEY, J. N. AND ANDERSON, E. S. 1973. R factor compatibility groups. *Molec. gen. Genet.*, **119**, 287.
- GRÜNEBERG, R. N. 1972. The choice of treatment for urinary infection according to the sensitivities of the causative organisms. In: Advances in antimicrobial and antineoplastic chemotherapy: proceedings of VII International Congress of Chemotherapy, Prague, 1971, vol. 1/2, p. 749. Urban and Schwarzenberg.
- HEDGES, R. W. AND DATTA, N. 1971. *fi*⁻ R factors giving chloramphenicol resistance. *Nature, Lond.*, **234**, 220.
- HEDGES, R. W. AND DATTA, N. 1973. Plasmids determining I pili constitute a compatibility complex. *J. gen. Microbiol.*, **77**, 19.
- HEDGES, R. W., DATTA, N., COETZEE, J. N. AND DENNISON, S. 1973. R factors from *Proteus morganii*. *J. gen. Microbiol.*, **77**, 249.
- HEDGES, R. W., DATTA, N. AND FLEMING, M. P. 1972. R factors conferring resistance to trimethoprim but not sulphonamides. *J. gen. Microbiol.*, **73**, 573.
- LACEY, R. W., GILLESPIE, W. A., BRUTEN, D. M. AND LEWIS, E. L. 1972. Trimethoprim resistant coliforms. *Lancet*, **1**, 409.
- LAWN, A. M., MEYNELL, E., MEYNELL, G. G. AND DATTA, N. 1967. Sex pili and the classification of sex factors in the Enterobacteriaceae. *Nature, Lond.*, **216**, 343.
- LEWIS, E. L. AND LACEY, R. W. 1973. Present significance of resistance to trimethoprim and sulphonamides in coliforms, *Staphylococcus aureus*, and *Streptococcus faecalis*. *J. Clin. Path.*, **26**, 175.
- LOWBURY, E. J. L., BABB, J. R. AND ROE, E. 1972. Clearance from a hospital of Gram-negative bacilli that transfer carbenicillin-resistance to *Pseudomonas aeruginosa*. *Lancet*, **2**, 941.
- MEYNELL, E., MEYNELL, G. G. AND DATTA, N. 1968. Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. *Bact. Rev.*, **32**, 55.
- MEYNELL, G. G. AND LAWN, A. M. 1968. Filamentous phages specific for the I sex factor. *Nature, Lond.*, **217**, 1184.
- MOORHOUSE, E. C. AND FARRELL, W. 1973. Effect of co-trimoxazole on faecal enterobacteria: no emergence of resistant strains. *J. med. Microbiol.*, **6**, 249.
- OKUBADEJO, O. A. AND MASKELL, R. M. 1973. Thymine-requiring mutants of *Proteus mirabilis* selected by co-trimoxazole in vivo. *J. gen. Microbiol.*, **77**, 533.
- OLSEN, R. H. AND SHIPLEY, P. 1973. Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. *J. Bact.*, **113**, 772.
- ROE, E. AND LOWBURY, E. J. L. 1972. Changes in antibiotic sensitivity patterns of Gram-negative bacilli in burns. *J. clin. Path.*, **25**, 176.
- STACEY, K. A. AND SIMSON, E. 1965. Improved method for the isolation of thymine requiring mutants of *Escherichia coli*. *J. Bact.*, **90**, 554.
- STOKES, E. J. 1968. Clinical bacteriology, 3rd ed., London, p. 179.
- WATANABE, T., NISHIDA, H., OGATA, C., ARAI, T. AND SATO, S. 1964. Episome mediated transfer of drug resistance in Enterobacteriaceae VII. Two types of naturally occurring R factors. *J. Bact.*, **88**, 716.