J. Hyg., Camb. (1983), 90, 27–32
Printed in Great Britain

Plasmid-determined antibiotic resistance in *Shigella flexneri* isolated in England and Wales between 1974 and 1978

By J. A. FROST and B. ROWE

Division of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

(*Received* 30 *August* 1982; *accepted* 10 *September* 1982)

SUMMARY

The majority of *Shigella flexneri* strains isolated in England and Wales are from infections contracted abroad. Most of these strains are drug-resistant, over 75% being resistant to streptomycin and sulphonamides, sulphonamides alone or streptomycin, sulphonamides and tetracyclines. A selection of resistant strains was tested for resistance transfer and the plasmids identified were characterized by compatibility grouping.

Streptomycin and sulphonamide resistance was usually determined by a non-autotransferring plasmid which may be mobilized by standard transfer factors, or by the plasmid which conferred tetracycline resistance where this was present. The remaining resistant strains were predominantly resistant to four or more drugs. These strains carried autotransferring plasmids of a variety of compatibility groups, of which groups B, I_1 and F_{II} were the most common.

INTRODUCTION

Of the four subgroups of the genus *Shigella*, only *Sh. sonnei* is indigenous to the United Kingdom, and in 1977 accounted for 90·8% of shigella infections in England and Wales (OPCS, 1979). The majority of infections with *Sh. flexneri*, *Sh. dysenteriae* and *Sh. boydii* are imported, predominantly from the Indian subcontinent and the Mediterranean countries of North Africa (Gross, Thomas & Rowe, 1979). Of these three serogroups, *Sh. flexneri* is the more prevalent, comprising 8·0% of shigellae isolated in England and Wales in 1978, while *Sh. dysenteriae* and *Sh. boydii* together accounted for approximately 1·2% of shigella isolations (OPCS, 1979).

A recent survey has shown that antibiotic resistance in shigellae other than *Sh. sonnei* has increased since 1974 such that by 1978 80% of 2370 strains examined were resistant to one or more drugs (Gross *et al.* 1981). The majority of strains in this survey were *Sh. flexneri*: 1867 strains (78·8%) were examined, of which 1513 (81·0%) were drug-resistant. These resistant strains can be divided into two groups. Over 75% of *Sh. flexneri* were resistant to streptomycin and sulphonamides, sulphonamides alone or streptomycin, sulphonamides and tetracyclines. Most of the remainder were resistant to three or more drugs, including the above, plus ampicillin and/or chloramphenicol.

A selection of these drug-resistant strains which reflected the distribution of resistances described above was tested for transferable drug resistance and the resistance plasmids were characterized. As many of the strains examined were from infections originating abroad, an attempt was made to assess the geographical distribution of the resistance plasmid compatibility groups.

MATERIALS AND METHODS

Strains examined

Three hundred and twenty-three strains of drug-resistant *Sh. flexneri* isolated in England and Wales between 1974 and 1978 were selected for study. This sample consisted of approximately 50 strains each of the three most common resistance patterns identified in the initial survey (Gross *et al.* 1981) plus a representative selection of strains from the next 12 most common R-types. All were R-typed again as some resistances may have been lost on storage. The distribution of resistance patterns in the original survey and in this sample are given in Table 1.

Drug resistance, transfer and mobilization

All strains were R-typed using the methods of Anderson & Threlfall (1974). Plasmids were transferred from the wild strains into *Escherichia coli* K12 resistant to nalidixic acid (DEP ref. 14R525) (Anderson & Lewis, 1965*a*, *b*). Where no direct transfer was observed, resistances were mobilized using the standard transfer factors Δ, of compatibility group I_1 and X, of group F_{II} (Anderson, 1965; Anderson & Threlfall, 1974).

Plasmid characterization

Auto and non-autotransferring resistance plasmids from multiresistant strains were tested for incompatibility with standard plasmids carried in a strain of *E. coli* K12 resistant to streptomycin, M.I.C. 500 μg/ml (DEP ref. 1R716). Standard plasmids carrying appropriate resistance markers were selected from the following compatibility groups: B, D, F_I, F_{II}, F_{IV}, H, I_1, I_2, J, K, M, N, P, W, X (Jacob *et al.* 1977); MP10 and F_I*me* (Anderson *et al.* 1977).

The techniques employed were based on those described by Grindley, Grindley & Anderson (1972) and Anderson & Threlfall (1974).

RESULTS

All of the 323 *Sh. flexneri* strains were tested for drug resistance transfer and mobilization (Table 1). One hundred and forty-six strains (45·2 %) transferred their resistances directly and resistances were mobilized from a further 74 strains (22·9 %). Neither direct resistance transfer nor mobilization was detected from the remaining 103 strains (31·9 %).

The distribution of transferable resistances showed considerable variation between the different resistance patterns. The majority of strains resistant to SSu or Su carried non-autotransferring resistance determinants which in some cases could be mobilized by the standard transfer factors used. Strains resistant to SSuT

Table 1. *Drug resistance and resistance transfer in* Sh. flexneri *isolated in England and Wales, 1974–1978*

R-type*	Total isolated†	Sample size	Resistance transfer: Direct	Resistance transfer: By mobilization	No transfer detected
SSu	387	64	2(3·1)	37(57·8)	25(39·1)
Su	355	40	4(10·0)	14(35·0)	22(55·0)
SSuT	310	31	15(48·4)	10(32·3)	6(19·4)
ACSSuT	112	61	22(36·1)	1(1·6)	38(62·3)
SuT	69	18	5	7	6
CSSuT	45	34	29	3	2
ASSu	44	25	25	0	0
T	34	15	9	2	4
ASSuT	27	7	7	0	0
CT	8	7	7	0	0
ACKSSuT	7	4	4	0	0
AKSSu	7	3	3	0	0
KSSuT	7	5	5	0	0
KSSu	7	3	3	0	0
CSuT	6	6	6	0	0
Other R-types‡	88				
Total	1513	323	146	74	103
% of sample			45·2	22·9	31·9

* *Drug resistance symbols:* A = ampicillin, C = chloramphenicol, K = kanamycin, S = streptomycin, Su = sulphonamides, T = tetracyclines.

† Strains referred to the Division of Enteric Pathogens 1974–1978 (Gross *et al.* 1981).

‡ The remaining 88 strains include 42 different R-types.

usually contained an autotransferring plasmid coding for tetracycline resistance and an independent SSu determinant.

Resistances in most of the multiresistant strains were determined by autotransferring plasmids. However, of the 61 resistant to ACSSuT, 38 (62·3%) carried resistances which were neither directly transferable nor mobilizable.

The 144 strains resistant to ampicillin and/or chloramphenicol were studied in further detail and the resistance plasmids present were classified by compatibility group. Two-thirds of these strains were from infections originating abroad, predominantly in the Indian subcontinent (Table 2). The distribution of plasmids between the different areas is shown in Table 3.

One hundred and three strains carried autotransferring resistance plasmids; five of these having two plasmids each. A further three strains isolated from Vietnamese immigrants, carried transfer deficient group H_1 plasmids.

Group B plasmids were found in 44 strains originating throughout the world. These, however, included 25 strains from one outbreak in a primary school in England in which the plasmids conferred resistance to ASSu. Elsewhere most group B plasmids conferred resistance to CT and their host strains also carried an independent SSu resistance determinant.

Twenty-four strains carried group I_1 plasmids, seven of which produced the Δ-type phage restriction pattern in *Salmonella typhimurium* (Anderson, 1965). A further ten strains carried plasmids of group F_{II}. These more common plasmid

Table 2. *Area of origin of drug-resistant* Sh. flexneri *selected for detailed study*

R-type	Total	Area of origin: Indian sub-continent	Far East	Middle East	Africa	Mediterranean	U.K.
ACSSuT	61	42	1	3	2	2	11
ASSu	25	0	0	0	0	0	25
CSSuT	32	6	6	4	7	0	9
ASSuT	7	1	0	1	1	1	3
CT	7	0	1	3	0	0	3
CSuT	6	1	3	0	0	1	1
ACKSSuT	4	1	1	1	0	0	1
AKSSu	2	1	0	0	0	1	0
Total	144	52	12	12	10	5	53

Table 3. *Plasmid compatibility groups identified in* Sh. flexneri *from different geographical areas*

Group	Total	Co-transferring SSu determinant	Plasmids identified in strains from: Indian sub-continent	Far East	Middle East	Africa	Mediterranean	U.K.
B	44	8	4	3	4	1	1	31
C	2	1	—	—	1	—	—	1
F_1	1	1	—	—	1	—	—	—
F_1me	4	—	2	—	1	—	1	—
F_{II}	10	2	3	1	2	2	—	2
F?	4	—	—	—	—	—	—	4
H_1	9	—	4	3*	—	—	—	2
H_2	1	—	—	1	—	—	—	—
$I_1\Delta$	7	6	1	—	—	4	—	2
I_1nr	17	3	8	1	—	1	1	6
M	2	1	—	1	—	1	—	—
N	2	—	1	1	—	—	—	—
P	2	—	2	—	—	—	—	—
X	3	3	—	—	2	—	—	1
Unclassified	3	2	1	—	—	—	—	2
Total	111	27	26	11	11	9	3	51

* These three plasmids were transfer defective.

types were distributed widely between the different areas. A further ten compatibility groups were represented by fewer than six plasmids each and three plasmids were found to be compatible with all of the standard groups tested.

DISCUSSION

Sh. flexneri is the most common of the three imported shigella serogroups in England and Wales (Gross *et al.* 1979) and the majority of strains are drug-resistant (Gross *et al.* 1981). However, of 323 resistant strains in this study which were tested for transfer and mobilization, direct resistance transfer was detected

https://doi.org/10.1017/S0022172400063798 Published online by Cambridge University Press

from only 146 (45·2%). The two most common resistance patterns, SSu and Su alone, were usually determined by non-autotransferring resistance plasmids. Many of these plasmids could readily be mobilized by one or both of the two standard transfer factors tested. Similar SSu resistance determinants were also present in some of the more resistant strains which also carried autotransferring plasmids.

Plasmids belonging to compatibility group B accounted for 39·6% of the autotransferring resistance plasmids characterized and were identified in strains originating from each of the geographical areas included in the present study. This group of plasmids has been implicated in a number of outbreaks of plasmid-determined antibiotic resistance in shigellae, particularly *Sh. dysenteriae* type 1, but is rarely encountered in salmonellae. Of especial importance was the extensive epidemic of Shiga dysentery in Central America between 1969 and 1972 (Mata *et al.* 1970). Multiresistant *Sh. dysenteriae* type 1 from Central Africa has been shown to carry plasmids of group X and group I_1 (Frost *et al.* 1981, 1982) and plasmids of these two groups have been identified in *Sh. flexneri* in the present study. Compatibility groups I_1 and F_{II} which were widespread in this sample are also widely distributed throughout the world in salmonellae, particularly *S. typhimurium* (Anderson, 1977).

A further ten compatibility groups were identified in a total of 28 strains. Although the number of isolations involved is small, the variations in geographical distribution between the different groups may be significant. For example, compatibility group F_I*me* is so called because it was first identified in *S. typhimurium* phage type 208 from the Middle East (Anderson *et al.* 1977). These plasmids have subsequently been found in a clone of *S. wien* which spread from North Africa across the Mediterranean and through Europe to Great Britain (McConnell *et al.* 1979) and in *S. typhimurium* phage types 66 and 122 in India (Rowe *et al.* 1980). In this survey F_I*me* plasmids were identified in *Sh. flexneri* originating from India, the Middle East and the Mediterranean suggesting that, in these areas, F_I*me* plasmids have spread to several different organisms.

Plasmids of compatibility group H_1 conferring resistance to CSSuT are particularly associated with *S. typhi* (Anderson, 1975) and have been indentified in strains from a number of outbreaks in India and the Far East. Of the six H_1 plasmids identified in this study, four were from India and two from Great Britain. Three transfer-defective H_1 plasmids were found in strains isolated from Vietnamese immigrants.

Thirty-eight strains of Indian origin, resistant to ACSSuT, failed to show resistance transfer either directly or by mobilization. However, although spontaneous loss of resistance to ACT *en bloc* could be demonstrated, this was not correlated with the loss of a plasmid DNA band on agarose gel electrophoresis (unpublished observations). Further study is necessary to determine whether or not these resistances are determined by extrachromosomal genes.

The present study demonstrates that multiple drug resistance in *Sh. flexneri* is plasmid-determined and that a wide range of compatibility groups is involved. Some of these compatibility groups are usually associated with particular host organisms, for example group H_1 with *S. typhi* (Anderson, 1975), group C with *Vibrio cholerae* 01 (Threlfall *et al.* 1980) and group B with shigellae, although they are not limited exclusively to these strains. In contrast, other groups, for example

$F_I me$, are found in organisms from a particular geographical area, whilst the more common groups such as I_1 and F_{II} are ubiquitous. Thus, especially in the investigation of imported infections, resistance plasmid characterization adds a further dimension to epidemiological studies.

REFERENCES

Anderson, E. S. (1965). Origin of transferable drug resistance factors in the Enterobacteriaceae. *British Medical Journal* ii, 1289–1291.

Anderson, E. S. (1975). The problems and implications of chloramphenicol resistance in the typhoid bacillus. *Journal of Hygiene* **74**, 289–299.

Anderson, E. S. (1977). The geographical predominance of resistance transfer systems of various compatibility groups in salmonellae. In *Topics in Infectious Diseases*, Vol. II. *R factors: Their Properties and Possible Control* (ed. J. Drews and G. Hogenauer), pp. 25–38.

Anderson, E. S. & Lewis, M. J. (1965*a*). Drug resistance and its transfer in Salmonella typhimurium. *Nature* **206**, 579–583.

Anderson, E. S. & Lewis, M. J. (1965*b*). Characterisation of a transfer factor associated with drug resistance in *Salmonella typhimurium*. *Nature* **208**, 843–849.

Anderson, E. S. & Threlfall, E. J. (1974). 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.

Grindley, N. D. F., Grindley, J. N. & Anderson, E. S. (1972). R factor compatibility groups. Molecular and General Genetics **119**, 287–297.

Frost, J. A., Rowe, B., Vandepitte, J. & Threlfall, E. J. (1981). Plasmid characterisation in the investigation of an epidemic caused by multiply resistant *Shigella dysenteriae* type 1 in Central Africa. *Lancet* ii, 1074–1076.

Frost, J. A., Rowe, B. & Vandepitte, J. (1982). Acquisition of trimethoprim resistance in epidemic strain of *Shigella dysenteriae* type 1 from Zaire. *Lancet* i, 963.

Gross, R. J., Thomas, L. V. & Rowe, B. (1979). *Shigella dysenteriae*, *Sh. flexneri* and *Sh. boydii* infections in England and Wales: the importance of foreign travel. *British Medical Journal* **280**, 744.

Gross, R. J., Rowe, B., Cheasty, T. & Thomas, L. V. (1981). Increase in drug resistance among *Shigella dysenteriae*, *Sh. flexneri* and *Sh. boydii*. *British Medical Journal* **283**, 575.

Jacob, A. E., Shapiro, J. A., Yamamoto, L., Smith, D. I., Cohen, S. N. & Berg, D. (1977). Appendix B. Bacterial Plasmids. In *DNA Insertion Elements, Plasmids and Episomes* (ed. A. I. Bukhari, J. A. Shapiro and S. L. Adhya), p. 607–670. Cold Spring Harbor Laboratory.

Mata, L. J., Gangarosa, E. J., Caceres, A., Perera, D. R. & Mejicanos, M. L. (1970). Epidemic Shiga bacillus dysentery in Central America. I. Etiologic investigations in Guatemala, 1969. *Journal of Infectious Diseases* **122**, 170–180.

McConnell, M. M., Smith, H. R., Leonardopoulos, J. & Anderson, E. S. (1979). The value of plasmid studies in the epidemiology of infections due to drug-resistant *Salmonella wien*. *Journal of Infectious Diseases* **139**, 178–190.

Office of Population Censuses and Surveys. (1979). *Statistics of Infectious Diseases*, December quarter, 1978. London: HMSO.

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* ii, 1070–1071.

Threlfall, E. J., Rowe, B. & Huq, I. (1980). Plasmid encoded multiple antibiotic resistance in *Vibrio cholerae* El Tor from Bangladesh. *Lancet* i, 1247–1248.