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Molecular epidemiology of shigella infection in Central Australia

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SUMMARY

Shigellosis is endemic in Central Australia and the infections are predominantly due to *Shigella flexneri* 6, *Shigella flexneri* 2a and *Shigella sonnei*. Plasmid profiles of isolates collected from 1985–9, suggested that infections caused by *Shigella flexneri* 6 were predominantly due to a single clone, whereas those caused by *Shigella flexneri* 2a and *Shigella sonnei* were due to several genetically diverse strains, although strains with identical plasmid profiles were found in widely separated geographical areas and in different years.

INTRODUCTION

Shigella infection is hyperendemic in areas where personal hygiene is poor. Since the Aborigial people in Central Australia live in a transitional society, shigellosis is also endemic in their settlements. For example, the shigella notifications received by the National Salmonella and Shigella Centre at the University of Melbourne, Melbourne, from the Northern Territory (of which Central Australia is a part) for 1987 suggest an infection rate of 133 per 100000 population for the Territory, which is approximately 30 times that of the rest of the country (calculated from the NSSS-Annual Report, issue 9/88). The majority of infections for the Territory are recorded in Aboriginal people in Central Australia.

Since the various species and serotypes of shigellae harbour plasmids, plasmid profile analysis has been used as a valuable epidemiological tool in studying the spread of strains in various geographical locations [1, 2]. We have noted that the isolates obtained from patients admitted to the Alice Springs Hospital, Alice Springs, Northern Territory, the only hospital serving Central Australia, are predominantly *Shigella flexneri* 6, *Shigella flexneri* 2a and *Shigella sonnei*. Moreover, all of the isolates have a similar antibiotic susceptibility pattern. This combined with the fact that Aboriginal people in Central Australia live in relatively isolated communities, cut off from the rest of the country, suggested the

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possibility of clonal spread of infection. To investigate this, we studied the plasmid profiles of 72 shigellae isolated in Central Australia and compared them with those of 26 isolates obtained from other parts of Australia.

MATERIALS AND METHODS

All the 98 isolates studied were from patients with clinical disease. The number of *Shigella flexneri* 6, *Shigella flexneri* 2a and *Shigella sonnei* isolates studied, their places and years of isolation are shown in Tables 1 and 2. The shigellae studied included all of the 62 strains isolated in Alice Springs between May 88 and February 89. The remaining 36 isolates studied were randomly picked from collections of shigellae obtained from different places including Alice Springs. Tennant Creek and Darwin are about 500 km and 1300 km north, Adelaide is about 1300 km south, Canberra is about 2300 km south-east and Brisbane is about 2000 km south-east to east of Alice Springs, respectively.

The strains isolated in Alice Springs, Tennant Creek and Darwin in 1988 and 1989 were stored in Alice Springs in brain heart infusion broth with 20% glycerol at −70 °C. The remaining isolates were stored in laboratories in Adelaide and Melbourne in minimal agar at room temperature. For the study, a subculture on nutrient agar slope was sent by airmail at ambient temperature to the University of Texas Health Science Centre, Houston, Texas, USA, where plasmid analysis was done. For plasmid analysis, lysates of bacteria were prepared by the method of Kado and Liu [3] and in some instances, by the methods of Birnboim and Doly [4] and Currier and Nester [5]. Plasmid DNA was detected by electrophoresis in 0·7% horizontal agarose gels [6]. Gels were stained with ethidium bromide and photographed with UV light illumination. The molecular sizes of plasmids were obtained by extrapolation of their relative mobilities to graphs constructed by using plasmids of known molecular sizes. The standards used were plasmids from enteroinvasive *Escherichia coli* 4750 (140, 50 and 6 megadalton (MDa)), enterotoxigenic *Escherichia coli* H10407 (56, 42, and 3·8 MDa), supercoiled ladder DNA molecules (mol. wts 10·6, 9·3, 8·0, 6·7, 5·3, 4·6, 4·0, 3·3, 2·6, 2·0 and 1·4 MDa) (Bethesda Research Laboratories, Gaithersburg, MD, USA) and plasmids PRC-357 (143 MDa), PRC-107 (112 MDa) and PRC-102 (96 MDa) (Plasmid Reference Centre, Stanford University, Stanford, CA, USA). Susceptibility to ampicillin (Am), sulphamethoxazole (Su), trimethoprim (TMP), trimethoprim-sulphamethoxazole (TMP-SMX), kanamycin (Ka), tetracycline (Tc) and chloramphenicol (Cm) were determined by disk diffusion [7] (Oxoid Laboratories, England).

RESULTS

Shigella flexneri *6*

The plasmid patterns and antibiograms are shown in Table 1. Twenty-five of 27 strains had identical plasmid patterns as well as antibiograms. This included 24 strains from Alice Springs and one from Adelaide isolated during 1986–8. One of the remaining two was isolated in Alice Springs and although it had an antibiogram identical with the majority of strains, its plasmid pattern was different. The other strain, isolated in Adelaide, had a different plasmid profile and

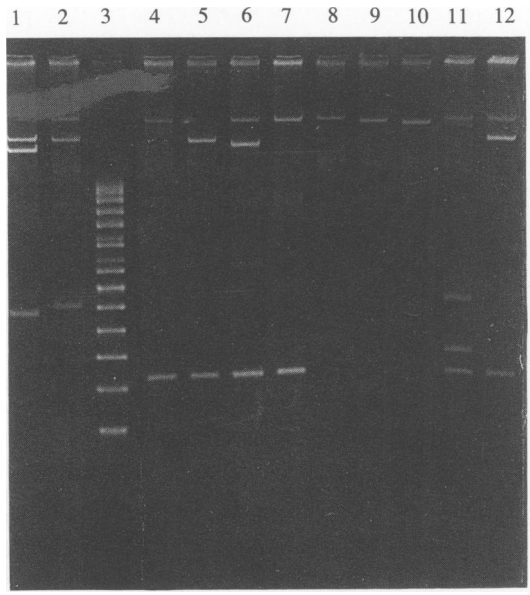

Fig. 1. Plasmid profiles of *Shigella flexneri* 2a. Plasmids were extracted by the method of Kado and Liu [3] and separated in 0·7% agarose gels by horizontal gel electrophoresis. Gels were stained by ethidium bromide and photographed with UV light illumination. The direction of migration is from top to bottom. Lanes 1–3: control plasmids from *Escherichia coli* H 10407 and *Escherichia coli* 4750, and supercoiled DNA molecules respectively. Lanes 4–7: Plasmid patterns 6, 2, 4 and 1 respectively of Table 1. Lanes 8–10: mol. wt standards PRC-357 (143 MDa), PRC-107 (112 MDa) and PRC-102 (96 MDa) respectively. Lanes 11 and 12: plasmid patterns 7 and 5 respectively of Table 1.

antibiogram. All strains irrespective of place of isolation had common size plasmids of 1·6 and 2 MDa.

Shigella flexneri *2a*

Thirty-one of the 37 strains studied had a common plasmid pattern (Table 1) although they had three different antibiograms and were isolated in different areas over a 4-year period. Of the 31 strains with an identical pattern, 23 were from Alice Springs. A strain of *Shigella flexneri* 2a isolated in Alice Springs from a tourist returning from the Philippines had a plasmid pattern and antibiogram different from Australian strains. In all, there were three plasmid patterns among Alice Springs strains. All except two strains (patterns 2 and 3) had a large plasmid, 140 MDa, the size of the invasive plasmid and all strains harboured a common size plasmid of 2 MDa. Some of the plasmid profiles are shown in Fig. 1.

Shigella sonnei

A total of 11 plasmid patterns was obtained among the strains isolated in Alice Springs, and the most common pattern remained unchanged over a 4-year period (Table 2). This pattern was also seen among strains isolated from Tennant Creek and Darwin in 1988 and 1989. Other strains isolated from Darwin and strains from Brisbane and Canberra had different plasmid patterns than those of Alice Springs strains. There was less diversity in antibiograms of strains than that indicated by

Table 1. *Plasmid profiles and antibiograms of* Shigella flexneri *6 and* Shigella flexneri *2a in Alice Springs and other areas*

Plasmid pattern*	No. of strains	Place †	Year	Antibiogram‡ Am	Su	TMP	TMP-SMX	Ka	Tc	Cm
Shigella flexneri 6										
(1) 1·6, 2, 4·8, 96	25§	ASP, ADEL	1986–8	S	R	R	R	S	S	S
(2) 1·6, 2, 4·8, 40, 96	1	ADEL	1988	R	R	R	R	S	S	S
(3) 1·6, 2, 3·2, 3·5, 4·8, 80	1	ASP	1988	S	R	R	R	S	S	S
Shigella flexneri 2a										
(1) 2, 140	31‖	ADP, TCK	1986–9	S	R	R	R	S	S	S
		DRWN		R	S	S	S	S	S	S
		ADEL		S	S	S	S	S	S	S
(2) 2, 50	1	BRIS	1988	S	R	R	R	S	S	S
(3) 2, 96	1	ADEL	1988	S	R	R	R	S	S	S
(4) 2, 40, 140	1	DRWN	1989	R	R	R	R	S	S	S
(5) 2, 50, 140	1	ASP	1988	R	R	R	R	S	S	S
(6) 2, 75, 100, 140	1	ASP	1988	S	R	R	R	S	S	S
(7) 2, 2·6, 6, 92, 140	1¶	ASP	1988	R	R	R	R	S	R	R

* Molecular weights are expressed in megadaltons.

† ASP: Alice Springs; TCK: Tennant Creek; DRWN: Darwin; ADEL: Adelaide; BRIS: Brisbane.

‡ Am: ampicillin; Su: sulphamethoxazole; TMP: trimethoprim; TMP-SMX: co-trimoxazole; Ka: kanamycin; Tc: tetracycline; Cm: chloramphenicol; R: resistant; S: sensitive.

§ 24 strains isolated in ASP (3 in '86, 2 in '87 and 19 in '88) and 1 strain in ADEL in '88.

‖ 21 strains isolated in ASP (2 in '87, 16 in '88 and 3 in '89), 5 strains in TCK (1 in '86, 1 in '87 and 3 in '88), 4 strains in DRWN in '89 and 1 strain in ADEL in '88.

¶ Isolated from a tourist returning from the Philippines.

plasmid patterns. All except two strains (patterns 3 and 4) had a common size plasmid of 1·6 MDa. None of the strains had the large invasive plasmid of 120 MDa. Some of the plasmid profiles are shown in Figure 2.

DISCUSSION

Twenty-four of 25 isolates of *Shigella flexneri* 6 studied from Alice Springs had identical plasmid profiles suggesting that *Shigella flexneri* 6 infections in Central Australia are predominantly due to a single clone. More diversity in plasmid profile was seen in strains of *Shigella flexneri* 2a although the majority of Alice Springs isolates (21/24) had the same plasmid pattern 1 (Table 1). The isolate obtained from the tourist returning from the Philippines had a different antibiogram from the local strains and its different plasmid profile also suggested that the organism was probably picked up overseas. Genetic diversity was more profound among *Shigella sonnei* strains since 11 different profiles were apparent in the isolates studied in Alice Springs. Among *Shigella flexneri* 2a, *Shigella flexneri* 6 and *Shigella sonnei* strains, the most common plasmid patterns remained stable for 3–4 years and among the latter two, strains with these plasmid profiles were

Table 2. *Plasmid profiles and antibiograms of* Shigella sonnei *in Alice Springs and other areas*

Plasmid pattern*	No. of strains	Place†	Year	Antibiogram‡ Am	Su	TMP	TMP-SMX	Ka	Tc	Cm
(1) 1·6, 4·3	17§	ASP, TCK	1985–9	S	R	R	R	S	S	S
		DRWN		S	R	S	R	S	S	S
(2) 1·6	2	ASP, DRWN	1989	S	R	R	R	S	S	S
(3) 10·5	1	CANB	1988	R	R	R	R	S	S	S
(4) 3·7, 140	1	ASP	1987	S	R	S	R	S	R	S
(5) 0·8, 1·6, 5·6	1	ASP	1988	R	R	R	R	S	S	S
(6) 1·6, 4·3, 9·4	1	ASP	1988	S	R	R	R	S	S	S
(7) 1·6, 4·3, 40	1	ASP	1988	S	R	R	R	S	S	S
(8) 1·6, 4·3, 20	2	DRWN	1989	S	R	R	R	S	S	S
(9) 1·6, 2·9, 4·3	1	ASP	1989	R	R	R	R	S	S	S
(10) 1·6, 4, 8	1	ASP	1985	S	R	S	R	S	S	S
(11) 1·6, 3·8, 4·3	2	ASP	1985,	S	R	S	R	S	S	S
			1986	R	S	S	S	S	S	S
(12) 1·6, 4·3, 4·7, 20	1	ASP	1987	S	R	S	R	S	S	S
(13) 1·6, 5·6, 8·5, 10·5, 50	1	ADEL	1988	R	R	R	R	S	S	S
(14) 1·6, 5·6, 10·5, 25, 30, 65	1	BRIS	1988	R	R	R	R	S	R	S
(15) 1·6, 1·8, 3·7, 4·3, 5·5, 90	1	ASP	1986	S	R	R	R	S	R	S

* Molecular weights are expressed in megadaltons.

† ASP: Alice Springs; TCK: Tennant Creek; DRWN: Darwin; ADEL: Adelaide; BRIS: Brisbane; CANB: Canberra.

‡ Am: ampicillin; Su: sulphamethoxazole; TMP: trimethoprim; TMP-SMX: co-trimoxazole; Tc: tetracycline; Cm: chloramphenicol; R: resistant; S: sensitive.

§ 12 strains isolated in ASP (2 each in '85, '86 and '87 and 6 in '88), 2 in TCK in '88 and 3 in DRWN in '89.

isolated from widely separated geographical areas. Similar observations have been made for isolates of *Shigella sonnei* studied in Mexico and the USA [2].

Although in both *Shigella flexneri* 2a and *Shigella sonnei* the predominant patterns were found in the majority of strains, the other patterns were each represented by only one or two strains. This could mean that the strains with less common profiles are either new strains introduced from outside, but unable to find a permanent niche due to competition from the established strains, or they are part of the established strains in the area whose true prevalence was underestimated in the present study by not including sufficient numbers of strains. Since the range of drug resistance in our isolates was rather limited, it was not possible to extrapolate antibiograms to plasmid profiles.

The invasiveness of shigella is mediated by plasmids; in *Shigella dysenteriae*, *flexneri* and *boydii* by 140 MDa plasmid and in *sonnei* by 120 MDa plasmid [8, 9]. However, none of the strains of *Shigella flexneri* 6 or *Shigella sonnei* studied had these plasmids. Instability of these plasmids has been documented in shigellae [8]. The failure to detect these plasmids is not due to faulty techniques. In the initial screening, plasmids were extracted by the method of Kado and Liu [3], but when we noted the absence of invasive plasmids, plasmid extractions were repeated by

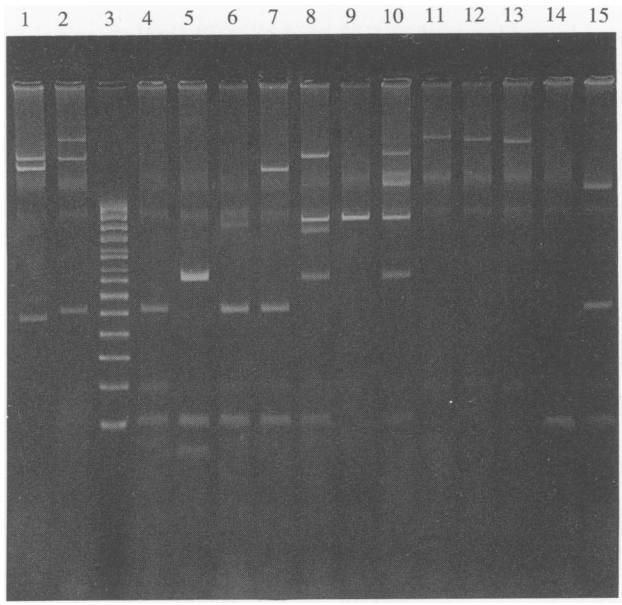

Fig. 2. Plasmid profiles of *Shigella sonnei*. Plasmids were prepared and separated as in legend to Fig. 1. Lanes 1–3: control plasmids from *Escherichia coli* H10407 and *Escherichia coli* 4750 and supercoiled DNA molecules, respectively. Lanes 4–10: plasmid patterns 1, 5, 6, 7, 13, 3 and 14 respectively of Table 2. Lanes 11–13: mol. wt standards PRC-357 (143 MDa), PRC-107 (112 MDa), and PRC-102 (96 MDa), respectively. Lanes 14–15: Plasmid patterns 2 ad 8, respectively, of Table 2.

the methods of Birnboim and Doly [4] and Currier and Nester [5] and still could not be demonstrated.

There have been reports that certain *Shigella* species and serotypes have core plasmids and that caution should be exercised while interpreting plasmid patterns [10, 11]. We have noted that all *Shigella flexneri* 6 had common plasmids of 1·6 and 2 MDa, *Shigella flexneri* 2a, plasmids of 2 MDa and *shigella sonnei* plasmids of 1·6 MDa. However, comparison of our data with those published from other parts of the world did not reveal universality of any plasmids (other than invasive plasmids) in these species and serotypes [2, 10].

The stability of certain plasmid profiles and clonal dissemination of shigellae, limit the ability to identify 'point sources' in outbreaks. Nevertheless, when the profiles of background strains are known for a given locale, identifying a distinctively different pattern would still be useful.

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