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Salmonella gold-coast from outbreaks of food-poisoning in the British Isles can be differentiated by plasmid profiles

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SUMMARY

Four distinctive plasmid profile types have been identified in strains of *Salmonella gold-coast* isolated in Britain. Strains of one type, designated plasmid profile type 4, caused an extensive outbreak of food-poisoning in 1984, and it has been confirmed that the vehicle of infection was imported French pâté.

INTRODUCTION

Salmonella gold-coast was first isolated in Ghana in 1953 from a child with diarrhoea (Lapage *et al.* 1966) and the first isolation in Britain was in 1972, from a traveller returning from West Africa. In Britain, this serotype was not an important cause of food-poisoning until 1984, when over 250 persons were infected in an outbreak in widely separated areas in England and Wales (Anonymous, 1985). There is no phage-typing scheme for *S. gold-coast* and to assist epidemiological investigations, an alternative method of strain discrimination was necessary. Plasmid profiles have been used for this purpose in salmonella outbreaks in the USA (Schmidt *et al.* 1982; Holmberg *et al.* 1984) and Norway (Olsvik *et al.* 1985). We report the use of this technique for the differentiation of *S. gold-coast* isolated in the British Isles in the 14-year period 1972–85, with special reference to the food-poisoning outbreak in 1984.

MATERIALS AND METHODS

Bacterial strains

Strains isolated from humans and food in England and Wales were referred to the Division of Enteric Pathogens (DEP) by laboratories of the Public Health Laboratory Service and by hospital laboratories. Strains from animals were identified at the Central Veterinary Laboratory of the Veterinary Investigation Service, New Haw, Weybridge, Surrey. Strains isolated in Scotland were identified at the Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow, and those isolated in France at the Institut Pasteur, rue du Dr Roux, Paris.

Identification

Strains were identified biochemically by the methods of Cowan & Steel (1974) and serotyped according to the methods of Kauffmann (1972).

Preparation of partially purified plasmid DNA and agarose gel electrophoresis

Partially purified plasmid DNA was prepared by the method of Birnboim & Doly (1979). Plasmid DNA samples were analysed by electrophoresis of 50 μl preparations on vertical slab gels containing 0·6 % agarose (w/v, Sigma, Type II) and were of approximate dimensions 16 × 18 cm. Electrophoresis was performed at 140 V for 4 h at room temperature, after which gels were stained for 30 min in distilled water containing 5 μg/ml of ethidium bromide. Molecular weights (MWs) were determined in relation to the mobility of reference plasmids ranging from 1·36 to 98 megadaltons (MDa). These were carried in standard strains of *Escherichia coli* K12.

RESULTS

Occurrence of S. gold-coast *in England and Wales, 1953–85*

Prior to 1977 there were only three isolations, all from patients who had recently returned from the west coast of Africa (Table 1). From 1977 to the end of 1981, 23 infections with this serotype were identified, 11 (47·8 %) of which were known to have been acquired abroad. The countries concerned included Nigeria, The Gambia, France, Spain and Portugal. Thus, before 1982, infections with *S. gold-coast* were uncommon and of those encountered, approximately 55 % involved persons with a history of travel in West Africa or Southern Europe. In 1981, *S. gold-coast* was also isolated from sewage sludge and from fish meal.

In 1982, 26 infections with *S. gold-coast* were identified, 8 of which had been contracted abroad and in 1983 there were 17 infections, of which 6 had been acquired in countries other than England, Wales and Scotland. In 1982 and 1983, the majority of persons infected with *S. gold-coast* and with a history of foreign travel had been infected in Spain (8 cases) but strains were also isolated from persons returning from Greece (3 cases), Portugal (2) and The Gambia (1). In 1982 *S. gold-coast* was also isolated from sea water, in 1983 from poultry feed and in 1982 6 incidents in food-producing animals were reported, 5 amongst cattle and 1 in sheep (Veterinary Investigation Service, 1983).

In the first 5 months of 1984 there was only one isolation of *S. gold-coast* from humans in England and Wales, from a patient in Hertfordshire, England, who, as far as is known, had not travelled abroad. However in June 1984 an outbreak of *S. gold-coast* occurred and by the end of the year this serotype had been identified in 236 patients, of whom 219 were known to have been infected in widely separated areas throughout England and Wales. The other 17 were thought to have been infected abroad. An increase in isolations of this serotype was also observed in Scotland. Investigations showed that many infections in England and Wales were associated with the consumption of imported French pâté which had been distributed by several large retail outlets. Although the product was withdrawn, sporadic cases continued to occur for the next few months and in August 1984 there was an apparently unrelated outbreak at a wedding in the northern part of England, at which imported French chicken was eaten. During 1984, *S. gold-coast* was also isolated from human food – imported pâté from France, from saddle of hare also from France, and from an abattoir in Devon, England.

In 1985, *S. gold-coast* has been isolated from 7 patients, 6 of whom were infected in Britain and 1 in Italy.

Table 1. *Isolations of* Salmonella gold-coast *in England and Wales, 1953–85*

(Numbers of strains isolated in England and Wales and referred to the division of enteric pathogens. Figures in parentheses indicate number of strains from infections acquired abroad.)

Year	Human	Non-human
1953–71	0	0
1972	1 (1)	0
1973	0	0
1974	1 (1)	0
1975	0	0
1976	1 (1)	0
1977	10 (3)	0
1978	2 (2)	0
1979	4 (3)	0
1980	2	0
1981	5 (3)	1, Fish meal 1, Sewage sludge
1982	26 (8)	2, Sea water
1983	17 (6)	1, Poultry feed
1984	236 (17)	25, Human food 1, Abattoir swab
1985	7 (1)	0
Totals	312 (46)	31

Table 2. *Plasmids in* S. gold-coast *isolated in England, Wales and Scotland*

Year of isolation	Source	Country of infection/origin	No. examined	MWs of plasmids identified (MDa)
1953*	Human	Ghana	1	120
1974	Human	Nigeria	1	120
1976	Human	Nigeria	1	—
1977	Human	Britain	5	—
1979	Human	France	2	—
		Nigeria	1	—
	Avian	Britain	1	—
1981	Human	Spain	2	—
		Spain	1	36†
		Portugal	1	3·0
		Britain	4	—
		Britain	1	50
	Fish meal	Britain	1	1·3

—, No plasmid species identified.
* Original isolate of *S. gold-coast*, isolated in Ghana.
† Codes for resistance to ampicillin.

Plasmids in S. gold-coast

1974–81

The original isolate of *S. gold-coast* and 21 strains isolated in England, Wales and Scotland between 1974 and 1981 were examined by agarose gel electrophoresis. The results are summarized in Table 2.

Plasmids were identified in six strains. The strain isolated in Ghana in 1953 and a strain isolated in 1974 from a person infected in Nigeria each carried a single

plasmid with a MW of approximately 120 MDa. A strain isolated in 1981 from a person infected in Spain carried a plasmid of 36 MDa which coded for ampicillin resistance, a strain from a patient infected in Portugal carried a plasmid of 3·0 MDa, and a patient in Manchester, England was infected with a strain which carried a plasmid of 50 MDa. Finally, a strain isolated in Hull, England, from fish meal carried a plasmid of 1·3 MDa.

1982–3

Twenty-seven strains isolated in 1982 were examined, 21 from infections acquired in Britain, 5 from travellers returning from Spain and 1 from a person infected in Greece (Table 3). Of the British strains, 10 carried a plasmid of 1·3 MDa; 1 of the strains with the 1·3 MDa plasmid carried 2 further plasmids of 4·2 and 35 MDa. Epidemiological investigations showed that patients infected with strains with the 1·3 MDa plasmid species were all from the Midland counties of Britain. The strain with the additional 4·2 and 35 MDa plasmids carried a non-conjugative streptomycin-sulphonamide resistance plasmid of 4·2 MDa and a conjugative plasmid of 35 MDa which did not confer antibiotic resistance. The plasmid profile of strains with the 1·3 MDa plasmid was indistinguishable from that of the strain isolated in 1981, from fish meal (Table 2).

Nine strains from infections contracted in Britain, 5 isolated in the South-west of England, 2 in Northern England and 2 in Scotland, did not carry plasmids. Two strains isolated in Edinburgh, Scotland, carried six plasmids with MWs of 64, 38, 5·4, 4·0, 3·8 and 3·4 MDa. These strains were resistant to kanamycin, streptomycin, sulphonamides, spectinomycin and trimethoprim and carried at least two drug-resistance plasmids. The five strains from travellers returning from Spain were plasmid-free but the strain from a person infected in Greece carried two plasmids of 16 and 3·0 MDa.

Fourteen strains isolated in 1983 were examined and plasmids were identified in 10 strains. Two strains from persons infected in Portugal, 2 strains from persons returning from Greece, 3 strains from persons from whom a history of travel was not available, and the strain from poultry feed had an identical profile and carried a plasmid of 3·0 MDa. A strain isolated from a person from Birmingham carried a plasmid of 1·3 MDa and had a profile indistinguishable from that of strains isolated in the same area in 1982, and a strain isolated from a patient in Northamptonshire carried 2 plasmids of 48 and 20 MDa. Four strains isolated from patients infected in other parts of Britain were plasmid-free.

1984

Fifty-two strains isolated in 1984 were examined (Table 4). These included 38 strains isolated in Britain and 14 strains isolated in France. The British isolations comprised as follows: 24 strains from humans, 7 animal strains, 5 strains from imported French pâté, 1 strain from saddle of hare imported from France and 1 strain from an abattoir swab. Of the human strains, 2 were from persons known to have been infected in Spain, 1 from an infection acquired in the Sudan, 1 from a person returning from Nigeria and 2 from travellers who were infected in France. Three strains were from persons who had travelled abroad but did not develop symptons until some time after their return to Britain. The remaining 15 strains were from infections contracted in Britain and included 2 strains from patients

Table 3. *Plasmids in* S. gold-coast *isolated in 1982 and 1983*

Year of isolation	Source	Country of infection/origin	No. examined	MW of plasmids identified (MDa)												
1982	Human	Britain	9	—	—	—	—	—	—	—	—	—	—	—	—	—
			9	—	—	—	—	—	—	—	—	—	—	—	—	1·3
			1	—	—	—	35	—	—	—	4·2	—	—	—	—	1·3
			2	64	—	38	—	—	—	5·4	—	4·0	3·8	3·4	—	—
		Spain	5	—	—	—	—	—	—	—	—	—	—	—	—	—
		Greece	1	—	—	—	—	—	16	—	—	—	—	—	3·0	—
1983	Human	Britain	4	—	—	—	—	—	—	—	—	—	—	—	—	—
			1	—	—	—	—	—	—	—	—	—	—	—	—	1·3
			1	—	48	—	—	20	—	—	—	—	—	—	—	—
			3	—	—	—	—	—	—	—	—	—	—	—	3·0	—
		Portugal	2	—	—	—	—	—	—	—	—	—	—	—	3·0	—
		Greece	2	—	—	—	—	—	—	—	—	—	—	—	3·0	—
	Poultry feed	Britain	1	—	—	—	—	—	—	—	—	—	—	—	3·0	—

Table 4. *Plasmids in* S. gold-coast *isolated in 1984 and 1985*

Year of isolation	Source*	Country of infection	No. examined	MW of plasmids identified (MDa)		
1984	Human	Britain	4	—	—	—
		Spain	2	—	—	—
		Sudan	1	—	—	—
		Nigeria	1	—	—	—
		Britain	14‡	5·0	3·6	—
		France	2	5·0	3·6	—
		France†	12§	5·0	3·6	—
	Food: Pâté	France	5	5·0	3·6	—
	Hare	France	1	5·0	3·6	—
	Abattoir swab	Britain	1	5·0	3·6	—
	Bovine	Britain	1	—	—	—
			2	5·0	3·6	—
	Porcine	Britain	1	—	—	—
		Iceland	1	—	—	3·0
	Canine	Britain	1	5·0	3·6	—
	Badger	Britain	1	5·0	3·6	—
	Water	France†	2	5·0	3·6	—
1985	Human	Britain	1	—	—	—
			5	5·0	3·6	—
			1	—	—	3·0
		Italy	1	—	—	—

* Unless otherwise stated, isolated in laboratories in England, Wales and Scotland.
† Isolated in France.
‡ Two strains from outbreaks associated with pâté.
§ Three strains from outbreaks associated with pâté.

infected in outbreaks in which the vehicle of infection was imported pâté, 2 strains from the wedding outbreak in the north of England and 11 strains from apparently sporadic cases. Of the strains from apparently sporadic cases, 1 was isolated prior to June 1984 and 10 in the last 7 months of the year. Of the 14 strains isolated in France, 12 were isolated from patients and 2 from water; the human isolations included 3 strains from outbreaks associated with pâté and 9 from patients infected in different geographical areas.

All strains isolated in France from humans and water had an identical plasmid profile and carried two plasmids with MWs of 5·0 and 3·6 MDa. Amongst the strains isolated in Britain, this profile was observed in the 5 strains from imported French pâté, in the strain from imported French saddle of hare, in 16 of 24 strains from humans, in 2 of 4 strains from cattle, and in isolations from a dog, a badger and an abattoir swab. Patients from whom *S. gold-coast* with this distinctive profile were isolated were all infected in the last 7 months of 1984 and included 2 travellers infected during visits to France, the 2 persons known to have consumed imported French pâté contaminated with *S. gold-coast*, and persons in the wedding outbreak at which imported French chicken was consumed. The strain isolated before June 1984, three other strains from patients infected in Britain and strains from infections contracted in Spain, the Sudan and Nigeria did not carry plasmids.

Table 5. *Profile types in* S. gold-coast

Plasmid profile type	MW of plasmids (MDa)	Occurrence
1	120	Ghana 1953 Nigeria 1972
2	1·3	Britain 1981, 1982, 1983
3	3·0	Portugal 1981, 1983, 1984 Greece 1982, 1983 Britain 1983, 1985 Iceland 1984
4	5·0, 3·6	France 1984 Britain 1984, 1985

1985

Of 7 strains isolated from humans in 1985, 5, all from patients known to have been infected in Britain in the first 9 months of the year, had a profile indistinguishable from that of the 1984 epidemic type. One strain from a person who did not provide a travel itinerary carried a plasmid of 3·0 MDa and the remaining strain, from a patient infected in Italy, was plasmid-free.

DISCUSSION

Four distinctive plasmid profile types of epidemiological significance have been identified in *S. gold-coast* isolated in Britain (Table 5). The first type, designated plasmid profile type 1 (PPT 1) is characterized by a plasmid of 120 MDa and has been observed in the original isolate of *S. gold-coast* and in a strain in 1972 from a traveller returning from West Africa. The second type, PPT2, is characterized by a plasmid of 1·3 MDa. The first strain of PPT2 identified was that isolated in 1981 from fish meal. Our results show that PPT2 caused an outbreak in Britain in 1982 and early 1983. Strains of the third type, PPT3, carry a plasmid of 3·0 MDa. This type has been identified in strains isolated from persons infected in Portugal in 1981 and 1983, in Greece in 1982 and 1983 and in Britain in 1983 and 1985. PPT3 has also been identified in a strain from poultry feed, isolated in 1983, and in a strain of porcine origin from Iceland, isolated in 1984. Strains of the fourth type, PPT4, carry two plasmids of 5·0 and 3·6 MDa. This type caused an extensive outbreak of food-poisoning in Britain in 1984.

Strains of PPT4 have been isolated from pâté imported from France for consumption in Britain, and from persons known to have consumed this product. PPT4 has also been observed in strains from pâté-associated outbreaks which occurred in France in 1984 but not in strains isolated in Britain before June 1984, nor in strains from infections contracted in Nigeria, Spain and the Sudan. These results confirm that imported French pâté was the vehicle of infection in the 1984 outbreak of *S. gold-coast* in the British Isles. PPT4 has also been identified in *S. gold-coast* isolated in Britain in the last 6 months of 1984 from cattle, a dog, a badger and an abattoir swab. This suggests that environmental contamination has occurred, possibly by surface water polluted with human sewage. From the epidemiological evidence available, it is unlikely that cattle were initially involved

in the British outbreak. However, in 1985, 5 of 7 human infections have been caused by strains of PPT4 and the patients gave no history of having eaten imported French pâté. It is possible that this type may now have become established in food animals in Britain.

These studies demonstrate that plasmid profiles can be used for the differentiation of *S. gold-coast*. This technique may be particularly useful in studying plasmid-carrying strains of salmonella serotypes which cannot be differentiated by the highly discriminatory and more rapid method of phage typing.

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