***Escherichia coli* 0142.H6; a drug-resistant enteropathogenic clone?**

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SUMMARY

For many years strains of *Escherichia coli* belonging to particular serotypes (EPEC) were a common cause of outbreaks of infantile enteritis in Europe and North America. *E. coli* 0142.H6 was first isolated from infants with diarrhoea in Indonesia in 1960 and a further outbreak occurred in Mexico in 1965. Between 1967 and 1972 outbreaks of infantile enteritis caused by *E. coli* 0142 were reported in hospitals in Scotland, England, Northern Ireland and Eire. Sporadic cases occurred in Canada in 1972 and a further outbreak occurred in Arizona, U.S.A. in 1975. Strains from all these incidents were examined by biochemical and serological methods. Their resistance to antimicrobial drugs was determined and their resistance plasmids characterized; their plasmid profiles were visualized by agarose gel electrophoresis. The cumulative evidence suggests that strains isolated in the British Isles all belonged to a single clone and were related to those isolated in Indonesia, the U.S.A. and Canada. However, the strains from Mexico appeared to be unrelated. This study demonstrates that single clones of enteropathogenic *E. coli* may spread throughout the world, causing outbreaks of diarrhoeal disease and acquiring resistance to antimicrobial drugs.

INTRODUCTION

Evidence accumulated over many years has demonstrated that members of certain serogroups of *Escherichia coli* have been the causative organisms in outbreaks of infantile enteritis. On the basis of epidemiological evidence obtained with the use of serotyping, by 1960 about seventeen O groups were regarded as enteropathogenic (Taylor, 1961). These strains of enteropathogenic *E. coli* (EPEC) usually do not produce either heat-labile or heat-stable enterotoxins when judged by widely used test systems (Gross, Scotland & Rowe, 1976; Levine *et al.* 1978) and in this respect differ from enterotoxigenic *E. coli* (ETEC). While the importance of ETEC as a cause of diarrhoea in tropical communities has been well documented in recent years, studies of EPEC have been less frequent.

Although EPEC were a common cause of hospital outbreaks of infantile enteritis in Europe and North America up until the early 1970's, EPEC enteritis now appears to be of minor importance in temperate areas with good standards of hygiene. Nevertheless surveys have shown that EPEC are still a common cause of enteritis in tropical countries (Maiya *et al.* 1977) and among communities with poor standards of hygiene (Gurwith & Williams, 1977).

E. coli 0142 was first isolated from infants with diarrhoea in Indonesia (Ørskov *et al.* 1960), and subsequently from an outbreak of diarrhoea among infants in a Mexico City hospital (Olarie & Ramos-Alvarez, 1965). In the first outbreak most cases were mild but in the second there was a high mortality (39%). In both outbreaks the *E. coli* 0142 strains possessed the flagella antigen H6.

Between 1969 and 1972 outbreaks of infantile enteritis caused by *E. coli* 0142 . H6 occurred in hospitals in Scotland, England and Northern Ireland. In the present study representative strains of *E. coli* 0142 . H6 isolated from these outbreaks and from sporadic cases in the United Kingdom (U.K.) between 1969 and 1972 were examined and compared. The methods used for strain identification were biochemical and serological; strains were further characterized by determination of their drug resistance spectrum, by characterization of their resistance plasmids and by comparison of their plasmid profiles, using agarose gel electrophoresis for the visualization of plasmid DNA. The same methods were used to characterize strains of the same O:H serotype that have caused outbreaks in other countries. In particular, evidence was sought to determine whether the outbreaks and sporadic cases in the U.K. have resulted from the spread of a single clone of *E. coli* 0142 . H6, and if so, whether this strain is related to those which have caused outbreaks in other countries.

MATERIALS AND METHODS

Bacterial strains

Strains of *E. coli* 0142 . H6 were obtained from the culture collection of the Division of Enteric Pathogens. Strains from the U.K. had been isolated during the period 1967–72 from the faeces of infants with diarrhoea, and had originally been referred by laboratories of the Public Health Laboratory Service and by hospital laboratories. Their origins and dates of isolation are shown in Table 1. Strains from Eire were received from Dr C. Keane, those from Indonesia and Mexico from Dr F. Ørskov, those from Canada from Dr J. M. S. Dixon and those from the U.S.A. from Dr K. M. Boyer. Their origins and dates of isolation are shown in Table 2.

Strains were biochemically characterized by the methods of Cowan (1974) and were serotyped using O and H antisera according to the methods of Ørskov & Ørskov (1975). Resistance to antimicrobial drugs was determined by the methods of Anderson & Threlfall (1974).

Resistance transfer and plasmid characterization

Resistant strains were tested for the ability to transfer resistance to a plasmid-free strain of *E. coli* K12 F⁻*lac*⁺, nalidixic acid-resistant (K12 *nal*^r). Crosses were incubated for 18 h at both 28 °C and 37 °C before plating on appropriate selective media. When no transfer was observed, the mobilization of resistance was attempted with conjugative plasmids of a range of compatibility groups. Resistance plasmids were characterized by examination of the properties described by Anderson & Threlfall (1974), and were assigned to compatibility groups on the basis of their inability to co-exist with representative plasmids of the compatibility groups listed by Willshaw *et al.* (1980). Non-conjugative plasmids were grouped in accordance with the scheme of Smith (1975).

Table 1. *E. coli* 0142 . H6 from cases and outbreaks of infantile enteritis in the U.K., 1967-72

<i>E. coli</i> 0142 . H6						
Source	Year	Patients with symptoms	No. isolated	No. examined	Resistance type†	Molecular weights of plasmids present (Megadaltons)
Dundee	1967	1	1	1	T	90 72 — — —
Paisley†	1969	9	2/4	1	AT	90 72 — — —
Glasgow (G1)	1969-70	4	4	2	ASuT	90 72 — — 5.5
Stoke-on-Trent†	1970	21	4/5	2	AT	90 72 — — 5.5
Kirkaldy	1970	6	6	2	AT	90 72 — — 5.5
Glasgow (G2)†	1970-1	30	14/15	6	AT (3)	90 72 — — 5.5
					ASuT (3)	90 72 62* — 5.5
Irvine (Irv 1)†	1970	7	7	2	AT	90 72 — — 5.5
Irvine (Irv 2)†	1971	4	4	2	AT	90 72 — — 5.5
Glasgow (G1)†	1971	10	10	2	ASuT	90 72 — — 5.5
Belfast†	1971	14	14	2	AT	90 72 — — 5.5
Glasgow (G3)†	1971	8	8	5	ASuT (4)	90 72 — — 5.5
					SuT (1)	90 72 — — 4.0
Leeds	1971	1	1	1	AT	90 72 — — 5.5
Manchester	1971	2	2	2	AST	90 72 — — 5.5
Liverpool	1971	6	6	6	AT	90 72 — — 5.5
Pontefract	1971	1	1	1	AT	90 79 62 — — 5.5

* in 1/3 strains.

† Outbreak.

‡ Resistance symbols: A, ampicillin; S, streptomycin; Su, sulphonamides; T, tetracyclines. Numbers of each resistance type in parentheses.

Table 2. *E. coli* 0142 . H6 from outbreaks of infantile enteritis in countries other than the U.K.

<i>E. coli</i> 0142 . H6						
Source	Year	Patients with symptoms	No. isolated	No. examined	Resistance type*	Molecular weights of plasmids present (Megadaltons)
Indonesia	1960	—	—	1	SuT	— 90 72 — — 34 26 — 4.2
Mexico	1965	—	—	2	CSuSpT	>95 — — — 44 — 29 — 4.8
Eire	1971	134	62	4	AKSSuT	98 90 72 65 — — — 5.5 4.0
Canada	1972	—	—	2	ASSuT	— 90 72 — — — 5.5 4.2
U.S.A.	1975	59	59	1	ASuT	— 90 — — — — 5.5 4.0

* Resistance symbols: C, chloramphenicol; K, neomycin-kanamycin; Sp, spectinomycin.

Preparation of partially-purified plasmid DNA and agarose gel electrophoresis

Partially-purified plasmid DNA from strains of *E. coli* 0142 . H6 and from strains of K12 *nal^r* into which resistance plasmids had been transferred or mobilized was prepared by the method of Birnboim & Doly (1979). Plasmid DNA samples were analysed by electrophoresis on vertical slab gels containing 0.6% agarose (w/v, Sigma, Type II) and were of approximate dimensions 16 cm × 18 cm. Electrophoresis was performed at 140 V for 4 h at room temperature. Molecular weights (MW's) were determined in relation to the mobility of reference plasmids ranging in size from 4.6 to 98 megadaltons (Md). These were carried in a strain of K12 *nal^r*.

OUTBREAKS

The *E. coli* 0142 . H6 from the U.K. were from cases and outbreaks of infantile enteritis chronologically listed in Table 1; those from other countries are listed in Table 2.

*Britain**Dundee, Scotland, 1967*

Prior to 1969 only one strain of *E. coli* 0142 . H6 had been identified in the U.K. in over 5000 strains studied. This strain was isolated in 1967 in Dundee, Scotland, from an infant with diarrhoea.

Paisley, Scotland 1969

In 1969 there was an outbreak of enteritis amongst neonates in a Paisley hospital in which nine babies were affected; there was one death. Of four strains examined, two were *E. coli* 0142 . H6.

Glasgow, Scotland, 1969

Between June 1969 and February 1970 *E. coli* 0142 . H6 was isolated from four children with enteritis in a Glasgow hospital (G1). One child died, but no outbreak occurred.

Stoke-on-Trent, England, 1970

In April 1970, 20 infants in a hospital ward developed enteritis after the admission of a 4-month-old child with diarrhoea. *E. coli* from five patients were serotyped and strains from four of them were of serotype 0142 . H6.

Kircaldy, Scotland, 1970

In May 1970, six babies in a paediatric ward developed diarrhoea. Strains from all six were found to be *E. coli* 0142 . H6.

Glasgow, Scotland, 1970

Between October 1970 and April 1971, 30 cases of infantile enteritis caused by *E. coli* 0142 . H6 occurred in a second Glasgow hospital (G2). The cases formed three distinct episodes involving three separated cubicle wards. In each episode, the admission of a community-derived case was followed by cross-infection within a ward (Love *et al.* 1972).

Irvine, Scotland, 1970

During December 1970 four babies in an Irvine hospital (Irv 1) developed diarrhoea following the admission of a 6-month-old infant with diarrhoea. All five babies and two members of staff were found to be excreting *E. coli* 0142 . H6 (Roberts *et al.* 1971).

Irvine, Scotland, 1971

In January 1971 cultures of *E. coli* 0142 . H6 were isolated from four infants with diarrhoea in a second Irvine hospital (Irv 2). The brother of a baby who died in hospital Irv 1 following infection with *E. coli* 0142 . H6 had been transferred to hospital Irv 2.

Glasgow, Scotland, 1971

In February 1971 a further outbreak of infantile enteritis affecting ten babies occurred in Glasgow hospital G1. This outbreak followed the admission of a 3-week-old baby with diarrhoea to a cubicle ward. Two other wards were affected following the transfer of patients between wards. *E. coli* 0142 . H6 was isolated from all patients (Kennedy *et al.* 1973).

Belfast, Northern Ireland, 1971

During an outbreak of infantile enteritis in a Belfast hospital in February and March 1971, 14 patients were found to be excreting *E. coli* 0142 . H6. The origin of the outbreak was not known, although *E. coli* 0142 . H6 strains were isolated subsequently from several patients who were admitted to the hospital from the community after developing diarrhoea.

Glasgow, Scotland, 1971

In May 1971 strains isolated from eight infants with diarrhoea in a third Glasgow hospital (G3) were found to be *E. coli* 0142 . H6.

England, 1971

In April 1971 antiserum for the identification of *E. coli* 0142 became widely available from commercial sources. Up until this time no strains of *E. coli* 0142 . H6 had been received from laboratories in England and Wales since the outbreak in Stoke-on-Trent a year earlier. In June 1971 a strain was received from a single case of infantile diarrhoea in Leeds. Later in 1971 strains of *E. coli* 0142 . H6 were isolated from patients in Manchester, Liverpool and Pontefract.

*Other countries**Indonesia, 1960*

In 1960 *E. coli* 0142 . H6 was isolated from cases of infantile enteritis in Indonesia (Ørskov *et al.* 1960).

Mexico, 1965

In 1965 *E. coli* 0142 . H6 caused an outbreak of diarrhoea amongst premature babies (Olarate & Ramos-Alvarez, 1965).

Eire (Dublin), 1971

Between April and October 1971 all patients under 6 years of age who were admitted to a Dublin hospital were screened for the presence of *E. coli* 0142. H6. Of 70 patients with diarrhoea on admission, 13 were excreting *E. coli* 0142. H6. During the trial period 49 of 64 patients who developed diarrhoea after admission to hospital were found to be excreting *E. coli* 0142. H6 (Hone *et al.* 1973).

Canada

Two strains of *E. coli* 0142. H6 were isolated in 1972 from the stools of infants with enteritis. No further epidemiological information was available.

United States of America (Arizona), 1975

In 1975 there was a nursery outbreak of enteritis in Arizona, U.S.A. Fifty-nine epidemiologically related patients were symptomatic and *E. coli* 0142. H6 was isolated from all of them (Boyer *et al.* 1975).

RESULTS

Serotyping

All strains were confirmed as members of *E. coli* 0 group 0142 and were shown to possess the flagella antigen H6.

Biochemical characters

All strains of *E. coli* 0142. H6 failed to produce indole, a result found in only 3.7 % of *E. coli* strains (Ewing *et al.* 1972). In other respects they were biochemically typical for *E. coli*.

Drug resistance studies

The drug resistance of strains of *E. coli* 0142. H6 isolated in the U.K. is shown in Table 1 and that of strains isolated in other countries in Table 2.

U.K.

All *E. coli* 0142. H6 isolated in the U.K. were resistant to tetracyclines (T) with a Minimal Inhibitory Concentration (MIC) of 250 µg/ml. Resistance to ampicillin (MIC: 2000 µg/ml) appeared in the small outbreak amongst neonates in Paisley in 1969 and, with the exception of 1 out of 5 strains from the Glasgow hospital G3, all subsequent *E. coli* 0142. H6 isolations were ampicillin-resistant. Strains from the Glasgow hospitals G1 and G3 were resistant to sulphonamides (MIC: > 2000 µg/ml) as were 3 of 6 strains from hospital G2. Streptomycin resistance was observed in 2 strains isolated in Manchester in 1971.

In all *E. coli* 0142. H6 isolated in Britain, T resistance was neither transferable nor mobilizable. Ampicillin (A) and sulphonamide (Su) resistance were specified by independent non-conjugative plasmids. The A plasmid belonged to group 1 of non-conjugative plasmids, but the Su plasmid was compatible with standard plasmids. In the strains from Manchester, streptomycin resistance (S) was encoded

by a conjugative plasmid, compatible with standard plasmids. One of six strains from the Glasgow hospital G2 and the strain isolated in Pontefract carried I_1 plasmids which did not confer drug resistance but had the ability to mobilize the A plasmids.

Other countries

The strain of *E. coli* 0142 . H6 isolated in Indonesia in 1960 was of R-type SuT; those isolated in Mexico in 1965 were of R-type CSSuSpT (C, chloramphenicol; Sp, spectinomycin). The strains from the Republic of Ireland were of R-type AKSSuT (A, ampicillin; K, neomycin-kanamycin), those from Canada, ASSuT, and those from the U.S.A., ASuT.

E. coli 0142 . H6 from Indonesia, Eire, Canada and the U.S.A. all possessed non-transferable T resistance. Strains from Indonesia, Eire and the U.S.A. carried non-conjugative Su plasmids and strains from Eire, the U.S.A. and Canada, non-conjugative group 1 A resistance plasmids. In addition, strains from Eire carried an F_1 plasmid which conferred streptomycin and tetracycline resistance and an I_1 plasmid which coded for kanamycin resistance. Although the strains from Canada were resistant to streptomycin and sulphonamides, neither the transfer nor mobilization of these resistances was detected.

The strains from Mexico carried an F_{II} plasmid which coded for resistance to CSSuSpT. When this plasmid was displaced from the wild-type strain by a F_{II} plasmid with a K resistance marker, the resultant strain was resistant to kanamycin only. Thus it was concluded that strains from Mexico carried a single resistance plasmid which coded for CSSuSpT, and did not possess non-transferable tetracycline resistance.

Molecular studies

The plasmid content of the *E. coli* 0142 . H6 strains was visualized by agarose gel electrophoresis; the results are presented in Tables 1 and 2.

U.K.

All strains from the U.K. carried two large plasmids with approximate MW's of 90 and 72 Md. Strains resistant to ampicillin carried a plasmid of 5.5 Md and those resistant to sulphonamides, a plasmid of 4.0 Md. K12 *nal^r* lines into which these A and Su plasmids had been mobilized carried plasmids of 5.5 and 4.0 Md which corresponded in size to those in the wild-type strains. Thus, in *E. coli* 0142 . H6 from the U.K., A resistance and Su resistance were specified by independent plasmids of 5.5 and 4.0 Md respectively. Four *E. coli* 0142 . H6 from Britain carried additional plasmids. The strain isolated in Pontefract in 1971 carried an I_1 plasmid of 62 Md, as did one of six strains isolated from the outbreak in Glasgow hospital G2. These I_1 plasmids did not confer drug resistance. The two strains from Manchester carried a plasmid of 50 Md which conferred resistance to streptomycin.

Other countries

E. coli 0142 . H6 from Indonesia, Eire and Canada possessed plasmids of 90 and 72 Md. However, strains from the U.S.A. carried a 90 Md plasmid species but not

one of 72 Md. Strains from Indonesia, Eire and the U.S.A. also carried plasmids of about 4.0 Md which coded for Su resistance. Strains from Eire, Canada and the U.S.A also possessed plasmids of about 5.5 Md which conferred A resistance and strains from Eire also carried plasmids of 98 and 65 Md which corresponded to plasmids of the F_I and I_I groups identified in these strains (see above). The F_I plasmids coded for streptomycin and tetracycline resistance and the I_I plasmids for kanamycin resistance. The Canadian strains also possessed plasmids of about 4.2 Md. These plasmids may have conferred resistance to streptomycin and sulphonamides (SSu) but, since SSu resistance was neither transferred nor mobile in these strains, this was not confirmed. Strains from Mexico carried three plasmids, of 95, 44 and 48 Md respectively. A CSSuSpT resistance plasmid of compatibility group F_{II} had previously been identified in these strains (see above), and a single plasmid species which corresponded in size to the 95 Md plasmid in the parent strain was identified in K12 exconjugants into which the F_{II} plasmid had been transferred. Thus, in *E. coli* 0142.H6 from Mexico, drug resistance was conferred by the 95 Md plasmid; as was evident from displacement studies, the plasmids of 44 and 4.8 Md did not confer drug resistance.

DISCUSSION

Strains of *E. coli* 0142 from outbreaks of infantile diarrhoea in several hospitals in Scotland, England and Northern Ireland between 1969 and 1972 had many properties in common: they all possessed the H6 flagella antigen and failed to produce indole in biochemical tests; all strains possessed non-transferable T resistance. With one exception, all strains isolated after 1969 carried non-conjugative ampicillin resistance plasmids and several strains also carried non-conjugative Su resistance plasmids. Plasmid profile studies demonstrated that all strains possessed two plasmids of 90 and 72 Md; those resistant to ampicillin carried an additional plasmid of 5.5 Md and those resistant to sulphonamides, a plasmid of 4.0 Md; in a few strains additional plasmids were also identified, some of which conferred resistance to other drugs.

These findings suggest that the strains of *E. coli* 0142 involved in these outbreaks are closely related and may well represent a single clone. Outbreaks were frequently preceded by the admission from the community of an infant with diarrhoea and it is possible that an *E. coli* 0142.H6 clone spread through communities in different areas of Britain between 1969 and 1972. A possible progenitor was the strain of *E. coli* 0142.H6 isolated in 1967 from a patient in Dundee, Scotland. This strain was resistant to tetracyclines only, but other resistance plasmids were acquired when the strain became disseminated in hospitals and presumably became exposed to selective pressures imposed by the use of antibacterial drugs. For example, ampicillin resistance first appeared in 1969, in the outbreak in Paisley, Scotland and the subsequent use of this drug in infants may have contributed to the establishment of the strain in hospital wards after its introduction from the community. Indeed, on one occasion infants in a hospital ward had been treated with ampicillin for respiratory infections prior to the spread of ampicillin-resistant *E. coli* 0142.H6 by cross-infection, following the admission of an excretor (Love *et al.* 1972). Sulphonamide resistance first appeared

in 1969, in strains from a Glasgow hospital (G1). In Britain, this resistance was subsequently observed only in outbreaks in Glasgow hospitals in 1970 and 1971 and these sulphonamide-resistant strains may represent a subclone which became disseminated in the Glasgow area. Streptomycin resistance plasmids were identified in *E. coli* 0142 . H6 from Manchester; these plasmids had probably been acquired from the normal intestinal flora of a patient as a consequence of antibiotic selective pressure.

The *E. coli* 0142 strains from the outbreaks of infantile enteritis in Indonesia in 1962, in Eire in 1971 and 1972, in Canada in 1972 and in Arizona, U.S.A. in 1975 had certain properties in common with the strains from the U.K. These strains all possessed the H6 antigen and were indole-negative. They all possessed non-transferable T resistance; the strains from Indonesia, Eire and the U.S.A. possessed non-conjugative Su plasmids and strains from Eire, Canada and the U.S.A., non-conjugative A plasmids. The Mexican strains were unique in that, although indole-negative and possessing the H6 antigen, they did not exhibit non-transferable T resistance nor did they carry non-conjugative A and Su plasmids.

Comparisons of plasmid profiles confirmed that the strains from Eire were probably closely related to those which caused outbreaks in the U.K. However these Irish strains had acquired additional plasmids which conferred resistance to kanamycin, and to streptomycin and tetracyclines, possibly as a consequence of the extensive use of antibiotics in the hospital in which they were isolated.

The plasmid profiles of strains from Indonesia, Canada and the U.S.A. had some similarities to those of strains isolated in the U.K. In particular, plasmids of 90 and 72 × Md were identified in the strains from Indonesia and Canada and the strains from the U.S.A. carried a 90 Md plasmid. However, the Indonesian strains carried two additional plasmids of 34 and 26 Md respectively, which did not appear to code for resistance and the American strains were lacking in the 72 Md plasmid. The plasmid profile and plasmid content of the Mexican strains were completely different from those of the strains from the U.K., Eire, Indonesia and the U.S.A. These strains carried a 95 Md group F_{II} resistance plasmid and two other plasmids of 44 and 4.8 Md, which did not code for resistance.

Studies on the pathogenicity mechanisms of *EPEC* have demonstrated the involvement of a 50–70 Md plasmid in the ability of strains to adhere to HEp-2 cells (Baldini *et al.* 1983). Outbreak strains of *E. coli* 0142 . H6 have been shown to possess the ability to adhere to HEp-2 cells (Cravioto *et al.* 1979) and it is possible that the 72 Md plasmid in strains from the U.K., Indonesia, Eire and Canada may correspond to that described by Baldini and her co-workers and this is being investigated. This plasmid was not observed in the strains from Mexico and the U.S.A., and may have been spontaneously lost during storage.

The location of the non-transferable T resistance characteristic of all strains of *E. coli* 0142 . H6, with the exception of those isolated in Mexico, was not determined. If plasmid-mediated, the most likely plasmid species is that of 90 Md. This plasmid was found in all strains except those from Mexico, which did not possess non-transferable T resistance.

The cumulative evidence suggests that outbreaks of infantile enteritis due to indole-negative *E. coli* 0142 . H6 in the U.K. and Eire were caused by a single clone

which was first recognised in Scotland in 1967. Thus the epidemiology of infection with this drug-resistant enteropathogenic *E. coli* strain may be similar to that of certain drug-resistant strains of salmonellae, notably *Salmonella typhimurium* type 66/122 in India (Rowe *et al.* 1980; Frost *et al.* 1982) and *S. wien* in North Africa and Southern Europe (Le Minor, 1972; McConnell *et al.* 1979). In outbreaks caused by these drug-resistant salmonellae, the epidemiology was that of person-to-person spread and hospital outbreaks were frequently preceded by cases in the community (Rangnekar *et al.* 1983; Bhat & Macaden, 1983). Moreover, the plasmid-profile studies demonstrated that *E. coli* 0142. H6 from Indonesia, the British Isles, the U.S.A. and Canada may have been derived from a common ancestor. Subsequent evolutionary diversification may have resulted from the loss and acquisition of plasmids. Apart from being indole-negative and carrying the H6 antigen, the 0142 strains from Mexico do not appear to be related to those from the U.K. and other parts of the world.

This study has demonstrated how biochemical and serological techniques for strain identification may be supplemented by the genetic and molecular characterization of plasmids. The results have shown that apparently unrelated hospital outbreaks caused by particular EPEC serotypes may be part of a single epidemic occurring over a wide geographical area.

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