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Plasmid pattern analysis of natural bacterial isolates and its epidemiological implication

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SUMMARY

Natural isolates of *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Providencia stuartii* were analysed to determine their plasmid content. This data allowed the identification of nosocomial strains of *K. pneumoniae* and *P. stuartii* and helped in the differentiation of epidemic strains of *E. coli* 0111 and *S. typhimurium*. Phenotypically similar isolates of *S. typhimurium* could be shown to be of independent origin using plasmid pattern analysis. The dissemination of a particular plasmid through different strains of *S. typhimurium* resulted in a simulation of a very widely distributed epidemic strain, because the plasmid interfered with the phage type of its host strain in addition to determining resistance properties. Plasmid pattern analysis disclosed two independently existing but interacting epidemic processes: a bacterial 'epidemic' strain may become disseminated over a large territory and may predominate there for a long time; a single plasmid, however, may also become distributed through many different bacterial strains and may spread over a large territory. Plasmid pattern analysis provides a valuable and universal epidemiological laboratory method.

INTRODUCTION

The presence of extrachromosomal DNA elements in natural isolates of bacteria is a commonly observed feature (Christiansen *et al.* 1973; Møller *et al.* 1976). It has been shown that many compatible plasmids may stably be maintained by one bacterial host cell (Barth, Richards & Datta, 1978). This is ensured by sophisticated mechanisms encoded in part by the plasmids themselves. There are several events that may result in a change of the plasmid content of a bacterial cell: the uptake of a new plasmid by conjugation or transduction, recombination between plasmids, or the spontaneous loss of a plasmid. Such events, however, are very rare compared to the normal reproduction of bacterial cells, that gives rise to identical progeny (clones). Thus the plasmid DNA content of a particular bacterial clone is mainly influenced by factors associated with plasmid biology. Under natural conditions bacteria must always respond to the given environmental situation. Since plasmids contribute to a major extent to the bacterial variation which is necessary for adaptation (Reanney, 1976), the plasmid content of a bacterial population in natural environments must be expected to be mainly influenced by ecological factors.

We analysed the plasmid patterns of a number of bacterial isolates belonging to different genera, which had previously been shown to be epidemiologically related by several 'markers' such as phage type or serotype. The plasmid pattern analysis consisted of the detection of extrachromosomal DNA in the bacterial wild type strains and additionally the genetic and molecular characterization of single plasmids transferred from the wild type strains to *Escherichia coli* K12. The investigations revealed that genetic relatedness between independent bacterial isolates may be recognized on the basis of their plasmid patterns and, in addition, that distinct plasmid species may be disseminated throughout different bacterial strains due to their own epidemic process.

MATERIALS AND METHODS

Bacteria

The various bacterial isolates subjected to plasmid pattern analysis are listed in Table 1. The sections of Table 1 each contain strains that appeared to be related on the basis of the epidemiological, clinical and microbiological data. The *Salmonella typhimurium* strains IE863–IE882 share a distinct phage pattern termed 'n.c. 1/72' (nc./n.c./204 according to the typing schemes of Felix and Callow/Lilleengen/Anderson – see Kühn *et al.* 1982). They have been selected in order to represent the plasmid profiles of more than 100 strains analysed with respect to their plasmid patterns. *S. typhimurium* n.c. 1/72 strains predominated in the G.D.R. between 1972 and 1980 causing food poisoning and severe gastroenteritis in animals and men (Kühn *et al.* 1982). The strains described in this paper were isolated from faeces of men except of strains IE875 and IE882 which originated from faeces of cattle. The *S. typhimurium* strains IE883–IE890 from septicaemia in children represent more than 100 isolates from different countries, that were identified in faecal samples as well as in blood cultures particularly from sick children (Pokrovski *et al.* 1982). They share the phage type 208 according to the Anderson-scheme (Anderson *et al.* 1977*b*) but are untypable using the phage typing sets of Felix and Callow (Callow, 1959) or Lilleengen (1948). The majority of these strains are of the same biochemotype and appear similar in their multiple drug resistance phenotype. Moreover, these *S. typhimurium* isolates causing septic diseases rather than gastroenteritis closely resemble those from the Middle East described by Anderson *et al.* (1977*a*). The *E. coli* strains IE913–IE930 represent 55 isolates from faecal samples of newborn babies suffering from diarrhoea and severe enteritis. The strains from different localities of the G.D.R. were identified and serotyped as 0111 : H2 by Dr H. Steinrück, The National Reference Laboratory of *E. coli* Infections, Berlin, G.D.R., and communicated to us for plasmid analysis. The *Providencia stuartii* strains IE897–IE903 represent 25 isolates which appeared to be identical with respect to their common multiple drug resistance phenotype and their biotype 5 (according to Penner, Hinton & Hennessy, 1975). These strains were isolated in various urological hospitals from clean-catch-urine specimens of patients suffering from post operative urinary tract infections (bacteriaemia and pyelonephritis). They were identified and biotyped by Dr K. Ziesché, Institut für Experimentelle Epidemiologie, Wernigerode, G.D.R., and Dr R. Fischer, Bezirkshygieneinstitut, Magdeburg, G.D.R., and communicated to us for plasmid

Table 1. *Origin and properties of the bacterial strains analysed*

Strain	Place	Origin: Disease	Origin: Date	Antibiogramme*
		Salmonella typhimurium n.c. 1/72		
IE863	Wismar	Gastroenteritis	1972	TcSu
IE867	Gera	Gastroenteritis	1974	TcSu
IE870	Bad Elster	Gastroenteritis	1980	TcSu
IE875	Angermünde	Gastroenteritis	1978	TcSuCmSm
IE877	Dresden	Gastroenteritis	1975	TcSuCmSmAp
IE882	Wittenberg	Gastroenteritis	1978	TcCmSmSuApKm
		Salmonella typhimurium 208		
IE883	Moscow, USSR	Septicaemia	1978	TcCmSmSuAp
IE885	Kecskemet, Hungary	Septicaemia	1978	TcCmSmSuAp
IE886	Erfurt, GDR	Septicaemia	1978	TcCmSmSuApKm
IE887	Armenia, USSR	Septicaemia	1976	TcSmAp
IE888	Moscow, USSR	Septicaemia	1979	TcCmSmSuApKm
IE890	Ulan-Bator, Mongolia	Septicaemia	1980	TcCmSmSuApKm
		Escherichia coli (0111:H2)		
IE913	Schwerin	Enteritis	1979	TcCmSmSuApKm
IE914	Tangermünde	Enteritis	1979	TcCmSmSuApKm
IE915	Rangsdorf	Enteritis	1980	TcCmSmSuApKm
IE929	Schwedt	Enteritis	1980	TcCmSmSuApKm
IE919	Budapest, Hungary	Enteritis	1980	TcCmSmSuApKm
IE917	Eberswalde	Enteritis	1980	TcCmSmSuApKm
IE918	Eberswalde	Enteritis	1980	TcCmSmSuApKm
IE930	Eberswalde	Enteritis	1981	TcCmSmSuApKm
		Providencia stuartii		
IE897	Halberstadt	Urinary infection	1980	TcCmSmSuApGmTp
IE898	Dessau	Urinary infection	1980	TcCmSmSuApGmTp
IE899	Erlabrunn	Urinary infection	1980	TcCmSmSuApGmTp
IE900	Magdeburg	Urinary infection	1980	TcCmSmSuApGmTp
IE901	Wernigerode	Urinary infection	1980	TcCmSmSuApGmTp
IE902	Osterwieck	Urinary infection	1980	TcCmSmSuApGmTp
IE903	Zwickau	Urinary infection	1980	TcCmSmSuApGmTp
		Klebsiella pneumoniae		
IE907	Cottbus	Meningitis	1980	TcSmSuAp
IE908	Cottbus	Septicaemia	1980	TcSmSuAp
IE909	Cottbus	Septicaemia	1980	TcSmSuAp
IE910	Cottbus	Meningitis	1980	TcSmSuAp
IE911	Cottbus	Septicaemia	1980	TcSmSuAp
IE912	Cottbus	Meningitis	1980	TcSmSuAp
IE939	Halle	Meningitis	1975	TcCmSmSuAp
IE940	Halle	Conjunctivitis	1975	TcSmSuAp
IE941	Halle	Pneumonia	1975	TcSmSuAp
IE942	Halle	Enteritis	1975	TcSmSuAp
IE943	Halle	Rhinitis	1975	TcSmSuAp
IE944	Halle	Enteritis	1975	TcSmSuAp

* Drug Resistance Symbols: Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline; Tp, trimethoprim.

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Table 2. *Reference plasmids for the molecular weight determination by agarose gel*

Plasmid	Md	Genetic characters	Source	Reference
pMB8	1·87	Col-imm	P. Bennett, Bristol	Meyers *et al.*, 1976
RSF1010	5·0	SmSu, IncQ	P. Bennett, Bristol	Guerry *et al.* 1974
RSF2124	7·4	ColE1::Ap	P. Bennett, Bristol	So *et al.* 1975
R388	21·5	TpSu, IncW	P. Bennett, Bristol	Bennett & Richmond, 1976
R6K	26	SmAp, IncX	N. Datta, London	Kontomichalou *et al.* 1970
RP1	39	TcApKm, IncP-1	P. Bennett, Bristol	Burkardt *et al.* 1978
R1drd19K-1	47	Km, IncFII	P. Bennett, Bristol	Beard & Conolly, 1975
R100-1drd	60	TcCmSmSu, IncFII	P. Bennett, Bristol	Ohtsubo *et al.* 1978
R40a	96	SuApKm, IncC	N. Datta, London	Chabbert *et al.* 1972
R27	112	Tc, IncH-1	N. Datta, London	Grindley *et al.* 1973
R478	166	TcCmKm, IncH-2	N. Datta, London	Hedges *et al.* 1975; Taylor & Grant, 1977

analysis. The *Klebsiella pneumoniae* strains IE907–IE912 originated from six newborn babies of one children's ward, which suffered from septicaemic illness and meningitis. They were isolated from blood cultures and cerebrospinal fluid, respectively and appeared identical in their antibiogrammes. The suggestion of hospital acquired infections by one *K. pneumonia* strain was the indication for Dr R. Siegmund, Bezirkshygieneinstitut, Cottbus, G.D.R., to send the strains to us for plasmid analysis. The *K. pneumoniae* strains IE939–IE944 exhibiting the same drug resistance phenotype also were assumed to be involved in hospital acquired infections in the children's ward of another hospital. They were isolated from cerebrospinal fluid, eye swab, respiratory secretions, faecal samples, nose swab, and a faecal sample, respectively, and characterized by Dr C. Höhne, Institut für Mikrobiologie und Epidemiologie, The Martin-Luther-University, Halle, G.D.R.

The antibiogrammes of all strains (see Table 1) were determined using a standard agar diffusion technique.

Plasmids

Reference plasmids used for incompatibility testing were those listed in Tschäpe & Tietze (1980, 1981). The plasmids employed as standards for the estimation of molecular weights by agarose gel electrophoresis are listed in Table 2.

Genetic characterization of conjugative plasmids

Transfer by conjugation into *E. coli* K12 and the subsequent characterization of plasmids by fertility inhibition of the F sex plasmid, pilus type and incompatibility behaviour were performed as described by Tschäpe, Tietze & Koch (1981). The plasmid pMLU1506 was sent to us in an *E. coli* K12 host already by Dr C. Höhne, Halle. The differentiation of IncH-1 and IncH-2 plasmids on the basis of their genetic properties will be published elsewhere (Tschäpe *et al.*, manuscript in preparation). IncI-4 is our preliminary designation for a new subgroup of the I-complex. FIme plasmids have been characterized by their incompatibility with IncFI reference plasmids, by a phage inhibition property conferred on their host cells (*E. coli*, *S. typhimurium*), and by their size of about 96 Md. They thus appear closely related to the FIme reference plasmid TP181 described by Anderson *et al.* (1977*a*).

Isolation of plasmid DNA

Two different methods were employed for the detection of extrachromosomal DNA in bacterial strains. A procedure involving alkaline lysis of the bacterial cells allowed the detection of even very large plasmids (monitored by isolating the reference plasmid R478 from *E. coli* K12). The method was as described by Kado & Liu (1981), except that a Tris-borate buffer gel was used for electrophoresis. Plasmid DNA up to 100 Md in size could be detected following the isolation protocol described by Meyers *et al.* (1976).

An initial screening for plasmid DNA and an approximate estimation of the size were carried out using a single colony lysis method as described by Eckhardt (1978). For a more precise molecular weight determination, the DNA of plasmids transferred to *E. coli* was prepared according to Meyers *et al.* (1976), or by the procedure of Kado & Liu (1981), if a large plasmid was involved.

Restriction enzyme analysis

Restriction enzyme analysis was carried out using plasmid DNA prepared according to Meyers *et al.* (1976). This procedure always gave a relatively higher yield of the smaller DNA species when strains containing several plasmids were analysed. Appropriate dilutions of such plasmid DNA preparations resulted in the 'purification' of the smaller DNA species. Therefore, using diluted samples, the restriction enzyme cleavage patterns of smaller plasmids could be evaluated immediately from whole cell plasmid DNA preparations of the original wild type strains. In order to perform restriction enzyme analysis, the ethanol precipitated DNA was redissolved in TM buffer (100 mM Tris, 10 mM-$MgCl_2$, pH 7·5). Any particular requirements for the reaction of the restriction enzymes *Eco*RI, *Bam*HI, *Pst*I or *Hind*III were added from concentrated solutions to 40 μl of DNA in TM buffer before 5 μl (between 10 and 20 units) of the respective enzyme were added. The basic reaction mixtures contained the following additions: for *Eco*RI, 40 mM-NaCl; for *Pst*I, 10 mM 2-mercapto ethanol; for *Hind*III, 70 mM-NaCl and 10 mM 2-mercapto ethanol. The reactions were carried out at 37 °C for 3 h. Samples were then heated to 65 °C for 10 min and 10 μl of a bromophenol blue solution containing 0·07 % bromophenol blue, 100 mM-Na_2EDTA and 40 % sucrose were added prior to electrophoresis.

Differentiation of oc and ccc conformations of smaller plasmids

The open circular (oc) conformations of larger plasmids were never observed in agarose gels. Plasmids smaller than 10 Md, however, were always obtained in the oc conformation in addition to the predominant covalently closed circular (ccc) conformation, when the DNA was prepared as described by Myers *et al.* (1976). In order to distinguish between these conformations, a DNA sample was extracted with acid phenol (pH 4·0) prior to electrophoresis. This procedure was found to remove oc and linear DNA molecules but not those present in the ccc conformation (Zasloff, Ginder & Felsenfeld, 1978), thus allowing the identification of the ccc conformation in the agarose gels.

Agarose gel electrophoresis and molecular weight determination

Electrophoresis was carried out in vertical slab gels (12 × 18 × 0·3 cm) of 0·7 or 1·0 % agarose in Tris borate buffer (89 mM Tris, 89 mM boric acid, 2·5 mM-Na_2EDTA, pH 8·0). DNA samples were mixed with 10 μl of a bromophenol blue solution prior to application to the gel. Electrophoresis was continued until the blue dye reached the bottom of the gel (about 3 h at 40 mA with small plasmids or with restriction enzyme-treated plasmid DNA). Gels were stained with ethidium bromide (0·5 μg/ml) and photographed in u.v. light.

Molecular weights were calculated from the mobility of the plasmid DNA in agarose gel electrophoresis as described by Meyers *et al.* (1976). Values obtained with wild type strains are only approximate because the four reference plasmids (R100, R6K, RSF1010, pMB8; see Table 2) usually included into such gels do not cover the whole range of molecular weights. Values determined with plasmids transferred to *E. coli* K12 were more precise, since all appropriate reference plasmids of similar size (see Table 2) were run in the same gel. Molecular weights of restriction enzyme-gene-rated fragments were determined using *Eco*RI or *Hin*dIII digested λ *cI 875* DNA as a standard (Thomas & Davies, 1975; Gottesman & Adhya, 1977). Additionally, RSF2124 DNA, cleaved once by *Eco*RI to generate a 7·4 Md linear DNA molecule (So, Gill & Falkow, 1975) was included as a reference for the molecular weight determination of restriction fragments.

RESULTS

The plasmid patterns of natural bacterial isolates belonging to different genera were studied in two ways: (i) the plasmid DNA contents of the various groups of wild type strains are listed in Table 3 together with the approximate molecular weights (Md-values) of the extrachromosomal DNA species observed; (ii) the molecular and genetic parameters of single plasmid species transferred by conjugation into *E. coli* K12 strains are summarized in Table 4.

Salmonella typhimurium *of phage type* '*n.c.* 1/72'

The numbers and sizes of extrachromosomal DNA species detected in *S. typhimurium* strains of phage type 'n.c. 1/72' are listed in the first section of Table 3. The presence of two DNA species (65 and 3·6 Md) in all but one (IE882) of these strains reflects the situation found with more than 100 *S. typhimurium* isolates of this phage type studied (Tietze *et al.* 1983). The respective plasmids of each strain could be shown to be identical with respect to several criteria. The 65 Md plasmids have been transferred into *E. coli* K12 using derivatives labelled with the Ap transposon Tn1821 (Tietze, Prager & Tschäpe, 1982; Tschäpe *et al.* manuscript in preparation). The plasmid pIE342 in Table 4 represents this type of plasmid and codes for LT enterotoxin synthesis. The small 3·6 Md plasmid appeared to be cryptic since no properties could be attributed to it by transduction, transformation and mobilization experiments using Tc or Sa for selection (data not shown). Comparative analysis was therefore performed by the digestion of suitable plasmid DNA preparations with various restriction enzymes (see Materials and Methods). This revealed the cleavage pattern of 1 *Eco*RI site 1 *Bam*HI site, 3 *Pst*I sites and

Table 3. *Plasmid DNA content of bacterial wild type strains*

Strain	Plasmid DNA species (Md)*										
	Salmonella typhimurium (n.c. 1/72)										
IE863			65				3·6				
IE867			65				3·6				
IE870			65			22	3·6				
IE875	100		65				3·6				
IE877	100		65				3·6				
IE882			75		36			1·9			
	Salmonella typhimurium (208)										
IE883		96				4·5	3·2		2·0		
IE885		96				4·5	3·2		2·0		
IE886		96				4·5	3·2		2·0		1·1
IE887		96						3·0			
IE888		96				4·7			2·0		
IE890		96	60		34	5·0					
	Escherichia coli (0111:H2)										
IE913		85	65	48					2·5	1·4	
IE914			65	48					2·5	1·4	
IE915			60	45	40	29	4·8			1·5	1·1
IE929			60	45	40	29	4·8			1·5	1·1
IE919			60	45	40	29	4·8			1·5	1·1
IE917			60	45	40	29	4·8			1·5	1·1
IE918			60	45	40	29				1·5	1·1
IE930			60	53	40		4·8			1·5	1·1
	Providencia stuartii										
IE897	100				38						
IE898	100				38	29					
IE899	100				38						
IE900	100				38						
IE901	100				38						
IE902	100				38						
IE903	100					28	22		2·8		
	Klebsiella pneumoniae										
IE907	130	100					5·0			2·1	
IE908	130	100							3·0	2·1	
IE909	130	100					5·0		3·0	2·1	
IE910	130	100							3·0	2·1	
IE911	130	100							3·0	2·1	
IE912	130	100							3·0	2·1	
IE939			60				7·0	3·6	3·1	2·5	
IE940		90	83		55			3·6	3·1	2·5	
IE941	120				55			3·6	3·1	2·5	
IE942			60		55			3·6	3·1	2·5	
IE943					55			3·6	3·1	2·5	
IE944					55					2·4	

* The values are approximate only (see Materials and Methods). For some plasmids more exactly determined Md (Megadalton) of values are given in Table 4.

no *Hind*III site which was probably identical for the 3·6 Md plasmid of all strains studied. From this we conclude that the molecular structure of these plasmids was identical. Plasmid DNA species of other sizes were occasionally present in some strains of this phage type (e.g. IE870 in Table 3). Among these a 100 Md DNA

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species was associated with the multiple drug resistance phenotype of the respective host strains. After conjugative transfer into *E. coli* K12 it was identified as an IncH-1 R plasmid (pIE531 and pIE535 in Table 4). Their particular properties suggest these plasmids to be of clonal rather than of independent origin. Thus it is tempting to assume the multiple drug resistant 'n.c. 1/72'-isolates to be a true subclone, that derived from the original TcSa resistant *S. typhimurium* strain by the acquisition of a particular R plasmid.

In contrast, the multiple resistant strain IE882 does not contain this IncH-1 R plasmid. Since the plasmid DNA content of IE882 differs from that of the other 'n.c. 1/72'-strains, one must consider it to be from a different source. An R plasmid could be isolated from this strain (pIE473 in Table 4), that represented a new type of 'I-like' plasmid. Beside the determination of multiple drug resistance, this plasmid exhibited a phage inhibition property that interfered with the propagation of the bacteriophages of the typing set employed for phage typing. Rabsch *et al.* (1982) demonstrated that some phage types of *S. typhimurium* could be converted to the 'n.c. 1/72' phenotype by the acquisition of plasmid pIE473. This phage type of *S. typhimurium* can therefore be encoded by different genetic determinants. Another example has been described by Willshaw *et al.* (1980).

Salmonella typhimurium *of phage type* 208

The plasmid DNA patterns of *S. typhimurium* 208 strains are listed in section 2 of Table 3. The presence of four plasmid DNA species (96, 4·5, 3·2 and 2·0 Md) in each of the strains IE883, IE885 and IE886 provided a strong argument in favour of a common origin of these isolates. The strains IE887, IE888 and IE890 differed considerably from the IE883 type strains and from each other with respect to their plasmid DNA content. This excluded any close genetic relationship between them. All of these strains however, revealed the presence of a 96 Md plasmid DNA species that could be identified as representing a particular IncFI subgroup (see pIE515 and pIE523 in Table 4) termed FIme (Anderson *et al.* 1977*a*). These plasmids were shown by a number of properties to be genetically closely related, whereas their differences with respect to the drug resistance determinants were considered to be nonspecific for a particular plasmid species (Tschäpe & Tietze, 1981). After conjugative transfer to several *S. typhimurium* recipients with different phage types, the very pronounced phage inhibition capacity of this FIme plasmid type (see Anderson *et al.* 1977*a*) resulted in the expression of a common phage type by the new host irrespective of the former phage type (Rabsch *et al.* manuscript in preparation). One can conclude, therefore, that the phage type of the obviously non-related *S. typhimurium* 208 strains is due to the carriage of a particular plasmid of the pIE523 type (see Table 4).

Escherichia coli *0111: H2 with a multiple drug resistance phenotype*

The plasmid DNA patterns of *E. coli* 0111 strains are listed in section 3 of Table 3. Two basically different types may be distinguished with respect to the pattern of DNA species present. Minor variations occurred even within strains of the same type (e.g. IE917, IE918 and IE930 in Table 3), but the basic pattern of DNA species seemed to be similar enough to put forward the hypothesis of common derivation. The conclusion drawn from the plasmid DNA patterns was supported

by the results of the more detailed characterization of some plasmids isolated from these strains. The *E. coli* 0111 type represented by IE913 or IE914 was characterized by the presence of an IncFI R plasmid (pIE565 in Table 4) that was responsible for the multiple drug resistance phenotype. The other *E. coli* 0111 type harboured a plasmid belonging to a new incompatibility group, IncZ (Tschäpe & Tietze, 1981), represented by pIE570 in Table 4. Additionally, typical exconjugants were obtained from these strains after matings with *E. coli* K12, that contained a 60 Md and a 40 Md plasmid DNA species. Phage P1 transduction experiments (data not given) revealed the 60 Md plasmid DNA species to be a 'F-like' sex plasmid, whereas the 40 Md DNA species appeared as a nonautotransferable R plasmid conferring the multiple drug resistant phenotype to its host. Thus the similar R^+ phenotypes of the genetically nonrelated *E. coli* 0111 strains were caused in two different ways.

Providencia stuartii *with a multiple drug resistance phenotype*

A number of providence isolates from patients of various urological hospitals were subjected to plasmid pattern analysis (section 4 in Table 3). A very large plasmids DNA and a smaller 38 Md DNA species were common to all providence isolates except strain IE903, which contained a large plasmid DNA together with several others, but not the 38 Md DNA species. After matings with *E. coli* K12 an IncC plasmid encoding Su resistance (pIE408 in Table 4) was identified as corresponding to the large plasmid DNA present in all of the providence strains. No transfer of the 38 Md plasmid DNA species was achieved into *E. coli* K12 by conjugation, transduction or mobilization experiments using appropriate antibiotics for selection. The digestion of sutable plasmid DNA preparations of these strains (see Materials and Methods) with the restriction enzyme *Eco*RI allowed the identification of six restriction fragments identical in size from each of these plasmid DNA species. This identical cleavage pattern strongly suggested that the 38 Md plasmid DNA species from each providence strains studied was identical. The presence of two distinct plasmids in each of the respective providence isolates makes it very likely, that the bacterial host strains are of common descendence. Strain IE903, however, was distinguished from the other strains on the basis of its different plasmid pattern, even though it harboured the pIE408 type plasmid.

Klebsiella pneumoniae *with Tc Sm Su Ap resistance phenotype*

K. pneumoniae isolates were collected from children's wards of two different hospitals (section 5 and 6 in Tables 1 and 3 respectively), that resembled each other with respect to their resistance patterns. Strains isolated from nosocomial infected children of one of these hospitals carried several different plasmid DNA species (section 5 of Table 3). Whereas two large plasmids were present in all of the strains, some variation occurred with respect to the smaller DNA species. After conjugative transfer into *E. coli* K12, one of the large plasmid DNA species from each of the strains could be identified as an IncC R plasmid (pIE564 in Table 4). The very similar plasmid pattern of these independent Klebsiella isolates supported the hypothesis of one strain being responsible for the nosocomial infections in the children's ward of the hospital at Cottbus.

The Klebsiella isolates from another hospital at Halle (last sections of Tables 1

Table 4. *Characteristics of the conjugative plasmids in* E. coli *K12 J53, which were isolated from the patient strains described in this paper*

Plasmid	Origin	Md*	Plasmid determined bacterial functions†	Genetic properties† of the plasmids Inc	Fi(F)	Dps
pIE342	IE863	63	Enterotoxin	FI	+	M13
pIE531	IE875	105	TcCmSmSu	H-1	+	−‡
pIE535	IE877	105	TcCmSmSuAp	H-1	+	−‡
pIE473	IE882	75	TcCmSmSuApKm, Phi	I-4	+	Ifm
pIE523	IE888	96	TcCm, Phi	FIme	+	M13
pIE515	IE887	96	Tc, Phi	FIme	+	M13
pIE565	IE914	65	TcCmSmSuApKm	FI	+	M13
pIE570	IE915	60	TcSmSu	Z	−	−
pIE408	IE901	110	Su	C	−	−
pIE564	IE907	100	TcSmSuAp	C	−	−
pMLU1506	IE941	53	TcSm	D	−	M13
pIE568	IE939	58	TcSmAp	D	−	M13
pIE569	IE939	60	CmSmSuAp	FII	+	M13

* The molecular weights (in Megadalton – Md) are averaged from 3 independent estimations in agarose gel electrophoresis as described in Materials and Methods. Values given here are more exactly determined than those symbolizing the distinct plasmid DNA species in Table 3, since more appropriate reference plasmids have been included in the gels (see Materials and Methods).

† The abbreviations and genetic symbols used are those of Datta (1979). Drug resistance symbols: Ap, ampicillin; Cm, Chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracyclines; Tp, trimethoprim. Genetic symbols: Inc, incompatibility; Fi, fertility inhibition; Dps, donor phage specificity for the phages indicated; Col-imm, colicin immunity; Phi, phage inhibition; Md, megadalton.

‡ Temperature sensitive (37 °C) transfer system.

and 3) also carried several different plasmid species, but no similarity was seen to the strain described above. The common pattern of four plasmid DNA species (55, 3·6, 3·1 and 2·5 Md) suggested a general relationship between strains IE931, IE932, IE933, IE934 and IE935, even though they differed from each other with respect to at least one plasmid DNA species. IE936 is included here as an example of another group of Klebsiella isolates from this hospital, phenotypically indistinguishable from the ones described here, that revealed a clearly different plasmid pattern. The only plasmid present in all strains was the 55 Md IncD plasmid pMLU1506 (Table 4). pIE568 (Table 4) from strain IE931 could be derived from pMLU1506 by the uptake of an Ap transposon from the R plasmid pIE569. Thus, beside a prevalence of certain nosocomial strains, this particular R plasmid is spread through many Klebsiella strains within one hospital.

DISCUSSION

The presence of extrachromosomal DNA-elements in natural bacterial isolates has been used to obtain information on the ecology and epidemiology of bacteria (Sewberg, Tompkins & Falkow, 1981; Willshaw *et al.* 1980; McHale, Keane & Dougan, 1981; Richards, Hughes & Datta, 1981). This assumes that the particular set of plasmids maintained by a certain bacterial clone (its plasmid pattern) remains unchanged, even if the progeny is exposed to different environmental

situations. Altering environmental conditions always requires some adaptational response from the affected bacterial population. Since plasmids are assumed to play a major role in the evolutionary adaptation of bacteria (Reanney, 1976; Tschäpe & Tietze, 1981), the question arises, how stable are plasmid patterns in conditions where independent evolution of the progeny of one original bacterial clone may be occurring.

We applied the plasmid pattern analysis method to a number of bacterial genera of clinical interest. For the respective *S. typhimurium*, *E. coli*, *P. stuartii* and *K. pneumoniae* strains, certain epidemic relationships had been previously postulated on the basis of classical epidemiological markers such as phage types, serotypes or biochemotypes (Table 1, see Materials and Methods). Our data revealed that a basic plasmid pattern may be conserved for many years during numerous generations of independent growth of subclones at distant places. Using plasmid pattern analysis we were able to define and descriminate epidemic strains of *S. typhimurium* and *E. coli* 0111 or nosocomial strains of *P. stuartii* and *K. pneumoniae* respectively. Occasional variants of such strains arose following the uptake of an additional plasmid (e.g. IE870 or IE898 in Table 3), and sometimes a clone resulted which gained some advantageous properties rendering it more fit in responding to certain environmental situations. This obviously happened to the *S. typhimurium* 'n.c. 1/72' epidemic strain, which segregated a subclone (IE875 or IE877 in Table 3) resulting from the acquisition of an IncH-1 R plasmid (pIE531 or pIE535 in Table 4). This subclone, exhibiting a multiple drug resistance phenotype, replaced the original Tc Su resistant variant of the epidemic strain to a considerable extent (Kühn *et al.* 1982).

The predominance of one nosocomial or epidemic strain for a long period of time gives rise to the question as to whether any particular biological properties were responsible. Since the plasmid pattern of such strains appears so highly conserved, one might assume that the harbouring of those plasmids was of some significance for the host's 'property' to occur as epidemic strain. Presumably the production of LT enterotoxin due to the presence of pIE342 enhanced the capability of the epidemic *S. typhimurium* 'n.c. 1/72' strain to cause food poisoning and the presence of an IncC plasmid of the pIE408 type (Table 4) in *P. stuartii*, which conferred an increased level of resistance to the bactericidal action of human serum to its bacterial host (Falkenhagen *et al.* 1983), might account for this clone's frequent association with severe urinary tract infection.

The *S. typhimurium* isolates displaying a phage type 208 shared many phenotypic characteristics, but they comprised several different strains without any close genetic relationships as revealed by their different plasmid patterns (Table 3). The results of plasmid pattern analysis, therefore, require a re-examination of the hypothesis that one epidemic *S. typhimurium* strain was responsible for the outbreak distributed over a large territory in East Europe and Asia (Anderson *et al.* 1977*a*; Pokrovski *et al.* 1982). All of these strains, however, shared the presence of a 96 Md R plasmid of a particular IncFI subgroup (represented by pIE523 in Table 4), which resembled those plasmids termed FIme (Anderson *et al.* 1977*a*; Willshaw, Smith & Anderson, 1978). It is this plasmid type, that appears to have spread through different bacterial strains and to have become distributed over a large territory. The apparent clonal distribution of one *S. typhimurium* strain

(Pokrovski *et al.* 1982; Anderson *et al.* 1977*a*) is simulated by the particular capability of this plasmid type to render different phage types to an identical phenotype.

The collection of data presented here discloses two independent, but interacting, epidemic processes: (i) the spread of one epidemic bacterial strain over a long period of time and a wide territory; (ii) certain plasmids are capable of reaching wide distribution among different bacterial strains. Such epidemic processes can be monitored by plasmid pattern analysis of the respective bacterial isolates. The question remains open as to whether the apparent stability of the plasmid pattern is a real 'property' of a strain, or results from the criteria used for the selection of the bacterial isolates. Nevertheless, the analysis of the plasmid pattern of bacterial strains must be considered as a useful and sensitive epidemiological laboratory method.

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