An investigation of the spread of gentamicin resistance in a district general hospital

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Summary. A cluster of infections caused by gentamicin-resistant gram-negative bacilli in a District General Hospital was investigated. The plasmids coding for gentamicin resistance in the infecting organisms and in isolates obtained from the ward environment and from the faeces of patients and staff were characterised. Six plasmids encoding gentamicin resistance were recognised amongst the organisms causing infection. Two of the plasmids were found in different serotypes of the same species and one plasmid was found in different genera. Three of the plasmids present in the organisms causing the infections were also present in the inanimate environment or in the bowel flora of patients and these also were found in different serotypes of the same species and in different genera.

Introduction

Most of the gram-negative bacilli responsible for infection are part of the normal intestinal flora; others are free-living organisms that have established themselves in moist places in the hospital environment, though it is generally accepted that the hospital environment does not have an important role as a source of gram-negative organisms causing infections in patients (Wenzel *et al.*, 1977; Maki *et al.*, 1982). Many are multi-resistant organisms and are selected by use of various antibiotics. The aminoglycosides remain antibiotics of first choice in the seriously ill infected hospital patient and the occurrence of gentamicin-resistant Enterobacteriaceae is still regarded with concern in the United Kingdom. Their isolation may lead to the introduction of infection-control measures to prevent further dissemination of the strain, although the source of the original isolate is seldom known. Recent understanding of the mechanisms of antimicrobial resistance and the knowledge that resistance can spread between species has led to an increased interest in the spread of resistance amongst microbial populations by spread of plasmids encoding multiple resistances (Rennie and Duncan, 1977; O'Brien *et al.*, 1980; Tompkins *et al.*, 1980; Rubens *et al.*, 1981). Although the source of these plasmids is rarely identifiable, one potential reservoir has been identified as *Klebsiella* strains

(Casewell *et al.*, 1981; Milch and Ke, 1981). Plasmid transfer is known to occur in the human gastrointestinal tract (Anderson *et al.*, 1973; Petrocheilou *et al.*, 1976) but little is known about the acquisition of plasmids in the environment.

We recently investigated a cluster of infections in a district general hospital caused by gentamicin-resistant klebsiellae and infections caused by various gentamicin-resistant gram-negative bacilli which had occurred in the same hospital in the preceding months. We identified several different gentamicin-resistance plasmids distributed amongst the isolates. We therefore decided to examine the ward environment not only for the species in which we had detected those plasmids, but also for similarly multi-resistant organisms and to study their plasmids. The possibility of intra-species plasmid transfer as well as cross infection was considered, and an investigation of the plasmid content of the clinical strains together with those of gentamicin-resistant strains from the inanimate environment and faeces of staff and patients of an affected ward was performed, in the hope of finding environmental reservoirs of the incriminated strains or their plasmids.

Materials and methods

Bacteria

One hundred and four isolates of gentamicin-resistant gram-negative bacilli were used in this study; 37 (table I) were isolated during a period of 6 months (Jun.–Dec., 1982) from infections occurring in 34 patients in eight wards of a district general hospital. The hospital has 450

Received 8 Mar. 1985; accepted 17 Sep. 1985.

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acute beds and is one of primary referral. The remainder of the organisms (table III) were isolated in Dec. 1982 from the environment of one of the wards (A) in which 16 members of staff, 16 patients and 75 inanimate sites were sampled.

Strains used as recipients in resistance-transfer experiments were *Escherichia coli* K12 14R525 F⁻lac⁺Nx^R (Frost *et al.*, 1982) and *E. coli* K12 J62.2 lac⁻pro⁻his⁻trp⁻Rf^R and *Pseudomonas aeruginosa* PU21 Rf^R (Datta *et al.*, 1980).

Media and antimicrobial agents

Commercial culture media used were Nutrient Broth (Oxoid no. 2) (NB), nutrient agar (NB+1.3% w/v New Zealand agar) (NA), Tryptone Soy Broth (Oxoid) (TSB), and Diagnostic Sensitivity Test Agar (Oxoid) (DST) to which lysed horse blood 5% v/v was routinely added. Antibiotics were obtained from commercial sources: amikacin, carbenicillin, chloramphenicol (Sigma); ampicillin (Beecham); gentamicin, cefotaxime (Roussel); cephadrine (Von Heyden); kanamycin, nalidixic acid (Winthrop); rifampicin (Ciba); streptomycin (Glaxo); trimethoprim, sulphamethoxazole (Burroughs Wellcome); tetracycline (Cyanamid) and tobramycin (Eli Lilly).

Maintenance and growth of bacteria

Acinetobacter sp. and *Pseudomonas* sp. other than *P. aeruginosa* were grown at 30°C, all others at 37°C, in NB and maintained at room temperature on NA slopes.

Isolation of bacteria from the ward environment

Swabs from inanimate objects and faecal samples (1 g) from staff and patients in ward A were cultured in TSB containing sodium deoxycholate (BDH) 0.05% w/v and gentamicin 4 µg/ml, at 37°C, overnight, to select for growth of gentamicin-resistant and moderately-resistant gram-negative bacteria. Colonies were isolated on the same medium solidified with New Zealand agar 1.3% w/v. Clones were selected on the basis of morphology and purified, then characterised by standard methods. *Klebsiella*, *P. aeruginosa* and multi-resistant *E. coli* isolates were further characterised by serological typing. Isolates were distinguished by species, type, source and antimicrobial sensitivity.

Antibiotic-sensitivity testing

Cultures were screened by a break-point method for resistance to a wide range of antibiotics, chosen to reflect various resistance mechanisms. Bacteria were grown in NB at 37°C for 18 h, diluted in saline and c. 10⁴ cfu inoculated on to paired DST plates containing two concentrations of each antibiotic (µg/ml): ampicillin 4,32; carbenicillin 16,64; cephadrine 8,32; cefotaxime 2,16; chloramphenicol 2,8; streptomycin 4,16; gentamicin 1,8; kanamycin 4,16; amikacin 8,32; tobramycin 2,8; tetracycline

4,16; sulphamethoxazole 16,64; and trimethoprim 1,8. Cultures that grew on plates containing both concentrations of an antibiotic were recorded as resistant, those that grew on the lower concentrations only were recorded as moderately resistant and those that grew only on the control plate containing no antibiotic were recorded as sensitive.

Minimum inhibitory concentrations (MICs) of gentamicin were determined similarly on DST containing a series of doubling concentrations of antibiotic. The MIC was the lowest concentration that prevented growth after overnight incubation.

Transfer of gentamicin resistance

Potential donor strains were tested for sensitivity to nalidixic acid 40 µg/ml and rifampicin 100 µg/ml. Sensitive donors were mated with nalidixic acid-resistant recipients and nalidixic acid-resistant donors with rifampicin-resistant recipients by mixing donors and recipients grown in NB for 18 h in 1:5 ratios (v:v) then incubating statically for a further 6 h at 37°C. Dilutions were then plated out and transconjugants selected on DST containing gentamicin 20 µg/ml plus either nalidixic acid 40 µg/ml or rifampicin 100 µg/ml to select against growth of the donor. Plates were incubated for 48 h at 37°C. A minimum of 10 clones were re-isolated on the same medium to ensure purity, grown in non-selective medium and tested for their antibiotic sensitivity to determine co-transferred resistances. The frequency of gentamicin-resistance transfer was calculated as the number of resistant transconjugants divided by the number of donor organisms. The ability of transconjugants themselves to transfer resistance was tested in a similar manner to determine whether or not plasmids were self-transferable.

Screening for presence of plasmid DNA

Plasmids were detected by agarose-gel electrophoresis by the method of Birnboim and Doly (1979).

Characterisation of plasmids

The approximate size of each plasmid was estimated from its migration in agarose gel relative to standards of known size. Plasmids were assigned to incompatibility groups by the methods given in Frost *et al.* (1982). Plasmid-encoded antibiotic resistances and gentamicin MICs were determined by testing for the resistance which they conferred on *E. coli* K12 recipients.

Results

Characterisation of isolates from infections

The isolation of 37 clinical isolates of gentamicin-resistant gram-negative bacilli from 34 patients in a 6-month period was unusual, in that only a single gentamicin-resistant isolate of *Citrobacter freundii*

had been detected in the previous 6 months. Several different species and serotypes were identified and these were grouped according to their place and time of isolation (table I). Most were isolated from urine, but three were isolated from sputum, two

from wounds and one each from bile and blood. They included 20 *Klebsiella* isolates, 18 of which were isolated within 1 month of each other. These were subdivided (table I) into *K. oxytoca* and *K. aerogenes* and were further distinguishable by being

Table I. Characterisation of gentamicin-resistant and moderately-resistant bacteria isolated from infections

Month of isolation	Species	Serotype	Antibiotic resistances*	Gentamicin MIC (μ g/ml)	Source
Ward A					
Jul.	<i>P. cepacia</i>	...	A Cb Cp C ^m S G K Ak To T Su Tm	512	urine
Jul.	<i>C. freundii</i>	...	A Cb Cp ^m C S G K To T Su Tm	16	urine
Jul.	<i>P. aeruginosa</i>	NT	A Cb Cp Ctx C S G K Ak ^m To ^m T Su Tm	512	urine
Sept.	<i>P. aeruginosa</i>	O1	A Cb Cp S G K To T Su Tm ^m	512	bile
Oct.	<i>Ent. cloacae</i>	O9	A Cb Cp C S G K T Su Tm	16	blood
Oct.	<i>Ent. agglomerans</i>	...	A Cb Cp S G K To T Su Tm ^m	64	urine
Nov.	<i>P. aeruginosa</i>	NT	A Cb Cp S G ^m K Ak ^m To ^m Su Tm	8	wound
Nov.	<i>K. oxytoca</i>	K35	A Cb Cp ^m S G K To T Su Tm	256	urine
Nov.	<i>K. oxytoca</i>	K3	A Cb Cp ^m C S G K To ^m T Su Tm	32	urine
Nov.	<i>K. oxytoca</i>	K3	A Cb Cp ^m C S G K To ^m T Su Tm	32	urine
Nov.	<i>K. oxytoca</i>	K3	A Cb Cp ^m C S G K To ^m T Su Tm	32	urine
Ward B					
Dec.	<i>K. aerogenes</i>	K6	A Cb Cp ^m C S G K T Su Tm	16	sputum
Dec.	<i>P. aeruginosa</i>	O1	A Cb ^m Cp ^m C S G K Ak To T ^m Tm ^m	512	sputum
Ward C					
Dec.	<i>K. aerogenes</i>	K2	A Cb Cp ^m C S G K To T ^m Su Tm	32	urine
Ward D					
Jun.	<i>K. oxytoca</i>	NT	A Cb Cp ^m S G K To T ^m Su Tm	128	urine
Oct.	<i>K. aerogenes</i>	K68	A Cb Cp ^m C S G K To T ^m Su Tm	32	urine
Nov.	<i>K. oxytoca</i>	K3	A Cb Cp ^m C S G K To ^m T Su Tm	32	urine
Nov.	<i>K. oxytoca</i>	NT	A Cb Cp ^m S G K To T Su Tm	128	urine
Nov.	<i>K. oxytoca</i>	NT	A Cb Cp ^m C S G K Ak ^m To ^m T Su Tm	16	urine
Nov.	<i>Ent. agglomerans</i>	...	A Cb Cp C S G K To T Su Tm	32	urine
Nov.	<i>Ent. agglomerans</i>	...	A Cb Cp C S G K To T ^m Su Tm	32	urine
Nov.	<i>Ent. agglomerans</i>	...	A Cb Cp S G K Ak ^m To T Su Tm	32	urine
Dec.	<i>K. aerogenes</i>	K2	A Cb Cp ^m C S G K To T ^m Su Tm	32	urine
Dec.	<i>K. aerogenes</i>	K2	A Cb Cp ^m C S G K To T ^m Su Tm	32	sputum
Ward E					
Sept.	<i>C. freundii</i>	...	A Cb Cp C S G K To T Su Tm	128	urine
Nov.	<i>K. oxytoca</i>	NT	A Cb Cp ^m S G K To T Su Tm	128	urine
Nov.	<i>K. oxytoca</i>	K35	A Cb Cp ^m S G K To T Su Tm	128	urine
Nov.	<i>K. oxytoca</i>	K35	A Cb Cp ^m S G K To T Su Tm	256	urine
Nov.	<i>Pr. morgani</i>	...	A Cb Cp C S G K Ak To T ^m Tm	64	urine
Nov.	<i>K. aerogenes</i>	K68	A Cb Cp ^m C S G K To T ^m Su Tm	32	urine
Nov.	<i>Ent. agglomerans</i>	...	A ^m Cp ^m S ^m G ^m K ^m Ak ^m To ^m T ^m Su ^m	8	urine
Dec.	<i>K. aerogenes</i>	K2	A Cb Cp ^m C S G K To T ^m Su Tm	32	urine
Dec.	<i>K. aerogenes</i>	K2	A Cb Cp ^m C S G K To T ^m Su Tm	32	urine
Dec.	<i>K. oxytoca</i>	NT	A Cb Cp ^m C ^m S G K Ak To T Tm ^m	128	urine
Ward F					
Nov.	<i>Pr. morgani</i>	...	Cp ^m C S G K Ak To T Su Tm ^m	64	urine
Ward G					
Oct.	<i>Pr. mirabilis</i>	...	A Cb Cp C S G K Ak ^m To T Su Tm	128	urine
Ward H					
Oct.	<i>Proteus</i> sp.	...	Cp ^m C S G K ^m Ak ^m To T Su ^m Tm ^m	16	wound

NT = non-typable

^m = moderately resistant

* A, ampicillin; Cb, carbenicillin; Cp, cephradine; Ctx, cefotaxime; C, chloramphenicol; S, streptomycin; G, gentamicin; K, kanamycin; Ak, amikacin; To, tobramycin; T, tetracycline; Su, sulphamethoxazole; Tm, trimethoprim.

K-serotype 2, 3, 6, 35, 68 or non-typable (NT). Some evidence of cross infection was suggested, in that three patients in ward A and one in ward D were infected by a *K. oxytoca* type-K3 strain and the latter patient had been transferred to ward D from ward A; two patients in ward E and one in ward A were infected by a *K. oxytoca* type-K35 strain, but there was no record of transfer of these patients between wards; one patient in ward D and one in ward E were infected by a *K. aerogenes* type-K68 strain and the latter patient had been transferred from ward D; and two patients in ward E, two in ward D and one in ward C were infected by a *K. aerogenes* type-K2 strain and the ward C patient and one of the ward E patients had been transferred from ward D. Cross infection did not, however, explain other gentamicin-resistant infections caused by a variety of organisms. All isolates were resistant to gentamicin 8 µg/ml at least, but MICs were in the range 16–256 µg/ml amongst *Klebsiella* isolates and were highest in *P. aeruginosa* and *P. cepacia* isolates. Multi-resistance was common, notably to other aminoglycosides and trimethoprim, though few isolates were resistant to amikacin and only one *P. aeruginosa* isolate was resistant to the third-generation cephalosporin, cefotaxime. Results are detailed in table I. Some similarities in resistance pattern were noted and, therefore, inter- and intra-generic relationships were explored by plasmid analysis.

Gentamicin-resistance plasmids in infecting organisms

Klebsiella isolates accounted for more than half the clinical gentamicin-resistant isolates and these were studied further. They were classified, tested for their ability to transfer gentamicin resistance to *E. coli* K12 and screened for the presence of plasmid DNA. Results are given in table II. Antibiotic-resistance pattern and plasmid content were remarkably similar amongst serologically related isolates and differed amongst non-typable isolates.

K. oxytoca serotype-K3, isolated from four different patients and two different wards, were further related by their resistance to the same set of antibiotics and, with one exception in which the smallest plasmid was missing, by their carriage of four similar-sized plasmids. Transferable gentamicin resistance was present in all and associated with only one of the plasmids (mol. wt 70×10^6). This was transferable at a moderate frequency of 10^{-4} to 10^{-5} and encoded further resistances (A Cb C S G K To^m T Su Tm). It was not, however, capable of self-transfer.

In contrast, *K. oxytoca* serotype-K35 isolates,

though similar in their antibiotic resistances, carried a pair of large plasmids which differed slightly in size between isolates, and none transferred gentamicin resistance.

Amongst the heterogeneous group of non-typable *K. oxytoca*, plasmid carriage differed. Only one isolate transferred gentamicin resistance and this was encoded by a plasmid of mol. wt 70×10^6 , further encoding a similar set of resistances to that encoded by the same sized plasmid carried by serotype-3 isolates, excepting low-level tobramycin resistance.

K. aerogenes serotype-K68 strains were isolated from different wards but were resistant to the same set of antibiotics and two of the three plasmids demonstrated in each isolate were of common size. In both, gentamicin resistance was transferable and encoded by 125×10^6 -mol. wt plasmids. Two plasmid-encoded antibiotic-resistance patterns were identified—one common to both isolates, the other present only in the isolate from ward D. Lack of tetracycline resistance determinants allowed us to characterise the plasmids further according to their incompatibility by use of tetracycline-resistance plasmids of known incompatibility groups and all three were related in belonging to incompatibility group C.

K. aerogenes serotype-K2 strains were isolated from five patients on three wards, but were resistant to the same set of antibiotics. Each carried three plasmids whose sizes were common to four isolates and only one plasmid differed in the fifth. In each isolate gentamicin resistance was transferable and encoded by a 125×10^6 -mol. wt plasmid and this further encoded resistance to the same nine antibiotics (A Cb C^m S G K To Su Tm) encoded by the same-sized plasmid (D) isolated from serotype-K68 isolates. Similarly, in three of the five isolates the plasmid belonged to incompatibility group C.

The single *K. aerogenes* serotype-6 isolate differed from the other *K. aerogenes* isolates not only in being isolated from ward B, but also in its pattern of antibiotic resistances, failure to transfer gentamicin resistance and lack of detectable plasmid DNA.

To determine whether these gentamicin plasmids occurred in other gram-negative bacteria isolated from infections, isolates with the same antibiotic-resistance patterns as those encoded by the plasmids were screened for transferability of gentamicin resistance and plasmid content. Table II shows that from seven such isolates, plasmids, 1–7 in number and $(1.2\text{--}125) \times 10^6$ mol. wt, were detected in five but transferable gentamicin resistance was detected only in one. In this *Enterobacter agglomerans* isolate, transfer of gentamicin resistance was

Table II. Characterisation of plasmids in infecting organisms

Isolate and source	Approximate mol. wt (10 ⁶) of plasmids detected	Frequency of gentamicin resistance transfer*	Gentamicin resistance plasmid				code
			Antibiotic resistances cotransferred†	mol. wt (10 ⁶)	incompatibility group		
<i>K. oxytoca</i> , K3							
Ward A	95 70 65	6.0 × 10 ⁻⁵	A Cb C S G K To ^m T Su Tm	70	—	A	
Ward A	95 70 65 1.5	1.0 × 10 ⁻⁴					
Ward A	95 70 65 1.5	6.0 × 10 ⁻⁴					
Ward D	95 70 65 1.5	4.0 × 10 ⁻⁵					
<i>K. oytoea</i> , K35							
Ward A	120 70	nt					
Ward E	100 70	nt					
Ward E	110 85	nt					
<i>K. oxytoca</i> , NT							
Ward D	NONE	nt					
Ward D	160 105	nt					
Ward D	110 70 1.8 1.5	1.6 × 10 ⁻⁵	A Cb C S G K T Su Tm	70	—	B	
Ward E	140 100	nt					
Ward E	140 100	nt					
<i>K. aerogenes</i> , K68							
Ward D	170 125 5.8	3.3 × 10 ⁻⁴	{ A Cb C G K To Su A Cb C ^m S G K To Su Tm	125	C	C	
Ward E	125 100 5.8	1.7 × 10 ⁻⁶		A Cb C ^m S G K To Su Tm	125	C	D
<i>K. aerogenes</i> , K2							
Ward C	170 125 5.8	8.3 × 10 ⁻⁶	{ A Cb C ^m S G K To Su Tm	125	C	D	
Ward D	125 100 5.8	2.4 × 10 ⁻⁴			C	D	
Ward D	125 100 5.8	2.6 × 10 ⁻⁶			?	E	
Ward E	125 100 5.8	8.9 × 10 ⁻⁵			C	D	
Ward E	125 100 5.8	3.2 × 10 ⁻⁶			?	E	
<i>K. aerogenes</i> , K6							
Ward B	NONE	nt					
<i>P. aeruginosa</i> , NT							
Ward A	15	nt					
<i>C. freundii</i>							
Ward A	120 45 20 18 13 7 1.8	nt					
Ward E	NONE	nt					
<i>P. cepacia</i>							
Ward A	NONE	nt					
<i>Ent. agglomerans</i>							
Ward D	125 110 70 5.0	1.0 × 10 ⁻⁶	A Cb C ^m S G K To Su Tm	110	C	F	
Ward D	125 110 5.0	nt					
<i>Pr. mirabilis</i>							
Ward G	1.2	nt					

NT = non-typable; nt = no transfer

* Frequency of transfer is the number of resistant transconjugants divided by the number of donor organisms.

† See footnote to table I.

accompanied by transfer of a plasmid (F), mol. wt 110×10^6 , which further encoded resistance to the same set of antibiotics and belonged to the same incompatibility group as the 125×10^6 -mol. wt plasmid (D) previously described in *K. aerogenes* serotypes 2 and 68.

In summary, six gentamicin-resistance plasmids were distinguished in infecting organisms and all conferred resistance to a core of five antimicrobials (A Cb G K Su). One plasmid (A) of mol. wt 70×10^6 , encoded resistance to A Cb C S G K To^m T Su Tm;

the second (B) was also of mol. wt 70×10^6 , but coded for resistance to A Cb C S G K T Su Tm; the third (C) was of mol. wt 125×10^6 encoding resistance to A Cb C G K To Su; the fourth (D) and fifth (E) were also of mol. wt 125×10^6 but coded for resistance to A Cb C^m S G K To Su Tm and whereas D belonged to incompatibility group C, E did not; and the sixth plasmid (F) was of mol. wt 110×10^6 encoding resistance to A Cb C^m S G K To Su Tm and belonging to incompatibility group C. No plasmid was self-transferable. Plasmid A was identi-

Table III. Characterisation of gentamicin-resistant and moderately-resistant bacteria isolated from ward A

Isolate	Serotype	Antibiotic resistances*	Gentamicin MIC ($\mu\text{g/ml}$)	Source
Inanimate environment				
<i>Acinetobacter lwoffii</i>	...	A ^m C ^m S G K Ak ^m To ^m T Su Tm ^m	128	kitchen sink
<i>Aeromonas</i> sp.	...	Cp ^m S G ^m K Ak ^m To ^m Tm ^m Su Tm	4	mop
<i>Aeromonas</i> sp.	...	A ^m C ^m C ^m S G ^m K Ak ^m To ^m T Su ^m	4	kitchen sink
<i>A. hydrophila</i>	...	A Cb Cp S G ^m K Ak ^m To ^m Tm ^m Su Tm ^m	4	kitchen sink
<i>A. hydrophila</i>	...	A Cb Cp S G ^m K Ak ^m To ^m Tm ^m Su ^m	2	flower vase (3)
<i>Ent. cloacae</i>	O27	A ^m C ^m S G K Ak ^m To ^m Tm ^m Su Tm	16	clean room sink
<i>Ent. cloacae</i>	O27	Cp S G K Ak ^m To ^m Tm ^m Su ^m	16	clean room sink
<i>Ent. cloacae</i>	O9	A Cb Cp ^m S G K Ak To Tm ^m Su ^m	16	ward hand basin (1)
<i>Ent. cloacae</i>	O9	A ^m C ^m S G ^m K Ak ^m To ^m Tm ^m Su ^m	8	w.c. (2)
<i>Ent. cloacae</i>	O9	Cp ^m S G K Ak ^m To ^m Tm ^m Su	16	bed pan washer
<i>K. oxytoca</i>	NT	A Cb ^m C ^m S G K Ak To Tm ^m Tm ^m	128	bathroom (B) bath
<i>K. oxytoca</i>	K68	A Cb Cp C ^m S G K Ak To T Su Tm	32	mop
<i>P. acidovorans</i>	...	A Cb Cp ^m C S G K Ak To Tm ^m Tm ^m	32	clean room hand basin
<i>P. acidovorans</i>	...	A Cb Cp ^m C S G K To ^m T Su Tm	512	flower vase (1)
<i>P. acidovorans</i>	...	A Cb Cp ^m C S G K To ^m T Su Tm	32	flower vase (2)
<i>P. acidovorans</i>	...	A Cb Cp ^m C S G K Ak To Tm ^m Tm ^m	256	bathroom (B) hand basin (1)
<i>P. acidovorans</i>	...	A Cb Cp ^m S G K To T Su Tm	256	bathroom (B) hand basin (1)
<i>P. acidovorans</i>	...	A Cb Cp ^m S G K To T Su Tm	256	sucker
<i>P. acidovorans</i>	...	A Cb Cp Ctx C S G K Ak To T Su Tm	512	sucker
<i>P. acidovorans</i>	...	A Cb Cp ^m C S G K Ak ^m To Tm ^m Su Tm	64	ward hand basin (2)
<i>P. acidovorans</i>	...	A Cb Cp C S G K To T Su Tm	256	flower vase (3)
<i>P. acidovorans</i>	...	A Cb Cp C S G K To ^m T Su Tm	128	side-ward hand basin
<i>P. aeruginosa</i>	O10	A Cb Cp ^m C S G K To T Su ^m Tm	512	w.c. (2)
<i>P. aeruginosa</i>	O10	A ^m Cb ^m Cp Ctx ^m C S ^m G ^m K T Su ^m Tm	2	ward hand basin (2)
<i>P. aeruginosa</i>	O1	A Cb Cp ^m C S G K To ^m T Su Tm	256	bed pan washer
<i>P. aeruginosa</i>	O1	A Cb Cp ^m C S G K To Tm ^m Su Tm	512	washroom sink
<i>P. aeruginosa</i>	O1	A Cb Cp Ctx C S G K To ^m T Su Tm	512	washroom sink
<i>P. aeruginosa</i>	O1	A Cb Cp Ctx C S G K Ak ^m To ^m T Su Tm	512	hopper
<i>P. aeruginosa</i>	O1	A Cb Cp Ctx C S G K To ^m T Su Tm	512	hopper
<i>P. aeruginosa</i>	O1	A Cb Cp Ctx C S G K To ^m T Su Tm	512	soaking elastic stockings
<i>P. alkaligenes</i>	...	A ^m Cb Cp Ctx ^m C ^m S ^m G ^m K To Su	4	w.c. (1)
<i>P. maltophilia</i>	...	A ^m Cp Ctx C S G K Ak To T So ^m Tm	16	clean room hand basin
<i>P. maltophilia</i>	...	A Cp Ctx ^m C S G K Ak To T Su Tm	16	clean room sink
<i>P. maltophilia</i>	...	A Cp Ctx C ^m S G K Ak To T Su Tm	16	bathroom (A) bath
<i>P. maltophilia</i>	...	A ^m Cp Ctx C S G K Ak To T Su Tm	32	flower vase (2)
<i>P. maltophilia</i>	...	A ^m Cp Ctx ^m C S G K Ak T Su Tm	256	bathroom (B) hand basin (2)
<i>P. maltophilia</i>	...	Cp C S G K Ak To T Su Tm	16	bathroom (B) sink
<i>P. maltophilia</i>	...	Cp C S G K Ak To T Su Tm	128	sucker
<i>P. maltophilia</i>	...	Cp Ctx ^m C S G K Ak To Su Tm	128	mop
<i>P. maltophilia</i>	...	A ^m Cp Ctx ^m C ^m S G K Ak To T Tm	32	ward hand basin (2)
<i>P. maltophilia</i>	...	A C ^m S G K Ak To	128	side-ward hand basin
<i>P. pseudoalkaligenes</i>	...	A Cb Cp Ctx ^m C S G K To Su Tm	16	washroom sink
<i>P. pseudoalkaligenes</i>	...	A Cb Cp Ctx ^m C S G k To Su Tm ^m	16	soaking elastic stockings
<i>P. putida</i>	...	A Cb Cp Ctx ^m C S G K T Su Tm	16	sucker
Staff				
<i>C. freundii</i>	...	Cp ^m Ctx ^m C ^m S ^m G ^m K Ak ^m To ^m Su ^m	4	faeces, staff 1
<i>E. coli</i>	...	A Cp ^m C ^m S G K Ak To Tm ^m Tm ^m	16	faeces, staff 2
<i>E. coli</i>	O92	A Cb Cp Ctx C S G K To ^m T Su Tm	16	faeces, staff 3
<i>E. coli</i>	...	Cp ^m S G K Ak ^m To ^m Tm ^m Su ^m	16	faeces, staff 4
<i>E. coli</i>	...	A ^m C ^m S ^m G ^m K Ak ^m To ^m Tm ^m Su ^m	2	faeces, staff 5
<i>E. coli</i>	...	A Cb Cp S G ^m K Ak ^m To ^m Tm ^m Su	4	faeces, staff 6
<i>E. coli</i>	...	Cp ^m S ^m G ^m K Ak ^m To ^m Tm ^m Su ^m	2	faeces, staff 7
<i>E. coli</i>	...	A Cb Cp ^m C ^m G ^m K Ak ^m To ^m T Su Tm	4	faeces, staff 8
<i>E. coli</i>	...	Cb Cp ^m S G K Ak ^m To ^m T Su Tm	16	faeces, staff 9
<i>E. coli</i>	...	A ^m Cp ^m S G Ak ^m To ^m Tm ^m S7 ^m Tm	16	faeces, staff 10
Patients				
<i>E. coli</i>	...	A C ^m S G K Ak To Tm ^m	16	faeces, patient 1
<i>E. coli</i>	...	A C ^m S G K Ak To Tm ^m	16	faeces, patient 2
<i>E. coli</i>	...	A Cb Cp Ctx C S G K To ^m T Su Tm	16	faeces, patient 3
<i>E. coli</i>	O77	A Cb Cp Ctx C S G K To ^m T Su Tm	16	faeces, patient 4
<i>E. coli</i>	NT	A Cb Cp Ctx C S G K To ^m T Su Tm	16	faeces, patient 5
<i>E. coli</i>	...	Cp ^m C ^m S G ^m K Ak ^m To ^m T Su ^m	2	faeces, patient 5
<i>E. coli</i>	...	Cp S ^m G K Ak ^m To Tm ^m Su ^m	16	faeces, patient 6
<i>E. coli</i>	...	A ^m Cp ^m C ^m S ^m G ^m K Ak ^m To ^m Tm ^m Su ^m	4	faeces, patient 7
<i>E. coli</i>	...	A Cb Cp ^m S G K Ak ^m To ^m T Su	16	faeces, patient 8
<i>E. coli</i>	...	A ^m Cp ^m S ^m G ^m K Ak ^m To ^m Tm ^m Su ^m	4	faeces, patient 9
<i>E. coli</i>	O19	A Cb Cp ^m C S G K To ^m T Su Tm	32	faeces, patient 10
<i>K. oxytoca</i>	K68	A Cb Cp C ^m S G K Ak To T Su Tm	32	faeces, patient 6
<i>P. aeruginosa</i>	O1	A Cb Cp Ctx C S G K Ak ^m To ^m T Su Tm	512	faeces, patient 10

NT = non-typable.

* See footnote to table I.

found in *K. oxytoca* serotype-K3, plasmid B in a non-typable *K. oxytoca*, plasmid C in a single *K. aerogenes* serotype K68, plasmid D in *K. aerogenes* serotypes K2 and K68, plasmid E in *K. aerogenes* serotype K2 and plasmid F in a single *Ent. agglomerans* strain. Thus, plasmid A was isolated from ward A, plasmid D from ward C, plasmids D and E from ward E and all six plasmids were isolated from ward D. Ward A was investigated further.

Characterisation of isolates from ward A

Characteristics of gentamicin-resistant and moderately-resistant gram-negative bacteria from ward A are given in table III. Their sources in the inanimate environment were, as expected, wet areas, e.g., sinks and baths, and none were obtained from medicines, creams, air fresheners, detergents, soap, sand buckets or food. Representatives of five genera—*Acinetobacter*, *Aeromonas*, *Enterobacter*, *Klebsiella* and *Pseudomonas*—renowned for their ability to colonise warm moist places, were identified. Their gentamicin MICs were in the range 2–512 µg/ml. The faecal sources were in 10 of the 16 patients and 10 of the 16 members of staff. Isolates were mostly *E. coli*, plus *K. oxytoca* from patient no. 6, *P. aeruginosa* from patient no. 10 and *C. freundii* from staff no. 1. Their gentamicin MICs were in the range 2–512 µg/ml. Amongst the 20 *E. coli* isolates, 13 had gentamicin MICs exceeding 8 µg/ml; additional multi-resistance was a common characteristic and four strains were resistant to all 12 antimicrobials tested, including one third-generation cephalosporin (cefotaxime). A comparison of data (tables I and III) shows that none of the strains causing infections resembled those isolated from either the inanimate environment or the gastro-intestinal tracts of staff and patients of ward A.

Gentamicin-resistance plasmids in staff, patients and environmental isolates of ward A

Isolates whose antibiotic-resistance patterns incorporated those encoded by plasmids from infecting organisms were examined further. Of 16 such isolates, plasmids were detected in nine isolates and gentamicin-resistance transferability in three (table IV). Transfer of gentamicin resistance was associated with transfer of 70×10^6 -mol. wt plasmids encoding resistance to the same set of antibiotics as the previously described 70×10^6 -mol. wt plasmids A and B. Plasmid A was detected in *K. oxytoca* serotype K68 isolated from a mop and plasmid B was detected in the faeces of two patients in *E. coli* serotype O19 and *K. oxytoca* serotype

K68. The occurrence of plasmids in *E. coli* from patient 4, which were of similar size to those (A, B and F) in infecting organisms, prompted us to investigate further. In a tri-parental cross involving the broad-host-range plasmid RP1, gentamicin resistance was mobilised and shown to be associated with transfer of a 110×10^6 -mol. wt plasmid which further encoded resistance to the same set of antibiotics and belonged to the same incompatibility group C as the previously described 110×10^6 -mol. wt plasmid (F) detected in an *Ent. agglomerans* isolated from ward D.

Discussion

Characterisation of isolates from infections and from ward A did not suggest the existence of a reservoir of gentamicin-resistant infecting strains within the hospital environment. The level of gentamicin resistance and other resistances in the environmental isolates was higher than would be expected from those species, with the possible exception of *P. acidovorans*. However, many of the species isolated are not normally regarded as primary hospital pathogens and therefore the multiple resistances observed and the levels of gentamicin resistance would not normally be regarded as cause for concern, unless they were plasmid encoded and capable of transfer to more pathogenic species. Plasmid transfer of resistance is probably normally a rarer event than cross infection involving a resistant strain, but though the frequency of plasmid transfer is probably not increased by antibiotic selection pressures within the hospital environment, survival of resistant recipients is greatly enhanced. Concern is less where strains merely act as "dead end" carriers of plasmids, and are incapable of transferring them, as for example the *E. coli* O20 isolated from patient 4, but increases with the realisation that, with the acquisition of a transfer factor as was demonstrated by insertion of another plasmid (RP1) into the donor strain, the resistance plasmid may then become transferable. In this study, six plasmids encoding gentamicin resistance, A B C D E and F, were identified amongst infecting organisms. They were not confined to a particular ward, nor did they owe their presence to that of a particular host serotype, species or genus. In *K. oxytoca*, plasmid A was detected in the different serotypes K68 and K3 and in *K. aerogenes*, plasmid D in serotypes K2 and K68. Plasmid B was detected in different genera in *E. coli* O19, *K. oxytoca* K68 and *K. oxytoca* NT.

Having identified transmissible gentamicin-resistance plasmids in infecting organisms, we ques-

Table IV. Characterisation of plasmids in isolates from ward A

Source	Isolate	Serotype	Mol. wt (10 ⁶) of plasmids detected	Frequency of gentamicin resistance transfer*	Transferable gentamicin resistance plasmid			
					antibiotic resistances co-transferred*	mol. wt (10 ⁶)	gentamicin MIC (mg L) conferred	
Inanimate environment								
Mop	<i>K. oxytoca</i>	K68	95 70 2	2.6 × 10 ⁻¹	A Cb C S G K To ^m T Su Tm	70	64	
WC2 (2)	<i>P. aeruginosa</i>	O10	56 14	nt				
Staff								
3	<i>E. coli</i>	O92	60 1.8	nt				
Patients								
3	<i>E. coli</i>	O77	95 3	nt				
4	<i>E. coli</i>	O20	110 70 2.7 1.8	nt				
5	<i>E. coli</i>	NT	33 17	nt				
10	<i>E. coli</i>	O19	95 70 16 2.2 1.6	2.5 × 10 ⁻⁴	A Cb C S G K T Su Tm	70	64	
6	<i>K. oxytoca</i>	K68	95 70 65	3.0 × 10 ⁻⁵	A Cb C S G K T Su Tm	70	128	
10	<i>P. aeruginosa</i>	O1	56 37 14	nt				

NT—non-typable; nt—no transfer

* See footnotes to tables I and II.

tioned whether these plasmids had an identifiable source in the ward environment, although the organisms themselves did not. Plasmid A was detected in the ward environment, i.e., in the isolate from a mop on ward A, and also in a different strain infecting three patients on ward A and one on ward D. Plasmid spread to ward D was probably via transfer of a patient carrying the infecting host strain from ward A to ward D. Plasmid B was detected on ward A, being present in two distinct organisms, *K. oxytoca* K68 and *E. coli* O19, carried in the gastro-intestinal tract of two patients; it was not in isolates from infections on ward A but was found in an infection on ward D caused by a non-typable *K. oxytoca* strain. No link was discovered for this, but the small difference, i.e., low level tobramycin resistance, between plasmids A and B may indicate that they are related and may partly explain some of the associations observed. Plasmid F was detected on ward A and was carried by a strain of *E. coli* present in the gastro-intestinal tract of one of the patients. It was not isolated from infections on ward A but was isolated from an infection on ward D caused by a different organism,

Ent. agglomerans. Plasmids C, D and E were detected neither in the environment nor in infections occurring on ward A. There thus appeared to be environmental reservoirs, both in the inanimate environment and in patients, for some but not all plasmids implicated in gentamicin-resistant infections and the reservoir might well comprise an organism different from the infecting strain. No environmental site for plasmid transfer was located, but it was interesting to note that plasmid D was clearly identified in two different serotypes of *K. aerogenes*, K2 and K68, isolated from the urine of a single infected patient on ward E.

We thank colleagues at the Central Public Health Laboratory, Colindale, London, Mr R. Gross, Dr J. Threlfall and Dr G. Smith (Division of Enteric Pathogens), for typing *E. coli* strains, for the gift of plasmid marker strains and for valuable advice, and Mr M. Gaston, Dr T. Pitt and Mr H. Todd (Division of Hospital Infection), for speciation of environmental isolates and typing of *P. aeruginosa* and *Enterobacter* isolates and we thank Dr P. Mortimer (Coventry, PHL), for typing *Klebsiella* strains. We are further grateful to Mr M. Gaston for his valuable assistance in sampling the ward environment and Mrs M. Gosling, Senior Nurse Infection Control.

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