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Control of infection with multiple antibiotic resistant bacteria in a hospital renal unit: the value of plasmid characterization

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SUMMARY

An outbreak of infections due to multiple antibiotic-resistant bacteria took place over a period of approximately 18 months in a renal unit. Strains of *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Citrobacter* spp. and *Pseudomonas* spp. were involved, and a variety of antibiotic resistances was encountered. Closely related plasmids encoding resistance to aztreonam, ceftazidime and piperacillin, possibly derived from an archetypal plasmid of 105 kb were found in the majority of isolates examined. After limiting the use of aztreonam the incidence of new patient isolates of multiple-resistant organisms was greatly reduced. This study demonstrated how molecular studies can contribute to the control of an outbreak situation in a hospital unit by providing an impetus to reduce the use of specific antibiotics.

INTRODUCTION

Hospital outbreaks of Gram-negative bacteria resistant to multiple antibiotics have been reported in a number of contexts, particularly in areas where seriously ill patients are treated, for example in intensive care and special care baby units [1, 2] and in oncology units [3], sometimes with spread to other wards [4]. In renal units, patients are often immunocompromised by underlying disease or by immunosuppressive therapy, but renal unit outbreaks of antibiotic-resistant bacteria have emphasized the role of Gram-positive organisms, such as vancomycin-resistant enterococci (VRE) [5] rather than of Gram-negative organisms. We report an outbreak of infections due to multiple-resistant Gram-negative bacteria in a renal unit which involved a number of different species and involved the spread of a single plasmid of 105 kb between several unrelated bacterial species.

MATERIALS AND METHODS

Background to the outbreak

The outbreak took place in a tertiary referral renal unit within an undergraduate teaching hospital over an 18 month period between March 1992 and August 1993.

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Table 1. *Antibiotic sensitivities of organisms isolated during the first year of the outbreak*

Organism	Antibiotic (Number sensitive/number tested)									
	Ami	Gen	Net	Cip	Aug	Cxm	Ctz	Azt	Pip	Imi
Citrobacter spp.	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2
E. coli	8/13	3/22	1/20	0/21	0/11	0/11	0/22	0/16	0/22	22/22
Enterobacter spp.	3/3	1/4	3/4	0/4	0/3	0/3	1/4	1/3	0/4	3/4
Klebsiella spp.	4/4	7/7	6/6	0/7	0/5	0/5	0/7	0/7	2/7	7/7
Total sensitive*	15/22	11/35	10/32	0/34	0/21	0/21	1/35	1/28	2/35	34/35
%	68	31	61	0	0	0	3	4	6	97

* Organisms not recorded as sensitive comprised those considered resistant and those showing reduced sensitivity.

Ami, amikacin; Gen, gentamicin; Net, netilmicin; Cip, ciprofloxacin; Aug, co-amoxyclav; Cxm, cefuroxime; Ctz, ceftazidime; Azt, aztreonam; Pip, piperacillin; Imi, imipenem.

The renal unit housed 33 beds out of a total hospital complement of 520 beds. The unit had a longstanding, although relatively minor, problem with VRE, but apart from a few sporadic and apparently unrelated isolates with moderate antibiotic resistance, there was no history of isolation of multiple-resistant Gram-negative microorganisms until March 1992. In March 1992 bacteria resistant to multiple antibiotics were recovered from the urine specimens of six patients. There were representatives of *Pseudomonas putidia*, *Enterobacter aerogenes* and *Escherichia coli*, and although the antibiograms differed between species, most strains were resistant to gentamicin, netilmicin, aztreonam, piperacillin, ceftazidime and ciprofloxacin; only imipenem was active against almost all isolates (Table 1).

Isolation and identification of antibiotic-resistant bacteria

Bacteria were isolated from clinical specimens using standard diagnostic methods. Organisms were identified by their API-20E biochemical properties (API Systems, La Balme Les Grottes, Montalieu-Vercieu, France). Representative isolates of *E. coli* were serotyped at the Laboratory of Enteric Pathogens (LEP), Central Public Health Laboratory, Colindale based on their somatic (O) and flagellar (H) antigens according to the method of Ørskov and Ørskov [6].

Screening for resistance to antibacterial drugs

Antibiotic susceptibility testing was performed by the method of Stokes [7] on all Gram-negative organisms isolated in the renal unit of St Mary's Hospital, London over the 18-month period March 1992–August 1993. Antibiotics screened included amikacin, aztreonam, ceftazidine, ciprofloxacin, gentamicin, imipenem, netilmicin and piperacillin. Coliforms resistant to these antibiotics were subsequently screened for resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulphonamides, tetracyclines, trimethoprim, nalidixic acid and rifampicin using the methods of Anderson and Threlfall [8] and Ward and colleagues [9].

Management of the outbreak

Standard infection control measures were applied to patients with multiple resistant bateria; these comprised isolation in single rooms whenever possible, and use of disposable aprons and gloves by staff in direct contact with the patient. The variety of bacterial species involved made the role of an environmental reservoir unlikely and so none was sought, and because of the large number of bacteriology specimens routinely submitted by the unit, case-finding was not considered necessary. As far as practicable, an attempt was made to limit the use of broad-spectrum antibiotics. The range of bacterial species involved in the outbreak prompted examination for plasmid-mediated antibiotic resistance. A selection of isolates representing the different species was therefore submitted to the LEP for molecular studies and plasmid characterization. Following the identification of a putative 'plasmid' outbreak, a survey of the hospital pharmacy records was initiated with particular reference to the use of aztreonam and ceftazidime.

Transfer of antibiotic resistance and plasmid characterization

Thirty-five multiple-resistant strains representative of isolates from different sites and different antibiograms (R-types) were tested for the ability to transfer resistance at both 28 °C and 37 °C to *E. coli* K12 F^- lac^- rifampicin-resistant (rif^r) (= 20R764) or when rifampicin-resistant, to a strain of *E. coli* K12 with high level resistance (> 500 mg/l) to streptomycin (Str^r) (= 1R716). The methods were those of Anderson and Threlfall [8] and counter-selection was exercised with rifampicin (100 mg/l) or streptomycin (500 mg/l) as appropriate. When no transfer of resistance was detected, mobilization of drug resistance determinants was attempted by standard methods [8], using the conjugative plasmids X (63 kb, *Inc* F_{II}) and Δ (90 kb, Inc I_1). Where possible, plasmids were tested for the ability to coexist with standard plasmids of defined compatibility groups [10] and on this basis were assigned to incompatibility groups in accordance with the scheme of Grindley and colleagues [11].

Extraction and fingerprinting of plasmid DNA

Plasmid DNA was extracted by a modification of the method of Kado and Liu [12] as described by Threlfall and colleagues [13]. Following electrophoresis at 100 V for 3 h on horizontal (BRL H5 Horizontal Gel Apparatus) Tris-borate agarose gels (0·7% w/v, Sigma Chemical Co. Ltd) and visualization under UV illumination after staining with ethidium bromide, relative molecular mass (M_r) was determined in relation to plasmids of 147, 63, 35·8 and 6·9 kb, carried in *E. coli* K12, strain 39R861 [14]. For restriction enzyme analysis, suitably purified plasmid DNA extracted as described by Threlfall and colleagues [15] was digested with the restriction enzymes *Eco*R I and *Pst* I in accordance with the instructions of the manufacturer (Gibco BRL).

RESULTS

Types of bacteria involved

In the 2 years following the isolation of the first multi-resistant organisms, 29 strains of *E. coli*, 4 of *Enterobacter cloacae*, 11 of *K. pneumoniae*, 2 of *Citrobacter* spp.

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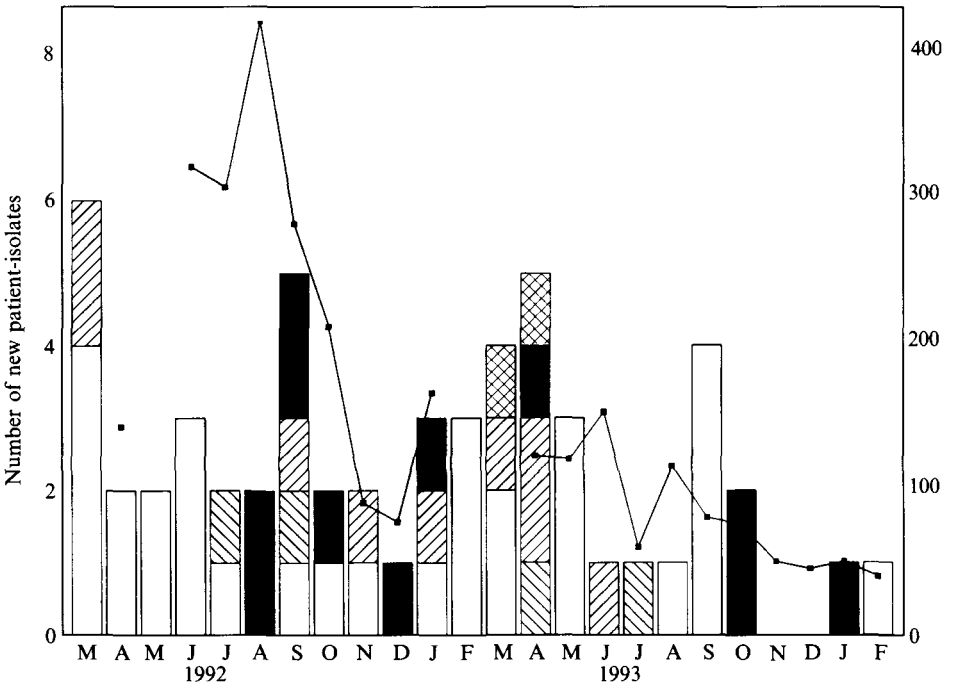

Fig. 1. Isolates of multiple resistant Gram-negative bacteria by month. Non-fermenters comprise *Acinetobacter* spp., *Pseudomonas* spp and *Xanthomonas* spp. AZ = aztreonam usage in grams (right hand scale), data not available for March and May 1992 nor for March 1993: □, *E. coli*; ▨, *Enterobacter* spp.; ▨, Non-fermenters; ■, *Klebsiella* spp; ⊠, *Citrobacter* spp.; —■—, AZ.

and 9 of *Pseudomononas/Xanthomonas* spp. were detected on the unit (Fig. 1). Twelve strains of *E. coli* were serotyped and these were found to comprise six of group O?:H31, three of group O?:H10 and one of each of groups O8:H16, O?:H5 and O?:H?.

Antibiotic usage

Pharmacy computer records provided details of the unit's use of antibiotics. The unit used little ceftazidime and piperacillin but substantial use was made of aztreonam. In the 6 months prior to the outbreak an average of 380 grams of aztreonam was used in each month and for the first 5 months of the outbreak between 300 and 400 grams of aztreonam were being used in the unit per month; however, after the identification of a putative 'plasmid' outbreak usage of this antibiotic was substantially reduced and from November 1992 to August 1993 less than 150 grams per month of aztreonam was prescribed for use in the unit (Fig. 1). The use of aztreonam was in pronounced contrast to all other areas of the hospital, very few of which ever used aztreonam although both ceftazidime and piperacillin had widespread occasional usage. The usage of ceftazidime in the unit, although never as substantial as that of aztreonam, was also reduced from between 100 and 150 grams per month in the early stages of the outbreak to between 50 and 100 grams per month for the 11 month period from October 1992 to August 1993 (data not shown).

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Table 2. *Plasmid profile patterns*

Serotype	M_r of plasmid DNA (kb)															PP	No.
Escherichia coli	—	—	—	—	—	105	—	—	—	—	—	—	4·5	—	—	Ai	5
($n = 24$)	—	—	—	—	—	105	—	75	—	—	—	—	4·5	—	—	Aii	2
	—	—	—	120	—	—	90	75	—	—	—	—	4·5	—	—	Aiii	4
	—	—	—	—	112	—	—	75	60	—	—	—	4·5	—	—	Aiv	1
	—	—	—	—	—	105	90	75	60	—	—	—	4·5	—	3·0	Av	3
	—	150	—	—	—	105	—	—	60	—	6·0	—	—	4·2	—	B	7
	—	150	135	120	—	105	—	—	—	—	—	5·0	—	—	—	C	2
Klebsiella spp.	180	150	—	—	—	—	—	—	—	—	—	—	—	—	—	Di	7
($n = 8$)	180	150	—	—	—	—	—	75	—	—	—	—	4·5	—	—	Dii	1
Citrobacter spp. ($n = 1$)	—	—	—	—	112	—	—	75	—	30	6·0	—	—	—	—	E	1
Enterobacter spp. ($n = 2$)	—	—	—	—	—	105	—	—	—	—	6·0	—	—	—	—	F	2

M_r, relative mobility; PP, plasmid profile designation; No., number of strains with the respective plasmid profiles.

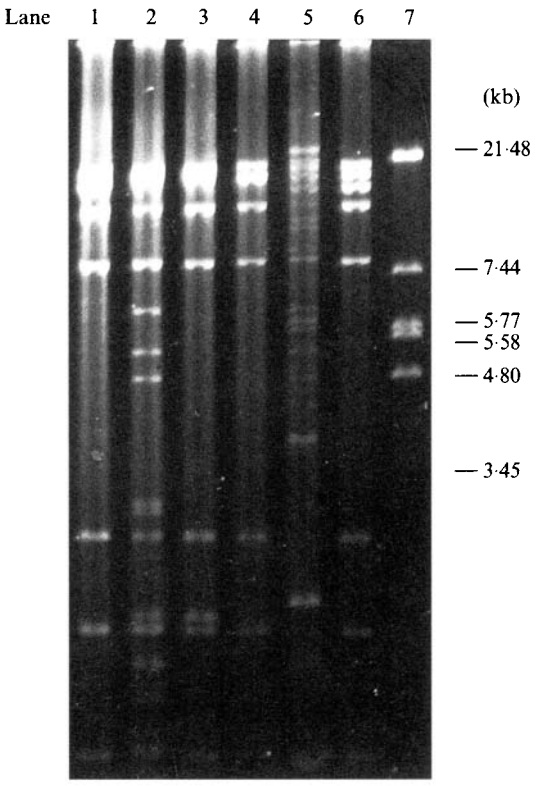

Fig. 2. *Eco*R I restriction enzyme fingerprints of ACP plasmids from strains of *Escherichia coli*, *Citrobacter* spp. and *Klebsiella* spp. Lanes 1–4: 105–120 kb plasmids from *Escherichia coli*, PPTs Ai, Aiii, Av and B; lane 5: 150 kb plasmid from *Klebsiella* spp., PPT Di; lane 6: 112 kb plasmid from *Citrobacter* spp., PPT E; lane 7: λ/*Eco*R I.

Plasmid analysis

Bacteria from the outbreak were found to harbour up to 6 plasmids and 11 plasmid profile types (PPTs) were identified (Table 2). For the *E. coli* isolates, these PPTs have been designated Ai through to Av (on the basis of a plasmid of 4·5 kb common to all strains), B and C; for *Klebsiella* spp., the PPTs have been designated Di and Dii, for *Citrobacter* spp., E and for *Enterobacter* spp., F. Analysis of plasmid content was confined to selected strains within these plasmid profile types which had been isolated in the period ending March 1993, and these are included in Figure 2. Of the 12 strains of *E. coli* that were serotyped, 1 strain of PPT Ai belonged to serotype O8:H16, 1 strain of PPT Aii to O?:H5, 3 strains of PPT Aiii to O?:H31, 1 of PPT Aiv to O?:H31, 3 strains of PPT B to O?:H10 and the 2 strains of PPT C to O?:H31.

Common to almost all strains of *E. coli*, *Citrobacter* spp. and *Enterobacter* spp. were plasmids of approximately 105–120 kb which, in conjugation experiments, were associated with transmission of resistance to aztreonam, ceftazidime, piperacillin (ACP), ampicillin and chloramphenicol, and in some strains to trimethoprim (Table 3). When tested for the ability to coexist with standard plasmids of defined compatibility groups, all of five 105–120 kb plasmids from

Table 3. *Resistances encoded by 105–150 kp plasmids from 35 strains of* Escherichia coli, Klebsiella *spp.*, Citrobacter *spp. and* Enterobacter *spp.*

Serotype	Plasmid M_r (kb)	PPs	No. of strains	Resistances encoded
Escherichia coli	105	Ai, Aii,	12	ACP, ACGSSuTTm
	—	Av, C	—	—
	120	Aiii	4	ACP, ACGSSuTTm
	112	Aiv	1	ACP, ACGSSuTTm
	105	B	7	ACP, ACGKSSuTTm (4)
	—	—	—	ACP, ACKSSuTTm (3)
Klebsiella spp.	150	Di, Dii	7	ACP, ACSSuTTm (6)
	—	—	1	ACP, ACGSSuTTm (1)
Citrobacter spp.	112	E	1	ACP, ACGKSSuTTm
Enterobacter spp.	105	F	2	ACP, ACSSuTTm

M_r, relative mobility; PP, plasmid profile.

Resistance symbols: ACP, aztreonam, ceftazidine, piperacillin; A, ampicillin; C, chloramphenicol; G, gentamicin; K, kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines; Tm, trimethoprim. All gentamicin-resistant strains were also resistant to netilmicin.

strains of *E. coli* tested, and the plasmids of 114 and 105 kb from strains of *Citrobacter* spp. and *Enterobacter* spp. were incompatible with standard plasmids of the F_2 incompatibility group.

Restriction endonuclease digests using the enzymes *Eco*R I and *Pst* I confirmed the molecular relatedness of these plasmids, and the *Eco*R I-generated fingerprints of plasmids of 105–120 kb from strains of *E. coli* of PPTs Ai, Aiii, Av and B, and from one strain of *Citrobacter* spp. of PPT E are shown in Figure 2. As a plasmid of 105 kb was found in 19 of 24 strains of *E. coli* and in the *Enterobacter* spp. strains, it is possible that this plasmid represents the archetypal plasmid involved in the outbreak. Although, as shown in Table 2, 105–120 kb plasmids were not found in strains of *Klebsiella* spp., these strains contained plasmids of approximately 150 kb also associated with transmission of ACP resistance. However, comparison of the *Eco*R I and *Pst* I fingerprints of one of the 150 kb *Klebsiella* spp. plasmids with those of plasmids of 105–120 kb from *E. coli* and *Citrobacter* spp. demonstrated a degree of molecular relatedness (Fig. 2). Furthermore, in incompatibility tests, some incompatibility with Inc F_{II} plasmids was demonstrable. A 105 kb plasmid was also detected in some strains of multiple-resistant *Pseudomonas* spp. involved in the outbreak; however this plasmid was not subjected to restriction endonuclease typing nor to incompatibility grouping.

Spread within the hospital

During the 18 months of the outbreak, no organisms resembling those reported in this study were isolated from patients outside the renal unit.

Results of interventions

Standard infection control precautions had no noticeable effect on the frequency of new patient-isolates of multiple-resistant bacteria. When molecular analysis revealed the existence of closely-related plasmids mediating resistance to ACP in a number of unrelated organisms, attempts were made to reduce the amount of

aztreonam used on the unit. Following this the incidence of isolates of multiple resistant organisms was greatly reduced (Fig. 1).

DISCUSSION

The progressive appearance of a number of bacterial species with multiple antibiotic resistance is characteristic of a 'plasmid' outbreak, in which a readily transmissible plasmid spreads between species. The subsequent acquisition by such plasmids, of resistance genes from other plasmids present within the bacterial cell has been described for non-fermenting Gram-negative rods [16] and recipient organisms may then display a variety of antibiograms. In this study, a putative archetypal Inc F_{II} plasmid of 105 kb was present in 19 of 24 strains of *E. coli* isolated from different patients and belonging to at least five serotypes, and also in strains of *Enterobacter* spp. from two further patients. Closely-related plasmids probably derived from this archetypal plasmid were identified in strains of *E. coli* from a further five patients and in a strain of *Citrobacter* spp. from a sixth patient. A plasmid of 150 kb from two strains of *Klebsiella* spp. also showed some degree of molecular relatedness and may also have been derived from the 105 kb plasmid during the course of the outbreak.

The 'plasmid outbreak' described here is of particular interest because within a relatively short period of time a 105 kb plasmid infected a range of bacterial species, in which *E. coli* played the major role. This contrasts with the emphasis placed by others on the central role of *Serratia* spp. and *Klebsiella* spp. in plasmid-mediated outbreaks [17]. We did not encounter *Serratia* spp., which is in any case an unusual cause of nosocomial infection in the UK, and although there were some isolates of *Klebsiella* spp., they did not appear until later in the outbreak.

The ability of the 105 kb plasmid in our outbreak to pass readily from one species to another presented an initially confusing picture. The near-simultaneous appearance of a variety of multi-resistant organisms diverted attention from a straightforward explanation of cross-infection, and suggested rather that several independent outbreaks of infection due to multiple-resistant bacteria were taking place simultaneously; the large number of interhospital transfers received by the unit made plausible such an hypothesis. The spread of the plasmid among the unit's bacterial flora, some of which contained several plasmids and possessed a variety of other antibiotic resistances (Table 3) prevented recognition that the transmission of linked resistance to aztreonam, ceftazidime and piperacillin was the underlying cause of the outbreak.

The inability of routine methods to demonstrate that patients were colonized with identical plasmids although in different bacterial hosts, hindered any epidemiological understanding and limited hypotheses that could be made concerning spread and potential reservoirs of infection. Outbreaks of antibiotic-resistant Gram-negative bacteria attributed to environmental reservoirs have often involved a single strain of environmental origin such as *Pseudomonas* spp., *Acinetobacter* spp. and *Serratia* spp. [3, 18–20]. The prominence of man-adapted *E. coli* and the involvement of the wide range of species in our outbreak, argued against an environmental source and suggested that investigation of patients and the environment was unlikely to be fruitful. In practice cross-infection control

measures were not compromised by the absence of an identified source of infection, as all patients with multiple-resistant bacteria were subjected to the same cross-infection control procedures.

Price and Sleigh provided evidence in 1970 that the reduction in use of an antibiotic to which bacteria are resistant may terminate an outbreak [21], although this strategy has not always been successful [22]. Success has also been claimed with other methods of control such as intestinal decontamination with non-absorbable antibiotics [4]. At the onset of our outbreak, the possible contributory role of antibiotic usage and the need to avoid unnecessary therapy was accepted as a matter of general principle. However, a wide-ranging limitation of antibiotic therapy was not possible because of the susceptibility to infection of many of the unit's patients and recommendations about specific agents were not possible in view of the large number of different resistances encountered. The dramatic reduction in the incidence of multiply-resistant Gram-negative organisms following change in aztreonam usage suggests that our outbreak was dependent on this antimicrobial and indicates that despite being a relatively narrow-spectrum agent – with useful activity only against facultative Gram-negative bacteria – aztreonam may disrupt the microbial environment as much as broader-spectrum agents.

The eventual confirmation that a single plasmid (or group of closely-related plasmids probably derived from a single archetypal plasmid) was present in the majority of multiply-resistant isolates, and the identification of its associated antibiotic resistances coupled with the unit's antibiotic usage data, led to a reasoned decision to limit use of aztreonam. However, this decision could be reached only after substantial work on the genetic composition of the bacteria and repeated examination of the pharmacy computer records. Whilst computerized pharmacy information is likely to be available in most modern hospitals, detailed investigation of antibiotic resistance mechanisms requires skills and resources available in few centres. Indeed, to our knowledge this study provides the first documented demonstration of how molecular studies can provide the impetus to reduce the usage of specific antibiotics in an outbreak situation. Regrettably, until more rapid means of characterizing transmissible resistance elements are available, it is likely that outbreaks such as the above will continue to be take much time to analyse and control.

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