Investigation of outbreaks of *Enterobacter aerogenes* colonisation and infection in intensive care units by random amplification of polymorphic DNA

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During a 4-month period, 41 isolates of *Enterobacter aerogenes* were cultured from different specimens from a 14-bed intensive care unit (ICU1). These were obtained from 12 patients out of a total of 187 patients admitted to the ICU. Sixteen *E. aerogenes* isolates were cultured from another ICU (ICU2) 6 months later. Six non-outbreak-associated strains were included as controls and all the isolates were compared by random amplification of polymorphic DNA (RAPD), with three different 10-mer oligonucleotide primers. The six non-outbreak-associated strains were distinguishable by RAPD with two of the three primers. RAPD fingerprinting with primer AP12h was as discriminatory as the combined results from all three primers and defined 22 different patterns for the 41 isolates from the ICU1. In nine instances, isolates with indistinguishable RAPD patterns were detected in two-to-five patients over a 3–15-day period, suggesting patient-to-patient transmission. During their stay in ICU1, patients harboured one-to-12 distinguishable isolates. Isolates from ICU2 were indistinguishable by RAPD analysis with the three different primers. These findings suggest that the cluster of colonisations and infections in ICU1 was a 'false outbreak', consisting of successive patient-to-patient transmission of different *E. aerogenes* strains. In contrast, the outbreak on ICU2 probably involved the extensive spread of a single strain.

Introduction

Until the 1980s, infections caused by *Enterobacter aerogenes* were relatively uncommon. However, according to recent National Nosocomial Infections Surveillance System data [1] *Enterobacter* spp. have replaced *Klebsiella pneumoniae* as the third leading cause of gram-negative nosocomial infections, particularly nosocomial pneumonias, after *Escherichia coli* and *Pseudomonas aeruginosa* [2–5]. Of particular concern is the ability of *E. aerogenes* to develop resistance to broad-spectrum β -lactam agents by the production of an extended-spectrum β -lactamase (ESBL) [6, 7]. This species is part of the normal flora of the gastrointestinal tract and is, in some cases, a stable skin resident [8]. Reports concerning opportunist infections with *E. aerogenes* in hospitalised or debilitated patients first appeared in 1986 [2, 7].

Consequently, all the studies concerning *E. aerogenes* nosocomial infections are recent [9–13]. Initial reports that described the spread of *E. aerogenes* strains involved two-to-five patients [14, 15]. The investigation of apparent outbreaks of infection with *E. aerogenes* has been hampered by the lack of a sufficiently discriminatory typing method. Traditional techniques for typing *E. aerogenes* are based on phenotypic characteristics and include biotyping, O-serotyping, bacteriocin and phage typing. Unfortunately, biochemical reaction patterns are usually invariable among clinical isolates. Phage typing and serotyping are usually performed with *K. pneumoniae* reagents and are poorly discriminatory for *E. aerogenes*. Bacteriocin typing is useful but labour intensive, time consuming and limited to a few specialised centres [11]. Although DNA-based typing methods such as plasmid-profile analysis [13, 16], ribotyping [17], small-fragment restriction endonuclease analysis and pulsed-field gel electrophoresis [18] have been used to differentiate isolates of *E. cloacae*, these techniques have not been applied to isolates from clusters of infection caused by *E. aerogenes*.

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A new DNA polymorphism assay was reported by Williams *et al.* [19] which is based on PCR amplification of random DNA fragments with short primers of arbitrary sequence. The resulting amplified fragments function as polymorphisms for DNA fingerprinting. In contrast to traditional target-specific PCR, no prior sequence information is required and the technique is potentially applicable to all bacteria. This technique has been applied successfully to epidemiological investigations of many bacterial genera and species [20]. In this report, the application of this random amplification of polymorphic DNA (RAPD) method to investigate the epidemiological relationship of isolates of *E. aerogenes* obtained from patients from two intensive care units is described.

Materials and methods

ICU1 study

Study population. An outbreak of *E. aerogenes* infection affecting 12 of 187 patients admitted to a 14-bed Intensive Care Unit (ICU1) in Sainte Marguerite Hospital, between 17 Nov. 1992 and 3 March 1993 was studied in detail (Table 1). In these patients, the mean duration of hospitalisation and mechanical ventilation

were 44 and 31 days, respectively, *versus* 8 and 5 days for non-infected or colonised patients. Infections were considered to be nosocomial when clinical source cultures were positive at least 48 h after admission.

Patient cultures. During the 4-month period of the study, 41 clinical isolates of *E. aerogenes* were obtained from the 12 patients on ICU1. The clinical specimens from which these were isolated included bronchial secretions, urines, closed cavity drainage specimens, catheters, vaginal samples, blood cultures and various wound swabs (from umbilicus, tracheostomy and drainage tubes). *E. aerogenes* was considered to be responsible for 10 infections: one bacteraemia, four pneumonias and five urinary tract infections and multiple colonisations. Between one and 16 isolates were studied from each patient, depending on the length of time the patient was hospitalised. Surveillance cultures were taken from patients within the first day of hospitalisation in the ICU and then once a week. Specimens from the rectum were obtained by Culturette swabs (Becton Dickinson and Co., Paramus, NJ, USA). Colonies were isolated on the selective medium Hektoen agar plates (bioMérieux, Marcy l'Etoile, France) as described previously [21]. All agar plates were incubated at 30°C for 24 h. Blood

Table 1. Clinical details of patients on ICU1

Patient no.	Duration of stay (days)	Predisposing factors	Age/sex	Underlying disease	Infected/colonised after (days)	Index of gravity	
						APACHE	SAPS
1	30	Ventilation CV catheter Corticosteroids	51/F	Asthma Cardio-respiratory failure	6*	28	14
2	38	Ventilation Urinary catheter	53/F	Hepatectomy	28*	19	18
3	45	Ventilation CV catheter	55/M	Oesophagus surgery	11*	30	11
4	8	CV catheter	65/F	Coma	4	25	10
5	51	CV catheter Corticosteroids Ventilation	43/M	Respiratory failure	39*	18	15
6	7	CV catheter Ventilation	81/M	Peritonitis	7*	35	17
7	58	CV catheter Haemodialysis Urinary catheter	58/M	Nephrectomy Cholecystectomy	13*	19	15
8	148	CV catheter Ventilation Urinary catheter	45/F	Peritonitis	5†	9	10
9	65	Ventilation Corticosteroids	13/F	Myasthenia	54*	8	10
10	77	CV catheter Haemodialysis Urinary catheter	45/M	Heart surgery Acute renal failure	49	12	13
11	38	Ventilation CV catheter Urinary catheter	57/M	Moschcowitz syndrome	30*	28	16
12	7	Ventilation CV catheter	65/M	Heart surgery	1	11	5

APACHE, acute physiology and health care evaluation.

SAPS, simplified acute physiology score.

CV catheter, central venous catheter.

*Patients considered to be infected.

†This patient had bacteraemia and pneumonia caused by *E. aerogenes* — isolates nos. 5 and 12 (Table 3), respectively.

cultures were performed in a Bactec culture apparatus with 6A medium (Becton Dickinson).

Environmental cultures. Environmental samples from ICU1 were obtained three times during the 4-month period (8 Dec. 1992, 10 Jan. 1993 and 6 Feb. 1993). Items used in patient care as well as those frequently handled by personnel were cultured. Samples were obtained with moistened Culturette swabs and plated on Bromo-Cresol Purple (BCP) agar plates (bioMérieux). Cultures from ICU1 health workers were obtained by pressing their fingers on to Mueller-Hinton agar supplemented with sheep blood 5% (bioMérieux).

Antibiotic susceptibility testing. Antimicrobial susceptibility to 42 antimicrobial agents was determined by the standard disk-diffusion method on Mueller-Hinton agar (bioMérieux) (Table 2). The presence of ESBL activity was determined by placing disks containing cefotaxime, ceftazidime and ceftriaxone near a disk containing a β -lactamase inhibitor (sodium clavulanate 10 mg and amoxycillin 20 mg). Extension of the zone of inhibition toward the disk containing clavulanate suggested the presence of an ESBL [22].

Identification. Biochemical reaction patterns (bio-profiles) were determined with the API 20E identification system (bioMérieux) according to the manufacturer's instructions.

Other isolates

Sixteen isolates from 10 patients associated with an outbreak of colonisation and infection with *E. aerogenes* between 21 Sept. and 14 Dec. 1993 in a different ICU (ICU2) in the same hospital were also investigated by RAPD analysis. These isolates were identified with the API20E and their antibiotic susceptibility was determined as above. As no regular epidemiological surveys were undertaken in ICU2, no environmental samples were obtained during this period.

Six non-outbreak-associated strains from our culture collection were included for comparison as controls for the RAPD method.

RAPD analysis

DNA preparation. Isolates were grown overnight at 37°C on Mueller-Hinton agar (bioMérieux) and lysed as described previously [23]. Total DNA was isolated by a phenol-chloroform extraction method as described previously [23]. Briefly, bacteria were suspended in a solution of sucrose 25%, 0.01 M Tris-HCl, 0.01 M ethylene-diamine-tetraacetate (EDTA) and lysozyme (Sigma) 5 mg/ml, pH 7.6, and incubated for 60 min at 37°C. Sodium dodecyl sulphate 1% and proteinase K (Sigma) 0.5 mg/ml were then added and the suspensions were incubated at 60°C for 60 min. Proteins were removed by four consecutive extractions with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and the total bacterial DNA was precipitated with ethanol 95% and dissolved in 10 mM Tris-HCl (pH 7.6), 0.1 mM EDTA. The DNA was finally dissolved in water and concentrations were estimated on agarose gels.

Primers. All isolates were compared by RAPD with three different 10-mer oligonucleotide primers. The primers were those described by Williams *et al.* [19]: AP4, 5'(TCACGATGCA)3'; AP12h, 5'(CGGCCCC TGT)3' and AP13, 5'(ATTGCGTCCA)3'.

Amplification conditions. Amplification reactions were performed in a total volume of 47 μ l containing 100 μ M each of dATP, dCTP, dGTP and dTTP, 0.2 μ M of primer, 25 ng of template DNA and 1.25 units of *Taq* polymerase (Perkin-Elmer/Cetus, Norwalk, CT, USA) in 1 $\times$ PCR buffer (20 mM Tris-HCl, pH 8.3; 50 mM KCl, 3 mM MgCl₂, gelatin 0.001% w/v). A negative control without template DNA was included in each experiment. The reaction mixtures were overlaid with mineral oil and amplified in a DNA thermal cycler (TR2, Cera-Labo, Aubervilliers, France) pro-

Table 2. Antibiotics used in disk-diffusion tests

benzyl penicillin	cephalothin	erythromycin
ampicillin	cefamandole	spiramycin
amoxycillin	cefoxitin	clindamycin
ticarcillin	cefotiam	rifampicin
cloxacillin	cefotaxime	neomycin
methicillin	cefsulodin	kanamycin
mezlocillin	cefmenoxime	gentamicin
piperacillin	ceftriaxone	tobramycin
	ceftazidime	netilmicin
ampicillin-sulbactam	cefpirome	amikacin
amoxycillin-clavulanate	cefoperazone	streptomycin
ticarcillin-clavulanate	moxalactam	fosfomycin
piperacillin-tazobactam		colistin
	ceftazidime-sulbactam	chloramphenicol
imipenem		doxycycline
meropenem	cefoperazone-sulbactam	ciprofloxacin
aztreonam		pefloxacin
		ofloxacin
trimethoprim-sulphamethoxazole		

grammed for 45 cycles of 1 min at 94°C, 1 min at 45°C and 1 min at 74°C. Amplification products were compared by electrophoresis of 10-µl samples in agarose 1.2% gels in Tris-acetate buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.2), stained with ethidium bromide and photographed on a UV light transilluminator. A molecular size standard (Marker VI, Boehringer-Mannheim, Mannheim, Germany) was included on each gel. Amplification patterns differing by two or more bands were considered to represent different strains. The reproducibility of the technique was determined by testing all isolates on at least three separate occasions with each RAPD primer.

Results

Typing

All isolates of *E. aerogenes* from ICU1 and the non-outbreak control strains exhibited the same biochemical reaction pattern (5105773). *E. aerogenes* strains from ICU2 exhibited the pattern (5105673). RAPD characterisation of the six non-outbreak-associated strains gave four distinct amplification patterns with primer AP4 (data not shown) and six distinct patterns with primers AP13 and AP12h (Fig. 1). The three primers successfully typed the 57 isolates from ICU1 and ICU2 and the reproducibility of the RAPD assays was good. No products were detected in the negative control

tubes. RAPD patterns with primers AP4, AP13 and AP12h classified the 41 clinical isolates from ICU1 in 11, 18 and 22 distinct types of *E. aerogenes* respectively (Figs. 2–5). There was good correlation between the results with the three primers, with AP12h providing the best discrimination between strains (Fig. 5). For example, strains 2 and 3 were identical with primers AP4 and AP13 but different with primer AP12h. Strains belonging to the same AP12h type always belonged to the same AP13 and AP4 type (e.g., isolates 28, 29 and 30 belonged to the same AP12h, AP13 and AP4 type). The 22 different types (types a–v) generated with AP12h were as discriminatory as the combined results from all three primers and were, therefore, used to compare isolates.

Between one and 12 strains, distinguishable by RAPD, were detected from each of the 12 patients on ICU1. The temporal and genotypic relationships between the different strains obtained from the 12 patients hospitalised in ICU1 are shown in Fig. 6. Nine different RAPD patterns were each associated with two-to-five isolates from different patients (Table 3), suggesting spread of these strains between patients. Cross-transmissions involved two to five patients over a 3–15-day period (Fig. 6). In one patient, mixed colonisation was observed. Patient 8 carried two or three different isolates on the same day, each at a different site (nasal fossae, abdominal drain and

Fig. 1. RAPD patterns obtained with primer AP13 (left, lanes 1–6) and AP12h (right, lanes 1–6) for the non-outbreak control strains of *E. aerogenes*. Lane M, molecular size standard. The sizes of the bands are shown in base-pairs.

Fig. 2. The 11 RAPD patterns obtained with primer AP4 for *E. aerogenes* isolates from ICU1 (I–XI). Lane M, molecular size standard. The sizes of the bands are shown in bp.

Fig. 3. The 18 RAPD pattern types obtained with primer AP13 for isolates of *E. aerogenes* from ICU1 (A–R). Lane M, molecular size standard. The sizes of the bands are shown in bp.

urinary tract). RAPD analysis of 10 separate colonies of *E. aerogenes* from single samples, showed that patients were colonised by a single clone of bacteria at a specific anatomical site at any one time. In all cases, the 10 colonies belonged to the same RAPD-type (data not shown).

An outbreak which had occurred in another ICU (ICU2) of the same hospital was also investigated. Which ever primer was used, the 16 strains studied gave very similar patterns (Fig. 4b). The RAPD patterns of the six non-outbreak control strains with primer AP12h were clearly different from

Fig. 4. Comparison between the RAPD patterns with primer AP12h of *E. aerogenes* isolates from ICU1 and ICU2. **a**, The different AP12h RAPD types of *E. aerogenes* isolates from ICU1, (a–v); **b**, RAPD profiles of *E. aerogenes* from ICU2 (lanes 1–16); **M**, molecular size standard; the sizes of the bands are shown in bp; **E**, environmental isolate from ICU1.

those obtained from the 41 strains from ICU1 and the 16 outbreak-related isolates from ICU2 (Figs. 1 and 4).

Surveillance cultures

Rectal colonisation with *E. aerogenes* was not found and *E. aerogenes* was not isolated from the hands of ICU1 health workers.

Clinical co-infections

In seven patients on ICU1, polymicrobial contamina-

tions or infections involving two to six different micro-organisms in a single patient were observed. In addition to *E. aerogenes*, *P. aeruginosa* was isolated in seven cases, *Enterococcus faecalis* in six, *Staphylococcus aureus* in three, coagulase-negative staphylococci from blood cultures in two and *Streptococcus pneumoniae* in bronchial secretions from two cases. In single cases, *Esch. coli*, *Candida albicans*, *Stenotrophomonas maltophilia*, *Serratia marcescens* (with ESBL) and *Mycobacterium tuberculosis* were isolated in addition to *E. aerogenes*. These results are similar to those obtained in a previous study [4].

Fig. 5. Classification of the 41 isolates of *E. aerogenes* from ICU1 by RAPD patterns with three primers. The patterns obtained with the three primers AP4, AP13 and AP12h are numbered in roman numerals (I–XI), capital letters (A–R) and small letters (a–v) respectively. Isolates are identified by arabic numbers from 1 to 41.

Environmental data

One strain of *E. aerogenes* was isolated from the humidifier of a mechanical respiratory device on ICU1, but this strain did not belong to an RAPD pattern found in clinical samples (Fig. 4, lane E).

Antibiotic susceptibility

The isolates from ICU1 had similar antibiotic susceptibility patterns, which indicated the presence of ESBL. Of the 41 isolates, 38 were susceptible only to imipenem, moxalactam, colistin and gentamicin. Three imipenem-resistant isolates were detected in urine and cutaneous samples in two patients. This resistance was associated with moxalactam resistance. Both urinary isolates appeared during the same week in two patients, but they were distinct by RAPD fingerprinting (isolates 25 and 27). Persistence of this type of resistance was not seen. The 16 isolates from ICU2 were susceptible

to imipenem, cefotaxime, cefmenoxime, moxalactam, amikacin, gentamicin and colistin. No ESBL activity was detected in these isolates.

Discussion

In recent years, *E. aerogenes* has been recognised increasingly as a cause of bacteraemia and other serious infections in hospitalised patients. However, the epidemiology of this organism is still poorly understood. Some studies have reported that the most common portals of entry are respiratory (34–40%), genitourinary (27%), wound (20–25%) and biliary (18%) [2, 4, 10]. One study considered the most common portal of entry to be abdominal (39%), particularly after abdominal surgery, which is consistent with the enteric origin of *Enterobacter* spp. [13]. For the 12 patients from ICU1 in the present study, 60% were probably first colonised in the respiratory

Fig. 6. Temporal and genotypic relationships between the 41 isolates of *E. aerogenes* obtained from the 12 patients hospitalised in ICU1. Strains with same API2h RAPD type are joined by line and illustrate a possible order of transmission.

tract and 40% in the urinary tract. Endotracheal tubes and indwelling urinary catheters have been shown to be associated with *Enterobacter* bacteraemia [5].

The increase in prevalence of *E. aerogenes* has paralleled the intensive use of the expanded-spectrum β -lactam agents, particularly cephalosporins, in intensive care units [12, 24]. Selection by these antibiotics favours the emergence of *Enterobacter* spp., whose presence in the gastrointestinal tract possibly serves as an endogenous source of infection [14, 22]. Therefore, antibiotic treatment may lead to colonisation of the tracheobronchial tree and the skin by *Enterobacter* spp. with subsequent invasion promoted by respiratory failure, gastrointestinal tract operation or open skin wounds [2, 6]. An endogenous source would explain why no common environmental source was detected in the present study. However, the protocol for collecting swab specimens from the rectum was not sufficiently sensitive to confirm this, despite the use of a selective medium for the isolation of enteric pathogens of the family Enterobacteriaceae. The use of rectal swabs rather than stool cultures to determine colonisation would certainly lead to the underestimation of the number of patients who were colonised at the time of admission. *E. aerogenes* was not isolated from the hands of staff on ICU1, suggesting that horizontal transmission could not be explained by hand-carriage alone in this study. In previous studies, *E. aerogenes* has been isolated from contaminated instruments or solutions [14] and the present study detected an isolate from a humidifier. This was shown to be different from the clinical

isolates by RAPD but the observation suggests that such items may act as a source of infection.

In the present study, three imipenem-resistant isolates were detected. The widespread use of imipenem in intensive care units is of concern as this selects imipenem-resistant isolates [9, 15]. This resistance may be associated with a modification of a major outer-membrane protein (OMP) [15, 25, 26] or changes in lipopolysaccharide [27]. De Champs *et al.* observed an associated imipenem-moxalactam resistance in some isolates and the loss of this resistance at the end of imipenem treatment [15].

The present study raises the question of whether multiple colonisation involved sequential exposure to different strains or whether genotypic variations within strains were induced *in vivo* in response to changes in therapy. It is difficult to determine the role, if any, of antibiotic therapy in the evolution of different isolates of *E. aerogenes*. In patient no. 8, isolation of 12 different strains of *E. aerogenes* was concomitant with successive and major modifications in antibiotic therapy (Table 3). However, despite constant antibiotic therapy during a 42-day period for patient no. 7, five strains of *E. aerogenes* with very different RAPD patterns were isolated. Therefore, it seems unlikely that the two phenomena are related.

Because of the large number of *E. aerogenes* isolates from each patient on ICU1 and the relatively rare previous isolation of this species, together with the similar antimicrobial susceptibility patterns of the

Table 3. Treatment and isolate details of patients in ICU1

Patient no.	Isolate no.	RAPD pattern*	Site of isolation	Antibiotic therapy before <i>E. aerogenes</i> isolation†	Antibiotic therapy during <i>E. aerogenes</i> isolation†
1	7‡	e	Tracheal aspiration	AMC-PEF, 8 days	IPM-GN, 10 days
	8	o	Vaginal sample	VA, 8 days	CS/IT, 16 days
2	9‡	e	Urine	IPM-CIP, 10 days	VA, 8 days
	11	t	Tracheal aspiration		AN-ATM, 4 days
3	2‡	b	Urine	CS/IT, 8 days	IPM-GN, 10 days
	6	d	Urinary catheter	AN, 3 days	CS/IT, 15 days
4	28	j	Umbilicus		
	29	j	Tracheal aspiration	AMC, 3 days	AMC, 4 days
5	30	j	Urine	IPM-AN-VA, 8 days	IPM, 8 days
				CTX-IPM, 15 days	CS/IT, 8 days
6	10	k	Tracheal aspiration	AN-S-CAZ, 10 days	CTX, 2 days
				S-CAZ, 3 days	
7	14, 16‡, 24	i, l, q	Tracheal aspiration	PIP-PEF, 6 days	Deceased on the same day
	17, 18	h, l	Nasal cavity	CAZ-CTX-MA, 3 days	PIP-S, 42 days
8	41	v	Bronchoalveolar lavage	PIP-S, 2 days	CL-IT, 10 days
	32	s	Urine		
9	19, 35	m, p	CV catheter		
	1‡, 12, 13, 21	a, h, l, f	Tracheal aspiration		PIP-PEF, 5 days
10	22, 36	g, p	Nasal cavity		PIP-AN, 2 days
	15, 25	l, q	Urine		IPM-AN, 15 days
11	23	f	Vaginal sample		ATM-TEC, 2 days
	4	d	CV catheter		ATM-TEC-S-AN, 12 days
12	5	n	Blood culture		ATM-TEC-S-GN, 12 days
	26	r	Intra-abdominal abscess		IPM-GN, 15 days
13	20, 33	f, u	Abdominal tapping		CS/IT, 15 days
	34	u	Drainage tube		
14	3	c	Tracheostomy swab		
	27	r	Urine	IPM, 10 days	
15	31‡	s	Nasal cavity	ATM-AN, 30 days	
	37	p	CV catheter	TEC, 4 days	PEF, 7 days
16	38‡	p	Urine	CLX-PEF, 11 days	
	40	p	Tracheal aspiration	AMC-PEF, 5 days	IPM-GN, 7 days
17	39	p	Nasal cavity	IPM-AN, 20 days	
				AM, 10 days	
18				CLX-PEF, 14 days	
				AMC, 6 days	IPM-GN, 7 days

CV catheter, central venous catheter.

*RAPD pattern with primer AP12h as shown on Fig. 5.

†AMC, amoxycillin plus clavulanic acid; AM, ampicillin; AN, amikacin; ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; CS/IT, colistin intra-tracheal; CLX, cloxacillin; MA, cefamandole; CIP, ciprofloxacin; GN, gentamicin; IPM, imipenem; PEF, pefloxacin; PIP, piperacillin; S, sulbactam; TEC, teicoplanin; VA, vancomycin.

‡Initial isolate from this patient.

isolates, it was thought initially that the outbreak was due to the spread of a single strain. However, the present study showed that several strains had spread between small numbers of patients. In some patients, isolates from different anatomical sites gave different RAPD patterns, suggesting carriage of multiple strains. We suggest that successive small outbreaks, as reported here, may be partly due to changes in the multiplication rate of strains. We suggest that the micro-organisms generally multiply at a low rate but this may increase for some reason, resulting in the spread of a micro-organism to several patients and possible infections. After transmission, the organisms may regress, leaving patients susceptible to colonisation and infection by other strains of *E. aerogenes*. In contrast to the situation in ICU1, a similar investigation in ICU2 revealed an outbreak that was probably caused by a single strain, although heterogeneity within this phenotype was not established and it is

possible that the RAPD pattern represented a common type or clone.

The PCR-based DNA fingerprinting method termed RAPD is being used increasingly to discriminate between isolates within different species to trace transmission during outbreaks of infection in hospitals. In this study, RAPD analysis distinguished between a cluster of co-incident but independent infections and an outbreak caused by a single strain. Therefore, RAPD is suitable for epidemiological studies of *E. aerogenes* and should also be applicable to investigations of other nosocomial infections.

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