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Note

Molecular and Antibiogram Relationships of *Acinetobacter* Isolates from Two Contrasting Hospitals in the United Kingdom and South Africa

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Abstract The aim of this study was to compare the molecular relationships and antibiograms of nosocomial isolates of *Acinetobacter* spp. from two acute-care hospitals in Nottingham, UK, and Soweto, South Africa, with different hospital infection control problems and procedures. In contrast to Nottingham, where randomly amplified polymorphic DNA fingerprinting demonstrated that a single multiresistant strain of *Acinetobacter baumannii* has predominated in the hospital intensive care unit over an 11-year period, the Soweto isolates formed a heterogeneous group of unrelated molecular clusters of different antibiograms, with numerous different strains of *Acinetobacter baumannii*, *Acinetobacter* sp. 3 and *Acinetobacter* sp. 13TU apparently being endemic throughout the Soweto hospital. The contrasting results illustrate the need to maintain exemplary infection control procedures in hospitals where high standards have been achieved and warn of what might result if such measures are diminished.

Introduction

Members of the genus *Acinetobacter*, particularly *Acinetobacter baumannii*, are implicated in a wide spectrum of nosocomial infections, including bacteraemia, secondary meningitis and urinary tract infection [1], but their most important role seems to be as agents of nosocomial pneumonia, particularly ventilator-associated pneumonia in patients confined to hospital intensive care units (ICUs) [2]. Although generally considered to be of low pathogenicity, these organisms seem to have a remarkable ability to acquire antibiotic resistance genes, to survive in the hospital environment and to spread easily from patient to patient [1]. Outbreaks of multiresistant *Acinetobacter* infection in European hospitals with good infection control policies and procedures have often been shown to be associated with the spread of particular 'local' strains [3–5]. Thus, in Nottingham, UK, surveillance of an adult ICU has demonstrated long-term persistence of a multiresistant strain of *Acinetobacter baumannii* [5].

The aim of the present study was to compare the Nottingham findings with the molecular relationships and antibiograms of nosocomial *Acinetobacter* isolates from an acute-care hospital with contrasting hospital infection control problems and procedures. The University Hospital Nottingham is a typical large European teaching hospital with approximately 1200 beds, while Chris Hani Baragwanath Hospital (located in the developing city of Soweto, part of the greater Johannesburg metropolitan area) has 3240 beds and is substantially under-resourced for the vast number of patients that it treats.

Materials and Methods

Bacteria were initially isolated by routine in-house methods and provisionally identified to the genus *Acinetobacter* by standard techniques [6] from patient or environmental samples sent to the diagnostic bacteriology laboratories of University Hospital Nottingham and Chris Hani Baragwanath Hospital, Soweto. All isolates from both centres were then tested in the Nottingham laboratory for their ability to grow on Leeds Acinetobacter medium [7]. Confirmed isolates of *Acinetobacter* were then identified to the genomic species level by the technique of tDNA fingerprinting [8]. *Acinetobacter* infections in the University Hospital Nottingham are relatively infrequent, but occasional outbreaks of infection occur, predominantly in the ICU. The 33 Nottingham isolates studied were obtained over the 11-year period 1985–1995, during which two significant outbreaks of infection occurred (7 years apart) [5]. In contrast, *Acinetobacter* infections in Chris Hani Baragwanath Hospital are endemic in many areas of the hospital, with varying numbers of infections

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depending on the stringency of control measures being applied in affected areas at a particular time. All of the Soweto isolates studied were obtained during 1996. The 75 isolates of *Acinetobacter* included in the study were considered to be a representative selection of isolates from the periods studied but were not consecutive, as some isolates had not been saved or could not be recovered.

Molecular relationships between the isolates were determined by computer analysis of randomly amplified polymorphic DNA (RAPD) fingerprints generated with DAF4 and M13 core primers as described previously [4]. For the purpose of this study, isolates with an RAPD fingerprint similarity coefficient (S_{AB} value) of ≥ 0.7 (a value shown previously to demonstrate good discrimination between genetically unrelated groups of *Acinetobacter* spp. [4]) were considered to belong to the same cluster. Antibiograms were determined by the disk inhibition zone method [9] for the selection of antibiotics shown previously to be useful for distinguishing *Acinetobacter* clusters [10]. Plates were incubated for 24 h at 30 °C and inhibition zones were measured from the edge of the disks. Antibiogram similarity coefficients were then calculated and analysed as described previously [10]. Isolates with an antibiogram S_{AB} value of ≥ 0.9 were considered to have indistinguishable antibiograms.

Results and Discussion

This study was not intended to be a formal assessment of the epidemiological aspects of *Acinetobacter* and the associated risk factors, clinical features and outcome, etc., of colonised or infected patients, but instead aimed to assess whether there were differences between the clustering relationships of isolates from two hospitals, one in the UK and one in South Africa, with differing health care structures and infection control resources. Table 1 lists the resistance phenotype patterns and their frequencies found amongst the isolates examined. Table 2 summarises the genotypic and phenotypic characteristics of the acinetobacters isolated in Nottingham and Soweto, respectively. Similar genotypic relationships were revealed following analysis of the RAPD fingerprints generated with both primers.

Despite the fact that the 33 Nottingham isolates represented those recovered over an 11-year period, only nine different clusters were recognised, with seven clusters comprising isolates with a single antibiogram (Table 2). A single multiresistant strain of *Acinetobacter baumannii* accounted for over 50% of the isolates and was recovered throughout the 11-year period, during which two significant outbreaks of infection occurred 7 years apart [5].

In contrast, although the 42 Soweto isolates were recovered during a single year, DNA fingerprinting revealed 19 unrelated molecular clusters of isolates (9 of which contained only a single isolate) belonging to genomic species 2, 3 and 13TU. In addition, eight of the ten clusters comprising more than one isolate contained isolates with more than one antibiogram (Table 2).

It is noteworthy that the Nottingham collection comprised clinical and environmental isolates, while the Soweto collection consisted of clinical isolates only. A greater species diversity, including "harmless" species, might be expected as a result of including environmental isolates. Indeed, if the environmental

Table 1 Summary of the resistance phenotype patterns and their frequencies found amongst the *Acinetobacter* isolates examined

Resistance phenotype designation[a]	No. isolates	Antibiogram (disk zone size, mm)[b]											
		Ap	Cd	Cf	Ct	Im	Cp	Gm	Tb	Ak	Te	Na	Rf
A	19	0	0	0	0	10	5	0	5	9	0	8	2
B	7	2	0	2	5	12	10	8	7	8	5	9	2
C	3	0	0	2	5	12	0	5	3	8	0	0	0
D	2	0	8	7	9	6	11	1	1	9	6	10	4
E	2	1	0	3	5	11	2	9	8	8	3	0	2
F	3	0	0	0	2	12	5	9	8	9	2	6	2
G	1	6	6	7	10	12	10	11	10	12	5	9	3
H	4	8	5	5	5	12	8	9	8	9	3	8	2
I	4	0	0	0	0	12	4	3	4	2	2	5	2
J	7	0	0	0	0	8	0	1	6	0	0	0	3
K	11	0	0	0	0	10	0	3	7	3	0	0	2
L	1	0	0	0	0	8	5	0	5	3	0	5	2
M	1	1	0	2	3	12	4	5	5	2	1	5	2
N	2	0	0	2	4	11	5	0	5	7	0	6	2
O	2	0	0	0	2	13	4	5	2	2	0	3	2
P	2	0	0	0	0	8	0	5	6	3	0	0	1
Q	1	3	0	4	7	11	0	5	5	1	0	0	2
R	1	0	0	0	0	8	0	0	1	2	0	0	1
S	2	0	0	0	0	9	4	3	3	8	0	3	1

[a] Antibiograms with a similarity coefficient of ≥ 0.9 were considered indistinguishable and were grouped together in a single resistance phenotype designation

[b] Disk antibiotic concentrations were as follows (μg/disk): Ap, ampicillin (10); Cd, cephradine (30); Cf, cefuroxime (30); Ct, cefotaxime (30); Im, imipenem (10); Cp, ciprofloxacin (1); Gm, gentamicin (10); Tb, tobramycin (10); Ak, amikacin (30); Te, tetracycline (10); Na, nalidixic acid (30); Rf, rifampicin (5)

Table 2 Summary of the molecular and antibiogram clusters found amongst nosocomial *Acinetobacter* isolates from hospitals in Nottingham, UK, and Soweto, South Africa

Genomic species	No. of isolates forming a cluster[a]	Antibiogram patterns in cluster[b]
Nottingham isolates ($n=33$)		
Acinetobacter baumannii (sp. 2)	19	A
	2	B, C
	2	C, D
	1	D
	1	C
Acinetobacter sp. 3	2	E
Acinetobacter johnsonii (sp. 7)	1	F
Acinetobacter radioresistens (sp. 12)	1	G
Uncharacterised species[c]	4	H
Soweto isolates ($n=42$)		
Acinetobacter baumannii (sp. 2)	5	I, J, K
	5	K
	3	J, K
	2	B, L
	2	B, M
	2	J
	1	N
	1	I
	1	B
	1	B
	1	N
Acinetobacter sp. 3	5	F, O, P
	3	J, Q
	2	F, R
	1	S
Acinetobacter sp. 13TU	4	J, K, S
	1	B
	1	K
	1	B

[a] Defined as isolates belonging to the same genomic species that have an RAPD fingerprint similarity coefficient of >0.7 [4]
[b] Antibiogram patterns are defined in Table 1
[c] Previously uncharacterised genomic species on the basis of tDNA fingerprinting [8]

isolates were excluded from the Nottingham collection to allow a direct comparison with the Soweto clinical isolates, an even greater proportion (86%) of the Nottingham clinical isolates would have comprised the predominant multiresistant strain of *Acinetobacter baumannii*.

Members of the genus *Acinetobacter* have a remarkable ability to develop resistance to even the most potent antimicrobial agents. Extensive and increasing use of broad-spectrum compounds in European hospitals has served to eliminate competing bacteria and create a vacant ecological niche that enhances the ability of particular resistant *Acinetobacter* clones to colonise and subsequently cause infection in susceptible patients. In Nottingham [5], as in other large European teaching hospitals [3, 4], it seems that particular *Acinetobacter* clones can persist in patients and the hospital environment for long periods of time, with outbreaks of infection occurring occasionally, perhaps as a consequence of a temporary breakdown or lapse in routine control of infection measures [5]. The predominance of a single strain in Nottingham may represent the effect of infection control practices to date (e.g., decontamination and antibiotic policies), which have resulted in the selection of a highly adapted strain that is capable of surviving in the environment and spreading occasionally between patients. In contrast, the prevailing conditions, large number of beds and high throughput of patients in Chris Hani Baragwanath Hospital make European standards of infection control difficult to achieve. A greater variety of strains seem to have achieved high levels of resistance to a wider range of antimicrobial combinations, possibly as a result of less restricted usage of antibiotics in the kind of patients (long-stay ICU, burns unit, trauma and surgical patients) who go on to acquire *Acinetobacter*. This has resulted in a difficult-to-control situation in which numerous endemic unrelated multiresistant strains of different *Acinetobacter* genomic species are circulating continuously amongst patients and, presumably, staff.

When outbreaks of *Acinetobacter* infection occur, it is essential to determine whether a single bacterial strain is involved in order to devise effective control strategies. If this is the case, such outbreaks can usually be controlled by monitoring patients and staff, by thorough cleansing of the environment, and by re-emphasising the need for handwashing before and after patient contact. In addition, periodic surveillance of patients and the environment at other times may help to identify persistent sources of infection and subop-

timal infection control procedures such as poor handwashing technique. The contrast in the strain diversity found in the two hospitals provides an important illustration of the continued need to maintain exemplary infection control procedures in hospitals where high standards have already been achieved and offers a warning of what might result if such measures are diminished. This is particularly relevant in view of the pressures for increased patient throughput in many European hospitals, coupled with a noticeable trend in some hospitals towards inadequate routine cleaning of clinical areas as a result of cuts in domestic cleaning budgets.

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