

Skin Carriage of *Acinetobacter* in Hong Kong

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We studied the carriage of *Acinetobacter* spp. at five superficial sites in 79 patients from two hospitals, in 133 healthy controls from the community (medical students and new nurses), and in 198 student nurses in different classes. A total of 431 isolates from 364 positive sites of 201 subjects and 124 blood culture isolates (1997 to 1998) were genospecies by amplified ribosomal DNA restriction analysis. Genospecies 3 was the most common species. The carriage rate of student nurses (42 of 131) was significantly lower than that of new nurses from the community (25 of 38) (chi-square test, $P = 0.0004$; odds ratio [OR], 4.08; 95% confidence limits, 1.78 to 9.41) but not significantly different ($P = 0.1$) from that of patients in the same hospital (20 of 42). Genospecies from blood cultures and subjects (acute patients and student nurses) from Prince of Wales Hospital were similar to one another but different from subjects from the community or from another hospital (chi-square test, $P < 0.0001$). Half of the subjects who were positive at at least two sites had different genospecies. Of the 28 sites examined, 68% showed strain variation among isolates of the same genospecies by random amplified polymorphic DNA analysis. Half of the 106 subjects who had samples taken again within 6 weeks or 6 months later were positive only once. In the 17 subjects who were positive on at least two occasions, each occasion yielded different genospecies in 13 subjects. Our results indicate that skin carriage in the majority of healthy subjects is characterized by low density, variation in genospecies and strains, short-term duration, and the typicality of a given locality.

Acinetobacter spp. are widely distributed in nature. Today, at least 19 genomic species established by DNA-DNA hybridization are recognized as constituting the genus (4, 5, 30). Genospecies 1, 2, 3, and 13TU are genotypically distinct but phenotypically similar DNA groups. They are grouped together as the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex (*Acb* complex) (2). Of these, *A. baumannii* (genospecies 2 or genomic group 2) is the most commonly reported species associated with hospital outbreaks and nosocomial infections, particularly ventilator-associated pneumonia in patients in intensive care units (2). *Acinetobacter* is an important nosocomial pathogen in Hong Kong, with a prevalence of infection higher than elsewhere (24, 27). As a result of *Acinetobacter* species' propensity to develop antibiotic resistance rapidly, multiple antibiotic resistance has often been a feature in clinical infections (2, 8, 9, 13, 16). Recently introduced molecular techniques provide acceptable genomic differentiation and are relatively easy to perform (11, 18, 26). This has given impetus to the investigation of the ecology and clinical significance of the genus. We report here our findings, based on amplified ribosomal DNA restriction analysis (ARDRA), of skin and mucous membrane carriage of *Acinetobacter* spp. in different groups of patients and healthy subjects and their comparison with blood culture isolates.

MATERIALS AND METHODS

Isolates from blood cultures. There were 124 blood culture *Acinetobacter* isolates from 79 individual patients during 1997 and the first 8 months of 1998 that met the criteria for genus identification (see below). These were stored on nutrient agar slants at room temperature until examination. After genospecies

tion, 91 isolates were used in this study, including 68 isolates from individual patients. In addition, 10 isolates obtained from five patients whose blood cultures were taken on different days, yielding isolates of either different genospecies or different novel patterns by ARDRA, and 3 isolates obtained similarly from a single patient were included.

Media for isolation. Leeds *Acinetobacter* Medium (LAM) was first used as described elsewhere (19). The concentration of vancomycin was later reduced from 10 to 3 $\mu\text{g/ml}$ to allow for growth of the 37 reference strains of 19 genospecies tested (supplied by T. L. Pitt, Laboratory of Hospital Infection, Central Public Health Laboratories, PHLS, Colindale, London, United Kingdom, with the permission of L. Dijkshoorn, Leiden Hospital, Leiden, The Netherlands). The modified LAM (MLAM) was then used in the carriage study of *acinetobacters*.

Subjects, sampling, and method of isolation. (i) **Medical students.** First-year medical students in 1997 who had been pursuing their studies solely at the campus of the Chinese University of Hong Kong were recruited.

(ii) **Student nurses.** Student nurses at different stages of training were recruited from the nursing school at the Prince of Wales Hospital (PWH), Shatin, Hong Kong. Their residential course was divided into periods of training, either as a 14- to 22-week ward block for clinical work or as a 7- to 12-week study block at the school. Samples were collected on the first day of a study block, immediately following the period of clinical training at the wards. Whenever a presumptive positive result was obtained, repeat specimens (recall samples) were obtained within 6 weeks.

(iii) **New nurses.** New student nurses commenced on a study block at the School of Nursing. Sampling was scheduled to be done within the first 48 h of their arrival at the school. Recall samples were obtained within 6 weeks whenever a presumptive positive result was obtained.

(iv) **Acute patients.** Patients from two acute medical wards at PWH who did not have a history of antibiotic therapy or hospital admission during the preceding 14 days were recruited within 2 days of their admission.

(v) **Chronic patients.** Patients at the Shatin Hospital, Shatin, Hong Kong, who had been hospitalized there for at least 7 days were studied. Shatin Hospital is a hospital for convalescence, and it receives patients from PWH.

Having obtained informed consent, cotton wool swabs (Venturi Transystem) moistened in sterile water (except swabs for the throat) were used to take samples from all subjects from five sites: throat, hairline on the forehead, anterior nares, groins, and fourth-toe webs. The same swab was used for both sides for each of the last three sites. When necessary, the swab was stored at 4°C until laboratory examination within 24 h. The samples were used for two studies: the carriage of *acinetobacters* and the carriage of multidrug-resistant organisms. Procedures relevant to the former are described here. The nasal swab was first used to inoculate a MacConkey plate before being placed in a methicillin salt

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TABLE 1. Isolation of acinetobacters from superficial sites in medical students, new nurses, student nurses, and patients

Group ^a	Period	No. of subjects ^b										
		F	M	With the following no. of positive sites:				Positive at the following site:				
				1	2	≥3	Total (%)	H	G	N	T	W
Patients												
Acute	July–Sept. 1997	21	21	10	4	6	20 (47.6)	9	10	2	5	13
Chronic	Aug.–Nov. 1997	22	15	17	5	7	29 (78.4)	24	6	4	10	10
Community (summer)												
N42 (41)	Oct. 1997	34	4	15	8	2	25 (65.8)	16	6	4	5	6
Medical students (98)	Oct. 1997	50	45	25	13	8	46 (48.4)	28	10	13	10	14
Total		84	49	40	21	10	71 (53.4)	44	16	17	15	20
Community (winter) N43 (60)												
Student nurses (summer)	Feb. 1998	56	3	14	5	0	19 (32.2)	14	6	0	0	4
N36 (41)	Oct. 1997	35	4	6	4	2	12 (30.8)	10	2	2	1	5
N39 (70)	Aug. 1997	50	8	13	4	2	19 (32.8)	10	7	2	3	6
N41 (34)	Aug. 1997	28	6	7	2	2	11 (32.4)	9	2	1	1	4
Total		113	18	26	10	6	42 (32.1)	29	11	5	5	15
Student nurses (winter)												
N39 (61)	Feb. 1998	47	4	12	3	0	15 (29.4)	11	2	0	3	2
N41 (29)	Mar. 1998	23	5	3	2	0	5 (17.9)	2	2	0	1	2
Total		70	9	15	5	0	20 (25.3)	13	4	0	4	4
Grand total		366	115	122	50	29	201 (41.8)	133	53	28	39	66

^a Values in parentheses indicate total number of students in the class.

^b H, hairline; G, groins; N, nares; T, throat; W, fourth-toe webs; F, female; M, male.

broth which was used for the study of multidrug-resistant organisms. For samples from other sites, the swab was vortexed in 1 ml of sterile peptone water for 1 min. Then 100 µl of the peptone water was used to inoculate a MLAM plate and other plates for the study of multidrug-resistant organisms. A glass rod was then used to spread the inoculum. The hairline swab was placed in an enrichment broth which was made with the same recipe as the MLAM but without the addition of agar. The broth was incubated at 30°C for 48 h before subculture on MLAM plates.

All MLAM and MacConkey plates used for nasal swabs were examined after 48 to 72 h of incubation at 30°C. Typical colonies were picked and examined further (32). The transformation assay of Juni was used to confirm the genus (22). Temperature tolerance was performed as described elsewhere (4). The amount of growth on a MLAM plate was scored as scanty for <20 colonies, moderate for 20 to 100 colonies, and heavy for >100 colonies. A positive culture only by subculture was also scored as scanty.

Method of genospeciation. Genospeciation was carried out by ARDRA, as described elsewhere (11), with two primers (5'-TGG CTC AGA TTG AAC GCT and 5'-TAC CTG TTA CGA CTT CA [Pharmacia, Uppsala, Sweden]) and enzymes (*CfoI*, *AluI*, *MboI*, *MspI*, and *RsaI* [Pharmacia] and *BfuI* and *BsmI* [New England Biolabs]). Restriction patterns were obtained by agarose (2%) gel electrophoresis and compared after ethidium bromide staining.

Random amplified polymorphic DNA (RAPD) analysis of groups of strains belonging to one genospecies was performed with Read-To-Go RAPD beads and primer 2 (both from Pharmacia) as recommended by the manufacturer. Products were analyzed by agarose (2%) gel electrophoresis.

Statistical analyses were carried out by using the chi-square test (Epi Info, version 5.01b, 1991) and the chi-square exact test (StatXact, version 2.05, 1991). Unclassified strains were grouped together for analysis.

RESULTS

The study was carried out from January 1997 to March 1998. Results from the first 6 months of the study involving three classes of nurses, two patient groups, and one class of medical students are excluded here because LAM containing 10 mg of vancomycin per liter was shown to not support the growth of all reference strains. Table 1 shows the month(s) of sampling, the number of subjects, and the number of subjects in each group

(studied from July 1997 on, when MLAM was used) with a positive result. The means and ranges of the ages in each group were as follows: 20.0 years and 19 to 22 years for medical students, 20.2 years and 17 to 33 years for new nurses (classes N42 and N43), 21.5 years and 19 to 30 years for student nurses at later stages of training (classes N36, N39, and N41), 67.9 years and 37 to 88 years for acute patients, and 78.9 years and 62 to 93 years for chronic patients, respectively. No clinical details were available on patients, but 10 acute patients had been admitted to PWH during the previous 9 months. Two classes (N39 and N41) were sampled twice, in August 1997 and February or March 1998.

Altogether, there were 2,845 site samples taken from 410 individuals, including recall samples from 88 subjects of all nurse classes because of presumptive positive results and winter samples of nurses in classes N39 and N41. Table 2 shows the distribution of different genospecies obtained from all sites in each group and those recovered from blood cultures. There were 280 sites which yielded one genospecies, 33 sites which yielded two genospecies, and 6 sites which yielded three or more genospecies. Five genospecies were isolated from five sites in one subject. The average number of positive sites per patient was 1.9, and the average number per healthy subject was 1.6. From the patient groups, 50% of the plates showed a light growth and 43% showed a heavy growth, whereas from the healthy groups, the corresponding figures were 79 and 8% (heavy growth: $P < 0.0001$; OR, 8.44; 95% confidence limits, 3.85 to 18.79; light growth: $P < 0.0001$; OR, 3.74; 95% confidence limits, 2.01 to 7.00). During the study period, no outbreak of infection caused by *Acinetobacter* spp. was observed in the wards.

Of the 133 positive hairline sites, 45.5% were positive after

TABLE 2. *Acinetobacter* genospecies obtained from all positive sites of patients, medical students, new nurses, and student nurses and blood culture isolates

Genospecies	No. of subjects (%) positive from the following group:					No. of positive blood culture isolates (%) (1997–1998)	Total no. (%)	
	Patients		Community (MS and N42) ^a	Student nurses (N36, N39, and N41) ^a	Community (N43) ^b			Student nurses (N39 and N42) ^b
	Acute	Chronic						
1	2 (4.7)		1 (0.8)	2 (2.9)		1 (3.3)	6 (1.4)	
2	15 (34.9)	5 (8.8)	5 (4.0)	10 (14.3)	3 (12.0)	1 (3.3)	57 (13)	
3	8 (18.6)	12 (21.1)	44 (35.5)	32 (45.7)		4 (13.3)	137 (31.1)	
4, 7, 14TU					2 (8.0)		2 (0.4)	
5				1 (1.4)			1 (0.2)	
5, 17			2 (1.6)	1 (1.4)			4 (0.9)	
7					1 (4.0)	9 (30.0)	10 (2.3)	
8, 9			2 (1.6)		3 (12.0)	2 (6.7)	8 (1.8)	
10	1 (2.3)				2 (8.0)		5 (1.1)	
11	1 (2.3)			1 (1.4)		2 (2.2)	2 (0.5)	
12	1 (2.3)		3 (2.4)	3 (4.3)	4 (16.0)		11 (2.5)	
16			2 (1.6)				2 (0.5)	
17	3 (7.0)		9 (7.3)	3 (4.3)	4 (16.0)	4 (13.3)	26 (5.9)	
13TU	4 (9.3)	26 (45.6)	19 (15.3)	8 (11.4)	1 (4.0)	2 (6.7)	74 (16.8)	
13BJ, 14TU					1 (4.0)		1 (0.2)	
14BJ			1 (0.8)				1 (0.2)	
15TU			7 (5.6)	1 (1.4)			8 (1.8)	
15BJ	1 (2.3)		5 (4.0)	1 (1.4)		1 (3.3)	8 (1.8)	
Unclassified	7 (16.3)	14 (24.6)	24 (19.4)	7 (10.0)	4 (16.0)	6 (20.0)	15 (16.5)	
Total ^c	43 (100.0)	57 (100.0)	124 (100.0)	70 (100.0)	25 (100.0)	30 (100.0)	91 (100.0)	

^a Groups tested during the summer. MS, medical students.

^b Groups tested during the winter.

^c Excluding 15 isolates from carriage study which failed to grow on subculture.

enrichment. There was no difference between the genospecies obtained by enrichment only and those obtained by plate culture ($P = 0.64$).

Sex. In the acute patients, female patients (14 of 21) were significantly more likely to be positive carriers than male patients (6 of 21) ($P = 0.031$; OR, 5.00; 95% confidence limits, 1.13 to 23.47), whereas in the chronic patient and medical student groups, no significant difference was found between the sexes.

Seasonal variation. The *Acinetobacter* carriage rate of class N42 (new nurses examined in the summer of 1997) was significantly higher than that of class N43 (new nurses examined in February 1998) (chi-square test, $P = 0.002$; OR 4.05; 95% confidence limits, 1.57 to 10.60), but a similar significant difference was not seen in student nurses N39 and N41 ($P = 0.38$). For both new and student nurses, there was a significant difference in the genospecies obtained in the summer and in the winter ($P = 0.0001$).

Locality. To avoid possible seasonal influence, data from different groups in the same season was used to examine the significance of locality (Table 1). There was no significant difference in the number of carriers positive at any site ($P = 0.01$) or in the genospecies obtained from medical students and new nurses (N42) ($P = 0.0523$). The carriage rate of student nurses (42 of 131) was not significantly different from that of acute patients (20 of 42) ($P = 0.1$) but was significantly lower than that of new nurses (25 of 38) ($P = 0.0004$; OR, 4.08; 95% confidence limits, 1.78 to 9.41). There was also a significant difference between the carriage rates of the two patient groups (20 of 42 versus 29 of 37; $P = 0.009$; OR, 3.99; 95% confidence limits, 1.34 to 12.17).

The distribution of genospecies in the community groups was significantly different from those of student nurses and acute patients ($P < 0.0002$). No significant difference was noted between the last two groups ($P = 0.897$). Isolates from

acute and chronic patients were also significantly different from each other ($P < 0.0001$).

Comparison of carriage isolates with those from blood cultures. The genospecies of isolates from blood cultures were similar to those from student nurses and acute patients ($P = 0.083$) but significantly different from those of the community groups ($P = 0.0001$). There was a significant difference between blood culture isolates and those from chronic patients ($P < 0.0001$).

Genospecies and sample sites. We examined the distribution of genospecies in all subjects according to the site and found no significant difference ($P = 0.008$). Genospecies 3 accounted for 33 to 38% of nasal and hairline isolates but only 9% of those from the throat, which harbored 13TU (23%) as the commonest genospecies.

Isolates of the same genospecies from the same sites. Of 18 randomly selected subjects, 69 isolates, representing 2 to 4 isolates of the same genospecies, obtained from 28 sites (hairline, 5 sites; groins, 8 sites; nares, 1 site; throat, 5 sites; and toeweb, 9 sites) were examined by RAPD analysis. Altogether, 45 patterns were obtained. Isolates from 19 (68%) sites yielded two or more patterns. No common pattern was found between any two subjects.

Multiple isolates obtained from different sites. Among the 162 positive subjects who were tested during the summer, there were 34 who were positive at two or more sites with different genospecies and another 35 who were positive with isolates of the same genospecies. Of these 35 subjects, 44 isolates from 9 subjects (4 patients and 5 controls) were examined by RAPD analysis. Altogether, 25 different RAPD patterns were obtained. A single pattern was seen only in isolates from three of the nine subjects examined.

Sequential samples. Before final genus identification could be concluded in the laboratory, nurses with a presumptive positive isolation from any site were recalled within 6 weeks for

TABLE 3. Results of repeat specimens from 106 nurses^a

Result ^b	No. of subjects sampled on the following no. of occasions:		
	2	3	4
All negative ^c	31	8	
Positive on 1 occasion	29	18	3
Positive on 2 occasions	3	9	2
Positive on 3 occasions		1	2
Total	63	36	7

^a Performed within 6 weeks as a result of recall on presumptive positive results or repeat sampling 6 months later.

^b Positive results were for carriage of acinetobacters at any site.

^c Presumptive positive results which proved negative after laboratory confirmation test.

all samples to be taken again. In addition, student nurses in classes N39 and N41 were sampled on two occasions 6 months apart (Table 1). As a result, 106 student nurses were sampled on two or more occasions (Table 3). Fifty nurses were positive on one occasion, and 14 nurses were positive on two occasions. Isolates from 11 of these 14 subjects belonged to different genospecies on both occasions. For the remaining three subjects, RAPD analysis showed that isolates of the same genospecies were different from each other in two of the subjects and similar in one. Different genospecies were obtained in two of the three subjects who were positive on three occasions. For the remaining subjects, the five isolates of genospecies 3 obtained on two occasions from two different sites had similar RAPD patterns. Genospecies 12 and 7 were isolated on the third occasion.

Unclassifiable isolates. Of the 79 isolates which yielded a total of 48 novel ARDRA patterns, 73 were tested for temperature tolerance at 30, 37, 41, and 44°C. Thirteen (18%) isolates grew at all the temperatures tested and therefore could belong to genospecies 2 or 13TU (32). The 28 (38%) isolates which grew in temperatures up to 41°C could belong to genospecies 3 or 5 (32). The remaining 32 (44%) isolates grew in temperatures up to only 37°C.

DISCUSSION

In the past decade, *Acinetobacter* has become an important opportunistic pathogen worldwide, associated with hospital outbreaks and nosocomial infections (1). *Acinetobacter* is of particular importance in Hong Kong, which has a hot, humid climate (24, 27). The number of *Acinetobacter* bacteremic episodes per 1,000 hospital admissions to our hospital from 1987 to 1994 ranged from 0.3 to 0.6 (24). In another hospital, the average annual incidence of isolation of *Acinetobacter* spp. from all clinical specimens was 29.8 per 1,000 hospital admissions and accounted for 5.5 to 6.2% of all positive blood cultures and 14.8% of those from intensive care units (27). These figures are higher than those reported elsewhere (7, 8, 28, 29).

The higher prevalence of skin carriage in the summer than in the winter (class N42 versus N43) has been attributed to the preference of the genus for moisture (7, 23, 24, 27). Once new nurses started working at the hospital, however, student nurse carriage rates fell significantly and remained the same irrespective of the length of training or the season. This may be explained by the fact that PWH is air-conditioned (22 to 25°C), with a controlled humidity all year round (55 to 60%). Patient carriage of acinetobacters has been associated with factors such as age, antibiotic therapy, underlying conditions, catheterization, mechanical ventilation, and duration of hospital stay (9, 27, 30, 33).

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Compared with what is known about *A. baumannii*, little information is available on the clinical importance of other genospecies. We used blood culture isolates as a guide to their relative clinical significance, although it is recognized that some isolates may be the result of contamination (24). Our results show that 41% of acinetobacters from blood cultures belonged to genospecies 3, the most common genospecies, whereas in Europe and North America, *A. baumannii* and DNA group 13TU are the most common species found (36 to 73%) in clinical specimens (2, 12, 25). Genospecies 3 was uncommon (2, 15, 25).

There are only two studies reported in the literature on skin and mucous membrane carriage of *Acinetobacter* with the new molecular classification scheme (3, 26). Both were carried out on European subjects. Berlau et al., examining three sites with enrichment techniques, described an overall positivity rate of 44% in 191 healthy subjects in London (3). Examining nine sites with enrichment techniques, Seifert et al. in Cologne, Germany, found that 75% of 40 patients and 43% of 40 healthy controls were positive at any site (26). Similar to the results of our experiment, 40% of their isolates grew only after enrichment and cultures from positive samples of healthy subjects were generally light in growth (23, 26). We compared the genospecies obtained by enrichment only with those obtained by plate culture and found them similar. Thus, enrichment seems to allow the recognition of acinetobacters present in small numbers only. The significance of such scanty carriage is uncertain. In the European studies, the most common genospecies isolated from skin and mucous membranes of patients and controls was *Acinetobacter lwoffii*, accounting for 44 to 61% of isolates, with genospecies 3 accounting for only 5 to 12%. DNA group 7 (up to 22%) and DNA group 5 (up to 10%) were reported in the German study but not found at all in the London study, which reported 15BJ as the next most common species after *A. lwoffii* (3, 26). The natural habitats of the most common clinical isolates, i.e., *A. baumannii* and genospecies 13TU, therefore remain a puzzle in these regions (26). In contrast, we found the ready presence of clinically significant genospecies (genospecies 2, 3, and 13TU) on superficial sites of both healthy subjects and patients. It is interesting to note that the distribution of genospecies of blood culture isolates was similar to that of skin and mucous membrane isolates of acute patients and student nurses but different from those from the community and from another hospital (Table 2).

It may be that locality plays a role in the genospecies carried by different groups of subjects. There was a significant difference between the community group (medical students and new nurses) and the PWH group (student nurses and acute patients). There was also a significant difference between patients in different hospitals (acute patients versus chronic patients). Once new nurses started working at PWH, the positivity rates were reduced (possibly as a result of working in an environment with controlled temperature and humidity) and the genospecies carried changed from the community type to that of the PWH type, i.e., the type of the student nurses. Studies have demonstrated the ready presence of *Acinetobacter* spp. in hospital environments (2, 13, 33). Resistance to desiccation is well recognized in isolates of the *Acb* complex and *Acinetobacter radioresistens* and considered to be one of the important attributes for transmission under dry conditions, e.g., via dust and fomites (17, 20, 21, 33). High rates of hand contamination have also been described (13). It may be that in an endemic area such as ours, each locality has its own resident acineto-

bacters whose characteristics vary according to the different ecological factors present.

There are few longitudinal studies on the skin carriage of acinetobacters. Kloos and Musselwhite (23) sampled six superficial sites of four healthy adults each at monthly intervals for a year, from 1971 to 1972. They found that the frequency of positive results varied greatly between sites and between individuals but was highest on the arms and legs and during the warm months of the year. Our results of repeat sampling of student nurses show similar variations. In addition, ARDRA and RAPD analysis demonstrated that *Acinetobacter* carriage was heterogeneous. For the 17 subjects who were positive on two or more occasions, each occasion yielded different genospecies from the same or different sites in 13 subjects and different strains of the same genospecies in another 2 subjects. Only in two subjects was the same strain found on different occasions (see section on sequential samples under Results).

Heterogeneity is also seen in isolates of the same genospecies obtained from the same sites (68% of the 28 sites examined). Among the 162 positive subjects examined in the summer, half (34 of 69) of those who were positive at multiple sites had different genospecies. We examined nine subjects who were positive at multiple sites with the same genospecies and found a single RAPD pattern present in three subjects only. In the literature, heterogeneity has been a feature in isolates of the same genospecies in studies undertaken in nonoutbreak situations (6, 7, 10). It is also found among nonoutbreak strains in outbreak investigations (15, 31). In contrast, outbreak strains were often associated with multiple sites in patients involved in the outbreak (28). These findings suggest the presence of bacterial characteristics associated with outbreak strains and highlight the importance of genospeciation and strain typing in the investigation of *Acinetobacter* outbreaks.

The above observations suggest to us that *Acinetobacter* carriage in the majority of healthy subjects in a nonoutbreak situation is characterized by low density, variation in genospecies and/or strains, short-term duration, and likelihood of being typical of a given locality. Such carriage is probably not the result of colonization as defined by Hierholzer (14): the multiplication of a microorganism at a body site or sites without evidence of infection but of constant exposure and frequent contamination from environmental sources. It is not certain how far our observations of healthy adults could be applied to patients as the latter were sampled only once. The role of exposure and contamination in clinical infections, therefore, merits further study.

ARDRA performs well in the discrimination of most genomic species, including those in the *Acb* complex (11). The present ARDRA classification, however, is largely based on clinical strains from Europe. This may explain why nearly 17% of our isolates were not classifiable by the scheme. They may represent new patterns of existing genospecies or new genospecies. Expansion of the ARDRA database is therefore required for isolates from different geographical regions. Fluorescent amplified fragment length polymorphism (FAFLP) has recently been used successfully for the fingerprinting of *Escherichia coli* (1). AFLP with ³²P primers has been validated for the discrimination of *Acinetobacter* genospecies (18). Because of its convenience, FAFLP may be a method of choice for the genospeciation of *Acinetobacter* after evaluation.

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