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Significance of Genomic DNA Group Delineation in Comparative Studies of Antimicrobial Susceptibility of *Acinetobacter* spp.

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There were significant differences in antimicrobial susceptibilities in isolates of genomic DNA groups 2 (Acinetobacter baumannii), 3, and 13TU collected from the same sources, e.g., patients in intensive care units and general wards, and in isolates of the same group collected from different sources. The delineation of genomic groups is important in comparative surveillance studies of antimicrobial susceptibilities.

The emergence and spread of antibiotic resistance among a wide variety of clinically important organisms have become an important public health issue in recent years (12). Regular surveillance and reporting of antimicrobial susceptibility in clinical isolates are carried out to monitor the emergence or evolution of resistance. This is of particular importance for bacterial genera which possess innate resistance to a range of antimicrobial agents, e.g., *Pseudomonas* spp. and *Acinetobacter* spp. Such data collected from different centers are often compared and contrasted to gain an insight into the trend of resistance to the newly available antimicrobial agents. *Acinetobacter* is a nosocomial pathogen endemic in Hong Kong and increasingly so in other parts of the world (1). The genus has only recently been delineated into 19 genomic DNA groups. The closely related genomic DNA groups 1, 2 (*Acinetobacter baumannii*), 3, and 13TU, often referred to as the *Acinetobacter calcoaceticus-A. baumannii* complex (*Acb* complex), are the most common among clinical isolates (1). We report here the significant differences found in antimicrobial susceptibilities among members of the *Acb* complex collected from the same sources and among isolates belonging to the same genomic DNA group but collected from different sources. Our results highlight the importance of delineating the genomic groups for a complex genus such as *Acinetobacter* when comparing susceptibility data from different studies.

For previous studies, collections of isolates were obtained from carriage sites of patients, healthy volunteers from the community, and members of the staff of Prince of Wales Hospital (Shatin, Hong Kong) and from blood culture isolates obtained in 1997 and 1998, clinical specimens obtained between 26 October and 6 December 1998 from patients in the intensive care unit (ICU) and general wards of Prince of Wales Hospital, and raw vegetables and soil samples obtained from 1997 to 1999 (3, 11). We did not have a documented outbreak of *Acinetobacter* infection in the hospital during the period when isolates were collected. The genomic DNA groups of these isolates were determined by amplified DNA ribosomal restriction analysis (3, 5, 11).

For the determination of antimicrobial susceptibility, single isolates from patients, volunteers, or environmental samples were randomly chosen from the collections (Table 1). For isolates obtained from carriage sites of community volunteers and hospital staff members and from food and soil samples, only genomic DNA group 3 included a sufficient number of isolates for comparison. The agar dilution method was used to determine the MICs of the major classes of agents: amikacin, gentamicin, ceftazidime, nalidixic acid, tetracycline, sulfamethoxazole, and rifampin (all from Sigma, St. Louis, Mo.), ciprofloxacin (Bayer, Leverkusen, Germany), imipenem (Merck Sharp and Dohme, Hoddesdon, United Kingdom), netilmicin (Schering-Plough Corporation, Kenilworth, N.J.), and sulbactam (Pfizer, Sandwich, United Kingdom). Inocula of 10^4 CFU/spot were inoculated onto Mueller-Hinton agar plates with a multipoint inoculator (Dynatech Laboratories, Alexandria, Va.), and the plates were incubated at 35°C for 18 h (15). The MIC was defined as the lowest concentration which inhibited visible growth. Control strains *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662 were included. Nonparametric tests (SPSS version 11) were used for statistical analysis.

The geometric means (GMs) of MICs and the MICs at which 50% of the isolates tested were inhibited (MIC_{50}) and at which 90% of the isolates tested were inhibited (MIC_{90}) for each genomic DNA group from the different sources are shown in Table 2. There was a general trend for GMs of MICs of all classes of agents tested to increase progressively for isolates from all three genomic DNA groups collected from the following sources: carriage sites of community volunteers, hospital staff members, and patients, specimens from patients in general wards, and specimens from patients in the ICU (Table 2). Other studies have shown that the increased use of antimicrobial agents, e.g., extended-spectrum cephalosporins and aminoglycosides, is associated with an increase in resistance in acinetobacters in the hospital (4).

Isolates of genomic DNA group 3 from food and soil were unexpectedly more resistant than those obtained from carriage sites of both community volunteers and hospital staff members

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TABLE 1. Sources of acinetobacters of genomic DNA groups 2 (*A. baumannii*), 3, and 13TU examined for antimicrobial susceptibilities

Genomic DNA group	No. of acinetobacters						
	Food or soil	Carriage sites			Specimens		Total
		Patients	Community volunteers	Hospital staff members	ICU	Wards	
2 (*A. baumannii*)		18			19	39	76
3	21	21	12	10	30	35	129
13TU		27			27	23	77
Total	21	66	12	10	76	97	282

to aminoglycosides, β-lactams, quinolones, and sulfonamide (Table 2). For gram-negative rods, including *Acinetobacter* spp., other studies have shown that there were significant differences in the susceptibilities of isolates obtained from waste effluents from hospitals or pharmaceutical plants or upstream or downstream from the wastewater discharge of a city (8, 9).

For previous studies at our hospital, isolates of genomic DNA groups 2, 3, and 13TU were collected over the same period of time and from the same sources (specimens from patients in the ICU and general wards and carriage sites of patients). The *P* values obtained with nonparametric tests when comparing the MICs of each antimicrobial agent for different members of the *Acb* complex collected from the same sources and for isolates of DNA group 3 collected from different sources are shown in Table 3. Because of the small number of isolates studied, only *P* values of <0.02 were regarded as significant. Table 3 shows that statistically significant differences (P, <0.02) are seen between one or more agents of each class of antimicrobials tested with genomic DNA groups 2, 3, and 13TU and between genomic groups 2 and 13TU (Table 3).

TABLE 2. GMs of MICs and MIC_{50}s and MIC_{90}s of 11 agents for isolates of genomic DNA groups 2, 3, and 13TU from clinical specimens from patients in ICU and general wards, carriage sites of patients, hospital staff members, and community volunteers, and food and soil samples[a]

Source of isolates	Genomic DNA group[b]	Value[c]	AMK	GEN	NET	CAZ	IPM	SUL	CIP	NAL	TET	SMX	RIF
ICU specimens	2 (19)	GM	2.4	3.6	0.9	34.4	0.3	4.1	5.2	39.8	26.7	39.8	2.5
		MIC_{50}	1	4	1	64	0.5	2	8	64	64	64	2.0
		MIC_{90}	64	64	2	64	1	32	16	128	64	64	8.0
	3 (30)	GM	4.0	1.0	1.0	29.9	0.6	1.1	0.5	4.8	2.2	28.5	3.7
		MIC_{50}	2	0.5	1	32	0.25	1	0.5	4	2	64	2.0
		MIC_{90}	64	4	8	64	8	4	4	32	8	64	64.0
	13TU (27)	GM	2.3	1.3	1.5	15.6	0.2	1.4	0.9	6.3	5.4	6.5	5.5
		MIC_{50}	2	1	1	16	0.13	1	0.5	4	4	4	4.0
		MIC_{90}	8	8	16	32	0.5	2	4	16	64	16	8.0
Ward specimens	2 (39)	GM	2.0	1.0	0.8	25.0	0.2	2.5	2.2	18.1	11.4	22.0	2.2
		MIC_{50}	2	1	1	32	0.5	2	1	1	4	16	2.0
		MIC_{90}	16	64	2	64	0.5	32	16	128	64	64	4.0
	3 (35)	GM	2.5	0.7	0.9	18.4	0.3	0.9	0.5	4.5	2.0	17.7	2.2
		MIC_{50}	2	0.5	1	16	0.13	1	0.5	4	2	8	2.0
		MIC_{90}	64	2	2	64	4	1	1	8	4	64	4.0
	13TU (23)	GM	2.6	1.0	1.1	21.6	0.2	1.4	0.8	6.7	4.9	9.9	3.7
		MIC_{50}	2	1	1	32	0.13	1	0.5	4	4	8	4.0
		MIC_{90}	4	2	2	32	0.25	4	2	16	64	32	4.0
Carriage sites of patients	2 (18)	GM	0.7	0.5	0.3	4.3	0.5	1.0	0.7	4.0	4.3	6.1	2.1
		MIC_{50}	0.5	0.5	0.25	4	0.5	1	0.5	4	2	4	2.0
		MIC_{90}	2	1	0.5	16	0.5	2	2	16	64	64	4.0
	3 (21)	GM	0.8	0.5	0.4	3.7	0.5	0.9	0.4	4.0	1.8	4.4	2.4
		MIC_{50}	0.5	0.5	0.25	4	0.5	1	0.5	4	2	4	2.0
		MIC_{90}	2	1	1	4	0.5	2	1	8	2	8	4.0
	13TU (27)	GM	1.7	1.2	0.9	4.2	0.4	0.6	0.5	3.9	3.1	4.9	3.9
		MIC_{50}	2	1	1	4	0.5	1	0.5	4	2	4	4.0
		MIC_{90}	2	4	2	8	0.5	1	1	8	64	64	8.0
Carriage sites of hospital staff members	3 (10)	GM	0.8	0.5	0.3	3.0	0.6	0.8	0.4	3.5	1.1	2.6	2.6
		MIC_{50}	0.5	0.5	0.5	4	0.5	1	0.5	4	2	4	2.0
		MIC_{90}	2	1	1	4	1	1	1	4	2	4	16.0
Carriage sites of community volunteers	3 (12)	GM	0.9	0.5	0.3	4.2	0.3	0.8	0.3	3.4	1.3	3.4	2.2
		MIC_{50}	1	0.5	0.25	4	0.25	1	0.5	4	2	4	2.0
		MIC_{90}	2	1	0.5	16	0.5	1	1	8	2	4	4.0
Food and soil samples	3 (21)	GM	1.4	0.7	0.6	14.0	0.1	0.5	0.6	4.9	1.9	11.1	2.8
		MIC_{50}	2	1	0.5	16	0.13	0.5	0.5	4	2	8	4.0
		MIC_{90}	2	1	1	16	0.25	0.5	1	8	2	64	4.0

[a] Abbreviations: AMK, amikacin; GEN, gentamicin; NET, netilmicin; CAZ, ceftazidime; IPM, imipenem; SUL, sulbactam; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; SMX, sulfamethoxazole; RIF, rifampin.

[b] Numbers in parentheses indicate the number of isolates examined.

[c] All values are given in micrograms per milliliter.

TABLE 3. *P* values determined by nonparametric tests for each antimicrobial agent for comparisons of different genomic DNA groups from the same sources and different sources of genomic DNA group 3[a]

Genomic DNA group(s)	Source(s)	*P*[b]										
		AMK	GEN	NET	CAZ	IPM	SUL	CIP	NAL	TET	SMX	RIF
2, 3, 13TU	ICU specimens	NS	0.018	NS	0.001	0.001	0.003	<0.001	<0.001	<0.001	<0.001	NS
	Ward specimens	0.010	NS	NS	NS	0.019	0.002	0.001	0.008	<0.001	0.016	<0.001
	Patient carriage sites	<0.001	<0.001	<0.001	NS	NS	NS	NS	NS	0.005	NS	0.001
2, 13TU	ICU specimens	NS	NS	NS	0.001	0.003	0.016	<0.001	<0.001	<0.001	<0.001	0.015
	Ward specimens	0.002	NS	0.015	NS	0.004	NS	NS	NS	NS	0.004	0.001
	Patient carriage sites	<0.001	<0.001	<0.001	NS	NS	NS	NS	NS	NS	NS	0.002
3	Specimens from ICU and wards	NS	NS	NS	0.016	NS	NS	NS	NS	NS	NS	NS
	Carriage sites of community volunteers and hospital staff members, food and soil samples	NS	0.020	0.001	<0.001	<0.001	0.002	NS	0.019	NS	<0.001	NS

[a] Abbreviations: AMK, amikacin; GEN, gentamicin; NET, netilmicin; CAZ, ceftazidime; IPM, imipenem; SUL, sulbactam; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; SMX, sulfamethoxazole; RIF, rifampin.

[b] Mann-Whitney test used for two independent samples; Kruskal-Wallis test used for three or more samples. NS, not significant ($P > 0.02$).

There are also significant differences in MICs for isolates belonging to genomic DNA group 3 that were collected from different nonclinical environments—carriage sites of community volunteers and of hospital staff members and food and soil samples (Table 3). For the individual genomic DNA group studied, with the exception of DNA group 3 for ceftazidime (Table 3), comparisons between ICU and general ward isolates did not show any significant difference for the 11 agents examined (data not shown).

The changing spectrum of nosocomial pathogens and the widespread emergence of antimicrobial resistance have made national and international surveillance an important means to monitor the prevalence of resistance. Such information can aid the prudent use of antimicrobial agents. Surveillance reports, however, often have not used the modern taxonomic scheme for *Acinetobacter* isolates (4, 6, 10, 13). Previous studies showed that there is a significant difference between Hong Kong and Europe in the distribution among genomic DNA groups of isolates obtained from blood cultures and various superficial carriage sites (3). For example, genomic DNA group 3 accounted for 40% of blood culture isolates in our hospital (3). The importance of locality for the distribution of genomic DNA groups in human carriage sites has also been demonstrated (3). The compositions of genomic DNA groups of acinetobacters may thus vary from center to center, and the reported susceptibility patterns of acinetobacters may therefore not be truly comparable.

The delineation of *Acinetobacter* spp. requires the use of molecular techniques which may not be available in routine clinical laboratories, where commercial kits based on phenotypic methods, e.g., API 20NE and Vitek (Biomerieux, Marcy l'Etoile, France), are often relied on for bacterial identification. Phenotypic methods cannot delineate members of the *Acb* complex fully (2, 7). In the only report on antimicrobial susceptibilities of acinetobacters based on modern taxonomy, Seifert et al. showed that acinetobacters belonging to the *Acb* complex are generally more resistant to antimicrobials than other species, e.g., *Acinetobacter lwoffii* and *Acinetobacter johnsonii* (14). The *A. baumannii* isolates reported, however, would have represented both genomic DNA groups 2 and 13TU as the phenotypic scheme used could not have distinguished them (2, 7, 14). Our studies showed that there were important differences between these groups. Table 3 shows that there were significant differences for the different classes of antimicrobials tested when isolates from the same sources were compared. Previous studies showed that genomic DNA group 13TU is particularly associated with human carriage and infection in the respiratory tract and is recovered only from the immediate clinical environment (3, 11). Genomic DNA group 13TU thus appears to possess distinct characteristics, and a readily usable method for its delineation should therefore be developed.

In conclusion, our data show that the delineation of genomic DNA groups and sources of acinetobacters is important for the compilation of susceptibility data for surveillance purposes so that information from different centers can be truly comparable.

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REFERENCES

1. **Bergogne-Berezin, E., and K. J. Towner.** 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. **9:**148–165.
2. **Bouvet, P. J. M., and P. A. D. Grimont.** 1987. Identification and biotyping of clinical isolates of *Acinetobacter.* Ann. Inst. Pasteur Microbiol. **138:**569–578.
3. **Chu, Y. W., C. M. Leung, E. T. S. Houang, K. C. Ng, C. B. Leung, H. Y. Leung, and A. F. B. Cheng.** 1999. Skin carriage of acinetobacters in Hong Kong. J. Clin. Microbiol. **37:**2962–2967.
4. **Courcol, R. J., M. Pinkas, and G. R. Martin.** 1989. A seven year survey of antibiotic susceptibility and its relationship with use. J. Antimicrob. Chemother. **23:**441–451.
5. **Dijkshoorn, L., B. van Harsselaar, I. Tjernberg, P. J. M. Bouvet, and M. Vaneechoutte.** 1998. Evaluation of amplified ribosomal DNA restriction analysis for identification of *Acinetobacter* genomic species. Syst. Appl. Microbiol. **21:**33–39.
6. **Fluit, A. C., M. E. Jones, F. J. Schmitz, J. Acar, R. Gupta, J. Verhoef, and the Sentry Participants Group.** 2000. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. Clin. Infect. Dis. **30:**454–460.
7. **Gerner-Smidt, P., I. Tjernberg, and J. Ursing.** 1991. Reliability of phenotypic tests for identification of *Acinetobacter* species. J. Clin. Microbiol. **29:** 277–282.
8. **Goni-Urriza, M., M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, and C. Quentin.** 2000. Impact of an urban effluent on antibiotic resistance of riverine *Enterobacteriaceae* and *Aeromonas* spp. Appl. Environ. Microbiol. **66:** 125–132.

9. **Guardabassi, L., A. Petersen, J. E. Olsen, and A. Dalsgaard.** 1998. Antibiotic resistance in *Acinetobacter* spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. Appl. Environ. Microbiol. **64:** 3499–3502.
10. **Hanberger, H., J. A. Garcia-Rodriguez, M. Gobernad, H. Goossens, L. E. Nilsson, M. J. Struelens, and the French and Portuguese ICU Study Groups.** 1999. Antibiotic susceptibility among aerobic Gram-negative bacilli in intensive care units in 5 European countries. JAMA **281:**67–71.
11. **Houang, E. T. S., Y. W. Chu, C. M. Leung, K. Y. Chu, J. Berlau, K. C. Ng, and A. F. B. Cheng.** 2001. Epidemiology and infection control implications of *Acinetobacter* spp. in Hong Kong. J. Clin. Microbiol. **39:**228–234.
12. **Levy, S. B.** 2002. Antibiotic and antiseptic resistance: impact on public health. Pediatr. Infect. Dis. J. **19**(Suppl.)**:**S120–S122.
13. **Ruiz. J., M. L. Nunez, J. Perez, E. Simarro, L. Martinez-Campos, and J. Gomez.** 1999. Evolution of resistance among clinical isolates of *Acinetobacter* over a 6-year period. Eur. J. Clin. Microbiol. Infect. Dis. **18:**292–295.
14. **Seifert, H., R. Baginski, A. Schulze, and G. Pulverer.** 1993. Antimicrobial susceptibility of *Acinetobacter* species. Antimicrob. Agents Chemother. **37:**750–753.
15. **Woods, G. L., and J. A. Washington.** 1995. Dilution and disk diffusion testing methods, p. 1327–1341. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology. ASM Press, Washington, D.C.