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Comparison of *Acinetobacter baumannii* Isolates from United Kingdom Hospitals with Predominant Northern European Genotypes by Amplified-Fragment Length Polymorphism Analysis

Richard P. Spence,[1] Tanny J. K. van der Reijden,[2] Lenie Dijkshoorn,[2] and Kevin J. Towner[1]*

Molecular Diagnostics and Typing Unit, Department of Microbiology, Queens Medical Centre, Nottingham NG7 2UH, United Kingdom,[1] and Department of Infectious Diseases, Leiden University Medical Center, 2300 RC Leiden, The Netherlands[2]

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Acinetobacter baumannii **isolates collected between 1999 and 2001 from 46 United Kingdom hospitals were compared with previously identified northern European genotypes by amplified-fragment length polymorphism (AFLP) analysis. Two predominant northern European genotypes associated with outbreaks in the mid-1980s had been superseded by new outbreak-associated genotypes.**

Acinetobacter baumannii is a major cause of nosocomial infections such as ventilator-associated pneumonia, septicemia, secondary meningitis, and urinary tract infections (1). Outbreaks of infection caused by *A. baumannii* are widely reported and are a significant burden in terms of prolonged hospital stays and increased morbidity and mortality (3, 9, 11, 13–15). A previous study of 287 *A. baumannii* isolates from 46 United Kingdom hospitals identified over 30 different genotypes by the technique of randomly amplified polymorphic DNA (RAPD) analysis (12) with most outbreak-associated infections caused by three predominant RAPD genotypes (12). This observation of a diverse population contrasted with a study published in 1996 that used a polyphasic approach, including amplified-fragment length polymorphism (AFLP) analysis (6–8), to examine the epidemiology of 14 outbreak-associated and 17 sporadic *A. baumannii* isolates in northern European countries. Four AFLP genotypes (clones) were identified, of which two genotypes, clones I and II, were responsible for most outbreak-associated infections in the countries examined (4). A further study in the Czech Republic published in 1999 also identified two predominant outbreak-associated clones, groups A and B, that corresponded to the two clones identified in other northern European countries (10).

In order to determine whether the current genotypes circulating in United Kingdom hospitals were related to the genotypes described in other northern European countries, the present study used AFLP analysis to directly compare the genetic diversity of 34 *A. baumannii* isolates, each representing an RAPD genotype identified in outbreaks and sporadic cases of infection in United Kingdom hospitals between 1999 and 2001, with 31 previously characterized isolates from six northern European countries (Belgium, Denmark, Ireland, The Netherlands, Sweden, and United Kingdom) (4, 5, 12). Isolates were classed as belonging to an outbreak on the basis of epidemiological and comparative local typing data (4, 12). Some of the RAPD genotypes identified in 1999 to 2001 were associated with separate outbreaks in hospitals located in different regions of the United Kingdom (12).

AFLP is a well-established species identification and genomic fingerprinting method that has been used successfully to study the epidemiology of *A. baumannii* (7, 8). Total cellular DNA was purified as described by Boom et al. (2), and 10 ng was digested with the restriction endonucleases *Eco*RI and *Mse*I. Adapters for *Mse*I and *Eco*RI were then ligated to the restriction fragments as described previously (6). Selective DNA amplification was performed with the Cy5-*Eco*RI+A primer (5′-Cy5-GACTGCGTACCAATTCA-3′) and the *Mse*I+C primer (5′-GATGAGTCCTGAGTAAC-3′) on 5 μl of the digested and ligated template DNA fragments. Amplification was performed in a Progene thermal cycler (Techne, Cambridge, United Kingdom) as follows: 2 min at 72°C; 2 min at 94°C; one cycle of 30 s at 94°C, 30 s at 65°C, and 60 s at 72°C; 12 cycles of 30 s at 94°C and 30 s at a temperature 0.7°C lower than the previous cycle, starting at 64.3°C, followed by 60 s at 72°C; 23 cycles of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C; and a final extension period of 10 min at 72°C. Amplified fragments were separated on a 5% (wt/vol) Reprogel high-resolution denaturing polyacylamide gel by using the ALFexpress II DNA analysis system (Amersham Biosciences, Roosendaal, The Netherlands) according to the manufacturer's instructions. Separation was for 500 min at 55°C and 30 W of constant power with 2-s sampling intervals. Strain LUH 1091 (Leiden University Medical Center, Leiden, The Netherlands) was run in the 1st lane and then every 10th lane for normalization purposes. Gel images were converted to the tagged image file (TIF) format before analysis with BioNumerics 2.50 software (Applied Maths, Kortrijk, Belgium). Levels of similarity between fingerprints were calculated with the Pearson product moment coefficient. Cluster analysis was performed by the unweighted pair group method with arithmetical averages (UPGMA).

The *A. baumannii* isolates included in this study were obtained between 1978 and 2001 from outbreaks and sporadic infections in 36 cities in six northern European countries (4, 12).

In the original European study, isolates that displayed ≥89% similarity by AFLP analysis were considered to be close-

* Corresponding author. Mailing address: Molecular Diagnostics and Typing Unit, Department of Microbiology, Queens Medical Centre, Derby Rd., Nottingham NG7 2UH, United Kingdom. Phone: 44-115-9709163. Fax: 44-115-9422190. E-mail: kevin.towner@mail.qmcuh-tr.trent.nhs.uk.

TABLE 1. Clustering of *A. baumannii* isolates following AFLP analysis

AFLP cluster[a]	United Kingdom nosocomial isolates[b]					Previously characterized (4) northern European isolates[f]				
	No. of isolates	No. of outbreak isolates[c]	Isolate	City	Date	No. of isolates	No. of outbreak isolates[c]	Isolate	City	Date
1	1	0	A1877	Berkshire	2000	1	0	PGS10086	Veile, Denmark	1990
2	NR[e]					2	0	TU133	Malmo, Sweden	1980
								TU144	Malmo, Sweden	1980
3	3	0	A886	Bristol	2000	1	1	RUH1752	Enschede, The Netherlands	1986
			A898	Ashford	2000					
			A1207	Derby	2000					
4	1	0	A1415	Salford	2000	1	0	RUH2688	Rotterdam, The Netherlands	1987
5	1	0	A1761	Chelmsford	2000	1	0	RUH1486	Rotterdam, The Netherlands	1985
6	1	0	A1233	Derby	1999	1	0	TU147	Malmo, Sweden	1980
7 (clone II)[d]	2	0	A1124	Leeds	2000	3	2	RUH0134	Rotterdam, The Netherlands	1982
			A1755	Chelmsford	2000			PGS189	Odense, Denmark	1984
								GNU1080	Salisbury, UK	1989
8	4	0	A375	Ashford	2000	1	1	GNU1086	Newcastle, UK	1989
			A732	Wrexham	2000					
			A1300	Stoke	2000					
			A2184	Chelsea	2000					
9	2	1	A791	London	2000	NR				
			A1850	Berkshire	2000					
10	4	2	A370	Ashford	1999	NR				
			A790	London	2000					
			A1637	London	2000					
			A1645	London	2000					
11 (clone I)[d]	NR					9	9	RUH0436	Utrecht, The Netherlands	1984
								RUH0510	Nijmegen, The Netherlands	1984
								RUH0875	Dordrecht, The Netherlands	1984
								RUH2037	Venlo, The Netherlands	1986
								GNU1078	Leuven, Belgium	1990
								GNU1079	Salford, UK	1990
								GNU1082	Basildon, UK	1989
								GNU1083	London, UK	1985–1988
								GNU1084	Sheffield, UK	1987

[a] Cluster relationships were calculated with BioNumerics software using the Pearson product moment coefficient and UPGMA method.
[b] Each isolate represented a separate RAPD genotype (12). Data are from 1999 to 2001.
[c] Outbreak isolates were representative of particular genotypes causing outbreaks, often in a number of geographically distinct hospitals. Outbreaks were defined according to epidemiological and comparative local typing data.
[d] As defined in previous northern European studies (4, 10).
[e] NR, not represented.
[f] Data are from 1978 to 1990.

ly related and to belong to the same genotype (clone) (4). In the present study, using a modified protocol, the cutoff value required to group isolates described previously as belonging to European clones I and II was found to be 87%. However, one isolate (GNU1086) that grouped originally with European clone II (4) only clustered at 78% similarity. This discrepancy could be the result of using different primers or may reflect problems with the storage of the isolate since the original study.

Using the identified cutoff value of ≥87%, 11 AFLP clusters containing 39 isolates were distinguished (Table 1). The remaining 26 isolates remained ungrouped. Of the 34 RAPD genotypes identified in 1999 to 2001, 19 grouped within nine AFLP clusters and 15 remained distinct. AFLP clusters contained between two and nine strains originating from one to nine cities in between one and three countries. Strains belonging to European clone I were located in AFLP cluster 11. AFLP cluster 7 contained strains belonging to European clone II, with the exception of isolate GNU1086, which was located in AFLP cluster 8. In previous studies, clone I and group A isolates were identified in four United Kingdom centers and were also identified in Belgium, the Czech Republic, and The Netherlands (4, 10). Clone II and group B isolates were located in three United Kingdom centers and were also identified in Denmark, the Czech Republic, and The Netherlands (4, 10). In the present study, six different *A. baumannii* AFLP clusters were associated with outbreaks of infection in between one and nine centers (Table 1). United Kingdom isolates were represented in all six of these AFLP clusters; however, the United Kingdom outbreak-associated isolates from 1999 to 2001 (12) did not cluster with the outbreak-associated United Kingdom isolates from the original study (4), which indicated that they did not belong to northern European clones I or II (Table 1). Thus, while northern European clones I and II were associated with a number of outbreaks of infection in the United Kingdom in the mid-1980s, this no longer appeared to be the case in 1999 to 2001. Nevertheless, two sporadic isolates of clone II were still detected in United Kingdom hospitals in 1999 to 2001.

Nonoutbreak-associated isolates belonged to 10 AFLP clusters and were found in between one and five centers, with those from the United Kingdom studied in 1996 belonging to six AFLP clusters. Similar isolates collected in 1999 to 2001 belonged to nine AFLP clusters (Table 1). A total of 26 isolates did not form AFLP clusters by the criterion used. These included 11 isolates from the original study and 15 isolates col-

lected in 1999 to 2001 (Table 1), each of which was representative of an individual RAPD genotype (12).

Overall, the study indicated that there is a diverse population of *A. baumannii* genotypes currently causing outbreaks and sporadic cases of infection in United Kingdom hospitals. Isolates belonging to the two predominant northern European clones identified previously were not associated with outbreaks of infection in the United Kingdom hospitals studied during 1999 to 2001. Nevertheless, a number of isolates in the current collection shared AFLP profiles with those from the previous study, indicating that some genotypes are spread across time and space and demonstrating that AFLP typing databases can be important tools for monitoring the distribution and prevalence of *A. baumannii* genotypes. The precise reasons for the epidemiological success and changes in predominance of particular genotypes have yet to be identified.

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