Antimicrobial resistance and clonality in Acinetobacter baumannii

Nemec, A.

CHAPTER 2

Nemec A, Dijkshoorn L, van der Reijden TJK.

Long-term predominance of two pan-European clones among multiresistant *Acinetobacter baumannii* strains in the Czech Republic.

J Med Microbiol 2004; 53: 147-153.

Long-term predominance of two pan-European clones among multi-resistant *Acinetobacter baumannii* strains in the Czech Republic

Alexandr Nemec,[1] Lenie Dijkshoorn[2] and Tanny J. K. van der Reijden[2]

[1]National Institute of Public Health, Šrobárova 48, 100 42 Prague, Czech Republic

[2]Department of Infectious Diseases, Leiden University Medical Center C5-P, PO Box 9600, 2300 RC Leiden, the Netherlands

Correspondence
Alexandr Nemec
anemec@szu.cz

Received 24 August 2003
Accepted 11 November 2003

In a recent study, a large proportion of multi-drug-resistant (MDR) *Acinetobacter baumannii* strains that were isolated from hospitalized patients in the Czech Republic was found to belong to two major groups (A and B). These groups appeared to be similar to epidemic clones I and II, respectively, which were identified previously among outbreak strains from north-western European hospitals. The aim of the present study was to assess in detail the genetic relatedness of Czech *A. baumannii* strains and those of epidemic clones I and II by using ribotyping with *Hin*dIII and *Hin*cII and by AFLP fingerprinting. The study collection included 70 MDR strains that were isolated in 30 Czech hospitals in 1991–2001, 15 susceptible Czech strains from 1991 to 1996 and 13 reference strains of clones I and II from 1982 to 1990. One major *Hin*dIII/*Hin*cIII ribotype (*R1-1*) was observed in 38 MDR Czech strains and eight reference strains of clone I, whereas another major ribotype (*R2-2*) was observed in 11 MDR Czech strains and in three reference strains of clone II. A selection of 59 Czech strains (representative of all ribotypes) and the 13 reference strains were investigated by AFLP fingerprinting. At a clustering level of 83 %, two large clusters could be distinguished: cluster 1 included all reference strains of clone I and 25 MDR Czech strains, whilst cluster 2 contained all reference strains of clone II and 11 MDR Czech strains. There was a clear correlation between the groupings by AFLP analysis and by ribotyping, as all strains with ribotype *R1-1* and four strains with slightly different ribotypes were found in AFLP cluster 1, whereas all strains with ribotype *R2-2* and seven strains with similar ribotypes were in AFLP cluster 2. Thus, 41 and 21 MDR Czech strains could be classified as belonging to clones I and II, respectively. The remaining eight MDR and 15 susceptible strains were highly heterogeneous and were distinct from clones I and II by both AFLP fingerprinting and ribotyping. These results indicate that the two predominant groups observed among MDR Czech *A. baumannii* strains from the 1990s are genetically congruent with the north-western European epidemic clones that were found in the 1980s. Recognition of these clinically relevant, widespread clones is important in infection prevention and control; they are also interesting subjects to study genetic mechanisms that give rise to their antibiotic resistance and epidemic behaviour.

INTRODUCTION

In an extensive review, Henriksen (1973) described acinetobacters as soil and water bacteria of widespread occurrence in the surroundings of man and animals, which have low pathogenic potential, but are opportunistic and capable of causing infection in individuals with reduced resistance. At the time, the genus *Acinetobacter* comprised only one species, *Acinetobacter calcoaceticus* (Lautrop, 1974). Today, acinetobacters are recognized as important nosocomial pathogens (Bergogne-Bérézin & Towner, 1996), but it is not yet well understood to what extent this is caused by increased susceptibility of the host or by expansion of specific strains.

Over the past three decades, considerable progress has been made in resolving the taxonomy of the genus *Acinetobacter* and in the development of methods to identify species and strains. With the inclusion of 10 recently described species, the genus now comprises 32 genomic species, 17 of which have validly published names (Nemec *et al.*, 2001, 2003; Carr

Abbreviation: MDR, multi-drug-resistant.

A table showing data on origin and properties of the strains used in this study is available in JMM Online.

et al., 2003). A few are of undisputed clinical relevance, whereas many others may be true environmental organisms, although the ecology of most species is as yet unrevealed. Among the clinically relevant species, *Acinetobacter baumannii* is the most common in clinical specimens and can give rise to severe infections in critically ill patients. Strains of this species that circulate on intensive-care units are frequently multi-drug-resistant (MDR) and combine this feature with the capacity to spread among patients and to persist in the hospital environment (e.g. Aygun *et al.*, 2002; Wang *et al.*, 2003).

In the mid 1990s, *A. baumannii* strains from 14 outbreaks and 17 sporadic strains from hospitals in different north-western European cities and countries were compared to assess the diversity among outbreak and non-outbreak strains. By using a combination of genotypic and phenotypic methods, the outbreak strains could be allocated to two main groups (designated clones I and II), whereas the sporadic strains were more heterogeneous (Dijkshoorn *et al.*, 1996). In a more recent study, phenotypic and genotypic properties of *A. baumannii* hospital strains from the Czech Republic were studied (Nemec *et al.*, 1999). It was found that MDR strains showed lower variability than susceptible strains. Most MDR strains were classified into two groups (designated A and B), each of which was characterized by a specific ribotype and similarity in other properties. The grouping of two reference strains of clones I and II with strains of groups A and B, respectively (Nemec *et al.*, 1999), and apparent similarities in ribotypes of strains of clones I and II with groups A and B, respectively (Pantophlet *et al.*, 2001), suggested that the respective clones and groups were congruent. Similarity of strains of group A and clone I was corroborated by their common reactivity with O-antigen-specific mAbs (Pantophlet *et al.*, 2001).

The panels of methods that were used to delineate clones I and II and groups A and B were different; therefore, definite conclusions on their genetic relatedness cannot be made until a representative sample of strains is subjected to common methods. The aim of the present study was to analyse in detail genotypic similarities between *A. baumannii* hospital strains from the Czech Republic and those that are representative of north-western European clones, in order to assess whether there is a pan-European presence of particular, genetically highly related, MDR strains (i.e. clones). For this purpose, the collection of Czech strains that was used in the previous study was enlarged with recent Czech MDR isolates. Strains were studied by ribotyping and by high-resolution AFLP fingerprinting, which has been found to be useful for the differentiation of *Acinetobacter* strains at the subspecies level (Dijkshoorn *et al.*, 1996; Janssen & Dijkshoorn, 1996; van Dessel *et al.*, 2003).

METHODS

Bacteria. Two sets of Czech *A. baumannii* strains were used in this study. Set ARC included 52 archive strains that were isolated in the Czech Republic between 1991 and 1999. These strains were selected from more than 700 clinical *Acinetobacter* isolates, in order to comprise hospital strains that were as heterogeneous as possible in terms of their time of isolation and geographical origin (18 cities were included). The ARC strains had been characterized in detail previously and were classified into group A ($n = 23$), group B ($n = 7$), a group of other MDR strains ($n = 7$) and a group of susceptible strains ($n = 15$) (Nemec *et al.*, 1999; Pantophlet *et al.*, 2001).

Set REC comprised 33 recent MDR *A. baumannii* strains from Czech hospitals that were selected, according to biochemical characteristics and susceptibility to antibiotics, from 250 clinical isolates that were referred to the National Institute of Public Health in 2000 and 2001. Strains were selected to be as geographically heterogeneous as possible (from 20 hospitals in 13 cities). Multiple isolates from the same hospital were not considered to be epidemiologically related, as assessed by biotyping (Bouvet & Grimont, 1987) and/or macrorestriction analysis of genomic DNA (Nemec, 1999). REC strains were recovered from sputum ($n = 9$), urine ($n = 7$), blood ($n = 7$), wound swabs ($n = 4$) and other clinical specimens, most of which were taken from intensive-care unit patients.

Reference strains of epidemic clones were RUH 436, RUH 510, RUH 875, RUH 2037, RUH 3238 (= GNU 1084), RUH 3239 (= GNU 1083), RUH 3242 (= GNU 1082), RUH 3247 (= GNU 1078) and RUH 3282 (= GNU 1079) for clone I, and RUH 134, RUH 3240 (= GNU 1086), RUH 3422 (= PGS 189) and RUH 3245 (= GNU 1080) for clone II. These strains were characterized in detail previously (Dijkshoorn *et al.*, 1996; Pantophlet *et al.*, 2001).

Phenotypic characteristics of all strains corresponded to those of the genus *Acinetobacter* (Juni, 1984). Strains were identified as *A. baumannii* according to *Eco*RI ribotypes (Nemec *et al.*, 1999) and were allocated to the biotypes of Bouvet & Grimont (1987) on the basis of utilization of laevulinate, citraconate, L-phenylalanine, phenylacetate, 4-hydroxybenzoate and L-tartrate.

Ribotyping. Ribotyping was carried out as described previously (Nemec *et al.*, 1999), with minor modifications. Total DNA was prepared by using SDS lysis, proteinase K treatment and phenol/chloroform extraction. Digestion was performed with *Hin*dIII and *Hin*cII in two separate steps. These enzymes were selected for the present study because they were found to show an optimal distribution of fragments for pattern analysis, compared to 15 enzymes tested [including *Eco*RI, which was used in previous studies (Nemec *et al.*, 1999; Pantophlet *et al.*, 2001)]. Electrophoretic separation of DNA fragments was done in 0·7 % (*Hin*dIII) or 0·8 % (*Hin*cII) agarose in TBE buffer (45 mM Tris/borate, 1 mM EDTA, pH 8·0) for 16 h. The voltage used was 45 and 35 V for *Hin*dIII and *Hin*cII, respectively. Fragments were blotted onto a nylon membrane, hybridized with a digoxigenin-labelled 16S–23S probe and visualized immunochemically. The resulting patterns were compared visually and distinct ribotypes were numbered arbitrarily. Each strain was characterized by a combined *Hin*dIII/*Hin*cII ribotype, e.g. *R1-1*. For cluster analysis, the presence or absence of a band at each position was scored as plus or minus, respectively. Percentage disagreement was used as a measure of dissimilarity between all pairs of *Hin*dIII/*Hin*cII ribotypes; it was expressed as the percentage of band position differences in a pair of ribotypes out of the total number of band positions (found in all ribotypes). Grouping was obtained by the UPGMA algorithm. All calculations were performed by using Statistica 5.1 software (StatSoft).

AFLP. AFLP fingerprinting was performed according to Nemec *et al.* (2001). Briefly, purified DNA was digested by using *Eco*RI and *Mse*I, while ligation of *Eco*RI and *Mse*I adaptors was performed simultaneously. PCR was done with a Cy5-labelled *Eco*RI + A primer and a *Mse*I + C primer (A and C represent selective nucleotides). The ALFexpress II DNA analysis system (Amersham Biosciences) was used for fragment separation. Fragments of 50–500 bp were subjected to

cluster analysis by using the BioNumerics software package, release 2.5 (Applied Maths), with an overall tolerance setting of 0·11 %. The Pearson product–moment coefficient (r) was used as the measure of similarity and UPGMA was used for grouping.

Antibiotic susceptibility testing. Antimicrobial susceptibility was determined by the disc diffusion method on Mueller–Hinton agar (Oxoid). Antimicrobial agents tested (Oxoid) were (μg per disc): ampicillin + sulbactam (10 + 10), piperacillin (100), ceftazidime (30), imipenem (10), gentamicin (10), tobramycin (10), amikacin (30), netilmicin (30), ofloxacin (5), cotrimoxazole (sulphamethoxazole + trimethoprim: 23·75 + 1·25) and tetracycline (30). Interpretative cut-off values for resistance were adjusted according to the known distribution of inhibition zone diameters among *A. baumannii* strains (Nemec, 1999). These values were identical to those recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2001) for intermediate categories except for tetracycline and piperacillin, for which the NCCLS values for resistance were used. Multi-resistance was defined as resistance to at least two antibiotics that represent different antibiotic classes.

RESULTS AND DISCUSSION

Ribotyping

Ribotyping of all 98 strains with *Hin*dIII and *Hin*cII separately revealed 33 and 25 different band positions, respectively. In total, 24 different *Hin*dIII ribotypes, 20 *Hin*cII ribotypes and 29 combinations of *Hin*dIII and *Hin*cII ribotypes were identified. Examples of *Hin*dIII and *Hin*cII ribotypes are shown in Fig. 1. The most frequent ribotype was *R1-1*, which was found in 38 MDR Czech strains and in eight reference strains of clone I. Czech strains with ribotype *R1-1* had previously been classified as group A. The second most frequent ribotype was *R2-2*, which was found in 11 MDR Czech strains and three reference strains of clone II. Czech strains with this ribotype had previously been classified as group B. Strain RUH 3242 (clone I) was of ribotype *R3-1*, whereas RUH 3240 (clone II) was of ribotype *R4-2*. These ribotypes were also found in MDR Czech strains. Each of the susceptible strains showed a unique *Hin*dIII/*Hin*cII ribotype.

AFLP

A selection of 72 strains, which included 59 Czech strains that were representative of all different ribotypes and the 13 reference strains of clones I and II, was studied by AFLP. Frequent ribotypes were represented by several strains, which differed mostly in other characteristics (biotype, plasmid profile and antibiotic susceptibility). Clustering of the strains according to their AFLP fingerprints is shown in Fig. 2. At a level of 83 %, two major clusters of MDR strains could be distinguished: cluster 1 included all strains with ribotypes *R1-1* and *R3-1* and one strain with the unique ribotype *R5-3*; whereas cluster 2 included strains with ribotype *R2-2* and four other ribotypes (*R2-4*, *R4-2*, *R2-5* and *R6-4*). AFLP patterns of all susceptible strains and other MDR strains were heterogeneous and clearly distinct from those of strains included in clusters 1 and 2.

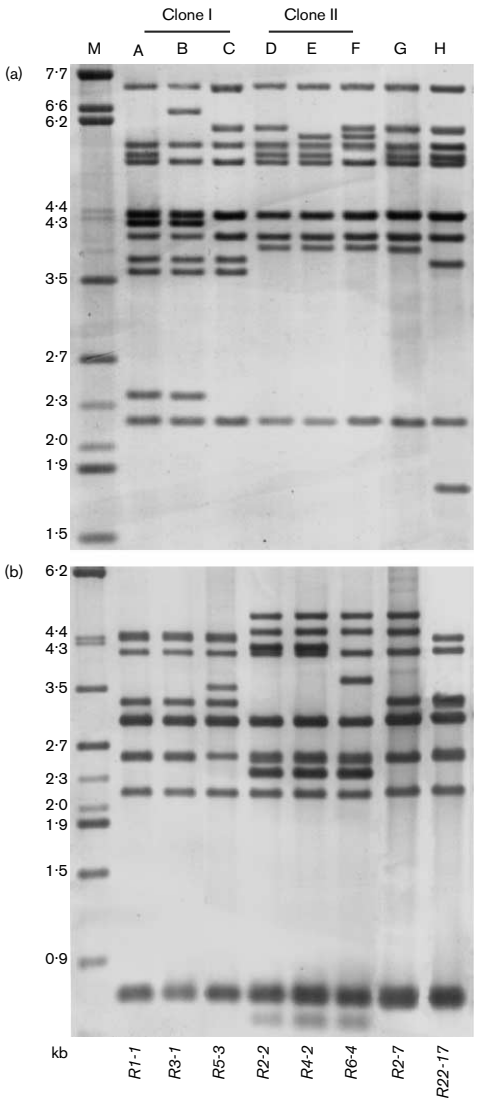

Fig. 1. Examples of (a) *Hin*dIII and (b) *Hin*cII ribotypes observed for *A. baumannii* strains. Strains are indicated by upper-case letters above the lanes: A, NIPH 7; B, NIPH 1605; C, NIPH 10; D, NIPH 24; E, NIPH 1362; F, NIPH 657; G, NIPH 301; H, NIPH 601. M, Molecular size marker (λ-phage DNA digested with *Hin*dIII and *Sty*I). Ribotype designations are given below the lanes.

Correlation between ribotyping and AFLP

There was a good correlation between AFLP and ribotyping results. Both AFLP clusters 1 and 2 contained strains of either identical or similar ribotypes that were specific for each of the

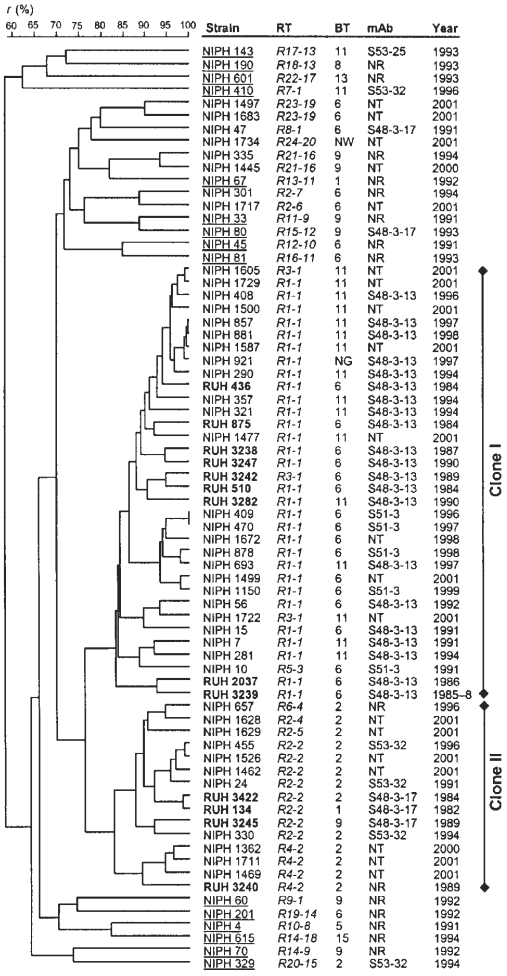

Fig. 2. Cluster analysis of AFLP fingerprints of 59 Czech *A. baumannii* strains (representative of different ribotypes) and 13 reference strains (RUH) for clones I and II. Strains NIPH 4–NIPH 657 belong to set ARC and strains NIPH 1362–NIPH 1729 belong to set REC. Susceptible strains are underlined. RT, *Hin*dIII-*Hin*cII ribotypes; BT, biotypes according to Bouvet & Grimont (1987); mAb, reactivity with O-antigen-specific mAbs (Pantophlet *et al.*, 2001). NG, No growth on mineral medium; NR, no reactivity with any of 20 antibodies tested; NT, not tested; NW, novel biotype.

clusters. This correlation was also found for strains linked in other clusters above 83 %, i.e. NIPH 1497 and NIPH 1683 or NIPH 335 and NIPH 1445 (Fig. 2). Clustering of *Hin*dIII/*Hin*cII ribotypes is shown in Fig. 3. Ribotypes *R1-1* and *R2-2*, which predominated among strains of AFLP clusters 1 and 2, respectively, were clearly distinct from each other (15 band differences in total). Differences between non-identical ribotypes of strains of the same AFLP cluster were small (Fig. 3). However, high similarity of some ribotypes was not confirmed by AFLP, e.g. strain NIPH 410, with a ribotype highly similar to *R1-1* (one band difference), was clearly different from clone I strains according to its AFLP pattern (Fig. 2) and other properties (Nemec *et al.*, 1999). This shows the limitation of ribotyping in estimating genetic relatedness of strains.

Relationship between the Czech groups and clones I and II

So-called epidemic clones I and II were distinguished originally among outbreak *A. baumannii* strains from north-western European hospitals on the basis of similarities in their genotypic and phenotypic properties (Dijkshoorn *et al.*, 1996). Within these clones, there was some intraclonal variability, but AFLP fingerprinting allowed unambiguous allocation of all strains to either clone I or clone II at a clustering level of 90 %. A further study showed that most MDR Czech strains belonged to two main groups, A and B, the delineation of which was based on identity in *Eco*RI ribotypes and supported by similarities in biochemical properties and plasmid profiles. It also appeared that groups A and B were similar to clones I and II, respectively, based on visual comparison of *Eco*RI ribotypes in studies that delineated these groups and clones, on inclusion of two reference strains of clones I and II in the study on Czech strains (Nemec *et al.*, 1999) and on common reactivity of clone I and group A strains with O-antigen-specific mAbs (Pantophlet *et al.*, 2001). However, as intraclonal variability of *Eco*RI ribotypes

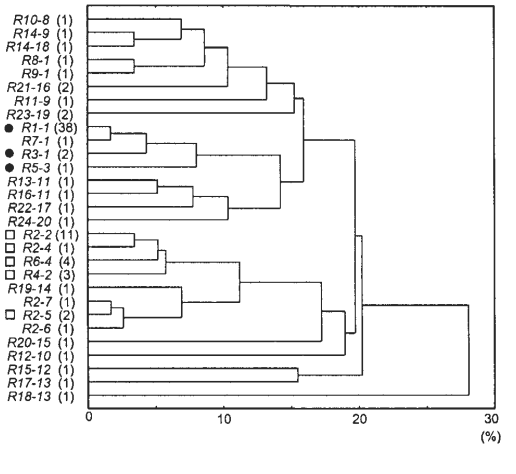

Fig. 3. Cluster analysis of *Hin*dIII/*Hin*cII ribotypes found in 85 Czech *A. baumannii* strains. No. strains with a respective ribotype is indicated in parentheses. Grouping was obtained by the UPGMA algorithm by using percentage disagreement. ●, Ribotypes of strains of AFLP cluster I (clone I); □, ribotypes of strains of AFLP cluster II (clone II).

was found in clone II (Dijkshoorn *et al.*, 1996) and could not be excluded for clone I, the relationship between the clones and some Czech strains remained unclear.

In the present study, a combination of ribotyping and AFLP results allowed the classification of 62 of 70 (89 %) MDR Czech strains into the north-western European clones. The current AFLP protocol was different from that used previously (Dijkshoorn *et al.*, 1996) with respect to the choice of restriction enzymes and selective primers and method of fragment separation. By this modified procedure, reference strains of clones I and II were linked at a level of 83 % in two major clusters. In total, 36 of 44 MDR Czech strains, including the strains allocated previously to groups A and B and strains with ribotypes that were highly similar to those of groups A and B, were found in these respective clusters. According to the positions and interrelatedness of strains in AFLP clusters 1 and 2 and overall similarity of their ribotypes and other characters (biotype, serotype defined by O-antigen-specific mAbs and plasmid content), we conclude that the Czech strains in these clusters belong to the previously described clones I and II (Fig. 2, Table 1). Similarity of AFLP and ribotypes are useful criteria to identify strains that belong to these clones.

Eight MDR and 15 susceptible strains were clearly distinct genotypically from clones I and II. These strains were highly heterogeneous in their AFLP pattern, ribotype (21 *Hin*dIII/*Hin*cII ribotypes), biotype (10 different biotypes), serotype (Pantophlet *et al.*, 2001) and plasmid profile (Nemec *et al.*, 1999). Similarly, remarkable heterogeneity of phenotypic and genotypic features was found among the strains from north-western Europe that were not allocated to clone I or II (Dijkshoorn *et al.*, 1996). These findings are suggestive of high genetic diversity in the general *A. baumannii* population.

Multi-drug resistance in Czech strains

Resistance of the Czech strains to 11 antibiotics is shown in Table 2. It is noteworthy that there was an apparent discontinuity in qualitative resistance between the susceptible and MDR strains, as shown in our previous study (Nemec *et al.*, 1999). Most susceptible strains were not resistant to any of the antibiotics tested, whereas 90 % of MDR strains showed resistance to five or more antibiotics. If susceptible to an antibiotic, MDR strains often had a smaller inhibition zone than susceptible strains (see Supplementary Table in JMM Online), which is indicative of their higher potential for being refractory to antimicrobial therapy.

Intraclonal diversity

Table 1 summarizes the ribotyping and biotyping results of the present study and those of biotyping, serotyping and plasmid analysis that were obtained previously (Nemec *et al.*, 1999; Pantophlet *et al.*, 2001). The data demonstrate some intraclonal variability in ribotype, biotype and serotype. Strains of clones I and II that were analysed in the present study were also heterogeneous in antibiotic resistance profile (see Supplementary Table in JMM Online) and plasmid profile (Nemec *et al.*, 1999). This intraclonal variation may result from ongoing diversification in space and time. One example of this diversification is the clone II strains that

Table 1. Properties of *A. baumannii* strains in clones I and II

Data are from this study, Nemec *et al.* (1999) and Pantophlet *et al.* (2001). Numbers in parentheses indicate no. strains with respective types. NT, Not tested; NR, no reactivity with any of the mAbs.

Clone/set of strains	Year of isolation	No. strains	*Hin*dIII/*Hin*cII ribotype	Biotype*	No. resistances per strain†	Reactivity with mAbs‡	No. of strains with 8·7 kb plasmid pAN1
Clone I:							
ARC	1991–1999	24	*R1-1* (23), *R5-3* (1)	6 (9), 11 (14)	7·1 [2–10]	S48-3-13 (18); S51-3 (6)	24
REC	2000–2001	17	*R1-1* (15), *R3-1* (2)	6 (6), 11 (10), 12 (1)	7·1 [5–10]	NT	NT
Reference strains	1984–1990	9	*R1-1* (8), *R3-1* (1)	6 (8), 11 (1)	6·6 [4–9]	S48-3-13 (9)	9
Clone II:							
ARC	1991–1997	10	*R2-2* (7), *R6-4* (3)	2 (10)	5·7 [3–8]	S53-32 (7); NR (3)	1
REC	2000–2001	11	*R2-2* (4), *R6-4* (1), *R4-2* (3), *R2-5* (2), *R2-4* (1)	2 (11)	5·8 [3–7]	NT	NT
Reference strains	1982–1989	4	*R2-2* (3), *R4-2* (1)	1 (1), 2 (2), 9 (1)	3·8 [1–5]	S48-3-17 (3); NR (1)	1

*Biotype according to Bouvet & Grimont (1987). One clone I strain (set ARC) was auxotrophic.

†Eleven antibiotics were tested (see Methods). Values are means, with range in square brackets.

‡Twenty mAbs against O-antigens were tested (Pantophlet *et al.*, 2001).

Table 2. Antibiotic resistance of Czech *A. baumannii* strains

Figures are percentages of resistant strains.

Antibiotic	Clone I ($n = 41$)	Clone II ($n = 21$)	Other multi-resistant strains ($n = 8$)	Susceptible strains ($n = 15$)
Ampicillin + sulbactam	61	57	25	0
Ceftazidime	41	67	13	0
Imipenem	0	10	25	0
Piperacillin	93	90	75	0
Amikacin	76	38	13	0
Gentamicin	95	76	100	0
Netilmicin	20*	10*	38	0
Tobramycin	39	5	75	0
Ofloxacin	95	71	75	0
Cotrimoxazole	95	57	63	13
Tetracycline	98	100	88	7

*The majority of non-resistant strains showed reduced inhibition zone diameters (15–19 mm) in comparison with the susceptible strains (23–26 mm).

shared ribotype *R4-2* and grouped in a distinct AFLP subcluster at a level of 88 %. Another example, although not reflected in the AFLP clustering pattern, is the Czech clone I strains of biotype 11. This biotype was the most frequent in Czech *A. baumannii* strains (Nemec *et al.*, 1999), but seems relatively rare in western Europe (Bouvet & Grimont, 1987; Seifert *et al.*, 1993). Most Czech strains of biotype 11 showed similarity in other properties (*Apa*I macrorestriction analysis profiles, inability to grow on L-arabinose and the presence of a 6 kb plasmid; data not shown) and are likely to represent a regional subclone. Thus, despite the noted similarity of strains that belong to the same clone, there are still characters that can be used to identify strains for epidemiological purposes.

Geographical spread of the clones

Our results and data from the literature indicate a pan-European spread of strains that are classifiable in clones I or II over a remarkable period of time. These strains were spread widely in Czech hospitals from at least 1991 to 2001 and were found in the Netherlands, the UK, Belgium and Denmark between 1982 and 1990 (Dijkshoorn *et al.*, 1996). They were also recognized by Brisse *et al.* (2000) and van Dessel *et al.* (2003) among quinolone-resistant *A. baumannii* isolates from different parts of Europe, including southern Europe, and one isolate from South Africa. Visual inspection of *Eco*RI ribotypes that were published by Seifert & Gerner-Smidt (1995) also suggests the occurrence of these strains in Danish and German hospitals. Finally, Pantophlet *et al.* (2001, 2002) have shown that serotypes found in strains of clones I and II (Table 1) are spread among *A. baumannii* strains from European countries, including Bulgaria and Hungary.

In conclusion, the results presented here confirm that MDR Czech strains of *A. baumannii* that were isolated from hospitalized patients belong mainly to two genetically distinct groups that were identified originally among strains in north-western Europe. These groups most probably represent old clones in a broad (evolutionary) sense, as can be judged from the noted intraclonal type variation and their wide distribution in space and time, as opposed to recent clonal lineages that are found in local outbreaks, which are usually relatively uniform in type characters. It is not yet known what properties have facilitated the wide spread of these MDR clones. It is possible that the capacity to develop or acquire antibiotic resistance was already an attribute of their ancestors and is a prerequisite for their success. Therefore, these clones, which are of undisputed clinical significance, are challenging targets for research on the evolution and spread of multi-drug resistance and of factors involved in *A. baumannii* epidemicity and pathogenicity.

Deposition of representative Czech strains in the CCM

The following strains were deposited in the Czech Collection of Microorganisms (CCM): CCM 7031 (= NIPH 7; clone I, the reference strain of group A), CCM 7032 (= NIPH 15; clone I/group A), CCM 7034 (= NIPH 281; clone I/group A), CCM 7116 (= NIPH 10; clone I), CCM 7033 (= NIPH 24; clone II, the reference strain of group B), CCM 7117 (= NIPH 657; clone II) and CCM 7118 (= NIPH 1362; clone II). The origin and properties of these strains are available in the Supplementary Table in JMM Online.

ACKNOWLEDGEMENTS

Part of this work was presented as poster P742 at the 13th European Congress of Clinical Microbiology and Infectious Diseases, Glasgow, UK, in 2003. We thank M. Maixnerová for her excellent technical assistance and E. Kodytková for her valuable help in preparation of the

manuscript. We also thank colleagues from Czech bacteriological laboratories for collection and provision of strains. This study was supported by research grant no. 310/01/1540 of the Grant Agency of the Czech Republic.

REFERENCES

Aygun, G., Demirkiran, O., Utku, T., Mete, B., Urkmez, S., Yilmaz, M., Yasar, H., Dikmen, Y. & Ozturk, R. (2002). Environmental contamination during a carbapenem-resistant *Acinetobacter baumannii* outbreak in an intensive care unit. *J Hosp Infect* **52**, 259–262.

Bergogne-Bérézin, E. & Towner, K. J. (1996). *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev* **9**, 148–165.

Bouvet, P. J. M. & Grimont, P. A. D. (1987). Identification and biotyping of clinical isolates of *Acinetobacter*. *Ann Inst Pasteur Microbiol* **138**, 569–578.

Brisse, S., Milatovic, D., Fluit, A. C., Kusters, K., Toelstra, A., Verhoef, J. & Schmitz, F.-J. (2000). Molecular surveillance of European quinolone-resistant clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. using automated ribotyping. *J Clin Microbiol* **38**, 3636–3645.

Carr, E. L., Kämpfer, P., Patel, B. K. C., Gürtler, V. & Seviour, R. J. (2003). Seven novel species of *Acinetobacter* isolated from activated sludge. *Int J Syst Evol Microbiol* **53**, 953–963.

Dijkshoorn, L., Aucken, H., Gerner-Smidt, P., Janssen, P., Kaufmann, M. E., Garaizar, J., Ursing, J. & Pitt, T. L. (1996). Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. *J Clin Microbiol* **34**, 1519–1525.

Henriksen, S. D. (1973). *Moraxella*, *Acinetobacter*, and the *Mimeae*. *Bacteriol Rev* **37**, 522–561.

Janssen, P. & Dijkshoorn, L. (1996). High resolution DNA fingerprinting of *Acinetobacter* outbreak strains. *FEMS Microbiol Lett* **142**, 191–194.

Juni, E. (1984). Genus III. *Acinetobacter* Brisou and Prévot 1954, 727^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 303–307. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Lautrop, H. (1974). *Acinetobacter*. In *Bergey's Manual of Determinative Bacteriology*, pp. 436–438. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

NCCLS (2001). Performance Standards for Antimicrobial Susceptibility Testing: 11th informational supplement (document M100-S11). Wayne, PA: NCCLS.

Nemec, A. (1999). Use of the disc diffusion test for epidemiological typing of multiresistant *Acinetobacter baumannii* strains. *Klin Mikrobiol Infect Lék* **5**, 287–297 (in Czech).

Nemec, A., Janda, L., Melter, O. & Dijkshoorn, L. (1999). Genotypic and phenotypic similarity of multiresistant *Acinetobacter baumannii* isolates in the Czech Republic. *J Med Microbiol* **48**, 287–296.

Nemec, A., De Baere, T., Tjernberg, I., Vaneechoutte, M., van der Reijden, T. J. K. & Dijkshoorn, L. (2001). *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* **51**, 1891–1899.

Nemec, A., Dijkshoorn, L., Cleenwerck, I., De Baere, T., Janssens, D., van der Reijden, T. J. K., Ježek, P. & Vaneechoutte, M. (2003). *Acinetobacter parvus* sp. nov., a small-colony-forming species isolated from human clinical specimens. *Int J Syst Evol Microbiol* **53**, 1563–1567.

Pantophlet, R., Nemec, A., Brade, L., Brade, H. & Dijkshoorn, L. (2001). O-antigen diversity among *Acinetobacter baumannii* strains from the Czech Republic and northwestern Europe, as determined by lipopolysaccharide-specific monoclonal antibodies. *J Clin Microbiol* **39**, 2576–2580.

Pantophlet, R., Severin, J. A., Nemec, A., Brade, L., Dijkshoorn, L. & Brade, H. (2002). Identification of *Acinetobacter* isolates from species belonging to the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with monoclonal antibodies specific for O antigens of their lipopolysaccharides. *Clin Diagn Lab Immunol* **9**, 60–65.

Seifert, H. & Gerner-Smidt, P. (1995). Comparison of ribotyping and pulsed-field gel electrophoresis for molecular typing of *Acinetobacter* isolates. *J Clin Microbiol* **33**, 1402–1407.

Seifert, H., Baginski, R., Schulze, A. & Pulverer, G. (1993). The distribution of *Acinetobacter* species in clinical culture materials. *Zentbl Bakteriol* **279**, 544–552.

van Dessel, H., Dijkshoorn, L., van der Reijden, T., Bakker, N., Paauw, A., van den Broek, P., Verhoef, J. & Brisse, S. (2003). Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. *Res Microbiol* (in press). http://dx.doi.org/10·1016/j.resmic.2003·10·003

Wang, S. H., Sheng, W. H., Chang, Y. Y. & 7 other authors (2003). Healthcare-associated outbreak due to pan-drug resistant *Acinetobacter baumannii* in a surgical intensive care unit. *J Hosp Infect* **53**, 97–102.