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**Diversity of strains of the genus *Acinetobacter*  
isolated from humans**

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**Summary of PhD thesis**

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## INTRODUCTION

The genus *Acinetobacter* includes non-motile, coccobacillary, strictly aerobic, Gram-negative bacteria that can be cultured on simple growth media (Juni, 1986). These organisms are widespread in nature, and can be recovered from water, soil and living organisms. In view of their low pathogenicity, acinetobacters remained out of focus of clinical microbiologists for a long time. However, over the last three decades they have increasingly been recognised as important causative agents of hospital infections. Acinetobacters cause severe infections including bronchopneumonia and septicaemia that can have a fatal course in debilitated patients and new-borns. Particularly problematic is the fact that multiple resistant strains can spread epidemically among patients, while due to their resistance to antibiotics, infections by these organisms are difficult to treat (Bergogne-Bérézin, 1995; Bergogne-Bérézin & Towner, 1996).

**Taxonomy.** The basis for the present classification of *Acinetobacter* was laid in 1986 by Bouvet & Grimont with the description of 12 DNA-DNA hybridisation groups (genomic species) within the genus. In the period 1986 up to 1999, this scheme was extended with 11 additional genomic species (Tjernberg & Ursing, 1989; Bouvet & Jeanjean, 1989; Gerner-Smidt & Tjernberg; 1993; Vaneechoutte *et al.*, 1999). Eight genomic species were given names (*A. calcoaceticus*, *A. baumannii*, *A. haemolyticus*, *A. junii*, *A. johnsonii*, *A. lwoffii*, *A. radioresistens*, and '*A. venetianus*') while the others are designated by numbers. Strains of *A. calcoaceticus*, *A. baumannii*, the unnamed groups 3 and 13TU are genetically closely related and difficult to separate phenotypically, and are therefore sometimes unified in the so-called *A. calcoaceticus*-*A. baumannii* (ACB) complex (Gerner-Smidt *et al.*, 1991). Apart from the known genomic species, additional strains of unknown taxonomic status were found in a number of studies (Tjernberg & Ursing, 1989; Bouvet & Jeanjean, 1989). The percentage of these strains ranged between 2 and 15%, depending on the origin of the organisms. These unidentifiable strains may indicate either the existence of additional genomic species or yet unrecognised intraspecies diversity.

**Ecology.** Strains of *A. baumannii*, and the unnamed groups 3 and 13 TU are recovered predominantly from clinical specimens, with *A. baumannii* being the most prominent as an agent able to colonise and infect severely ill, hospitalised patients (Horrevorts *et al.*, 1995; Bergogne-Bérézin and Towner, 1996; McDonald *et al.*, 1999). Strains of the latter species can persist in hospitals and give rise

to outbreaks; they are usually highly resistant to antibiotics (Seifert *et al.*, 1993b). Most other (genomic) species of *Acinetobacter* have been found in different environments, e.g. *A. calcoaceticus* was isolated predominantly from soil, and *A. johnsonii* was found in activated sludge and frozen food, although representatives of these and other species have also been recovered occasionally from human specimens (Seifert *et al.*, 1993a; Bergogne-Bérézin & Towner, 1996). Overall, the natural habitats of most *Acinetobacter* (genomic) species have not been well-studied.

**Species identification.** DNA-DNA hybridisation is the gold standard for identification of *Acinetobacter* strains, but this method is not applicable in most laboratories. A phenotypic identification scheme, including nutritional tests and growth at different temperatures, was devised by Bouvet & Grimont (1987). However, it has been shown that some species are difficult to identify by these tests (Gerner-Smidt *et al.*, 1991). Similarly, commercial phenotypic identification systems show only moderate performance (Bernards *et al.*, 1995; Bernards *et al.*, 1996). Several genotypic methods have been proposed for identifying acinetobacters to genomic species level, including ribotyping (Gerner-Smidt, 1992), tDNA fingerprinting (Ehrenstein *et al.*, 1996), amplified ribosomal DNA restriction analysis (ARDRA) (Vanechoutte *et al.*, 1995; Dijkshoorn *et al.*, 1998), and AFLP<sup>TM</sup> fingerprinting (Janssen *et al.*, 1997). Nevertheless, identification to genomic species using one method is still problematic (Dijkshoorn *et al.*, 1998; Jawad *et al.*, 1998) and the development of practical reliable methods for identification within the genus *Acinetobacter* remains a challenging task.

**Population structure at the subspecies level.** Virtually all currently available typing methods have been used for epidemiological typing of hospital strains of *A. baumannii*. These methods include phenotypic (biotyping, cell envelope protein electrophoresis, antibiogram typing *etc.*) or genotypic techniques (plasmid typing, ribotyping, pulsed-field gel electrophoresis (PFGE), PCR fingerprinting and AFLP analysis), often used in combination (Dijkshoorn *et al.*, 1993; Seifert and Gerner-Smidt, 1995; Janssen & Dijkshoorn, 1996; Grundmann *et al.*, 1997). The application of the methods has revealed high diversity at the strain level. However, most of these studies dealt with geographically limited collections of isolates and results obtained in different laboratories were difficult to compare because of variations in the methods used. A recent study has shown that epidemic strains from different cities and countries in Europe are highly similar (Dijkshoorn *et al.*, 1996), which may indicate that the emergence of epidemic *A. baumannii* at different locations is associated with the occurrence of specific clones (i.e., isolates showing so many identical phenotypic and genotypic traits

that the most likely explanation for this identity is a common origin [Ørskov & Ørskov, 1983]).

## **OBJECTIVES**

The present study was conceived as a taxonomic analysis of a large group of clinical isolates of the genus *Acinetobacter* isolated at different locations in the Czech Republic with the aim to establish the prevalence of different (genomic) species, and to analyse the strain (clonal) diversity among hospital isolates of the clinically most relevant species, *A. baumannii*. In the course of the study, two novel phenotypically and genotypically distinct groups were discovered, including most strains that could not be identified to any of the hitherto known species. At the same time, it was found that Czech multiresistant *A. baumannii* strains belong almost exclusively to two phenotypically and genotypically homogeneous groups (clones). That is why collaboration with several European laboratories was sought to analyse the groups discovered in more detail by additional methods and to compare them with other strains from various European locations.

In summary, the steps undertaken are as follows:

- Study of the (genomic) species diversity of clinical isolates of the genus *Acinetobacter* originating from the Czech Republic.
- Classification of hitherto unidentifiable strains and definition of their taxonomic status.
- Study of the diversity of *A. baumannii* hospital isolates at the subspecies (strain, clonal) level.
- Study of characteristics of *A. baumannii* epidemic clones.
- Development of a practical method for epidemiological typing of *A. baumannii* hospital isolates.

## THESIS CHAPTERS

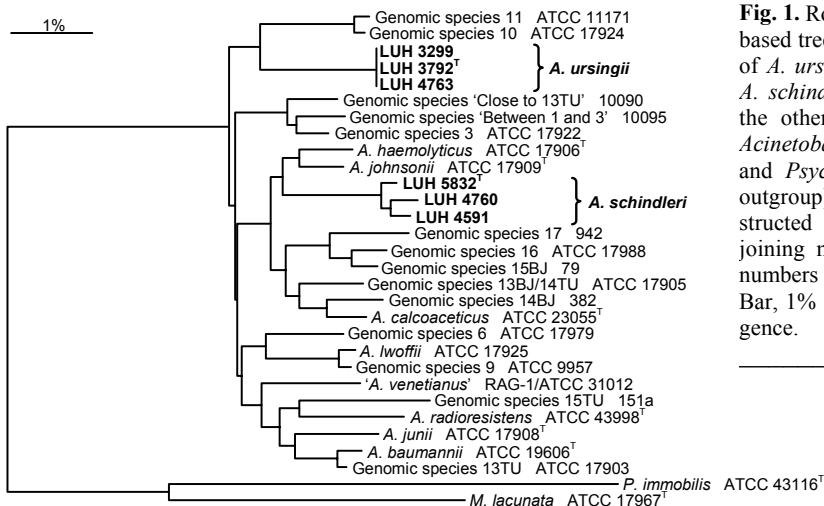
The results of the PhD thesis have been published as follows:

1. **Nemec A.** [Taxonomy of the genus *Acinetobacter*.]. *Epidemiol Mikrobiol Imunol* 1996;45:23-29. [Czech]
2. **Nemec A**, Urbášková P, Grimont F, Vránková J, Melter O, Schindler J. [Identification and typing of hospital strains of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex.] *Epidemiol Mikrobiol Imunol* 1996;45:71-82. [Czech]
3. **Nemec A**, Janda J, Melter O, Dijkshoorn L. Genotypic and phenotypic similarity of multiresistant *Acinetobacter baumannii* isolates in the Czech Republic. *J Med Microbiol* 1999;48:287-296.
4. **Nemec A.** [Use of the disk diffusion test for epidemiological typing of multiresistant *Acinetobacter baumannii* strains.] *Klin Mikrobiol Inf Lék* 1999;5:287-297. [Czech]
5. **Nemec A**, Dijkshoorn L, Ježek P. Recognition of two novel phenons of the genus *Acinetobacter* among non-glucose-acidifying isolates from human specimens. *J Clin Microbiol* 2000;38:3937-3941.
6. Pantophlet R, **Nemec A**, Brade L, Brade H, Dijkshoorn L. O-antigen diversity among *Acinetobacter baumannii* strains from the Czech Republic and Northwestern Europe, as determined by lipopolysaccharide-specific monoclonal antibodies. *J Clin Microbiol* 2001;39:2576-2580.
7. **Nemec A**, De Baere T, Tjernberg I, Vaneechoutte M, van der Reijden TJK, Dijkshoorn L. *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* 2001;51:1891-1899.
8. Pantophlet R, Severin JA, **Nemec A**, Brade L, Dijkshoorn L, Brade H. 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* 2002;9:60-65.
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10. **Nemec A**, van der Reijden TJK, Dijkshoorn L. [Multiresistant clones of *Acinetobacter baumannii* in the Czech Republic.] *Klin Mikrobiol Inf Lék* 2003 (in press) [Czech]

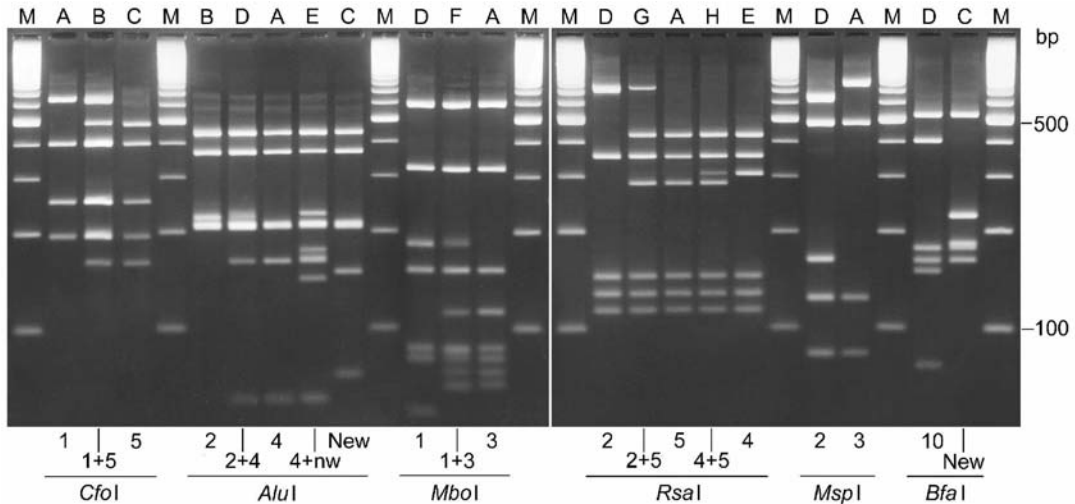
## RESULTS

### Species diversity

▪ **Genomic species diversity** was studied among 700 *Acinetobacter* clinical isolates obtained between 1991 and 1999 from diagnostic laboratories in the Czech Republic (*Chapter 5*). The isolates were recovered from a variety of specimens from patients in hospitals and general practice and represented different levels of clinical significance, ranging from colonisation to life-threatening infections. All isolates were analysed by biochemical tests of Bouvet and Grimont (1987) and identified by numerical probabilistic identification using two reference probability matrices. If not identified concordantly by both matrices as belonging to the *ACB* complex, the strains were further analysed by ARDRA. The final identification was then derived from the combined biochemical and ARDRA results ("consensus identification"). Identification of 700 isolates yielded the following results: the *ACB* complex ( $n=553$ ), *A. lwoffii* ( $n=63$ ), genomic species 13BJ ( $n=9$ ), *A. johnsonii* ( $n=7$ ), *A. haemolyticus* ( $n=6$ ), *A. junii* ( $n=5$ ), and other genomic species (<5 isolates each). Forty-five isolates could not be identified as belonging to any known (genomic) species. Among the unidentified isolates two large groups of non-glucose-acidifying, non-hemolytic and non-gelatinase-producing isolates were distinguished. These groups, designated phenon 1 ( $n=17$ ) and phenon 2 ( $n=15$ ), had distinctive phenotypic features and novel ARDRA profiles, which suggested that they represented hitherto undescribed *Acinetobacter* species.



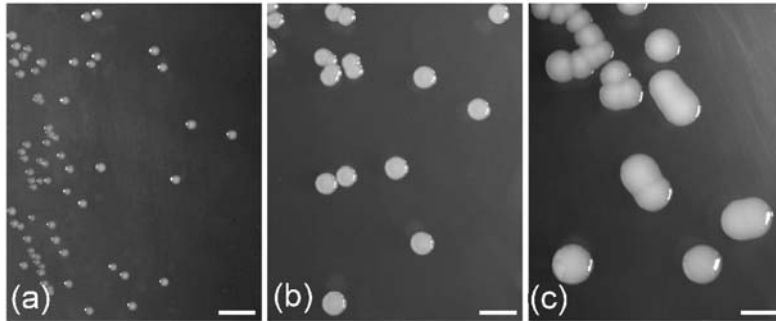
**Fig. 1.** Rooted 16S rDNA sequence based tree showing the relationship of *A. ursingii* sp. nov. (phenon 1), *A. schindleri* sp. nov. (phenon 2), the other members of the genus *Acinetobacter*, *Moraxella lacunata* and *Psychrobacter immobilis* (an outgroup). The tree was constructed using the neighbour-joining method. EMBL accession numbers are given in parentheses. Bar, 1% estimated sequence divergence.



**Fig. 2.** Overview of the ARDRA patterns found in *A. ursingii* sp. nov. and *A. schindleri* sp. nov. strains. Strains are indicated by the upper-case letters above the lanes: A, LUH 3792<sup>T</sup> (*A. ursingii*); B, LUH 4594 (*A. schindleri*); C, LUH 4765 (*A. schindleri*); D, LUH 5832<sup>T</sup> (*A. schindleri*); E, LUH 3793 (*A. ursingii*); F, LUH 4761 (*A. ursingii*); G, LUH 4613 (*A. ursingii*); H, LUH 4618 (*A. ursingii*). Lanes M, molecular size markers (100-bp ladder). Pattern designations for the respective enzymes are given below the lanes.

▪ **Taxonomic positions of phenon 1 and phenon 2** were studied in collaboration with three European laboratories (*Chapter 7*). For this purpose, the collection of Czech strains was enlarged with strains from other European countries that showed characters similar to those of the two phenons. These additional strains ( $n=21$ ) were selected from a set of *c.* 100 archive *Acinetobacter* strains that remained unidentifiable. Polyphasic taxonomic analysis (analysis of biochemical characteristics, ARDRA, AFLP fingerprinting, DNA-DNA hybridisation, 16S rRNA gene sequence analysis) showed that phenons 1 and 2 represented two novel species of the genus *Acinetobacter*, for which the names *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov. were proposed (Fig. 1). *A. ursingii* and *A. schindleri* differed in their distribution in patients. While the majority of the *A. schindleri* strains were isolated from non-sterile body sites of outpatients, *A. ursingii* comprised mainly clinically significant isolates from seriously ill, hospitalised patients. Almost half of the *A. ursingii* strains were isolated from blood cultures and at least some of them were recovered from patients with diagnosed bacteraemia or septicaemia. Practical identification of both species based on specific biochemical characteristics and ARDRA profiles (Fig. 2) was proposed. An analogous procedure was used for defining the taxonomic position of *Acinetobacter* strains ( $n=7$ ) showing unusually small colonies on agar plates (Fig. 3) and low biochemical activity (*Chapter 9*). Polyphasic analysis confirmed,

that these strains represent a new species, for which the name *Acinetobacter parvus* sp. nov. was proposed.



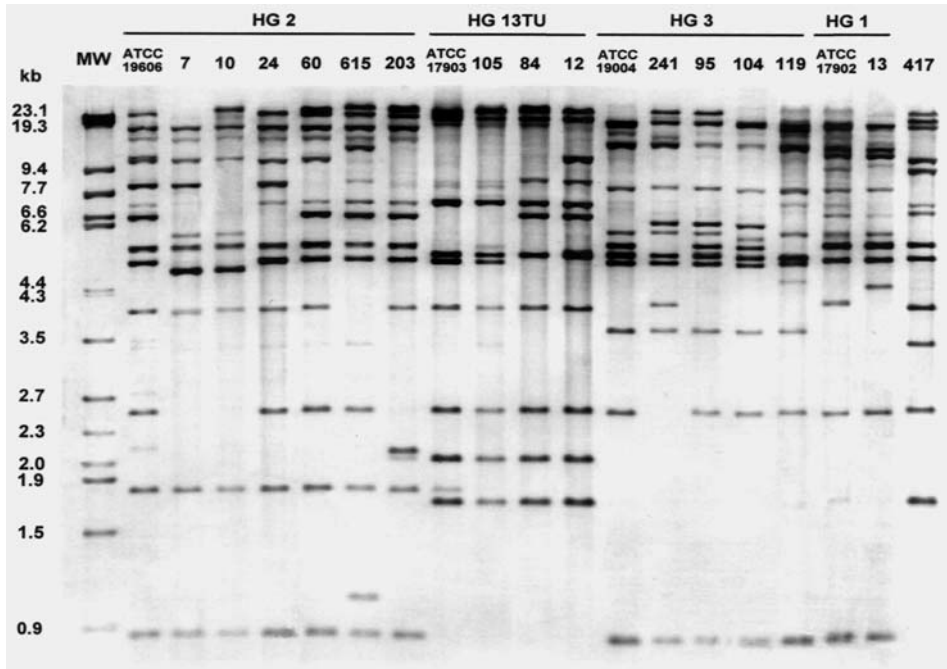
**Fig. 3.** Colonies of the type strains of (a) *Acinetobacter parvus* sp. nov., (b) *Acinetobacter ursingii* sp. nov. and (c) *Acinetobacter schindleri* sp. nov. The strains were grown on tryptic soya agar at 30 °C for 24 h. Bar, 2 mm.

▪ **Genomic species diversity within the *ACB* complex** was studied among 103 clinical isolates obtained between 1991 and 1997 from 17 Czech hospitals (*Chapter 3*). These isolates were selected from *c.* 400 *ACB* complex isolates to include strains as heterogeneous as possible in time and place of isolation. According to the *EcoRI* ribotypes, all but one of these isolates were identified to species: 77 (75 %) isolates were allocated to *A. baumannii*, 14 to genomic species 3, 10 to genomic species 13TU and one to *A. calcoaceticus*. The remaining isolate could not be unambiguously identified as any of these (genomic) species; its *EcoRI* ribotype was closest to that of one of two additionally described groups of the *ACB* complex (Gerner-Smidt & Tjernberg, 1993). All multiresistant and epidemic strains were found to belong to *A. baumannii*.

#### **Subspecific classification of *Acinetobacter baumannii* strains**

▪ **Diversity at the subspecific level** (strain, clonal) was studied in the 103 Czech isolates of the *ACB* complex (*Chapter 3*) by ribotyping, plasmid analysis, biotyping and antibiotic susceptibility testing. *EcoRI* ribotyping showed high genotypic diversity (50 different ribotypes); most of the isolates had a unique ribotype (Fig. 4). However, more than 50% of the *A. baumannii* isolates belonged to two ribotypes only. The isolates with identical ribotypes were also similar in other characteristics such as plasmid profile, biotype and susceptibility to antimicrobial agents. These two genotypically and phenotypically relatively homogeneous groups were designated as groups A and B and it was hypothesised that they represented two discrete clonal lineages of *A. baumannii* spread in Czech hospitals. Group A comprised 37 isolates of *EcoRI* ribotype I belonging to biotypes 11 or 6 and containing an 8.7 kb cryptic plasmid (designated pAN1; Fig. 5).





**Fig. 4.** Examples of *Eco*RI ribotypes showing those for 15 Czech clinical isolates and four reference strains of the *A. calcoaceticus* - *A. baumannii* complex. Isolates (NIPH) 7 and 24 represent clone I (group A) and clone II (group B), respectively; isolate 417, ungrouped. HG, DNA hybridisation group (= genomic species); HG 2, *A. baumannii*; HG 1, *A. calcoaceticus*; MW, mol. wt markers (phage  $\lambda$  DNA digested with *Hind*III and *Sty*I).

Group B consisted of eight isolates of *Eco*RI ribotype II, belonging to biotype 2, in most of which plasmid DNA was not detected. Both groups contained epidemic and sporadic isolates resistant to more antibiotics than the remaining isolates (groups A and B included 85% of all multiresistant isolates).

- A taxonomically and epidemiologically well-defined set comprising representative Czech hospital strains of 1991-1999 ( $n=52$ ) and reference strains for epidemic clones I ( $n=9$ ) and II ( $n=4$ ) taken from the original study of Dijkshoorn *et al.* (1996) was selected for **further analysis of *A. baumannii* clonal groups** (Chapter 6). The Czech collection comprised strains of groups A ( $n=23$ ) and B ( $n=7$ ) and other multiresistant ( $n=7$ ) and susceptible ( $n=15$ ) strains. In collaboration with Dr. R. Pantophlet (Borstel, Germany), the strains were characterised using newly developed monoclonal antibodies (*MABs*) against acinetobacter O-antigens. The results confirmed homogeneity of both Czech clonal groups A and B and their difference from the other Czech strains. All group A strains reacted with *MAB* S48-3-13 or S51-3 and all group B strains reacted with *MAB* S53-32, while most of the other resistant and susceptible strains did not show reactivity with any of 20 *MABs* tested. The group A characteristics were congruent with

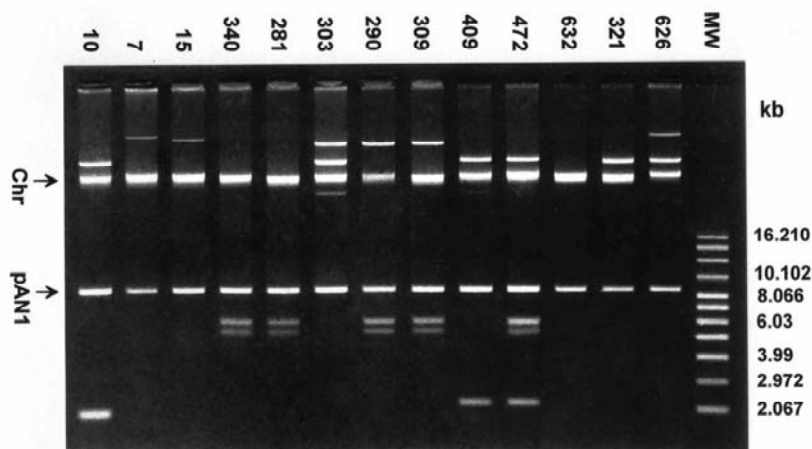


Fig. 5. Plasmid profiles of *A. baumannii* isolates of clone I characterised by the presence of 8.7 kb plasmid (pAN1). MW, mol. wt marker; Chr, chromosomal DNA.

clone I (identical *EcoRI* ribotype, biotypes 6 or 11, reactivity with *Mab* S48-3-13, presence of plasmid pAN1) and most group B characteristics were congruent with clone II (identical *EcoRI* ribotype, biotype 2), although reactivity with *MABs* was different since clone II strains reacted with S48-3-17, and not with S53-32. The results of the following study (*Chapter 8*) showed that reactivity with the above *MABs* is frequently found in hospital *A. baumannii* isolates from other European countries as well, which is indicative of pan-European spread of the epidemic clones.

- Ribotyping with enzymes *HincII* and *HindIII* and AFLP fingerprinting were used for **quantitative analysis of genotypic similarity among *A. baumannii* strains** of the previous collection completed with recent multiresistant isolates ( $n=33$ ) from the Czech Republic (*Chapter 10*). Numerical analysis of combined *HincII/HindIII* ribotypes and AFLP profiles confirmed genotypic congruency between group A and clone I and group B and clone II. Furthermore, the results showed that both clones included some strains with slightly different ribotypes, that initially had not been classified into groups A or B. Comparison of recent isolates (2001-2002) with strains of 1991-1999 revealed that the structure of the Czech *A. baumannii* population had not undergone any significant change in the last decade.

- **A practical method for epidemiological typing of multiresistant *A. baumannii* strains** was introduced, based on numerical analysis of inhibition zone diameters in the disk diffusion test (*Chapter 4*). Eleven antibiotics representative of the major groups of effective antimicrobial drugs were selected for typing: ampicillin+sulbactam, piperacillin, ceftazidime, imipenem, co-trimoxa-zole, ofloxacin, gentamicin, tobramycin, amikacin, netilmicin and tetracycline. Each isolate was characterised by a set of inhibition zone diameters for different antibi-

otics and these sets were compared by cluster analysis with the Euclidean distance as a similarity coefficient. Compared to ribotyping and biotyping, the discriminatory power of quantitative antibiogram typing was highest for the multiresistant strains. As chromosomal DNA macrorestriction analysis (pulsed-field gel electrophoresis) did, this method effectively differentiated genotypically similar strains belonging to epidemic clone I. Theoretical limits to the method are given by the instability of the resistance markers and phenotypic convergence, and therefore, definitive conclusions on strain identity should be confirmed by genotypic methods.

▪ **Strain and clonal diversity of the *ACB* complex in an endemic situation** was studied in 95 isolates obtained between 1991 and 1993 in the Prague Burn Centre (*Chapter 2*). Classification based on restriction fragment length polymorphism of total DNA, plasmid profiles, ribotypes, antibiograms and biotypes allowed distinguishing of 12 relatedness groups (each including isolates of the same strain) and 19 single strains. *EcoRI* ribotyping of 28 strains yielded the following results: 18 strains of *A. baumannii*, 5 strains of genomic species 13TU and 5 strains of genomic species 3. Two multiresistant *A. baumannii* strains belonging to epidemic clone I persisted in the burn centre throughout the whole study period and one of them caused an outbreak in summer 1993. Isolates of these two strains ( $n=39$ ) presented 41 % of all isolates studied. Short-term occurrence and patient-to-patient transmission were also made conceivable on the basis of epidemiological typing for a strain belonging to epidemic clone II. Illustrative of the significance of the polyphasic description is that three almost simultaneously isolated multiresistant strains belonged to epidemic clone I and were distinguishable only by a combination of at least two of the methods used (e.g. ribotyping and biotyping).

## CONCLUSIONS

In summary, the present study has significantly contributed to the current knowledge of species and subspecies diversity within the genus *Acinetobacter*. A method termed "consensus identification" was developed, using a combination of practical, complementary methods, that together allowed for reliable species identification. Furthermore, groups of hitherto unidentifiable strains were defined and *A. baumannii* multiresistant/epidemic clones were described. Cooperation with other European laboratories enabled to complete the study set with additional strains from other geographically distinct localities and to analyse the most relevant groups by supplementary taxonomic methods (polyphasic taxonomy). This collaboration resulted in description of three novel *Acinetobacter* species, i.e. *A.*

*ursingii* sp. nov., *A. schindleri* sp. nov., and *A. parvus* sp. nov. *A. ursingii* is a clinically important taxon isolated from severely ill, hospitalised patients. The results of the polyphasic subspecific analysis of strains from the Czech Republic and northwestern Europe showed the existence of pan-European clonal lineages of *A. baumannii*. Strains belonging to these clones are usually multiresistant and can spread epidemically within hospitals, but they also occur at different locations in Europe. Multiresistant strains isolated in the Czech Republic were found to belong almost exclusively to these two clones. The distinguished multiresistant/epidemic clones will be the subject of future research addressing the factors involved in pathogenicity, epidemicity and multiresistance of *A. baumannii*.

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## PROFESSIONAL BIOGRAPHY OF ALEXANDR NEMEC

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**General interests.** Molecular-biology and phenotypic methods in classification and identification of bacteria; numerical taxonomy; typing of multiresistant nosocomial strains; taxonomy and epidemiology and of the genus *Acinetobacter*; genetic basis of resistance to antibiotics in *A. baumannii*.

### Principal investigator of the following research grants

- No. 0968-2 of the Internal Grant Agency of the Ministry of Health Agency of the Czech Republic: "Epidemiological significance of molecular markers in nosocomial strains of the genus *Acinetobacter*" (1992-1993).
- No. 3486-3 of the Internal Grant Agency of the Ministry of Health Agency of the Czech Republic: "Polyphasic classification of multiresistant isolates of the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex" (1996-1998).
- No. 310/98/1602 of the Grant Agency of the Czech Republic: "Phenetic diversity of the strains of the genus *Acinetobacter* isolated from humans" (1998-2000).
- No. 310/01/1540 of the Grant Agency of the Czech Republic: "Evolution of multiresistance in *Acinetobacter baumannii*: the relationship between clonality and aminoglycoside resistance." (2001-2003).

### Fellowships abroad

- Study of the secondary structure of ribosomal protein S20 by comparative analysis of the primary structure of the homologous genes in selected eubacterial species, Department of molecular biology of the University of Western Ontario, London, Canada (1992, three months).
- Ribotyping of nosocomial strains of the *Acinetobacter calcoaceticus* - *baumannii* complex - FEMS fellowship, Unité des Entérobactéries, Institut Pasteur, Paris (1994, one month).
- Complex genomic typing of unclassified strains of the genus *Acinetobacter*, University Medical Centre, Leiden, the Netherlands (1997, one month).

### Organisation of international meetings

- The international conference "The Taxonomy and automated identification of bacteria". (Prague, 20.-24.7. 1992)", a member of the organising committee.
- The symposium: "Classification methods and classification in epidemiology", the 7th IUMS international congress of bacteriology and applied microbiology (Prague, 3.-8.7. 1994), a chairman.

## LIST OF PUBLICATIONS

### *Original articles in peer reviewed journals and book chapters*

1. Nemec A, van der Reijden TJK, Dijkshoorn L. Multirezistentní klony *Acinetobacter baumannii* v České republice. [Multiresistant clones of *Acinetobacter baumannii* in the Czech Republic.] *Klin Mikrobiol Inf Léč* 2003 (in press)
2. Nemec A, Dijkshoorn L, Cleenwerck I, De Baere T, Janssens D, van der Reijden TJK, Ježek P, Vanechoutte M., *Acinetobacter parvus* sp. nov., a small colony-forming species isolated from human clinical specimens. *Int J Syst Evol Microbiol* 2003 (in press)
3. Melter O, Hercik K, Weyant RS, Janeček J, Nemec A, Mecera J, Gonzorová L, Branny P. Detection and characterization of feline *Bartonella henselae* in the Czech Republic. *Vet Microbiol* 2003; 93: 261-273.
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5. Volf P, Kiewegová A, Nemec A. Bacterial colonization in the gut of *Phlebotomus duboscqi* (Diptera: Psychodidae): transtadial passage and the role of female diet. *Folia Parasitol* 2002; 49:73-77.
6. Pantophlet R, Severin JA, Nemec A, Brade L, Dijkshoorn L, Brade H. 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* 2002; 9: 60-65.
7. Nemec A, De Baere T, Tjernberg I, Vanechoutte M, van der Reijden TJK, Dijkshoorn L. *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* 2001; 51: 1891-1899.
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9. Nemec A, Dijkshoorn L, Ježek P. Recognition of two novel phenons of the genus *Acinetobacter* among non-glucose-acidifying isolates from human specimens. *J Clin Microbiol* 2000; 38: 3937-3941.
10. Nemec A. Využití diskového difúzního testu v epidemiologické typizaci multirezistentních kmenů *Acinetobacter baumannii*. [Use of the disk diffusion test for epidemiological typing of multiresistant *Acinetobacter baumannii* strains.] *Klin Mikrobiol Inf Léč* 1999; 5: 287-297.
11. Nemec A, Janda J, Melter O, Dijkshoorn L. Genotypic and phenotypic similarity of multiresistant *Acinetobacter baumannii* isolates in the Czech Republic. *J Med Microbiol* 1999; 48: 287-296.
12. Aldová E, Schindler J, Šourek J, Nemec A, Urbášková P. Detection and isolation of *Citrobacter sedlakii*. *Zentralbl Bakteriol* 1997; 285: 389-396.
13. Nemec A, Urbášková P, Grimont F, Vránková J, Melter O, Schindler J. Identifikace a typizace nemocničních kmenů komplexu *Acinetobacter calcoaceticus* - *Acinetobacter baumannii*. [Identification and typing of hospital strains of the *Acinetobacter calcoaceticus* - *Acinetobacter baumannii* complex.] *Epidemiol Mikrobiol Imunol* 1996; 45: 71-82.
14. Nemec A. Taxonomie rodu *Acinetobacter*. [Taxonomy of the genus *Acinetobacter*.] *Epidemiol Mikrobiol Imunol* 1996; 45: 23-29.
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17. Urbášková P, Toršová V, Nemec A. Antibiotika u nozokomiálních nákaz. [Antibiotics in patients with nosocomial infections.] In: Šrámová H *et al* Nozokomiální nákazy [Nosocomial infections]. Praha, Maxdorf-Jessenius, 1995: 135-154.
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19. Urbášková P, Schindler J, Aldová E, Nemec A. Antibiotic susceptibility of mesophilic aeromonads isolated in Czechoslovakia. *Med Microbiol Lett* 1993; 2: 152-158.

#### **Posters**

1. Nemec A, Dijkshoorn L, Cleenwerck I, De Baere T, Janssens D, van der Reijden TJK, Ježek P, Vanechoutte M. *Acinetobacter parvus* sp. nov., a small colony-forming species isolated from human specimens. The 1st FEMS Congress of European Microbiologists, Ljubljana, Slovenia, 29.6.-3.7. 2003. (*accepted*)
2. Nemec A, van der Reijden T, Dijkshoorn L. Dissemination of structurally related class I integrons among *Acinetobacter baumannii* clones. The 13th European Congress of Clinical Microbiology and Infectious Diseases. Glasgow, UK, 10.-13.5. 2003. (*accepted*)
3. Pantophlet R, Nemec A, Brade H, Brade L, Dijkshoorn L. Serological characterization of *Acinetobacter* isolates from the Czech Republic and NW Europe using monoclonal antibodies against the O-antigen of the lipopolysaccharides [abstract P128]. In: Abstracts of the 5th International Meeting on Bacterial Epidemiological Markers. Noordwijkerhout, The Netherlands, 2000: 207-208.
4. Nemec A, De Baere T, Tjernberg I, Vanechoutte M, Dijkshoorn L. *Acinetobacter ursingii* sp. nov., a new species associated with nosocomial infections [abstract 22]. In: Abstracts of the 5th International Symposium on the Biology of *Acinetobacter*. Noordwijkerhout, The Netherlands, 2000: 22.
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7. Nemec A, Melter O, Mohelská E. Genotypic and phenotypic characterization of clinical isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex [abstract P882]. In: Abstracts of the 8th European Congress of Clinical Microbiology and Infectious Diseases. *Clin Microbiol Infect* 1997; 3(suppl 2): 215.
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*Other 13 articles and notes in non-peer reviewed journals or abstract books*



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