589

Extended Description of Three Novel *Acinetobacter* Species of Possible Clinical Relevance, *Acinetobacter ursingii, Acinetobacter schindleri* and *Acinetobacter parvus*.

Lenie Dijkshoorn,[1] Alexandr Nemec,[2,3] Tanny J. K. van der Reijden,[1] Thierry De Baere,[4] Peterhans J. van den Broek,[1] Petr Ježek[5] & Mario Vaneechoutte[4]

[1]Department of Infectious Diseases, Leiden University Medical Center, The Netherlands; [2]National Institute of Public Health and [3]3rd Faculty of Medicine, Charles University, Prague, Czech Republic; [4]Department of Clinical Chemistry, Microbiology and Immunology, University Hospital, Gent, Belgium; [5]Department of Clinical Microbiology, Příbram, Czech Republic.

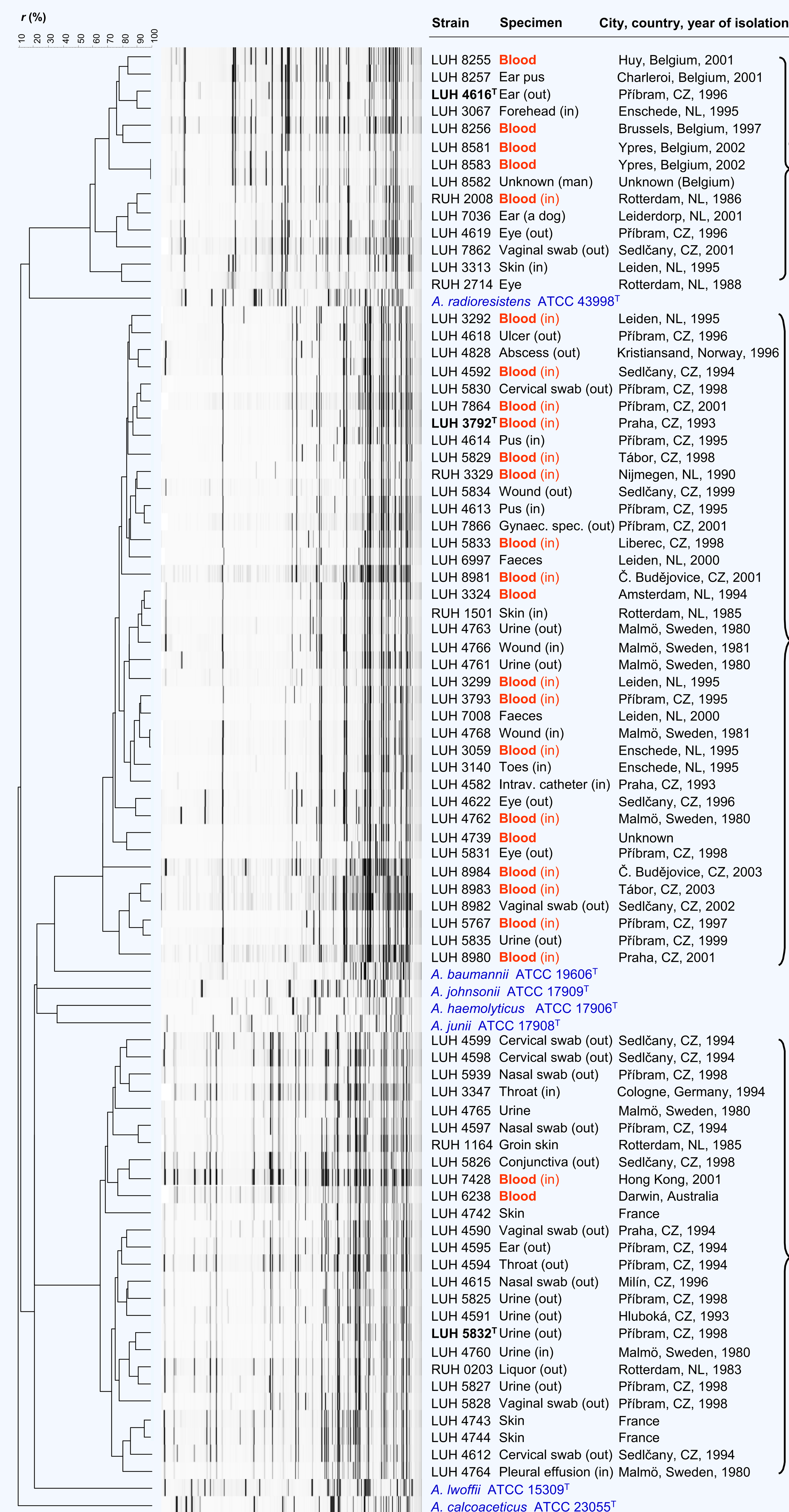

Fig. 1. Dendrogram of cluster analysis of AFLP fingerprints of the strains of *A. ursingii* (*n*=38), *A. schindleri* (*n*=26) and *A. parvus* (*n*=14), and of the type strains of 7 validated species of the genus *Acinetobacter*.
Fingerprints were generated using automated laser fluorescence detection and cluster analysis was performed with the BioNumerics software package using Pearson's product for similarity calculation and UPGMA for clustering. CZ, Czech Republic; NL, the Netherlands; in, inpatient; out, outpatient.

Objective

Recently, three novel *Acinetobacter* species from human clinical specimens have been described, i.e., *Acinetobacter ursingii, Acinetobacter schindleri* and *Acinetobacter parvus* (4,5,6).

The aim of the study is to provide a description and identification criteria of the species using an extended set of strains.

Results

AFLP (Fig. 1). *A. ursingii*, *A. schindleri* and *A. parvus* strains formed three distinct clusters at a grouping level of 67 %, 65 % and 58 %, respectively, which are above the 50 % level seen in previous studies for the delineation of *Acinetobacter* species (5).

Biochemical properties (Table 1) and **colony morphology** (Fig. 2). Striking features of *A. parvus* were its lack of metabolic activity and its small colony growth which is unusual in *Acinetobacter*.

ARDRA restriction profiles were characteristic for each species with few exceptions (Table 2).

Antibiotic susceptibility (Table 3). *A. schindleri* strains were mostly fully susceptible to the antibiotics tested whereas many *A. ursingii* strains showed intermediate susceptibility or resistance to some β-lactams and chloramphenicol. Notably, all but one *A. ursingii* strains had either no inhibition zones with penicillin or this zone was less than 10 mm, while all but one *A. schindleri* strains had inhibition zones >13 mm (range 14 – 25 mm).

Phenotypic tests allowed for **presumptive identification** of the novel species (Table 4), which can be improved by combination of these tests with ARDRA (Table 2).

Strains

A total of 78 strains were investigated: *A. ursingii* (*n*=38), *A. schindleri* (*n*=26) and *A. parvus* (*n*=14) (Table 1). All but one strains were from human specimens; 18, 2 and 5 isolates of *A. ursingii*, *A. schindleri* and *A. parvus*, respectively, were from blood.

Methods

AFLP™ fingerprinting (5)
Phenotypic characterization (4,5)
Susceptibility testing using disk diffusion (3)
Amplified rDNA restriction analysis (ARDRA) (1,5)

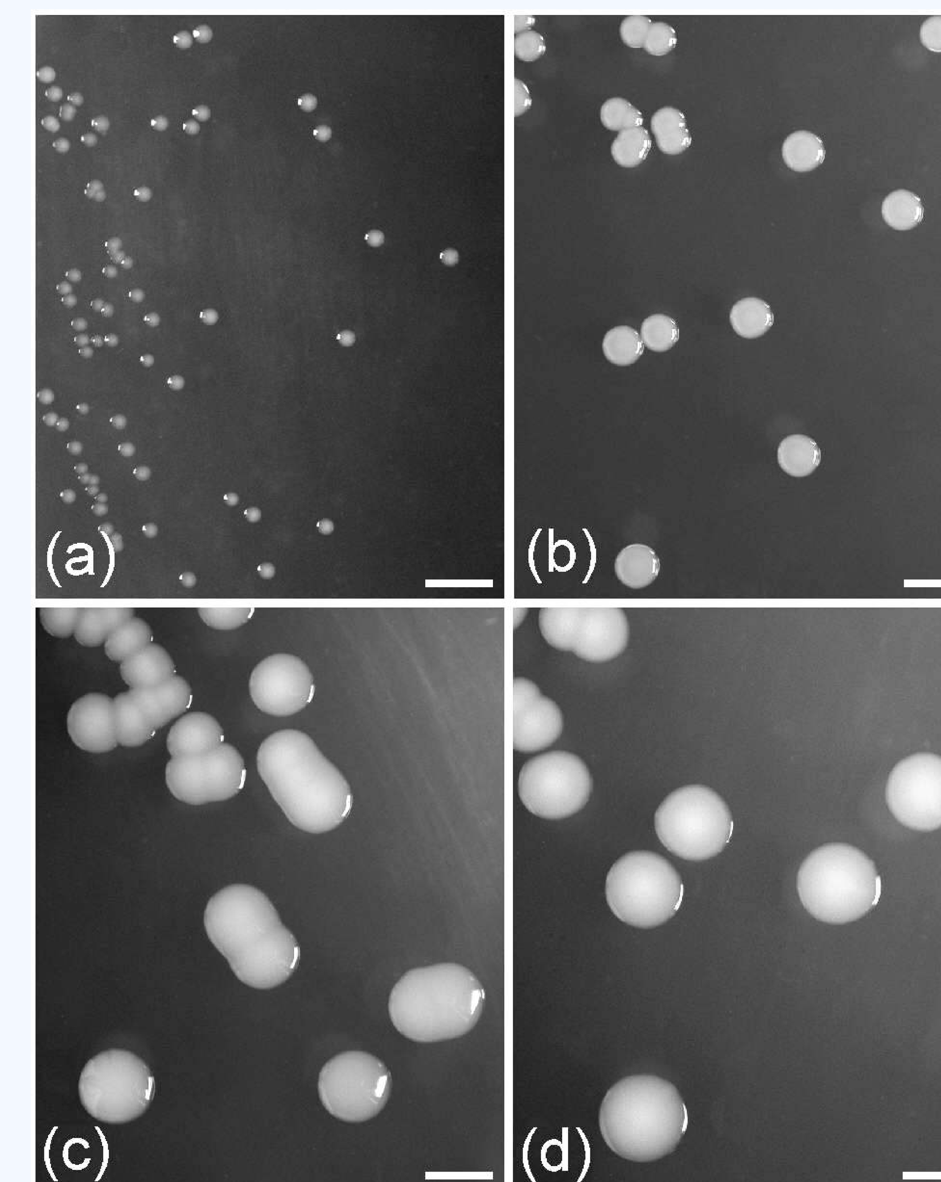

Fig. 2. Colonies of the type strains of (a) *A. parvus* (LUH 4616T), (b) *A. ursingii* (LUH 3792T), (c) *A. schindleri* (LUH 5832T) and (d) *A. lwoffii* (ATCC 15309T).
The strains were grown on Tryptic Soya Agar (Oxoid) at 30 °C for 24 h. Bar, 2 mm.

Table 2. ARDRA patterns
The patterns designations as published previously (1,5). Typical combinations are in bold.

Species	Restriction pattern with enzyme					
	Cfo I	*Alu* I	*Mbo* I	*Rsa* I	*Msp* I	*Bfa* I
A. ursingii	**1**	**4**	**3** or 1+3	**5**	**3**	
	1	4 or 4+nw	3 or 1+3	4 or 4+5	3	
	1	4	3 or 1+3	2+5	3	
A. schindleri	**1+5**	**2 or 2+4**	**1**	**2**	**2**	**10**
	5	4	1	2	2	10
	5	New	1	2	2	New
A. parvus	**1 or 1+5**	**2**	**1**	**2**	**3**	

Table 3. Antibiotic susceptibility
Susceptibilities of *A parvus* were difficult to obtain due to poor growth, but were in the range of 71-100 % for these antibio tics. AMP, ampicillin; SAM, ampicillin-sulbactam; CAZ, ceftazidime; PIP, piperacillin; TET, tetracycline; CHL, chloramphenicol; KAN, kanamycin; GEN, gentamicin; NAL, nalidixic acid; OFX, ofloxacin; SXT, trimethoprim-sulfamethoxazole.

Species	Percentages of fully susceptible strains										
	AMP	SAM	CAZ	PIP	TET	CHL	KAN	GEN	NAL	OFX	SXT
A. ursingii	26	97	53	77	95	24	92	95	92	100	97
A. schindleri	100	100	100	88	92	100	100	100	92	100	85

Table 4. Phenotypic characteristics useful for the differentiation of *A. ursingii*, *A. schindleri* and *A. parvus* from each other and from phenotypically similar species.
Data for *A. junii*, *A. johnsonii and A. lwoffii* from (2). +, Positive for 90-100% strains; -, positive for 0-10% strains; D, positive for 11-89% strains.

Characteristic	*A. ursingii*	*A. schindleri*	*A. parvus*	*A. junii*	*A. johnsonii*	*A. lwoffii*
Colony diameter in mm after 24 h at 30 °C (TSA)	>1	>1	0.3-0.6	>1	>1	>1
Inhibition zone diameter in mm with penicillin 10 U disk	<10	>13				
Growth at 41 °C (BHI)	-	+	-	D	-	-
Growth at 37 °C (BHI)	+	+	+	+	-	D
Utilization of						
Glutarate	+	+	-	-	-	-
L-Aspartate	D	-	-	-	D	-

Conclusions

- **➢ *A. ursingii*, *A. schindleri* and *A. parvus* are difficult to identify by phenotypic tests, but combination with ARDRA allows for their reliable identification.**
- **➢ The noted differences in susceptibility to antibiotics, especially to β-lactams, may be useful for provisional separation of *A. ursingii* and *A. schindleri*, while *A. parvus* can be recognized on the basis of colony morphology.**
- **➢ A substantial proportion of the *A. ursingii* and *A. parvus* isolates and some *A. schindleri* isolates were from blood, which indicates the clinical relevance of these species. Therefore,**
- **➢ knowledge of their identification in the clinical laboratory is required.**

Table 1. Biochemical properties
Growth on the carbon sources was evaluated after 2 and 6 days, the other tests after 2 days of incubation. +, Positive for all strains; -, negative for all strains; numbers, percentage of strains giving positive reactions.

Character	*A. ursingii* (*n*=38)	*A. schindleri* (*n*=26)	*A. parvus* (*n*=14)
Growth in BHI broth (Oxoid #) at:			
44°C	-	-	-
41°C	-	96*	-
37°C	97*	+	93*
Acid from D-glucose	-	-	-
Gelatinase	-	-	-
Hemolysis of sheep blood	-	-	-
Utilization of:			
DL-Lactate	+	+	-
DL-4-Aminobutyrate	-	-	-
trans -Aconitate	-	-	-
Citrate (Simmons)	97	54*	-
Glutarate	97	92	-
L-Aspartate	87†	-	-
Azelate	+	58	-
β-Alanine	-	-	-
L-Histidine	-	-	-
D-Malate	87†	73†	-
Malonate	-	-	-
Histamine	-	-	-
L-Phenylalanine	-	-	-
Phenylacetate	-	-	-
L-Ornithine	-	-	21
2,3-Butanediol	-	35	-
Etnanol	+	88	+
Acetate	+	+	+

BHI from other manufacturers may give slightly different results.
* Weak growth of some strains.
† Week growth of most strains.

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Correspondence. Lenie Dijkshoorn, Department of Infectious Diseases, Leiden University Medical Center C5-P, P.O. Box 9600, 2300 RC Leiden, the Netherlands . Tel. +3171 5262946; ldijkshoorn@lumc.nl.

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