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Species identification and molecular characterization of *Acinetobacter* spp. blood culture isolates from Norway

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Objectives: The study investigated the species distribution, antibiotic susceptibility patterns and genotypic resistance characteristics of 113 consecutive blood culture isolates of *Acinetobacter* species collected between 2005 and 2007 throughout Norway.

Methods: Species identification was performed by partial *rpoB* sequence analysis, and verified by 16S rDNA and *recA* sequence analyses. Susceptibility testing was performed by agar disc diffusion and Etest. Distribution of OXA carbapenemase genes and epidemic clonality of *Acinetobacter baumannii* isolates were detected by PCR assays. Analyses of $bla_{\text{OXA-51-like}}$ variants and quinolone resistance-determining regions (QRDRs) were done by sequencing.

Results: The most prevalent species in the collection were *Acinetobacter* genomic species (gen. sp.) 13TU (46.9%) and *Acinetobacter* gen. sp. 3 (19.5%), followed by *A. baumannii* (8.8%) and *Acinetobacter lwoffii*/*Acinetobacter* gen. sp. 9 (7.1%). Carbapenem resistance was observed in one $bla_{\text{OXA-23-like}}$-positive *A. baumannii* isolate. Quinolone resistance was detected in five isolates from the *Acinetobacter calcoaceticus–A. baumannii* complex, of which two had point mutations in the QRDRs, including one novel ParC mutation. None of the *A. baumannii* isolates belonged to European/international clones I, II or III. Six $bla_{\text{OXA-51-like}}$ variants, including two novel variants, were identified.

Conclusions: *Acinetobacter* gen. sp. 13TU and *Acinetobacter* gen. sp. 3 were predominant in Norwegian blood cultures, in contrast to in other countries where *A. baumannii* has dominated. The study demonstrated the importance of genotypic identification to determine the exact epidemiology of non-*baumannii Acinetobacter* species.

Keywords: *rpoB*, *Acinetobacter* genomic species 13TU, *Acinetobacter soli*, *parC*

Introduction

Acinetobacter are strictly aerobic Gram-negative coccobacilli that are widely distributed in soil and water, but also commonly found in the hospital environment.[1] Thirty-three genomic species (gen. sp.) of the *Acinetobacter* genus have so far been identified.[1–4] Of these, *Acinetobacter baumannii*, *Acinetobacter* gen. sp. 3 and *Acinetobacter* gen. sp. 13TU have been considered the clinically most relevant species.[1]

Our knowledge on the ecology and epidemiology of many *Acinetobacter* species is limited, mostly due to the lack of accurate methods for routine identification of *Acinetobacter* isolates to the species level.[5] While phenotypic identification of *Acinetobacter* species has been found insufficient,[6] several molecular methods have been shown to be adequate for this purpose.[1] Sequence analysis of the highly discriminative zone 1, between positions 2900 and 3250 of the *rpoB* gene, has been found to represent a reliable and rapid method for identification of *Acinetobacter* species.[7] This method has recently been validated on both a collection of *Acinetobacter* reference strains and a collection of *Acinetobacter* clinical isolates.[5] In addition, a modified *rpoB* zone 1, spanning 352 bp between positions 2916 and 3267, has been included

in the sequence-based delineation of species within the genus *Acinetobacter*.[3,8]

The occurrence of class D carbapenemase genes in *Acinetobacter* represents vertical inheritance in specific species, but horizontal acquisition in other species.[9] The genes $bla_{OXA-51-like}$, $bla_{OXA-23-like}$ and $bla_{OXA-134}$ are, for instance, intrinsically present in *A. baumannii*, *Acinetobacter radioresistens* and *Acinetobacter lwoffii*, respectively.[9,10]

The aim of this study was to investigate the species distribution of a national blood culture collection of *Acinetobacter* spp. along with molecular characterization of the isolates.

Materials and methods

Bacterial isolates

The study included 113 consecutive blood culture isolates of *Acinetobacter* species collected between 2005 and 2007 from 111 patients by 19 diagnostic microbiology laboratories throughout Norway. Four isolates recovered from two patients were included in the study, since they belonged to different species.

Identification of Acinetobacter species

Species identification of isolates was performed by partial *rpoB* gene sequence analysis (zone 1, 352 bp).[3,7] An *rpoB* sequence type (*rpoB* seqtype) was defined as a unique sequence of zone 1 (between positions 2916 and 3267) of the *rpoB* gene, in a comparable way to what has previously been described.[5] A cut-off of ≥94%–95% nucleotide similarity was used for partial *rpoB* gene sequence-based identification of *Acinetobacter* spp.[11] Sequence analysis of 1379 bp of the 16S rRNA gene was performed for *A. baumannii* and *A. radioresistens* isolates, and for all isolates with *rpoB* sequences showing <99% nucleotide identity to type/reference strains in GenBank (http://www.ncbi.nlm.nih.gov/Genbank).[12] Sequence analysis of 382 bp of the *recA* gene was performed for one isolate with a 16S rDNA sequence most related to more than one *Acinetobacter* species.[13] PCR products were purified by ExoSAP-IT (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) treatment, according to the manufacturer's instructions. Sequencing of PCR products was performed using BigDye 3.1 technology (Applied Biosystems, Foster City, CA, USA). Primers used for PCR amplifications and sequencing are listed in Table S1 (available as Supplementary data at *JAC* Online). Nucleotide sequence homology search was performed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were aligned and compared with published sequences of *Acinetobacter* type strains (Table S2, available as Supplementary data at *JAC* Online), using the Lasergene software package (DNASTAR, Madison, WI, USA). PFGE on selected *Acinetobacter* gen. sp. 13TU and *Acinetobacter* gen. sp. 3 isolates was performed as previously described.[14]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for meropenem, imipenem, gentamicin, amikacin, tobramycin, nalidixic acid, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole was performed by the agar disc diffusion method, as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/). Etest (bioMérieux, Solna, Sweden) was used to confirm reduced susceptibilities. Results were interpreted using clinical breakpoints as defined by EUCAST (http://www.eucast.org/). The disc diffusion breakpoints used for nalidixic acid were those recommended by EUCAST for Enterobacteriaceae.

PCR assays for molecular characterization

PCR assays and sequencing were performed to: (i) investigate the distribution of four groups of OXA carbapenemase genes ($bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$ and $bla_{OXA-58-like}$);[15] (ii) detect IS*Aba1* upstream of $bla_{OXA-51-like}$ and $bla_{OXA-23-like}$ genes;[16] (iii) detect mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes;[17,18] (iv) determine full-length sequences of $bla_{OXA-51-like}$ genes;[16,19] and (iv) determine the clonal lineage of *A. baumannii* isolates.[20]

GenBank accession numbers

The nucleotide sequences of $bla_{OXA-179}$ from *A. baumannii* isolate K50-13, $bla_{OXA-180}$ from *A. baumannii* isolate K50-71, zone 1 of the *rpoB* gene from *Acinetobacter soli* isolate K50-54 and the QRDR of *parC* from *A. baumannii* isolate K51-7 were deposited in the GenBank nucleotide database under accession numbers HM570035, HM570036, HM570037 and HM570038, respectively.

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics, reference number 2010/371-4.

Results

Acinetobacter species isolates

Isolates were obtained from patients in: internal medicine wards ($n=54$); intensive care units ($n=23$); surgery wards ($n=17$); cancer wards ($n=7$); paediatric/neonatal wards ($n=6$); burns wards ($n=2$); an ear, nose and throat ward ($n=1$); and an orthopaedic ward ($n=1$). The age distribution of the patients was between <1 year and 93 years, with a median age of 69. Altogether, 67% of the patients were males and 33% were females.

Identification of Acinetobacter species

Sequence analysis of zone 1 of the *rpoB* gene assigned isolates into 35 *rpoB* seqtypes (Figure 1 and Table 1). One hundred and five isolates (*rpoB* seqtypes 1–28) showed between 97.7% and 100% nucleotide identity to *Acinetobacter* type/reference strains in GenBank, and were identified as: *Acinetobacter* gen. sp. 13TU ($n=53$); *Acinetobacter* gen. sp. 3 ($n=22$); *A. baumannii* ($n=10$); *A. lwoffii*/*Acinetobacter* gen. sp. 9 ($n=8$); *Acinetobacter ursingii* ($n=3$); *A. radioresistens* ($n=3$); *Acinetobacter* gen. sp. 'close to 13TU' ($n=2$); *Acinetobacter calcoaceticus* ($n=1$); *Acinetobacter* gen. sp. 'between 1 and 3' ($n=1$); *Acinetobacter* gen. sp. 14BJ ($n=1$); and *Acinetobacter guillouiae* ($n=1$). 16S rDNA sequence analysis confirmed the *rpoB* identification for all *A. baumannii* and *A. radioresistens* isolates, and for 11 isolates of other species showing <99% *rpoB* nucleotide identity to *Acinetobacter* type/reference strains (Table 1). Isolates showed ≥99.6% 16S rDNA nucleotide identity to corresponding *Acinetobacter* type/reference strains in GenBank. PFGE typing of 43 isolates of *Acinetobacter* gen. sp. 13TU ($n=32$) and *Acinetobacter* gen. sp. 3 ($n=11$) assigned them into 32 distinct pulsotypes, with each pulsotype comprising ≤3 isolates (data not shown).

One isolate (*rpoB* seqtype 29) showed 98.9% nucleotide identity to the reference strain of *Acinetobacter* gen. sp. 'close to 13TU' (Table 1). However, according to the 16S rDNA sequence,

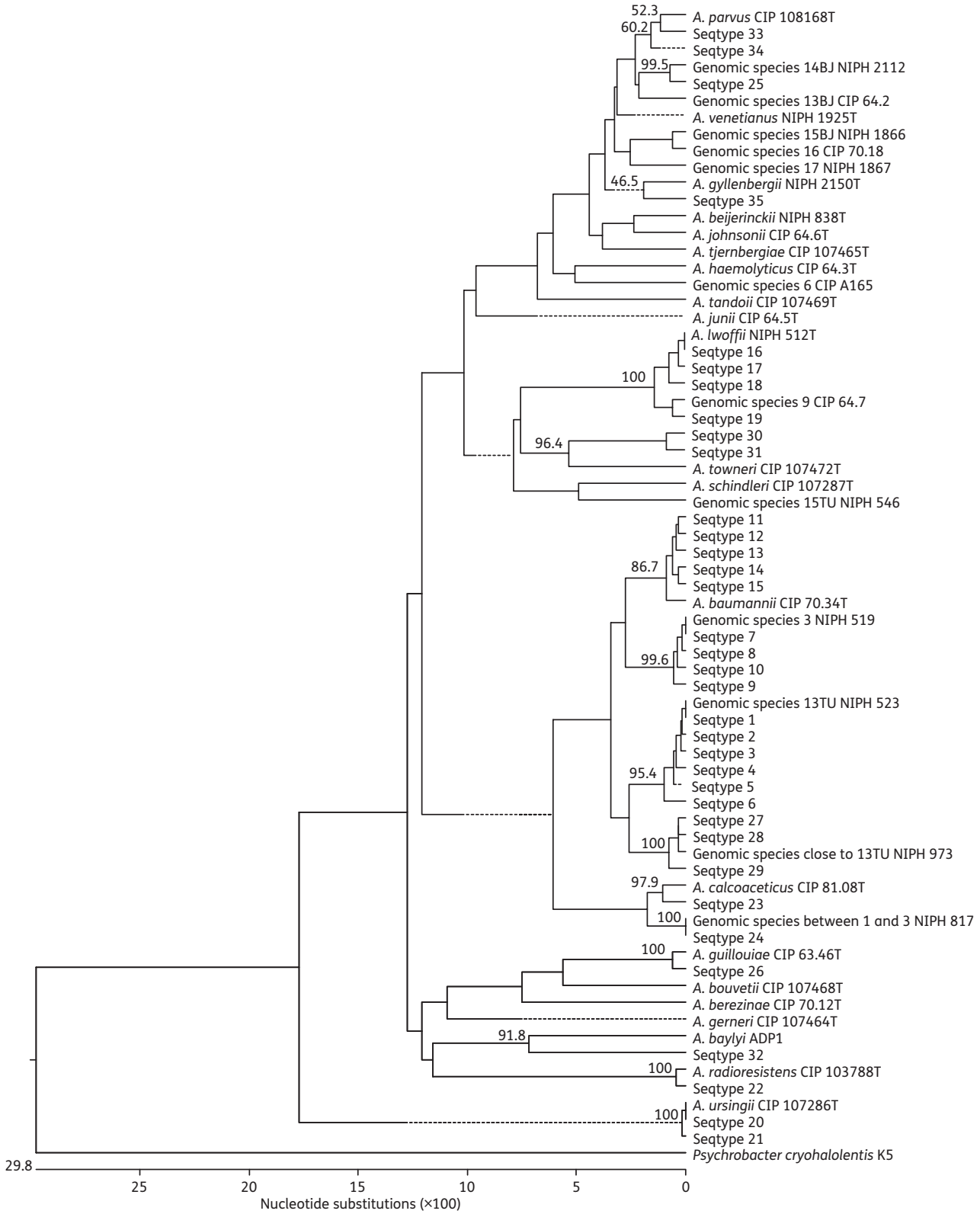

Figure 1. Dendrogram representing phylogenetic relationships between clinical seqtypes and type/reference strains of *Acinetobacter* species. The tree was derived from sequences of 352 bp of the *rpoB* gene between positions 2916 and 3267. The Lasergene MegAlign software (DNASTAR, Madison, WI, USA) was used to construct the tree using the Clustal W method. One thousand replicates were used for bootstrap analysis. The bootstrap support of branches representing our *rpoB* seqtypes is indicated by values at the nodes. A broken line on the tree indicatives a negative branch length.

Table 1. Results of partial *rpoB* gene and near-complete 16S rDNA sequence analyses

		rpoB identification		16S rDNA identification
rpoB seqtype	No. of isolates	first nearest species (nucleotide similarity with type/reference strains)	second nearest species (nucleotide similarity with type/reference strains)	first nearest species (nucleotide similarity with type/reference strains)
1	16	*Acinetobacter* gen. sp. 13TU (100%)	*Acinetobacter* gen. sp. 'close to 13TU' (96%)	ND
2	2	*Acinetobacter* gen. sp. 13TU (99.7%)	*Acinetobacter* gen. sp. 'close to 13TU' (95.7%)	ND
3	3	*Acinetobacter* gen. sp. 13TU (99.7%)	*Acinetobacter* gen. sp. 'close to 13TU' (95.7%)	ND
4	19	*Acinetobacter* gen. sp. 13TU (99.4%)	*Acinetobacter* gen. sp. 'close to 13TU' (95.5%)	ND
5	11	*Acinetobacter* gen. sp. 13TU (99.4%)	*Acinetobacter* gen. sp. 'close to 13TU' (96%)	ND
6	2	*Acinetobacter* gen. sp. 13TU (98.6%)	*Acinetobacter* gen. sp. 'close to 13TU' (95.2%)	*Acinetobacter* gen. sp. 13TU (99.9%)
7	13	*Acinetobacter* gen. sp. 3 (100%)	*A. baumannii* (94.9%)	ND
8	5	*Acinetobacter* gen. sp. 3 (99.7%)	*A. baumannii* (95.2%)	ND
9	1	*Acinetobacter* gen. sp. 3 (99.4%)	*A. baumannii* (94.3%)	ND
10	3	*Acinetobacter* gen. sp. 3 (99.1%)	*A. baumannii* (94.6%)	ND
11	2	*A. baumannii* (99.1%)	*Acinetobacter* gen. sp. 13TU (96%)	*A. baumannii* (100%)
12	1	*A. baumannii* (98.6%)	*Acinetobacter* gen. sp. 13TU (96%)	*A. baumannii* (99.9%)
13	2	*A. baumannii* (98.3%)	*Acinetobacter* gen. sp. 13TU (96.3%)	*A. baumannii* (≥99.9%)
14	4	*A. baumannii* (98.3%)	*Acinetobacter* gen. sp. 13TU (96.9%)	*A. baumannii* (≥99.9%)
15	1	*A. baumannii* (97.7%)	*Acinetobacter* gen. sp. 13TU (96.9%)	*A. baumannii* (100%)
16	1	*A. lwoffii*/*Acinetobacter* gen. sp. 9 (100%)	*Acinetobacter* gen. sp. 15TU (87.8%)	ND
17	1	*A. lwoffii*/*Acinetobacter* gen. sp. 9 (99.4%)	*Acinetobacter* gen. sp. 15TU (88.4%)	ND
18	4	*A. lwoffii*/*Acinetobacter* gen. sp. 9 (98.3%)	*Acinetobacter* gen. sp. 15TU (88.1%)	*A. lwoffii*/*Acinetobacter* gen. sp. (≥99.6%)
19	2	*A. lwoffii*/*Acinetobacter* gen. sp. 9 (98.9%)	*Acinetobacter* gen. sp. 15TU (87.5%)	*A. lwoffii*/*Acinetobacter* gen. sp. 9 (99.6%)
20	2	*A. ursingii* (100%)	*Acinetobacter venetianus*/*Acinetobacter gerneri* (85.5%)	ND
21	1	*A. ursingii* (99.7%)	*A. venetianus*/*A. gerneri* (85.2 %)	ND
22	3	*A. radioresistens* (99.1%)	*Acinetobacter* gen. sp. 15TU (84.1%)	*A. radioresistens* (≥99.6%)
23	1	*A. calcoaceticus* (98%)	*Acinetobacter* gen. sp. 'between 1 and 3' (96.3%)	*A. calcoaceticus* (99.9%)
24	1	*Acinetobacter* gen. sp. 'between 1 and 3' (100%)	*A. calcoaceticus* (96.9%)	ND
25	1	*Acinetobacter* gen. sp. 14BJ (98.6%)	*A. venetianus*/*Acinetobacter* gen. sp. 13BJ (96%)	*Acinetobacter* gen. sp. 14BJ (99.9%)
26	1	*A. guillouiae* (98.9%)	*Acinetobacter bouvetii* (89.8%)	*A. guillouiae* (99.6%)
27	1	*Acinetobacter* gen. sp. 'close to 13TU' (99.4%)	*Acinetobacter* gen. sp. 13TU (96%)	ND
28	1	*Acinetobacter* gen. sp. 'close to 13TU' (99.4%)	*Acinetobacter* gen. sp. 13TU (96%)	ND
29	1	*Acinetobacter* gen. sp. 'close to 13TU' (98.9%)	*Acinetobacter* gen. sp. 13TU (95.5%)	*Acinetobacter* gen. sp. 13TU/ *Acinetobacter* gen. sp. 'close to 13TU' (99.4%)
30	1	*A. towneri* (90.6%)	*A. venetianus* (88.9%)	*A. towneri* (97.6%)
31	1	*A. towneri* (89.8%)	*A. venetianus* (88.9%)	*A. towneri* (97.8%)
32	2	*A. baylyi* (87.2%)	*Acinetobacter tandoii* (86.6%)	*A. soli* (≥99.9%), *A. baylyi*[a] (98.6%)

Continued

Table 1. *Continued*

rpoB seqtype	No. of isolates	*rpoB* identification		16S rDNA identification
		first nearest species (nucleotide similarity with type/reference strains)	second nearest species (nucleotide similarity with type/reference strains)	first nearest species (nucleotide similarity with type/reference strains)
33	1	*Acinetobacter parvus* (97.7%)	*A. venetianus* (97.2%)	*Acinetobacter junii* (98.8%)
34	1	*A. parvus*/*A. venetianus* (97.4%)	*Acinetobacter* gen. sp. 13GJ (97.2%)	*Acinetobacter* gen. sp. 13BJ (98.9%)
35	1	*Acinetobacter gyllenbergii* (96.3%)	*A. venetianus* (96%)	*A. gyllenbergii* (99.1%)

ND, not determined.
[a]The second nearest species according to 16S rDNA sequence analysis.

the isolate was most related to *Acinetobacter* gen. sp. 13TU (99.4% nucleotide identity) and *Acinetobacter* gen. sp. 'close to 13TU' (99.4% nucleotide identity). Of note, the isolate showed 99.9% 16S rDNA nucleotide identity to *Acinetobacter* sp. RUH 1139, for which a nosocomial outbreak of infection has previously been reported.[21] Partial *recA* sequence analysis confirmed the *rpoB* identification, showing 100% nucleotide identity to the reference strain of *Acinetobacter* gen. sp. 'close to 13TU' compared with 93.2% nucleotide identity to the reference strain of *Acinetobacter* gen. sp. 13TU.

Four isolates (*rpoB* seqtypes 30–32) were most related to *Acinetobacter towneri* ($n=2$) and *Acinetobacter baylyi* ($n=2$). However, the isolates showed ≤90.6% *rpoB* nucleotide identities to the corresponding type strains in GenBank (Table 1). 16S rDNA sequence analysis confirmed the *rpoB* genotypic closeness of the four isolates to *A. towneri* ($n=2$) and *A. baylyi* ($n=2$) species, though with ≤99% 16S rDNA nucleotide identities to the corresponding type strains. Two isolates (*rpoB* seqtypes 30 and 31) were recognized in this article as 'close to *towneri*'. However, for the other two isolates (*rpoB* seqtype 32), 16S rDNA sequence analysis showed ≥99.9% nucleotide identity to the type strain of *A. soli* (GenBank accession number EU290155) and these isolates were accordingly assigned into this species.[2] No *rpoB* sequence for the type strain of *A. soli* was found in GenBank.

Three isolates (*rpoB* seqtypes 33–35) were most related to more than one species (Table 1) and were identified as 'unclassified'. The three isolates showed ≤97.7% *rpoB* and ≤99.1% 16S rDNA nucleotide identities to *Acinetobacter* spp. type strains in GenBank. One of the three isolates showed 99.9% 16S rDNA nucleotide identity to *Acinetobacter* sp. phenon 8 strain LUH 4713 (GenBank accession number AJ633634).

Antimicrobial susceptibility testing

Five out of the 113 (4.4%) isolates showed reduced susceptibility to one or more of the antimicrobial agents: *A. baumannii* ($n=2$); *Acinetobacter* gen. sp. 13TU ($n=2$); and *Acinetobacter* gen. sp. 3 ($n=1$). One *A. baumannii* isolate was resistant to all the investigated antimicrobial agents, whereas the other *A. baumannii* isolate was resistant to ciprofloxacin, nalidixic acid, trimethoprim/sulfamethoxazole and gentamicin, and intermediately susceptible to amikacin. One *Acinetobacter* gen. sp. 13TU isolate was resistant to all aminoglycosides and ciprofloxacin, whereas the other *Acinetobacter* gen. sp. 13TU isolate and the *Acinetobacter* gen. sp. 3 isolate were resistant only to nalidixic acid.

Molecular characterization

The gene $bla_{\text{OXA-51-like}}$ was detected in all 10 *A. baumannii* isolates, while it was absent in all other species. None of the isolates harboured an IS*Aba1* element upstream of the $bla_{\text{OXA-51-like}}$ genes. The gene $bla_{\text{OXA-23-like}}$ was present in all three *A. radioresistens* isolates and in the carbapenem-resistant *A. baumannii* isolate. An IS*Aba1* element was detected upstream of $bla_{\text{OXA-23-like}}$ only in the *A. baumannii* isolate and not the *A. radioresistens* isolates. The $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ genes were absent in all isolates.

Sequence analysis of the QRDRs of the five isolates showing resistance to fluoroquinolones and/or nalidixic acid identified double mutations (Ser-83→Leu in GyrA and Ser-80→Tyr in ParC) in one *A. baumannii* isolate and a single mutation (Ser-83→Leu in GyrA) in the *Acinetobacter* gen. sp. 3 isolate, whereas no mutations were detected in the other three isolates.

None of the *A. baumannii* isolates belonged to any of the three previously described European/international clones of *A. baumannii*.[20] Six different variants of $bla_{\text{OXA-51-like}}$ genes were detected: $bla_{\text{OXA-65}}$ ($n=5$); $bla_{\text{OXA-64}}$ ($n=1$); $bla_{\text{OXA-78}}$ ($n=1$); $bla_{\text{OXA-69}}$ ($n=1$); and the novel variants $bla_{\text{OXA-179}}$ ($n=1$) and $bla_{\text{OXA-180}}$ ($n=1$).

Discussion

Partial sequence analysis of the *rpoB* gene assigned *Acinetobacter* spp. isolates of the Norwegian blood culture collection into ≥13 different species (Table 1). Interestingly, the most prevalent species were *Acinetobacter* gen. sp. 13TU and *Acinetobacter* gen. sp. 3. The clinical significance of these two species has been indicated before, both in individual case reports and in epidemiological studies.[6,22–24] The isolates were collected by 19 diagnostic microbiology laboratories throughout Norway, indicating a low probability of large-scale cross-transmission among patients. Furthermore, PFGE indicated that it is unlikely that a major outbreak involving *Acinetobacter* gen. sp. 13TU or *Acinetobacter* gen. sp. 3 has taken place in Norway.

Although *A. baumannii* has been considered the most clinically relevant *Acinetobacter* species,[1] only 8.8% of the isolates in our collection belonged to this species. The finding that *Acinetobacter* species—other than *Acinetobacter* gen. sp. 13TU, *Acinetobacter* gen. sp. 3 and *A. baumannii*—together accounted

for 24.8% of the isolates (about three times more than *A. baumannii*) further demonstrated the limited contribution of *A. baumannii*. Two of our isolates were assigned by 16S rDNA sequencing into *A. soli* species. To our knowledge, this study is the first to report the clinical relevance of *A. soli*, since this species has formerly been isolated only from forest soil.[2]

A recently published study has described the identification of three isolates that did not cluster closely enough with any of the currently described *Acinetobacter* species.[25] According to *rpoB* sequencing, the closest currently described species for the three isolates was *A. towneri*.[25] Similarly, two of our isolates showed low *rpoB* and 16S rDNA nucleotide identities to any of the known species, and the two isolates were found to be most related to *A. towneri* species. All or part of the five isolates from the two studies may represent a new species genotypically close to *A. towneri*.

The capability of *rpoB* zone 1 sequencing for accurate species allocation of *Acinetobacter* clinical isolates was supported by bootstrap values ≥86.7% for 13 branches (comprising 32 *rpoB* seqtypes and 110 isolates) in the *rpoB* phylogenetic tree (Figure 1). Moreover, the parallel results of 16S rDNA (Table 1) and/or *recA* sequence analyses maintained the validity of partial *rpoB* sequencing for the identification of 25/25 (100%) isolates of the *A. calcoaceticus–A. baumannii* complex, *A. lwoffii*/*Acinetobacter* gen. sp. 9, *A. radioresistens*, *Acinetobacter* gen. sp. 14BJ and *A. guillouiae* species.

On the other hand, partial *rpoB* sequencing was not able to precisely assign three isolates (seqtypes 33–35). This could initially be anticipated by the low bootstrap values for their corresponding branches in the *rpoB* phylogenetic tree (Figure 1) and, furthermore, by the low nucleotide identities of their 16S rDNA sequences to type/reference strains in GenBank (Table 1). Further study is needed to determine precise species identification of these three isolates.

The $bla_{\text{OXA-51-like}}$ gene was present in all *A. baumannii* isolates, whereas $bla_{\text{OXA-23-like}}$ was present in all *A. radioresistens* isolates and in 1 out of 10 *A. baumannii* isolates. This maintained the proposals that $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ are intrinsic genes in *A. baumannii* and *A. radioresistens*, respectively, and that *A. radioresistens* represents the source for dissemination of $bla_{\text{OXA-23-like}}$ into other *Acinetobacter* species.[26,27]

The occurrence of specific amino acid substitutions in the QRDRs of DNA gyrase and topoisomerase IV has represented a major mechanism of bacterial resistance to quinolones.[28] A novel mutation, Ser-80→Tyr, in the QRDR of ParC was identified in one *A. baumannii* isolate with high levels of resistance to ciprofloxacin, levofloxacin and nalidixic acid. Further investigations are required to determine the exact role of the newly detected ParC mutation in conferring resistance to quinolones.

One *Acinetobacter* gen. sp. 3 isolate showing a high level of resistance to nalidixic acid (MIC 128 mg/L) but susceptibility to ciprofloxacin (MIC 1 mg/L) and levofloxacin (MIC 0.5 mg/L) contained only a single amino acid substitution, Ser-83→Leu, in the QRDR of GyrA. The occurrence of this mutation in *A. baumannii* isolates has generally resulted in ciprofloxacin MICs of ≥4 mg/L.[17,29,30] However, consistent with our finding, a study conducted by Wisplinghoff *et al.*[29] has reported the occurrence of this mutation in one *A. baumannii* isolate with a ciprofloxacin MIC of 1 mg/L. The MIC of nalidixic acid for that isolate was not reported.[29]

Based on partial sequences of *ompA*, *csuE* and $bla_{\text{OXA-51-like}}$ genes, the majority of epidemic strains of *A. baumannii* have been found to belong to three major European/international clones (I, II and III).[20] None of our *A. baumannii* isolates belonged to any of these three prevalent groups. The sporadic nature of our isolates was predictable, since most of them were susceptible to all antibiotics tested, whereas multidrug resistance has generally been a signal feature of *A. baumannii* European/international clones I, II and III.[31] In accordance with a recently published study,[32] the $bla_{\text{OXA-69}}$-positive isolate in our collection did not belong to European/international clone I, even though it yielded a positive $bla_{\text{OXA-51-like}}$ amplicon in the group 2 multiplex PCR. However, in contrast to the same study,[32] $bla_{\text{OXA-65}}$-positive isolates in our collection did not belong to European/international clone II, since they did not yield *csuE* amplicons in the group 1 multiplex PCR.

In conclusion, the present study revealed the predominance of *Acinetobacter* gen. sp. 13TU and *Acinetobacter* gen. sp. 3 in Norwegian blood cultures, indicating the significance of molecular identification of *Acinetobacter* species clinical isolates for precise determination of the epidemiology of non-*baumannii* species.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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