

# Endemic acinetobacter in intensive care units: Epidemiology and clinical impact

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## Abstract

**Aims**—To assess whether *Acinetobacter* isolates obtained over 20 months in a tertiary care hospital were epidemiologically related; to establish the clinical importance of the organisms; and to identify the isolates according to the recent taxonomy.

**Methods**—Fifty eight *Acinetobacter* isolates from 49 patients collected during 1984 and 1985 were investigated. Most isolates were from respiratory tract specimens from intensive care patients. The organisms were typed by cell envelope protein electrophoresis and by a quantitative carbon source growth assay; patients' charts were reviewed to differentiate between colonisation and infection; representative isolates were identified to species level by DNA-DNA hybridisation.

**Results**—Twelve protein profiles were distinguished in the isolates. Forty two isolates were of the same protein profile (profile I); other profiles were observed in a few or single isolates. Cluster analysis of carbon source growth divided profile I isolates into two groups—one of isolates from 1984 and one from 1985. They were identified as *A baumannii* and associated with infections in eight patients. Four other infections were caused by acinetobacters with other protein profiles (three of *A baumannii*; one of the unnamed DNA group 3).

**Conclusions**—Apart from sporadic strains, two strains of the same protein profile, but distinguishable by carbon source growth, were successively endemic. Cluster analysis was a valuable tool in the interpretation of typing and epidemiological data. The 12 (28%) infections of *Acinetobacter* in 43 patients in intensive care suggest that the presence of these organisms in wards of severely ill patients should be a cause of concern.

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*Acinetobacter* have been occasionally involved in serious infections in hospitals.<sup>1</sup> Many outbreaks of colonisation of patients in intensive care units with highly resistant *Acinetobacter* strains, however, have been reported.<sup>2-5</sup>

Several methods have been used for

epidemiological typing of acinetobacters, including biotyping,<sup>6</sup> antibiotic susceptibility and plasmid typing,<sup>5</sup> whole cell and cell envelope protein electrophoresis,<sup>7,8</sup> a quantitative carbon source growth assay<sup>9</sup> and phage typing.<sup>10</sup> Differences between strains can be small and the combined use of methods for epidemiological typing has been advocated.<sup>10</sup>

Protein electrophoresis<sup>8</sup> and carbon source growth<sup>9</sup> were used to investigate a large number of isolates from intensive care units to establish whether a single strain was predominant. The clinical impact of the outbreak was also analysed and representative isolates were identified by DNA-DNA hybridisation according to the recent classification.<sup>11</sup>

## Methods

*Acinetobacter* strains were isolated from clinical specimens from 43 patients in four intensive care units and six patients in other wards of a tertiary care hospital. The 58 isolates (37 respiratory; 12 urine; nine other specimens) were identified to the genus *Acinetobacter* by conventional methods<sup>12</sup> and were glucose acidifying.

Cell envelope protein sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described before<sup>8</sup> with minor modifications.<sup>13</sup>

The quantitative bacterial dot method described by Tjernberg *et al* was used.<sup>14</sup> The reference strains for the DNA groups were those of a previous DNA hybridisation study,<sup>15</sup> as were the procedures for isolation of reference DNA and labelling with<sup>125</sup>I.

The strains were differentiated according to their ability to grow on 10 different carbon sources in a standardised, quantitative micro-assay, which has already been described.<sup>9</sup>

Cluster analysis reveals the most striking pattern of variation. The mean city block distance<sup>16</sup> was used to calculate similarities between isolates in carbon source utilisation, and Ward's agglomerative method<sup>17</sup> was used for forming clusters. The groupings obtained were compared with groupings based on other features, including origin, moment of sampling, and protein profile.

Charts of patients from whom *Acinetobacter* were isolated during their stay in intensive care were inspected retrospectively for differentiation between colonisation and infection using criteria developed by the Centers for Disease Control (Atlanta).<sup>18</sup>

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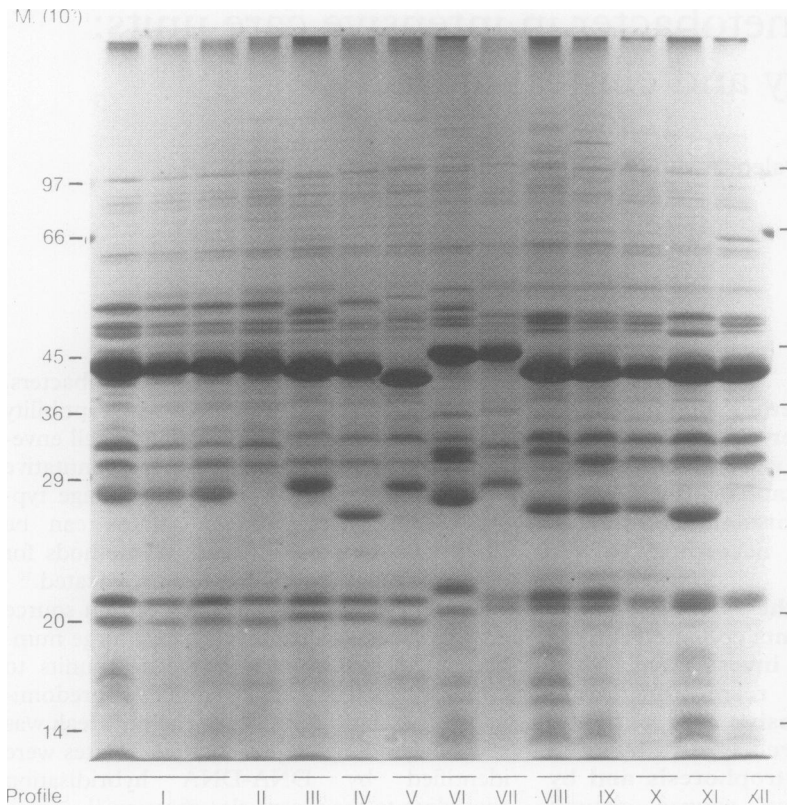


Figure 1 Cell envelope protein SDS-PAGE profiles of *Acinetobacter* isolates from different patients.  $M_r$ , relative apparent molecular weight.

### Results

Fifty isolates of *Acinetobacter* were from 41 patients in three of the four intensive care units and most were from bronchial secretions.

The protein profiles were characterised by 30–50 bands of different staining intensity in apparent molecular weight range ( $M_r$ ) 14–100 kilodaltons. Twelve different profiles were observed, coded I–XII (fig 1). Profiles I and II were indistinguishable, except for one band ( $M_r$  28 kilodaltons) missing in profile II. Profiles VIII–XII were very similar but not identical. The prevalence of the profiles and their distribution in the specimens is shown in the table. Forty two isolates belonged to protein profile I and most of these were observed in bronchial secretions. Other

profiles were seen in single or in a few strains.

Seven isolates allocated to protein profile I and one isolate of each of the other profiles were identified by DNA-DNA hybridisation. The isolates of profiles I–V tested belonged to DNA group 2 (*A baumannii*). The isolate of profile VI was identified as DNA group 1 (*A calcoaceticus*), those of profiles VII–XII as DNA group 3 (table). The results of the cluster analysis of the carbon source growth data are shown in fig 2. Three clusters were distinguished: 27 isolates (cluster I) of protein profile I were isolated in 1984 in the intensive care units and in other wards, 12 isolates (cluster II), also of protein profile group I, were recovered in 1985 from eight patients during their stay in intensive care; cluster III comprised isolates of a variety of protein profiles obtained in 1984 and in 1985 in different wards. These results indicate that two different protein profile I strains were successively endemic. This was corroborated by the finding that the 1985 isolates were more resistant to antibiotics (quinolones) than the 1984 isolates (derived from minimal inhibitory concentrations; data not shown).

In 31 of the 43 patients in intensive care the presence of the acinetobacters was interpreted as colonisation, while for the remaining 12 (28%) patients, the organisms were associated with infection: one isolate with bacteraemia (*A baumannii*, profile V), one isolate with meningitis (*A baumannii*, profile I), three with pneumonia (*A baumannii*, profiles I (n = 2) and III (n = 1), and seven with tracheobronchitis (*A baumannii*, profile I (n = 5), profile IV (n = 1), and the unnamed DNA group 3 (n = 1)).

### Discussion

Numerous reports of epidemic spread of *Acinetobacter* in hospital wards have appeared,<sup>2–5</sup> and in two outbreaks the concurrent spread of different strains has been notified.<sup>3,5</sup> Our results show that protein electrophoresis, although found to be useful in previous studies,<sup>4,9</sup> may not always be sufficient for the identification of *Acinetobacter* strains. Except for strains with rare protein profiles, combinations of typing methods may be needed, and results have to be judged in narrow association with epidemiological data. For this, cluster analysis could be a valuable tool.

At least 17 DNA hybridisation groups (genomic groups) can be distinguished within the genus *Acinetobacter*.<sup>11</sup> The groups 1 (*A calcoaceticus*), 2 (*A baumannii*), and the unnamed groups 3 and 13 are genetically and phenotypically closely related.<sup>15</sup> For groups 1–12, a phenotypic identification scheme has been described.<sup>6</sup> DNA-DNA hybridisation, however, is needed for unambiguous identification of acinetobacters to DNA group level.<sup>19</sup> The seven protein profile I isolates tested were all DNA group 2 (*A baumannii*). It was assumed that isolates of a common, indistinguishable protein profile belong to the same DNA group and, therefore, only one

Prevalence of cell envelope protein SDS-PAGE profiles in specimens and DNA-DNA hybridisation groups of *Acinetobacter* associated with profiles

Profile	Specimens					DNA group*
	Blood	Cerebrospinal fluid	Bronchial secretion	Urine	Other specimens†	
I		1	27	9	5	2 ( <i>A baumannii</i> )
II			3	1		2
III			1			2
IV			1			2
V	1					2
VI					1	1 ( <i>A calcoaceticus</i> )
VII			1			3
VIII				1		3
IX			2			3
X				1		3
XI					1	3
XII			2			3

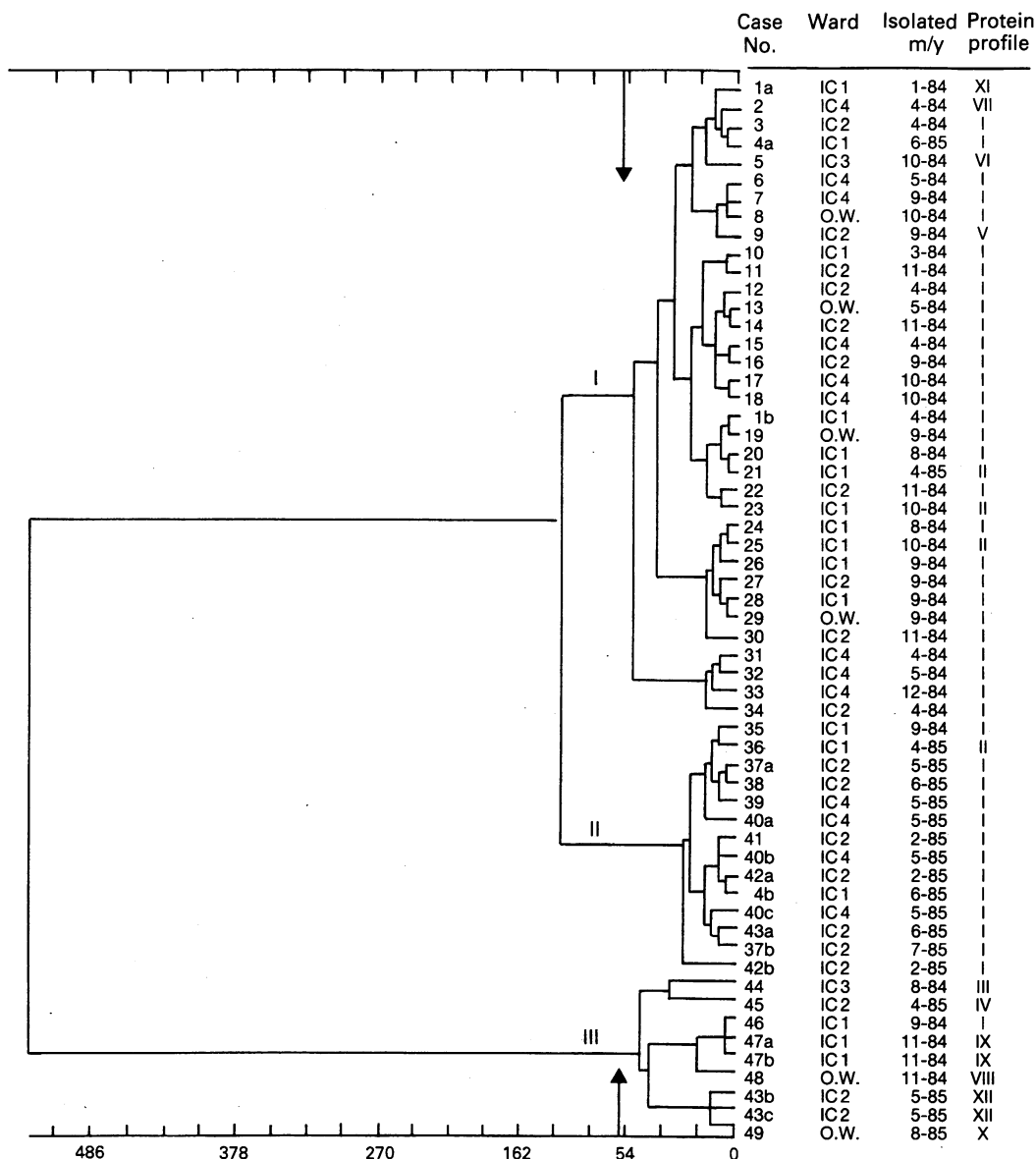
†Six from wounds, one insertion site drain.

\*Investigated for seven isolates of profile I and for one isolate of each other profile.

Figure 2 Dendrogram of cluster analysis of carbon source growth data.

Horizontal axis: dissimilarities at which strains are grouped, determined by Ward's criterion and based on mean city block distance. Arrows denote the cutting level for separating main clusters.

Vertical axis: isolates arranged according to their C-growth similarities. Patients are numbered 1-49 and suffixes a, b, and c indicate multiple Acinetobacter isolates from single patients. IC: intensive care units; OW, other wards; m/y, month and year of isolation.



isolate of the other profiles was investigated by DNA-DNA hybridisation. The organisms of profiles II-V were also identified as DNA group 2 (*A. baumannii*), the group most commonly associated with nosocomial infections.<sup>11</sup> With the exception of one isolate, isolates of the remaining profiles were identified as DNA group 3. This group was rare in France,<sup>6</sup> but was common in Sweden.<sup>15</sup>

Acinetobacters are, typically, opportunistic organisms of low virulence, but they may contribute, in mixed culture, to the virulence of other species.<sup>20</sup> Severe infections with these organisms are more common than usually appreciated, especially in very ill inpatients.<sup>1</sup> In the present study 12 of 43 (28%) patients in intensive care with *Acinetobacter* were actually clinically infected, but seven of these had tracheobronchitis, which is a minor infection related to endotracheal intubation. Eight of the 12 infections were associated with endemic multiresistant organisms of protein profile I of DNA group 2 (*A. baumannii*). The findings suggest that the presence of these organisms in wards should be a cause of concern.

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