Identification of Acinetobacter baumannii Strains with Monoclonal Antibodies against the O Antigens of Their Lipopolysaccharides

RALPH PANTOPHLET, LORE BRADE, AND HELMUT BRADE*

Division of Medical and Biochemical Microbiology, Research Center Borstel, Center for Medicine and Biosciences, Borstel, Germany

Received 6 November 1998/Returned for modification 19 January 1999/Accepted 24 February 1999

Despite the emergence of Acinetobacter baumannii strains as nosocomial pathogens, simple methods for their phenotypic identification are still unavailable. Murine monoclonal antibodies specific for the O-polysaccharide moiety of the lipopolysaccharide (LPS) of two A. baumannii strains were obtained after immunization with heat-killed bacteria. The monoclonal antibodies were characterized by enzyme immunoassay and by Western and dot blot analyses and were investigated for their potential use for the identification of A. baumannii strains. The antibodies reacted with 46 of the 80 A. baumannii clinical isolates that were investigated, and reactivity was observed with 11 of 14 strains which were isolated during outbreaks in different northwestern European cities; no reactivity was observed with Acinetobacter strains of other genomic species, including the closely related genomic species 1 (Acinetobacter calcoaceticus), 3, and 13 sensu Tjernberg and Ursing, or with other gram-negative bacterial strains. The results show that O-antigen-specific monoclonal antibodies such as the ones described are convenient reagents which can be used to identify Acinetobacter strains in clinical and research laboratories.

The genus Acinetobacter belongs to the family Moraxellaceae (36), and its members are ubiquitous in the natural and clinical environments (1, 20, 38, 39, 44, 47). However, Acinetobacter strains have also been recognized as opportunistic nosocomial pathogens in recent years (2, 3). Urethritis, pneumonia, meningitis, and septicemia are the major diseases caused by these bacteria (3, 43), which, in addition, are usually highly resistant to a large spectrum of antibiotics (43). Acinetobacter baumannii (genomic species 2) is the most frequently occurring Acinetobacter sp. among clinical Acinetobacter isolates, and most hospital outbreaks are attributed to this species (3). Although other Acinetobacter species such as genomic species 3, A. johnsonii (genomic species 7), A. wodlii (genomic species 8), and A. radioresistens (genomic species 12) may also be found among clinical Acinetobacter isolates, these species are usually considered to represent contamination or colonization rather than infection when they are isolated from clinical specimens (38, 40), particularly since these species are also present on the skin and mucous membranes of humans as part of their normal bacterial flora (39). Unfortunately, there is still lack of simple methods for the rapid identification of Acinetobacter strains in clinical laboratories (3, 6, 43). This is partly due to the confused taxonomic status associated with the genus (6, 11, 48) and also to the diversity of the strains, which is reflected in the different pheno- and genotypic groups that have been described (3, 6). To date, DNA-DNA hybridization studies have resulted in the delineation of 20 DNA homology groups, of which only 7 have received a formal species name (4, 5, 12, 42). Many strains described so far have remained unclassified (4, 5, 42).

Gram-negative bacteria express lipopolysaccharides (LPSs) at their outer surface (24, 25, 32). These LPSs consist of a polysaccharide covalently linked to a lipid component, termed lipid A, which anchors the LPS in the outer membrane (18, 31–33, 35). In enterobacteria, the polysaccharide is divided into the core oligosaccharide (linked to lipid A) and the O polysaccharide or O antigen (32, 33, 35). This type of LPS is referred to as the smooth- or S-form phenotype (33–35); the O antigens are characteristic for a given LPS and the parental bacterial strain, a fact on which serotyping schemes for various enteric and also nonenteric gram-negative bacteria are based (21, 27, 30, 32, 34, 35). Since all recently investigated LPSs from Acinetobacter strains have been shown to be of the smooth phenotype (13–17, 45, 46), a serotyping scheme for identification of members of this genus may also be possible. Recently, we reported on the specificity of hyperimmune rabbit sera against Acinetobacter LPS to examine the feasibility of such an identification scheme for Acinetobacter strains (29). Although they were shown to be useful (29), such antisera have certain disadvantages which make them unsuitable for routine applications, such as the presence of core-reactive antibodies as well as protein and possible capsular antibodies, which may lead to false-positive reactions when unabsorbed sera are used for O serotyping (23, 29, 37). Thus, to overcome this problem, we decided to generate monoclonal antibodies (MAbs) against the O antigens of various clinical and environmental Acinetobacter isolates.

In this report, we describe the generation and characterization of two MAbs specific for the O antigen of A. baumannii LPS and show that they can be used for the identification of A. baumannii strains, particularly for the tracing in hospital wards of strains originating from two epidemiologically important A. baumannii clonal groups.

MATERIALS AND METHODS

Bacteria. The A. baumannii strains (n = 82) investigated in this study are listed in Table 1. They consisted mostly of clinical isolates from different parts of...
| Strain | Source | City (country) | Position | Reactivity with MAb | Pattern obtained after acid hydrolysis |
|--------|--------|---------------|----------|--------------------|---------------------------------------|
|        |        |               |          | S48-3-13 | S48-3-17 |                                  |
| ATCC 17904 | Urine | Not known | E4 | – | – | – |
| ATCC 19606T | Urine | Not known | E3 | – | – | – |
| NCTC 7844 | Sputum | Malmö (SE) | G7 | – | – | – |
| 10° Wound | Rotterdam (NL) | G5 | – | + | ND |
| 11° Wound | Rotterdam (NL) | G2 | – | + | ND |
| 12° Toe web | Rotterdam (NL) | E6 | – | + | ND |
| 13° (I) Bronchus | Nijmegen (NL) | F3 | + | – | ND |
| 14° Not known | Not known | F1 | – | – | – |
| 16° Urine | Rotterdam (NL) | E12 | – | + | ND |
| 17° Wound | Rotterdam (NL) | G1 | – | + | ND |
| 18° Urine | Rotterdam (NL) | G3 | – | + | ND |
| 19° Pus | Leiden (NL) | G6 | – | – | A |
| 20° Sputum | Rotterdam (NL) | F7 | – | + | ND |
| 21° Wound | Malmö (SE) | E5 | – | – | – |
| 22° Urine | Dordrecht (NL) | E9 | + | – | ND |
| 23° Drain | Rotterdam (NL) | F6 | – | + | ND |
| 24° (I) Urine | Rotterdam (NL) | N1 | + | – | ND |
| 26° Wound | Nijmegen (NL) | E11 | + | – | ND |
| 27° Sputum | Dordrecht (NL) | F8 | + | – | ND |
| 28° Urine | Dordrecht (NL) | E10 | + | – | ND |
| 29° (I) Sputum | Utrecht (NL) | F2 | + | – | ND |
| 30° Urine | Rotterdam (NL) | F4 | – | – | B |
| 31° (II) Urine | Rotterdam (NL) | F11 | – | + | ND |
| 32° Blood | Dordrecht (NL) | F9 | + | – | ND |
| 33° (I) Urine | Dordrecht (NL) | C12 | + | – | ND |
| 34° (I) Urine | Rotterdam (NL) | N1 | – | + | ND |
| 36° Sputum | Rotterdam (NL) | D12 | – | – | D |
| 37° Blood | Rotterdam (NL) | E2 | – | + | ND |
| 38° Sputum | Rotterdam (NL) | F10 | – | + | ND |
| 39° Sputum | Rotterdam (NL) | F12 | – | + | ND |
| 40° Sputum | Rotterdam (NL) | G4 | – | + | ND |
| 41° Catheter tip | Utrecht (NL) | E1 | + | – | ND |
| 42° Ear | Leiden (NL) | F5 | – | – | A |
| RUH 508h | Bronchus | Nijmegen (NL) | D9 | + | – | ND |
| RUH 513h | Bronchus | Nijmegen (NL) | D10 | + | – | ND |
| RUH 733h | Bronchus | Nijmegen (NL) | D11 | + | – | ND |
| RUH 735h | Bronchus | Nijmegen (NL) | D8 | – | – | – |
| RUH 937h | Urine | Nijmegen (NL) | D4 | + | – | ND |
| RUH 975h | Pus | Nijmegen (NL) | D5 | + | – | ND |
| RUH 1027h | Urine | Nijmegen (NL) | D6 | + | – | ND |
| RUH 1202h | Bronchus | Nijmegen (NL) | D7 | + | – | ND |
| RUH 1205h | Bronchus | Nijmegen (NL) | D3 | – | – | E |
| RUH 1277h | Bronchus | Nijmegen (NL) | D2 | – | – | ND |
| RUH 3204h | Tube | Nijmegen (NL) | D1 | – | – | F |
| RUH 1093h | Sputum | Rotterdam (NL) | C6 | – | – | D |
| RUH 1486h | Navel | Rotterdam (NL) | C7 | – | – | G |
| RUH 1752h (ug) | Bronchus | Enschede (NL) | C8 | – | – | D |
| RUH 1907h | Bronchus | Rotterdam (NL) | C9 | – | – | J |
| RUH 2180h | Sputum | Nijmegen (NL) | C10 | – | – | H |
| RUH 2688h | Pharynx | Rotterdam (NL) | C11 | – | – | ND |
| GNU 1078h (I) | Rectal mucosa | Leuven (BE) | B10 | + | – | ND |
| GNU 1079h (I) | Tracheal site | Salford (UK) | B12 | + | – | ND |
| GNU 1080h | Catheter urine | Salisbury (UK) | C1 | – | | |
| GNU 1081h (ug) | Tracheal aspirate | Cork (IE) | C2 | – | – | – |
| GNU 1083h (I) | Urine | London (UK) | C3 | + | – | ND |
| GNU 1084h (I) | Burn wound | Sheffield (UK) | C4 | + | – | ND |
| GNU 1086h (II) | Respiratory tract | Newcastle (UK) | C5 | – | – | B |
| MB 142 | Skin | London (UK) | B7 | – | – | – |
| SH 9/MB 264h | Skin | London (UK) | B8 | – | – | – |
| SH 26/MB 288h | Nail fold | London (UK) | B9 | – | – | J |
| 189h | Crural ulcer | Odense (DK) | B6 | – | + | ND |
| 9771h | Urine | Næstved (DK) | B11 | – | – | G |

Continued on following page
TABLE 1—Continued

| Strain* | Sourceb | City (country)c | Positiond | Reactivity with MAAb | Pattern obtained after acid hydrolysisd |
|---------|----------|----------------|-----------|---------------------|---------------------------------------|
| 10074a  | Urine    | Vejle (DK)     | A1        | –                   | –                                     |
| 10086a  | Urine    | Vejle (DK)     | A2        | –                   | I                                     |
| 2032    | Sputum   | Venlo (NL)     | A3        | +                   | ND                                    |
| 2033    | Sputum   | Venlo (NL)     | A4        | +                   | ND                                    |
| 2034    | Urine    | Venlo (NL)     | A5        | +                   | ND                                    |
| 2036    | Pus      | Venlo (NL)     | A6        | –                   | ND                                    |
| 2037(1) | Sputum   | Venlo (NL)     | A7        | +                   | ND                                    |
| 3242(1)| Burn wound | Basildon (UK) | A8        | +                   | ND                                    |
| 3344    | Burn wound | Basildon (UK) | A9        | +                   | ND                                    |
| 3347    | Burn wound | Basildon (UK) | A10       | +                   | ND                                    |
| 3348    | Burn wound | Basildon (UK) | A11       | +                   | ND                                    |
| 3349    | Burn wound | Basildon (UK) | A12       | +                   | ND                                    |
| 3370    | Respiratory tract | Newcastle (UK) | B1        | –                   | –                                     |
| 3371    | Respiratory tract | Newcastle (UK) | B2        | –                   | B                                     |
| 3372    | Respiratory tract | Newcastle (UK) | B3        | –                   | B                                     |
| 3373    | Respiratory tract | Newcastle (UK) | B4        | –                   | B                                     |
| 3374    | Respiratory tract | Newcastle (UK) | B5        | –                   | B                                     |
| 133m    | Wound    | Malmo (SE)     | E7        | –                   | –                                     |
| 147m    | Wound    | Malmo (SE)     | E8        | –                   | –                                     |

* ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, United Kingdom; T, type strain; (I) and (II), outbreak strains belonging to clonal groups I and II, respectively; (7); (ag), outbreak strains which could not be allocated to a particular clonal group (7); •, strains used to prepare monoclonal antibodies.

b Source or specimen from which the strain was originally isolated.

c City and country where the strain was originally isolated. SE, Sweden; NL, The Netherlands; BE, Belgium; UK, United Kingdom; IE, Ireland; DK, Denmark.

d Position of the bacterial strains in dot blots (see also Fig. 4); NI, not included (homologous antigen).

e Position of the bacterial strains in dot blots (see also Fig. 4); ND, not determined.

Scandinavia, the Netherlands, and the United Kingdom. Acinetobacter strains belonging to other genomic species were also examined (genomic species 1 [A. calcoaceticus; n = 5], genomic species 3 [A. haemolyticus; n = 7], genomic species 5 [A. junii; n = 5], genomic species 6 [n = 1], genomic species 7 [A. johnsonii; n = 5], genomic species 8/9, which is considered a single entity [42] [A. hovorii; n = 13], genomic species 10 [n = 3], genomic species 11 [n = 6], genomic species 12 [A. radioresistens; n = 7], genomic species 13 sensu Tjernberg and Ursing [42] [n = 11], and genomic species 14 [n = 4]. All Acinetobacter strains had previously been identified to the species level by DNA-DNA hybridization and other methods and were from the culture collection of L. Dijkshoorn (Leiden University Medical Center, Leiden, The Netherlands). The strains were originally obtained from A. Horrevorts (Canisius Wilhelmina Ziekenhuis, Nijmegen, The Netherlands), P. Gerner-Smidt (Statens Seruminstitut, Copenhagen, Denmark), T. L. Pitt (Central Public Health Laboratory, London, United Kingdom), J. Tjernberg and J. Ursing (Malmo University Hospital, Malmo, Sweden), and P. Janssen (University of Ghent, Ghent, Belgium). The non-Acinetobacter strains investigated in this study were obtained from R. Podschn (National Reference Center of Klebsiella species, Kiel, Germany) or from our own culture collection (Salmonella spp. [n = 10], Escherichia coli [n = 4], Shigella sonnet [n = 8], Enterobacter spp. [n = 10], Pseudomonas spp. [n = 6], Stenotrophomonas maltophilia [n = 6], Serratia spp. [n = 10], Burkholderia cepacia [n = 2], Hafnia spp. [n = 10], and Proteus spp. [n = 20]).

Bacterial LPSs, whole-cell lysates, and proteinase K digestion. The Acinetobacter strains against which MAbs were prepared were grown in a fermentor (10 liters), and the cells were killed with phenol and centrifuged. LPS was extracted from the sedimented cells by the hot phenol-water method (49) and was lyophilized. Preparation of whole-cell lysates (undiluted or diluted 1:4 in sample buffer [45]) and proteinase K digestion were performed as described previously (29).

MAbs. MAbs were prepared by conventional protocols after immunization of mice with heat-killed bacteria. A. baumannii 24 and 34, against which rabbit immune sera have been produced in a previous study (29), were selected as immunogens. BALB/c mice (four mice per antigen) were injected intravenously on days 0, 7, 14, and 21 with 20, 20, 60, and 120 μg of antigen, respectively. Animal sera were screened on day 28 for antibodies against the respective immunogens by a dot blot assay with purified LPS as the antigen (see below). The animal whose serum had exhibited the strongest reactivity was given a booster intravenous injection on day 125 and booster intraperitoneal injections on days 126 and 127, with 200 μg of antigen administered in each injection. Two days after the last injection, the animals were exsanguinated and the spleens were removed. Spleen cells were prepared and fused at a ratio of 1:1 with mouse myeloma X63Ag8 cells by using polyethylene glycol 1500 (Boehringer Mannheim) according to conventional protocols. Primary hybridomas were screened by dot blot and enzyme immunoassay (EIA) with isolated LPS as the antigen. Relevant hybridomas were cloned three times by limited dilution, isolated with a commercially available isotyping kit (Bio-Rad), and purified by affinity chromatography on Protein G (Pharmacia). The antibodies were checked for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent Coomassie staining. The purified MAbs were stored at −20°C until further use.

Serological assays. EIA and Western blotting were performed as described previously (29) with LPS and proteinase K-digested whole-cell lysates, respectively, as antigens. For Western blotting, 1:4-diluted bacterial lysates were treated with proteinase K, separated by SDS-PAGE with a 10% separating gel, electrotransferred overnight onto a polyvinylidine difluoride (PVDF) membrane, and immunostained as described previously (29, 45). For dot blotting, proteinase K-digested lysates were diluted 1:3 in distilled water, of which 1 μl was dotted onto nitrocellulose. For the screening of animal sera and primary hybridoma supernatants, 0.5 μg of purified LPS was applied to the nitrocellulose membrane. After drying (37°C, 2 h), the membranes were blocked in blot buffer (45) supplemented with 10% nonfat dry milk and were immunostained as described above for the Western blots (29, 45).

Acid hydrolysis of membrane-bound LPS. Membrane-bound LPS was hydrolyzed under acidic conditions as described in a previous study (28). Briefly, lysates (undiluted) were digested with proteinase K, separated by SDS-PAGE with a 10% separating gel, and transferred onto PVDF membranes. The membranes were then incubated at 100°C for 1 h in heat-resistant glass containers containing 0.1 M HCl. After extensive washing in blot buffer (at least six times), they were blocked in blot buffer supplemented with 10% nonfat dry milk and were immunostained with lipid A-specific MAbs as described previously (28). Parallel gels were stained with alkaline silver nitrate as reported elsewhere (45).
RESULTS

Immunization of mice and preparation of MAbs. BALB/c mice were successfully immunized with heat-killed bacteria from *A. baumannii* 24 or 34. The primary hybridomas (*n* = 864) were tested for antibody reactivity by dot blotting and EIA with purified LPS as the antigen. Eleven hybridomas reacted with strain 34, whereas only 1 was observed to react with strain 24. None of the hybridomas reacted with both strains. Among the 11 hybridomas which were found to react with strain 34, the 1 with the highest reactivity was selected for further studies. The antibodies were cloned by limiting dilution (three times), isotypized, and subsequently purified by chromatography on Protein G; purity was ascertained by Coomassie staining following SDS-PAGE (data not shown). MAb S48-3-13 against strain 24 was of the immunoglobulin G3 (IgG3) isotype, and MAb S48-3-17 against strain 34 was of the IgG1 isotype. The results described below were obtained with affinity-purified MAbs.

Specificities of MAbs. The antibodies were tested by EIA with LPS (5 µg/ml; 50 µl/well) as the antigen. MAbs S48-3-13 and S48-3-17 reacted (optical density at 405 nm, >0.2) at concentrations of 5 and 40 ng/ml, respectively, with the homologous antigen (strains 24 and 34, respectively). No heterologous reactivity (concentration of antibody yielding an optical density at 405 nm of >0.2, >5,000 ng/ml) was observed. Next, checkerboard titrations were performed with antigen concentrations of between 32 and 4,000 ng/ml (1.6 to 200 ng of antigen per well) and antibody concentrations of between 0.5 and 1,000 ng/ml. The binding curves showed that both antibodies bind to the homologous LPS over a broad range of antigen concentrations (Fig. 1). Proteinase K-treated bacterial lysates or LPSs from *A. baumannii* 24 and 34 were separated by SDS-PAGE, blotted onto PVDF membranes, and immunostained with the homologous or heterologous antibody. A banding pattern characteristic of that of an O-polysaccharide chain could be observed for both strains (Fig. 2 and 3, lanes 1). Identical patterns were observed when LPS was used. No heterologous reactivity was observed, and no reaction with the core lipid A region was observed when the LPSs were separated on a 15% gel (data not shown). To show that both antibodies were indeed directed against the LPS and not another polysaccharide, the following experiment was performed (28); proteinase K-treated whole-cell lysates from both strains were separated by
SDS-PAGE, transferred onto a PVDF membrane, and subjected to hydrolysis in 0.1 M HCl. The free 4'-monophosphoryl lipid A (41), which remained membrane bound, could be detected in situ by MAb S1, which recognizes this partial structure (22). For both strains, the pattern was indistinguishable from that observed when the LPS was stained with the homologous antibody, thus indicating that the antibodies were indeed directed against the O polysaccharide (data not shown).

The MAbs were subsequently tested by dot blotting with proteinase K-treated lysates from 80 *A. baumannii* clinical isolates. They were found to react with 46 strains (Fig. 4; Table 1); none of the strains reacted with both antibodies. The specificities of the reactions could be confirmed by Western blotting (Fig. 2 and 3, lanes 2 to 6). For both antibodies, the ladder patterns obtained were indistinguishable from those observed after immunostaining of the respective homologous LPS. No reactivity was observed when the two antibodies were tested with the *Acinetobacter* strains of other genomic species or with the non-*Acinetobacter* strains (data not shown).

**DISCUSSION**

LPSs are amphiphilic molecules imbedded in the outer membranes of gram-negative bacteria (24, 25, 32). Serological and chemical analyses of the LPSs from several *Acinetobacter* strains have shown they are of the smooth phenotype (13–17, 45, 46). Since S-LPS has been used as a basis for serotyping schemes for various bacterial species in the past (27, 32, 34, 35), such LPS antibodies could also be expected to be of value for the differentiation of *Acinetobacter* strains. This technique could then also be implementable in clinical microbiology laboratories, which lack simple methods for the phenotypic identification of *Acinetobacter* strains (3, 6, 43). By using rabbit antisera, this hypothesis was proven to be correct (29). However, although they were shown to be highly specific, the antisera have the disadvantage that they contain core-reactive and non-LPS antibodies, e.g., capsular and protein antibodies, which would lead to false-positive results when the sera are used for identification purposes (29). MAbs, however, which react only with the O antigen of the LPS can be generated, and this specific reactivity thus makes them more suitable for such a scheme. Moreover, the latter approach offers the advantage that virtually unlimited amounts of antibodies of homogeneous specificity can be made available.

Since numerous studies have now confirmed that *A. baumannii* is the most prevalent species associated with outbreaks (3, 43), MAbs were generated against the LPSs of two *A. baumannii* strains and were investigated for their potential use for the identification of strains belonging to this species. Two antibodies, S48-3-13 (IgG3) and S48-3-17 (IgG1), were se-
clonal group II. This hypothesis is supported by the ladder pattern which was observed following acid hydrolysis of the LPS (see below), which differed from that observed for the LPS of strain 34 when the LPS was immunostained with MAb S48-3-17.

Strains of clones I and II have been proposed to have virulence factors related to invasiveness, transmissibility, or an enhanced ability to colonize immunocompromised patients and therefore should be a cause of concern in hospitals (7). Thus, since the two MAbs reacted with strains having characteristics of clones I and II, respectively, they provide an easy way of tracing such strains in the hospital environment (7), e.g., by means of a simple (latex) agglutination test. The other two isolates, strains GNU 1081 and RUH 1752, could not be grouped in the previously mentioned study (7) and, as postulated (7), may represent additional clones or groups within this species.

Since no staining with alkaline silver nitrate was observed for those strains which had failed to react with MAb S48-3-13 or MAb S48-3-17, we used a method in which lipid A, which is membrane bound following acid hydrolysis of LPS which has been blotted onto a PVDF membrane, is detected with specific MAbs, thus allowing LPS phenotype determination. Ten additional banding patterns were identified. None of the patterns were identical, and they also differed from the patterns observed for the LPSs from strains 24 and 34 when the LPSs were immunostained with the homologous antibody; thus, they may represent additional serotypes within this species. Some strains did not show a banding pattern, which may be due to a reduced level of O-antigen expression or the natural production of LPS which is of the rough phenotype. The lack of staining with alkaline silver nitrate has also been observed with other Acinetobacter strains, and possible reasons for this phenomenon have extensively been discussed elsewhere (13, 14, 17, 45, 46).

The possibility of the use of a scheme for the identification of Acinetobacter strains based on their O antigens is clearly demonstrated in this report. The two antibodies described herein are highly specific for the O antigen of the LPS from a large number of A. baumannii strains; no reactivity was observed with Acinetobacter strains of other genomic groups, including the pheno- and genotypically closely related genomic species 1 (A. calcoaceticus), 3, and 13 sensu Tjernberg and Uprising (42), or with strains of other gram-negative bacterial genera and species, such as Salmonella, E. coli, Serratia, Pseudomonas, S. maltophilia, or B. cepacia (data not shown). The LPSs of the A. baumannii strains which did not react with either of the two antibodies as well as the LPSs of strains belonging to genomic species which are not clinically relevant will be characterized in our laboratory, and MAbs against the O antigen will be generated in the future to fill the present gaps.

ACKNOWLEDGMENTS

We gratefully thank L. Dijkshoorn (Leiden University Medical Center, Leiden, The Netherlands) and R. Podschun (National Reference Center of Klebsiella species, Kiel, Germany) for providing the strains investigated in this study and V. Susott, D. Brötzmann, S. Cohrs, and S. Ruttkowski for excellent technical assistance. C. P. A. van Boven and L. Dijkshoorn are also thanked for their suggestions and critical review of the manuscript.

REFERENCES

1. Baumann, P. 1968. Isolation of Acinetobacter from soil and water. J. Bacteriol. 96:39–42.
2. Bergogne-Berezin, E. 1995. The increasing significance of outbreaks of Acinetobacter spp.: the need for control and new agents. J. Hosp. Infect. 30:441–452.
