JOURNAL OF CLINICAL MICROBIOLOGY, May 1989, p. 1086–1089
0095-1137/89/051086-04$02.00/0

Vol. 27, No. 5

Laboratory Investigation of Hospital Outbreak Caused by Two Different Multiresistant *Acinetobacter calcoaceticus* subsp. *anitratus* Strains

J. VILA,* M. ALMELA, AND M. T. JIMENEZ DE ANTA

Servei de Microbiologia i Parasitologia, Hospital Clinic i Provincial de Barcelona, and Facultat de Medicina, Universitat de Barcelona, Villarroel, 170, 08036 Barcelona, Spain

Received 28 November 1988/Accepted 9 February 1989

During a 7-month period, from December 1986 to June 1987, multiresistant strains of *Acinetobacter calcoaceticus* subsp. *anitratus* were isolated from 25 patients in a respiratory intensive care unit. The biochemical characteristics defined two groups of strains, group 1 (14 strains) and group 2 (11 strains). Both groups had the same biochemical characteristics, but group 2 strains could assimilate adipate and phenyl acetate. Moreover, of 16 antibiotics tested only netilmicin and imipenem had some inhibitory activity for group 1 strains; group 2 strains were susceptible to mezlocillin, piperacillin, and ticarcillin. Plasmid profiles of the groups were also different. The results of a laboratory investigation (biochemical characteristics, antibiotic susceptibility, and plasmid isolation) identified two different *A. calcoaceticus* subsp. *anitratus* strains as the causes of the outbreak.

During the past decade, several reports of outbreaks of *Acinetobacter calcoaceticus* subsp. *anitratus* in hospitalized patients, often in association with contamination of hospital equipment or the hands of personnel, have been described (9, 17, 28).

A. calcoaceticus subsp. *anitratus* is a nonfermentative aerobic gram-negative rod that is widely dispersed in nature, and it is commonly part of the normal flora of humans (2, 29). *A. calcoaceticus* subsp. *anitratus* was previously called *Bacterium anitratum*, *Achromobacter anitratum*, *Herella vaginicola*, and other names (19).

The most common site of infection with *A. calcoaceticus* subsp. *anitratus* is the lower respiratory tract; the infection occurs most frequently in patients with tracheostomies or endotracheal tubes (8).

During a 7-month period, 25 patients admitted to an intensive care unit became infected with *A. calcoaceticus* subsp. *anitratus*, defining an outbreak of respiratory tract infection. We report here the results of the laboratory investigation, which identified two different *A. calcoaceticus* subsp. *anitratus* strains as the causes of the outbreak.

MATERIALS AND METHODS

Bacteria. A total of 25 clinical isolates of *A. calcoaceticus* subsp. *anitratus* were used for this study. All were derived from specimens submitted to the Clinical Laboratory of Microbiology of the Hospital Clinic of Barcelona, Spain, and grown in MacConkey agar (Oxoid Ltd., Basingstoke, England). Isolates were identified as *A. calcoaceticus* subsp. *anitratus* by using standard biochemical procedures and the API 20NE system.

Antimicrobic susceptibility. Susceptibility to antimicrobial agents was determined by two methods. First, we used the disk diffusion method with Mueller-Hinton medium (Oxoid) as advocated by the National Committee for Clinical Laboratory Standards (24). Disks were obtained from Oxoid (ampicillin, 10 μg; mezlocillin, 75 μg; piperacillin, 100 μg; ticarcillin, 75 μg; cephalothin, 30 μg; cefamandole, 30 μg; cefoxitin, 30 μg; cefuroxime, 30 μg; cefotaxime, 30 μg; chloramphenicol, 30 μg; gentamicin, 10 μg; amikacin, 30 μg; netilmicin, 30 μg; tobramycin, 10 μg; trimethoprim-sulfamethoxazole, 1.25/23.75 μg; and imipenem, 10 μg). Second, MICs were determined by using the Sceptor system (BBL Microbiology Systems, Cockeysville, Md.). Sceptor system gram-negative MIC panels were used (18). A suspension of the organism was prepared in sterile saline to a 0.5 McFarland standard, and 10 μl was transferred to the Sceptor gram-negative broth with a disposable loop. Panels were inoculated according to the directions of the manufacturer by using the automated preparation station.

Plasmid isolation. Plasmid DNA of isolates was obtained by a modification of the method of Bennett et al. (4). All centrifugations were performed in Eppendorf tubes by using an Eppendorf microcentrifuge at 14,000 × *g*. After an overnight incubation in LB broth at 37°C, bacteria contained in 1.5-ml samples were collected by a brief centrifugation. Sedimented cells suspended in 100 μl of lysis buffer (1 mg of lysozyme per ml in 50 mM glucose, 10 mM EDTA, and 25 mM Tris hydrochloride [pH 8.0]) were incubated at room temperature for 15 min. Then, 200 μl of alkaline sodium dodecyl sulfate (1% sodium dodecyl sulfate in 0.2 M NaOH) was added, inverted several times, and incubated on ice for 5 min. The addition of 150 μl of 3 M sodium acetate was followed by incubation on ice for 5 min more. The mixture was centrifuged, and the supernatant was extracted once with 500 μl of phenol-chloroform (1:1). The aqueous upper layer was transferred to a fresh tube, and DNA was precipitated with 2.5 volumes of 95% ethanol at −70°C for 10 min. After centrifugation for 10 min, the DNA pellet was air dried and dissolved in 40 μl of TE buffer (10 mM Tris hydrochloride [pH 7.4], 0.1 mM EDTA). The solution was then incubated at 37°C with 10 μl of 0.1 mg of pancreatic RNase (Boehringer GmbH, Mannheim, Federal Republic of Germany) per ml for 30 min. The sample was analyzed by horizontal electrophoresis in 0.7% agarose, and plasmid DNA bands were visualized under a UV lamp after being stained with ethidium bromide.

Restriction endonuclease digestion. Rapidly isolated DNA

* Corresponding author.

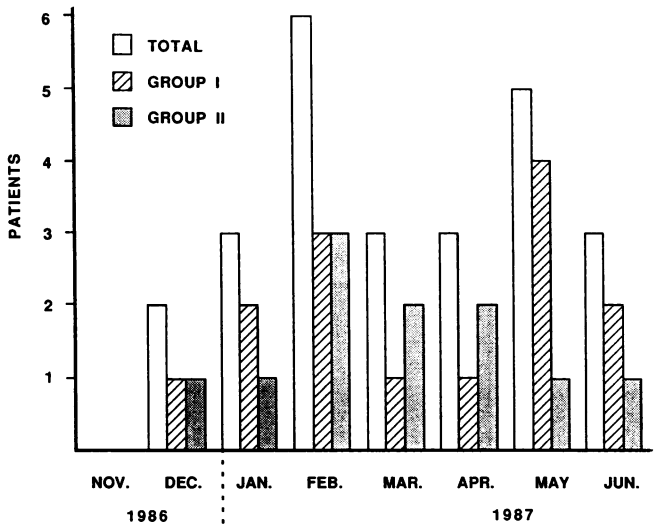

FIG. 1. Time course of outbreak showing the number of patients infected with multiresistant *A. calcoaceticus* subsp. *anitratus* from December 1986 to June 1987.

samples were cleaved with restriction endonucleases (*Bam*HI, *Hin*dIII, *Eco*RI, *Pst*I, *Bgl*III, *Eco*RV, and *Pvu*I) (Boehringer) for 2 h, as described previously (23), and analyzed by 1.0% (wt/vol) agarose gel electrophoresis.

RESULTS

In the period between December 1986 and June 1987, 25 patients in the respiratory intensive care unit had positive cultures for *A. calcoaceticus* subsp. *anitratus*. These patients were relatively evenly distributed throughout the epidemic period (Fig. 1). The patients were divided into two groups, group 1 (14 patients) and group 2 (11 patients) on the basis of characteristics of the *A. calcoaceticus* subsp. *anitratus* strains isolated, as described below.

The biochemical characteristics of the strains isolated from patients are shown in Table 1. The biotypes of the epidemic isolates (as defined by the API biochemical profile number) defined two groups, 1 and 2. Both groups had the same biochemical characteristics except that the strains included in group 2 could assimilate adipate and phenyl acetate; group 1 strains could not.

Antimicrobial susceptibility data were reviewed for all 25 isolates of *A. calcoaceticus* subsp. *anitratus*. Table 2 shows the antibiotic susceptibilities of and MICs for the strains isolated. The MIC study allowed the strains to be separated into two groups. The more-resistant group coincided with group 1 established by biochemical characteristics. In this group, of 16 antibiotics tested, only netilmicin and imipenem had some inhibitory activity. The antibiotic susceptibility pattern of group 2 isolates (Table 2) differed slightly from that of isolates from patients who made up group 1.

Plasmid DNA from the clinical isolates of *A. calcoaceticus* subsp. *anitratus* was analyzed to determine the genetic difference between the groups. Plasmid profiles of selected strains of both groups are shown in Fig. 2. Rapidly isolated DNAs of the strains from group 1 (Fig. 2A, lanes 1 and 2) showed two plasmid bands, whereas the plasmid profile from group 2 (lanes 3 and 4) showed only one band; therefore, the plasmid pattern was common to all the strains with similar antibiograms.

TABLE 1. Biochemical characteristics of *A. calcoaceticus* subsp. *anitratus*

Test	Result for[a]:	
	Group 1	Group 2
Morphology	CC	CC
Glucose fermentation	−	−
Glucose oxidation	+	+
Growth on MacConkey agar	+	+
Nitrate reduction	−	−
Indole	−	−
Arginine dihydrolase	−	−
Urea, Christensen	−	−
Esculin hydrolysis	−	−
Gelatin hydrolysis	−	−
β-Galactosidase	−	−
Assimilation of:		
Glucose	−	−
Arabinose	+	+
Mannose	−	−
Mannitol	−	−
N-Acetylglucosamine	−	−
Maltose	−	−
Gluconate	−	−
Caprate	−	−
Adipate	−	+
Malate	−	−
Citrate	−	−
Phenyl acetate	−	+
Catalase	+	+
Oxidase	−	−

[a] CC, Coccobacillus; −, negative reaction; +, positive reaction.

To examine the relatedness of plasmids found in both groups, rapidly isolated plasmid DNA was subjected to restriction endonuclease digestion and analyzed on agarose gel. Comparison of the restriction patterns showed a similarity between the DNAs of the strains from groups 1 and 2, but the fingerprints were clearly different between the groups (Fig. 2B).

DISCUSSION

Over the past two decades, *A. calcoaceticus* subsp. *anitratus* has become increasingly recognized as an organism of

TABLE 2. Antimicrobial susceptibility patterns of *A. calcoaceticus* subsp. *anitratus*

Antimicrobial agent	MIC (μg/ml) for[a]:	
	Group 1	Group 2
Ampicillin	>16 (R)	>16 (R)
Mezlocillin	>256 (R)	16 (S)
Piperacillin	>256 (R)	16 (S)
Ticarcillin	>256 (R)	16 (S)
Cephalothin	>32 (R)	>32 (R)
Cefamandole	>32 (R)	>32 (R)
Cefuroxime	>32 (R)	>32 (R)
Cefoxitin	>32 (R)	>32 (R)
Cefotaxime	>32 (R)	>32 (R)
Chloramphenicol	>16 (R)	>16 (R)
Gentamicin	16 (R)	16 (R)
Amikacin	>64 (R)	>64 (R)
Netilmicin	≤1 (S)	>64 (R)
Tobramycin	>16 (R)	>16 (R)
Trimethoprim-sulfamethoxazole	>16 (R)	>16 (R)
Imipenem	≤0.5 (S)	≤0.5 (S)

[a] R, Resistance; S, susceptibility.

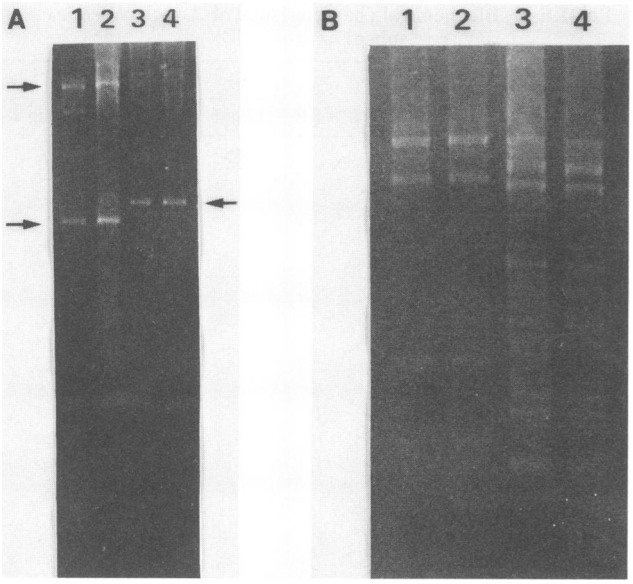

FIG. 2. (A) Agarose gel electrophoresis of plasmid DNA obtained from selected strains of group 1 (lanes 1 and 2) and group 2 (lanes 3 and 4). Plasmids are indicated by arrows. (B) Restriction endonuclease digests of plasmid DNA from group 1 strains (lanes 1 and 2) and group 2 strains (lanes 3 and 4).

some nosocomial importance. It is capable of causing a broad range of clinical disease syndromes, especially in patients with factors that impair normal host resistance, such as surgery, prior treatment with antibiotics, malignancy, and instrumentation (10, 13, 21). It has been established as a cause of pneumonia and tracheobronchitis (8, 15), urinary tract infection (12, 16), meningitis (7), cellulitis (13), wound infections and other skin infections (13), septicemia (27), and endocarditis (26; W. R. Thompson, Letter, J. Am. Med. Assoc. **215**:982, 1971). It has been predicted that *A. calcoaceticus* subsp. *anitratus* will become more and more prevalent and important as a cause of disease among compromised hosts as well as normal individuals (25). We have described a 7-month outbreak of infections caused by this organism in an intensive care unit. In this outbreak, the presence of two different strains of *A. calcoaceticus* subsp. *anitratus* with multiresistance, a feature not previously reported in outbreaks of infection with this bacterium, was demonstrated. This conclusion is supported by the comparison between groups of biochemical characteristics, antimicrobic susceptibility patterns, and plasmid DNA profiles.

The distribution according to biotype established two groups that were biochemically different on the basis of adipate and phenyl acetate assimilation. Baumann et al. (3) differentiated seven phenotypic groups of *A. calcoaceticus*; of those, only groups A_1 and A_2 could assimilate adipate, but group A_2 could not grow on arabinose as a carbon source. Therefore, our group 2 must correspond to phenotypic group A_1. We do not know where to include group 1, since there is no phenotypic group of strains that grow on arabinose and do not assimilate adipate.

In the beginning of the 1970s, *Acinetobacter* infections were treated with gentamicin, nalidixic acid, ampicillin, carbenicillin, and cephalosporins, either as single agents or as combined therapy, with generally satisfactory results (6). A rapid increase in the resistance of clinical strains has been reported (5, 11). In vitro synergy with an aminoglycoside and carbenicillin has been demonstrated for strains susceptible or moderately resistant to aminoglycosides (14), and two-drug combination therapy has been recommended for patients with severe infection, such as pneumonia or septicemia (22). However, with the problem of emerging resistance to aminoglycosides, other regimens must be evaluated.

Cefuroxime has appreciable in vitro activity against *Acinetobacter* spp. (20) and has been used successfully in two seriously ill patients with *Acinetobacter* pneumonia (1). We found both strains of *A. calcoaceticus* subsp. *anitratus* to be resistant to cefuroxime. In contrast, the in vitro activity of imipenem was very good (MIC, ≤ 0.5 μg/ml). Therefore, we can use this antibiotic as an alternative in light of the multiresistance of these strains.

The nosocomial reservoirs and mode of spread of this organism were not studied, since we did a retrospective laboratory investigation of the outbreak.

LITERATURE CITED

1. **Applebaum, P. C., J. Tamin, and J. Stairtz.** 1981. Sensitivity of *Acinetobacter calcoaceticus* strains to seven cephalosporins. Lancet **ii**:472.
2. **Baumann, P.** 1968. Isolation of *Acinetobacter* from soil and water. J. Bacteriol. **96**:39–42.
3. **Baumann, P., M. Doudoroff, and R. Y. Stainer.** 1968. A study of the *Moraxella* group. II. Oxidative-negative species (genus *Acinetobacter*). J. Bacteriol. **95**:1520–1541.
4. **Bennett, P. M., J. Heritage, and P. M. Hawkey.** 1986. An ultra-rapid method for the study of antibiotic resistance plasmids. J. Antimicrob. Chemother. **18**:421–424.
5. **Bergogne-Berezin, E., M. L. Joly, N. Moreau, and F. Le Goffic.** 1980. Aminoglycosides-modifying enzymes in clinical isolates of *Acinetobacter calcoaceticus*. Curr. Microbiol. **4**:361–364.
6. **Bergogne-Berezin, E., N. Zechovsky, M. Piechaud, J. F. Vieu, and A. Bordini.** 1971. Sensibilité aux antibiotiques de 240 souches de *Moraxella* (*Acinetobacter*) isolées d'infections hospitalières. Evolution sur 3 ans. Pathol. Biol. **19**:21–22.
7. **Berk, S. L., and W. R. McCabe.** 1981. Meningitis caused by *Acinetobacter calcoaceticus* variant *anitratus*. Arch. Neurol. **38**:95–98.
8. **Buxton, A. E., R. L. Anderson, D. Werdegar, and E. Atlas.** 1978. Nosocomial respiratory tract infection and colonization with *Acinetobacter calcoaceticus*. Am. J. Med. **65**:507–513.
9. **Cunha, B. A., J. J. Klimek, J. Gracewski, J. C. McLaughlin, and R. Quintiliani.** 1980. A common source outbreak of *Acinetobacter* pulmonary infections traced to Wright respirometers. Postgrad. Med. J. **56**:169–172.
10. **Daly, A. K., B. Postic, and E. H. Kass.** 1962. Infections due to organisms of the genus *Herella*. Arch. Intern. Med. **110**:580–591.
11. **Duval, J., C. J. Soussy, B. Koumare, C. Juliet, and L. Deforges.** 1982. Evolution des bacteries hospitalieres. Pathol. Biol. **30**: 405–414.
12. **French, G. L., M. W. Casewell, A. J. Roncoroni, J. Knight, and I. Phillips.** 1980. A hospital outbreak of antibiotic-resistant *Acinetobacter anitratus*: epidemiology and control. J. Hosp. Infect. **1**:125–132.
13. **Glew, R. H., R. C. Moellering, Jr., and L. Kunz.** 1977. Infections with *Acinetobacter calcoaceticus* (*Herella vaginicola*): clinical and laboratory studies. Medicine (Baltimore) **56**:79–97.
14. **Guerisse, P.** 1981. Cefuroxime for *Acinetobacter* infection. Lancet **ii**:96.
15. **Guerrero, M. L.** 1980. Community-acquired *Acinetobacter* pneumonia. Chest **78**:670.
16. **Hoffmann, S., C. E. Mabeck, and R. Vejlsgaard.** 1982. Bacteriuria caused by *Acinetobacter calcoaceticus* biovars in a normal population and in general practice. J. Clin. Microbiol. **16**: 443–451.
17. **Holton, J.** 1982. A report of a further hospital outbreak caused by a multi-resistant *Acinetobacter anitratus*. J. Hosp. Infect. **3**:305–309.

18. **Jones, R. N., C. Thornsberry, A. L. Barry, and T. L. Gavan.** 1981. Evaluation of the Sceptor microdilution antibiotic susceptibility testing system: a collaborative investigation. J. Clin. Microbiol. **13**:184–194.
19. **Juni, E.** 1984. Genus III. *Acinetobacter* Brison and Prévot 1954, 727^{AL}, p. 303–307. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
20. **Lang, S. D. R., D. J. Edwards, and D. T. Durack.** 1980. Comparison of cefoperazone, cefotaxime, and moxalactam (LY127935) against aerobic gram-negative bacilli. Antimicrob. Agents Chemother. **17**:488–493.
21. **Larson, E.** 1984. A decade of nosocomial *Acinetobacter*. Am. J. Infect. Control **12**:14–17.
22. **Maderazo, E. G., R. Quintiliani, R. C. Tilton, R. Bartlett, N. C. Joyce, and V. T. Andriole.** 1975. Activity of minocycline against *Acinetobacter calcoaceticus* var. *anitratus* (syn. *Herellea vaginicola*) and *Serratia marcescens*. Antimicrob. Agents Chemother. **8**:54–57.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **National Committee for Clinical Laboratory Standards.** 1984. Performance standards for antimicrobial disk susceptibility tests, M2-A3, 3rd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
25. **Ramphal, R., and R. M. Kluge.** 1979. *Acinetobacter calcoaceticus* var. *anitratus*: an increasing nosocomial problem. Am. J. Med. Sci. **227**:57–66.
26. **Rao, K. N. A., M. Kotian, and S. G. S. Prabhn.** 1980. Infective endocarditis due to *Acinetobacter calcoaceticus*. Postgrad. Med. J. **26**:186–191.
27. **Raz, R., G. Alroy, and J. D. Sobel.** 1982. Nosocomial bacteremia due to *Acinetobacter calcoaceticus*. Infection **10**:168–171.
28. **Sherertz, R. J., and M. C. Sullivan.** 1985. An outbreak of infections with *Acinetobacter calcoaceticus* in burn patients: contamination of patients' mattresses. J. Infect. Dis. **151**:252–258.
29. **Taplin, D., G. Rebell, and N. Zaias.** 1963. The human skin as a source of *Mima-Herella* infections. J. Am. Med. Assoc. **186**: 952–955.