Search for *Acinetobacter calcoaceticus* subsp. *anitratus*: Enrichment of Fecal Samples

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None of 50 healthy individuals and only 2 of 50 hospitalized patients (with *Acinetobacter anitratus* elsewhere) yielded *A. anitratus* on enrichment from fecal samples.

Publications reporting isolates of *Acinetobacter calcoaceticus* subsp. *anitratus* (*A. anitratus*) from human feces have been rare (3). One reason for this scarcity could be the purposeful omission of the recovery of *A. anitratus* in final reports of stool cultures, an omission justified by the fact that earlier reports linking the organism to diarrhea (8) were never confirmed later. On the other hand, we have not been able to isolate *A. anitratus* from feces in the past 2 years.

The natural habitat of *A. anitratus* is soil and water (3). Nosocomial infections have been reported with increasing frequency (2, 3). Although part of the strains originated either from moist hospital sources or from the skin (6), the source of many other strains has remained unknown. *A. anitratus* is not known to be part of the normal intestinal flora (3, 8). Since the intestinal tract of humans, however, is the reservoir for many gram-negative bacteria causing nosocomial infection, an attempt was made to establish whether the intestine of nonhospitalized and hospitalized individuals may be colonized with *A. anitratus* in quantities too small to be detected by routine stool cultures. The use of selective and enrichment media was contemplated for this purpose.

To test the suitability of selective agar media, seven strains of *A. anitratus*, two strains of *Escherichia coli*, and one strain each of *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Klebsiella ozaenae*, and *Pseudomonas aeruginosa* were used as challenge organisms, since these bacteria occur with reasonable frequencies in stools of hospitalized patients. Plating was done with a 0.001-ml loop from a 24-h tryptic soy broth culture (Difco Laboratories, Detroit, Mich.).

Initially, *A. anitratus* was selected by means

of chloramphenicol, to which most strains are resistant (2). Endo agar, MacConkey agar, and deoxycholate agar (all from Difco), without and with 5, 10, and 20 µg of chloramphenicol (Parke Davis & Co., Westboro, N.J.) per ml, were used. Whereas all strains grew on the antibiotic-free agar plates, only five of the seven *A. anitratus* strains grew on all of the chloramphenicol plates. *P. aeruginosa* and *S. marcescens* grew likewise. These species are also largely chloramphenicol resistant (10, 11).

Next, an attempt was made to utilize the ability of *A. anitratus* to grow with sodium hippurate as the sole carbon source (7). A mineral medium with ammonium sulfate as the only nitrogen source (5), with 0.2% (wt/vol) sodium hippurate (Mallinckrodt Inc., Hazelwood, Mo.) and 1.0% Ionagar (Oxoid; Flow Laboratories, Rockville, Md.), was tested. All seven strains of *A. anitratus* grew, but so did *S. marcescens*, *K. ozaenae*, *E. aerogenes*, and *P. aeruginosa*, albeit with smaller colonies. Some strains of these bacteria are known to utilize hippurate (9).

A third attempt was made by using herellea agar (Difco), which supposedly helps in recognizing *A. anitratus* colonies (4). All of our *A. anitratus* strains grew on this medium, but so did *P. aeruginosa*, *C. freundii*, and several strains of *Proteus mirabilis* and *P. rettgeri*, some of them yielding lavender colonies said to be characteristic of *A. anitratus* (4).

Since these selective agar media proved unsatisfactory, enrichment was tried. The medium of Baumann, consisting of 0.2% (wt/vol) sodium acetate, 0.2% KNO₃, and 0.02% MgSO₄·7H₂O in 0.04 M KH₂PO₄-Na₂HPO₄ buffer (pH 6.0) containing 20.0 ml of Hutner mineral base, has previously been used to enrich *A. anitratus* from soil samples (1). The medium utilizes the ability of *A. anitratus* to grow with acetate as the sole carbon source (7), with nitrate, an incubation temperature of 30°C, and aeration favoring *A. anitratus* over *Enterobacteriaceae*.

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A suspension of 0.15 g of feces in 5.0 ml of 0.9% NaCl was gently centrifuged to remove coarse particles. A 1.0-ml amount of the supernatant was mixed with 20 ml of Baumann medium and incubated at 30°C for 48 h in a shaking water bath. Samples were removed, diluted, and plated on deoxycholate agar before and after incubation. Non-lactose fermenter colonies were identified according to a method described earlier (11).

To check the efficiency of the medium, six stool samples from normal individuals were inoculated with *A. anitratus*, two each with different strains. The initial ratio of coliforms to *A. anitratus* was held at 100:1 to 1,000:1 (per g of feces, 10^6 to 10^7 : 10^4 colony-forming units). The uninoculated samples yielded no *A. anitratus*. In every inoculated sample, the coliform/*A. anitratus* ratio was reversed 1:10 to 1:100 after incubation. This was considered sufficient to use the medium for enrichment purposes.

Fifty stools of healthy, nonhospitalized volunteers and 50 stools of hospitalized, ill patients under antimicrobial agent therapy were checked for *A. anitratus* with the enrichment method. The age range of both groups was between 2.5 and 75 years; the hospitalized group had, for the most part, previously received ampicillin and/or cephalothin, which are largely ineffective against *A. anitratus* (2). Twenty patients were from the medical, 20 from the surgical, seven from the pediatric, two from the gynecological, and one from the dermatological service. None of the individuals in the two groups had been checked systematically for extraintestinal carriage of *A. anitratus*, but 10 of the hospitalized patients had yielded the organism from other samples (four each from wounds and respiratory secretions) before the stool culture was taken.

None of the 100 subjects yielded *A. anitratus* on direct plating of stool suspensions. None of the 50 healthy individuals and only 2 of the 50 hospitalized individuals yielded *A. anitratus* from the enrichment cultures. Both positive patients had previously yielded *A. anitratus* from wound and sputum specimens, respectively; the antibiograms of their strains were identical to those of the intestinal isolates. Both patients had been hospitalized for more than 4 weeks, but earlier stool cultures had not been taken.

Even if identity of the intestinal and extraintestinal strains is assumed, one cannot be sure that the intestine was the primary site of colonization; it may have been secondarily colonized.

The hospitalized patients with *A. anitratus* isolates from extraintestinal sources carried the organism significantly more often ($P < 0.05$) than the hospitalized patients without extraintestinal isolates (2 of 10 versus 0 of 40), although the lack of a systematic investigation of all possible extraintestinal sites limits the value of these data. Nevertheless, our results indicate that *A. anitratus* at best rarely colonizes the normal intestine and that in hospitalized patients under antimicrobial agent treatment, a group in which *A. anitratus* infections usually occur (2), the intestinal tract is not, as a rule, colonized either. It is, therefore, not an important reservoir for nosocomial infections even in cases where *A. anitratus* is found elsewhere in the body.

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