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Urinary Tract Infection Caused by a Capnophilic *Proteus mirabilis* Strain

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From a urine sample from a patient with a urinary tract infection, a carbon dioxide-dependent *Proteus mirabilis* strain was isolated. It is important to perform urine cultures in 5% carbon dioxide and an anaerobic atmosphere if bacteria prominent in Gram stains do not grow on routine media in ambient air.

A 70-year-old male visited his general physician with lower abdominal pain, dysuria, and malaise. A urine sample was submitted for staining and culture. The Gram stain showed many erythrocytes, no leukocytes, and many Gram-negative rods. A Columbia sheep blood agar (CBA) plate and a MacConkey agar plate without salt (Oxoid, Landsmeer, The Netherlands) were each streaked with 1 μ l of the urine sample and incubated at 35°C in ambient air. After 24 h, the plates showed no growth. Subsequently, this procedure was repeated, but additionally, a Chocolate agar plate was streaked with 1 μ l of urine and incubated at 35°C in a 5% carbon dioxide incubator and a CBA plate was streaked with 1 μ l of urine and incubated at 35°C in an anaerobic jar. The next day, aerobic cultures again showed no growth but heavy growth of gray waxy colonies was observed on the chocolate agar incubated with 5% carbon dioxide and on the CBA plate incubated in the anaerobic jar. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (BioTyper; Bruker Daltonics, Hamburg, Germany) identified the isolate as *Proteus mirabilis* (score, 2.552).

To determine the effects of media and environmental conditions on the growth of this strain, we performed subcultures on CBA, chocolate agar, and MacConkey agar, in ambient air, in 5% carbon dioxide, and in anaerobic and microaerophilic jars; this was performed simultaneously for *P. mirabilis* ATCC 7002. The patient's strain grew on all of the media under all of the atmospheric conditions used, except ambient air. No swarming growth was observed on any medium under any atmospheric condition. *P. mirabilis* ATCC 7002 showed swarming growth on all of the media under all of the atmospheric conditions used. After five subcultures incubated in 5% carbon dioxide, the isolate did not regain the ability to grow in ambient air.

The isolate was submitted to the National Institute of Public Health and the Environment (RIVM, Bilthoven, The Netherlands) for phenotypic and genotypic identification. The strain was again identified as *P. mirabilis* on the basis of 100% sequence similarity of a 500-bp fragment of the 16S rRNA gene, cell wall lipid content measured by high-performance liquid chromatography, and biochemical tests. Growth conditions and lack of swarming growth were completely reproduced.

Disk diffusion susceptibility tests were performed according to EUCAST guidelines (available at <http://www.eucast.org>), except that plates were incubated in a 5% carbon dioxide incubator. The isolate proved susceptible to all of the compounds tested except nitrofurantoin (Table 1), supporting the identification result. The

adjacent nitrofurantoin and norfloxacin disks showed an antagonistic effect of nitrofurantoin on norfloxacin, with a D-shaped zone of inhibition around the norfloxacin disk.

Empirically, the patient was treated with ciprofloxacin administered orally at 500 mg twice daily for 7 days and reported full resolution of symptoms.

Failure of aerobic Gram-negative rods to grow in ambient air has been sporadically reported. Given the fact that the isolate reported here grew well in carbon dioxide-enriched and anaerobic atmospheres, it is to be considered capnophilic, not microaerophilic. To date, four capnophilic *Escherichia coli* isolates have been reported as causative agents of human disease; three caused urinary tract infections (1–3), and one caused pleural empyema (4). This phenomenon has also been reported for *Klebsiella pneumoniae* subspecies *ozaenae* isolates but without clinical data (5).

Recently, a single report of a capnophilic *P. mirabilis* strain was published in Japan (6). That isolate, too, was the causative agent of a urinary tract infection and showed growth characteristics similar to those of the isolate reported here. The isolate in Japan was not tested for growth in a microaerophilic atmosphere, and while it did grow in 5% carbon dioxide, its growth was less than that of reference strains. Our isolate grew as well as the type strain in 5% carbon dioxide. The isolate reported from Japan showed pinpoint colonies without swarming at atmospheric CO₂ contents of 1 and 2% and swarming growth at an atmospheric CO₂ content of \geq 3% (6), which we did not observe in our strain. Whether the capnophilicity and lack of swarming result from a single mechanism warrants further investigation.

The key role of carbon dioxide in the growth of bacteria, including Gram-negative facultative aerobic rods, was demonstrated in the 1970s. Repaske and Clayton (7) showed that addi-

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TABLE 1 Results of disk diffusion drug susceptibility testing^a

| Compound(s) | Zone diam (mm) | Interpretation ^c |
|-----------------------------|-----------------|-----------------------------|
| Amoxicillin | 36 | S |
| Amoxicillin-clavulanic acid | 34 | S |
| Cefuroxime | 32 | S |
| Co-trimoxazole | 36 | S |
| Trimethoprim | 22 | S |
| Nitrofurantoin | 6 | R |
| Norfloxacin | 42 ^b | S |
| Ciprofloxacin | 50 | S |
| Gentamicin | 28 | S |
| Fosfomycin | 28 | S |

^a Tests were performed with Mueller-Hinton medium incubated in 5% carbon dioxide.

^b D-shaped inhibition zone, antagonism with nitrofurantoin.

^c S, susceptible; R, resistant.

tion of carbon dioxide to *E. coli* grown on minimal medium under controlled atmospheric conditions shortens the lag phase and induces prompt growth. The mechanism behind this effect of carbon dioxide is unknown, as is the mechanism behind the carbon dioxide dependency of the previously reported capnophilic isolates (7).

Results of antimicrobial susceptibility tests should be interpreted with caution when they are performed in a 5% carbon dioxide atmosphere, which acidifies the medium. Nitrofurantoin-norfloxacin antagonism is specific to *P. mirabilis* (8).

Routine incubation of urine samples on chocolate medium in a 5% carbon dioxide atmosphere can aid in the recovery of carbon dioxide-dependent organisms, as well as *Haemophilus* species, which are also capable of causing urinary tract infection (9).

In conclusion, the occurrence of carbon dioxide-dependent

bacteria reinforces the common practice of performing urine cultures with 5% carbon dioxide in an anaerobic atmosphere if bacteria prominent in Gram stains of clinical samples do not grow on routine media in ambient air. The mechanisms underlying the capnophilicity and lack of swarming growth observed warrant further investigation.

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