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Method for Differentiating *Klebsiella planticola* and *Klebsiella terrigena* from Other *Klebsiella* Species

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Conventional methods usually fail to identify *Klebsiella planticola* and *Klebsiella terrigena*, which represent up to 19% of clinical *Klebsiella* isolates. By combining four carbon substrate assimilation tests and two conventional tests, the method identified these species with a specificity and a sensitivity of 100%. Overall, sensitivity for *Klebsiella* identification was 94.7%.

Correct identification of some *Klebsiella* strains to the species level remains difficult in the clinical microbiology laboratory. *Klebsiella planticola* and *Klebsiella terrigena* (1, 7, 12), which were primarily considered environmental species (2, 5, 7, 9), have been isolated from clinical specimens. In fact, *K. planticola* may represent 8 to 19% of clinical *Klebsiella* isolates (11, 12) and has been associated with various infections, including septicemia (6). *K. terrigena*, which represents only 0.4% of clinical *Klebsiella* isolates, has been isolated from clinical specimens only in conjunction with other bacteria and has never been proven to be responsible for infections (15). However, it is generally misidentified as *Klebsiella pneumoniae* or *Klebsiella oxytoca* because commercialized systems, such as the API 20E (bioMérieux, Marcy l'Etoile, France), usually fail to correctly identify these two species (11), as they are not included in the systems' data bases. On the other hand, most of the proposed additional tests, such as growth at 4, 10, or 44°C, gas production from lactose at 44.5°C, and pectate liquefaction (3–5, 8, 9, 14), are usually not performed in routine practice. A third recently described species, *Klebsiella ornithinolytica* (17), can be identified by commercial identification systems, but its clinical significance remains unknown. In Europe, carbon substrate assimilation systems, such as the API 50 CH, API 50 AO, and API 50 AA galleries, have identified *Klebsiella* isolates to the species level (11), but the great number of tests that have to be performed is time-consuming and the galleries are expensive. To achieve a better and quicker identification of *Klebsiella* strains in the clinical microbiology laboratory, we developed a combination of two conventional tests and four carbon substrate assimilation tests that can easily be prepared and performed, in addition to conventional identification systems.

We tested a collection of 198 nonmotile strains of clinical origin previously identified with the API 20E, API 20EC, API 50 CH, API 50 AO, and API 50 AA systems (bioMérieux) (13, 15, 16) and growth tests at 4 and 44°C (3, 5, 6) as the following species and subspecies: *K. pneumoniae* subsp. *pneumoniae* (101 strains), *K. pneumoniae* subsp. *ozaenae* (2 strains), *K. pneumoniae* subsp. *rhinoscleromatis* (2 strains), *Klebsiella oxytoca* (61 strains), *K. planticola* (17 strains), *K. terrigena* (1 strain), and *K. ornithinolytica* (14 strains). Repartition within species was identical to that observed in clinical practice, with the exception of *K. ornithinolytica*, which was overrepresented. We also tested a total of 11 reference strains of the following species and subspecies: *K. pneumoniae* subsp. *pneumoniae* ATCC 13882 (American Type Culture Collection, Rockville, Md.); *K. pneumoniae* subsp. *ozaenae* ATCC 11297; *K. pneumoniae* subsp. *rhinoscleromatis* ATCC 6908; *K. oxytoca* ATCC 13182; *K. planticola* ATCC 33531; *K. terrigena* ATCC 33257, ATCC 33628, ATCC 33629, ATCC 33630, and ATCC 33631; and *K. ornithinolytica* ATCC 31898. We selected four carbon substrates from a previous study (11): ethanolamine, histamine, D-melezitose, and DL-3-hydroxybutyrate (Sigma Chemical Co., St. Louis, Mo.). Aqueous solutions of 10 g of carbon per liter (ethanolamine and histamine) and 20 g of carbon per liter (D-melezitose and DL-3-hydroxybutyrate) were prepared, sterilized by filtration on 0.22-μm-pore-size Millex filters (Millipore Corp., Bedford, Mass.), and stored at 4°C, as sterile 50-μl aliquots, in 5-ml capped sterile test tubes. A fifth tube, without a carbon substrate, was used as a growth control. Two hundred microliters of AUX Medium (bioMérieux) was added to the aliquoted tubes and control tube just before use. Substrates and the control were inoculated with 100 μl of a suspension (3×10^8 CFU/ml) in 0.85% NaCl, and readings were taken after 24 and 48 h of incubation at 30°C. Presence or absence of bacterial growth was observed visually and compared with growth in the control. The results of the indole and ornithine decarboxylase tests were taken from the API 20E (bioMérieux). One reference strain of each *Klebsiella* species and subspecies (Table 1) was tested each week for 2 months, with the 4°C-stored aliquots, to determine the reproducibility of the tests and to study the stability of the four carbon substrates. The reproducibility of indole production and ornithine decarboxylase tests of the API 20E was assumed to be correct.

The reproducibility assay always gave the same identification patterns for each reference strain tested; however, carbon substrate aliquots were not usable after 2 months because of desiccation. Identification results for 209 strains and the number and percentage of positive reactions for each test and species are shown in Table 1. Overall, the method, using the four carbon substrate assimilation tests and the two conventional tests, identified 198 *Klebsiella* strains to the species level (sensitivity, 94.7%), including most of the *K. pneumoniae* (sensitivity, 92.6%) and *K. oxytoca* (sensitivity, 95.2%) strains and all of the *K. planticola*, *K. terrigena*, and *K. ornithinolytica*

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TABLE 1. Percentage of positive reactions for each test and *Klebsiella* species

Species and subspecies	No. of strains tested	No. (%) of positive reactions					
		Assimilation tests				Other tests	
		Ethanolamine	Histamine	D-Melezitose	DL-3-Hydroxybutyrate	Indole production	Ornithine decarboxylase
K. pneumoniae subsp. *pneumoniae*	102	98 (96)	0	1 (1)	101 (99)	0	0
K. pneumoniae subsp. *ozaenae*	3	1 (33)	0	0	1 (33)	0	0
K. pneumoniae subsp. *rhinoscleromatis*	3	3 (100)	0	0	3 (100)	0	0
K. oxytoca	62	58 (94)	0	46 (74)	1 (2)	60 (97)	0
K. planticola	18	0	18 (100)	0	18 (100)	11 (61)	0
K. terrigena	6	0	6 (100)	6 (100)	6 (100)	0	0
K. ornithinolytica	15	0	15 (100)	0	15 (100)	15 (100)	15 (100)

strains (sensitivity, 100%). Specificity was 100% for these five species.

The method is suitable for identifying most *Klebsiella* species and subspecies, with the exception of *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *rhinoscleromatis*, for which growth was generally weak. Two of three *K. pneumoniae* subsp. *ozaenae* strains failed to grow in the assimilation medium, and when growth was observed, the panel of carbon substrates was unable to differentiate the three subspecies of *K. pneumoniae* (Table 1). Conventional systems, such as the API 20E gallery, generally correctly identify these subspecies (13). New *Klebsiella* species have been isolated from clinical specimens, and the most frequent new species, *K. planticola*, must be considered an opportunistic pathogen (4, 6). However, most of conventional identification systems are not able to identify these new species that are misidentified in the clinical microbiology laboratory. A new identification system using a great number of carbon substrates, Biolog, might be able to identify these new species, but unfortunately, this system has not been tested for *K. planticola*, *K. terrigena*, or *K. ornithinolytica* (10). We recommend that the method described here be used to detect *K. planticola* and *K. terrigena* among strains previously identified in routine practice as *K. pneumoniae*, *K. oxytoca*, or a *Klebsiella* sp. and also used as a preliminary step before epidemiologic studies of clinical *Klebsiella* strains. Another alternative could be routine histamine assimilation testing to detect *K. planticola* or *K. terrigena* among *Klebsiella* isolates, followed by use of the method described here to test histamine-positive *Klebsiella* strains only. In conclusion, this method appears to be a sensitive, specific, and reproducible alternative method to correctly identify clinical *Klebsiella* strains to the species level.

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