Commercial Identification Systems Often Fail To Identify
Providencia stuartii

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Received 15 July 1987/Accepted 14 October 1987

We tested 145 clinical isolates in an attempt to evaluate some of the most widely used commercial
identification systems in Europe in terms of their ability to identify Providencia strains. Two manual
miniaturized systems (API 20E and Enterotube II) and three mechanized-automated systems (Cobas-Bact,
Sceptor System, and Titer tek-Enterobac-Rapid Automated System) were evaluated. Providencia alcalifaciens
and Providencia retgeri strains were correctly identified by all systems in all cases, and in most cases
identification was achieved without the aid of supplementary tube tests. By contrast, Providencia stuartii was
identiﬁed without the aid of supplementary tube tests for only 42.5% (API 20E), 37.5% (Enterotube), 68.7%
(Sceptor), and 71.2% (Cobas-Bact) of the isolates. The overall misidentiﬁcation rates were 16.3, 11.3, 11.3, and
10%, respectively. The Titer tek-Enterobac-Rapid Automated System failed to identify only 1 of 80 strains
(1.3%) and required supplementary tests in 2 other cases (2.5%). Since four of the multitest systems examined
often failed to correctly identify P. stuartii, we conclude that supplementary conventional tube tests should
always be used to distinguish this species from the other taxa of the Proteae tribe.

MATERIALS AND METHODS

Bacterial strains and reference identiﬁcation. In our study we used 145 Providencia strains (1 per patient) freshly
isolated from a variety of specimens from patients. All the isolates were identiﬁed to species level by the following test
battery: indole and H2S production; Voges-Proskauer test; urea, gelatin, and esculin hydrolysis; Simmons citrate; phenylalanine deaminase; lysine and ornithine decarboxylase; arginine dihydrolase; fermentation of adonitol, L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, glycerol, myoinositol, lactose, maltose, D-mannitol, D-mannose, melibiose, α-methyl-D-glucoside, rafﬁnose, L-rhamnose, salicin, sorbitol, sucrose, trehalose, and D-xyllose; and the o-nitrophenyl-β-D-galactopyranoside (ONPG) test. All tests were
performed by using test tubes and conventional methods (6, 16).

To calculate the percent identiﬁcation (12), we developed a matrix of probabilities which included the members of the family Enterobacteriaceae cited by Farmer et al. (3) as taxa and the positivity percentages reported by the same authors for the tests we had performed as the data base.

Identifications to species level were also carried out by using the reduced battery of tests proposed by Farmer et al. for
differentiation within the genus Providencia (3), including urea hydrolysis and fermentations of myo-inositol, adonitol, D-arabitol, trehalose, and D-galactose. The last test could not be included in the matrix we described above, because data on the probabilities of its results were not available for all the species of Enterobacteriaceae.

Commercial identiﬁcation systems. The same strains were also identiﬁed by two manual miniaturized systems (API 20E
[API Systems S.A., La Balme-les-Grottes, Montalieu-Vercieu, France] and Enterotube II [Roche Diagnostics, Basel,
Switzerland]) and three mechanized-automated systems.
RESULTS

On the basis of the reference identification, the organisms included in the study could be assigned to all the Providencia species which are of interest in human pathology, namely P. rettgeri (47 strains), P. stuartii (80 strains), Providencia alcalifaciens (16 strains), and Providencia rustigianii (2 strains). In all cases, the identifications at species level coincided perfectly with those obtained by using the reduced test battery proposed by Farmer et al. (3). The strains identified by the reference matrix as P. alcalifaciens, and only these strains, were all galactose positive.

The species P. rustigianii is not included among the taxa of any of the systems evaluated, and as a consequence, a correct identification for the two strains we examined was not achievable. These strains were identified as P. stuartii by Enterotube II and as P. alcalifaciens by all the other systems. Table 1 shows the percentages of correct and incorrect identifications given for the remaining Providencia species by the commercial systems considered.

The identifications of P. alcalifaciens and P. rettgeri strains by all the systems generally proved correct and, in most cases, were achieved without the aid of supplementary tube tests. These tests were required only with Enterotube II for four strains of P. alcalifaciens (25.0% of the total) and with both Cobas-Bact and API 20E for four strains of P. rettgeri (8.5%).

The percent correct identifications registered for P. stuartii were less satisfactory than those for P. alcalifaciens and P. rettgeri, especially if we consider the results obtained without the aid of tube tests. The identifications given by API 20E that required no additional tests proved correct for 34 of 80 strains (42.5%) and incorrect for 11 strains; when we performed the supplementary tests required to obtain a higher-confidence identification for a further 33 isolates, 33 strains (41.2% of the total) were correctly identified and 2 were not, thus bringing the misidentification rate of this system up to 16.3%.

Table 2 lists the P. stuartii strains which were misidentified, along with the wrong identifications. Most of the misidentifications were confined to the Providencia genus or, at most, to the Proteaceae tribe. To assess whether these errors might depend on the different percent positivity attributed to the individual tests by different data bases, we checked the identifications on a data matrix developed for each system by excluding from our reference matrix the tests not present in the system considered. In practice, these control matrices yielded the same misidentification rates as the respective systems (data not shown).

A few strains were misidentified as species outside the Proteaceae tribe, namely Tatumella pyseos, Yersinia pseudotuberculosis, Yersinia enterocolitica, and Enterobacter agglomerans. Only one strain (PV 48) was misidentified by all the systems: it was an atypical indole-negative, sucrose-positive strain; the reference matrix identified it as P. stuartii or T. pyseos (with P = 87.6 and 12.7%, respectively); the ultimate diagnosis of this strain as P. stuartii resulted from

| TABLE 1. Identification of Providencia strains by the five commercial systems |
|-----------------------------|-----------------------------|-----------------------------|
| Identification system       | P. alcalifaciens            | P. rettgeri                 | P. stuartii                |
|                             | No. (%) correct (−STT)*     | No. (%) correct (+STT)*     | No. (%) incorrect*        | No. (%) correct (−STT)*     | No. (%) correct (+STT)*     | No. (%) incorrect*        | No. (%) correct (−STT)*     | No. (%) correct (+STT)*     | No. (%) incorrect*        |
| API 20E                     | 16 (100.0)                  | 0 (0.0)                     | 0 (0.0)                   | 43 (91.5)                  | 4 (8.5)                      | 0 (0.0)                   | 34 (42.5)                  | 33 (41.2)                  | 13 (16.3)                   |
| Enterotube II               | 12 (75.0)                   | 4 (25.0)                    | 0 (0.0)                   | 47 (100.0)                 | 0 (0.0)                      | 0 (0.0)                   | 30 (37.5)                  | 41 (51.2)                  | 9 (11.3)                    |
| Sceptor                     | 16 (100.0)                  | 0 (0.0)                     | 0 (0.0)                   | 47 (100.0)                 | 0 (0.0)                      | 0 (0.0)                   | 55 (68.7)                  | 16 (20.0)                  | 9 (11.3)                    |
| TTE-RAS                     | 16 (100.0)                  | 0 (0.0)                     | 0 (0.0)                   | 47 (100.0)                 | 0 (0.0)                      | 0 (0.0)                   | 77 (96.2)                  | 2 (2.5)                    | 1 (1.3)                     |
| Cobas-Bact                  | 16 (100.0)                  | 0 (0.0)                     | 0 (0.0)                   | 43 (91.5)                  | 4 (8.5)                      | 0 (0.0)                   | 57 (71.2)                  | 15 (18.8)                  | 8 (10.0)                    |

* Correct (−STT), Identification agreeing with the reference identification without the aid of supplementary tube tests.

* Correct (+STT), Identification agreeing with the reference identification after supplementary tube tests.

* Incorrect, Identifications not agreeing with the reference identification even after supplementary tube tests (when required).
the fact that it was weakly citrate positive after 48 h and glycerol positive and actively motile at 37°C. The last two tests ruled out the diagnosis of *T. pyseos* for another strain (PV 113, which was citrate negative within 72 h), which could not be identified by API 20E and Enterotube II. Finally, a few isolates were misleading because they were D-xylose positive, and these were attributed by Enterotube II to the *Enterobacter* genus; they were correctly identified by the other systems.

**DISCUSSION**

Our results show that *P. alcalifaciens* and *P. rettgeri* strains were adequately identified by all the systems examined, even without the aid of supplementary tube tests. The tests included in the standard system configurations alone were sufficient for a correct identification of all *P. alcalifaciens* strains by API 20E and Cobas-Bact and of all *P. rettgeri* strains by Enterotube II. The identifications obtained by Sceptor and TTE-RAS alone proved correct for all the strains of both species. Finally, all the systems assured 100% correct identifications when supplementary tube tests were performed.

*P. rustigianii* is not included among the taxa of any of the systems examined, but it is worth mentioning that the identifications obtained for our two *P. rustigianii* isolates accorded with their former taxonomy. Before the formal name *P. rustigianii* (9) was proposed, these strains were classified as *P. alcalifaciens* biogroup 3 and were not differentiable by simple tests from *P. stuartii* strains previously known as *P. alcalifaciens* biogroup 4, although they were already considered to belong to a different species (1).

Thus, the only true misidentifications were of *P. stuartii*. Both the rate and nature of the errors varied from system to system. The misidentification rates decrease in the following order: API 20E (16.3%), Sceptor and Enterotube II (11.3%), Cobas-Bact (10.0%), and TTE-RAS (1.3%).

As regards the nature of the errors, identifications outside the *Proteaeae* tribe were confined to a few strains that had uncommon biochemical traits and were not easy to identify even by conventional methods. Misidentifications deriving from the inability to differentiate among the three genera within the *Proteaeae* tribe or the three species within the genus *Providencia* were much more frequent. In some cases, errors were caused by negativity or delayed positivity of tests such as citrate and *myo*-inositol and could be attributed to a poorer sensitivity of the micromethods (19): most of the strains that were misidentified proved positive by standard tube procedures. These errors enhanced the probabilities that these strains would be identified as *Proteus vulgaris*, *Proteus penneri* (8), or ornithine-negative *Morganella morganii* (7) or, within the genus *Providencia*, as *P. alcalifaciens*.

When the results of the micromethods agreed with those of the tube tests, the errors could be attributed to the presence or absence in the miniaturized system of the various tests allowing identification of the *P. stuartii* isolates to both genus and species level. This correlation was investigated by developing a control matrix for each system.
Since the frequency of the errors in each matrix was substantially the same as in the respective system (the only difference between the reference and control matrix being the selection of tests), it could be argued that the misidentifications depended on test selection and not on the different percent positivity attributed to the individual tests by the system data bases.

Besides urease production, which is tested in all systems and therefore cannot account for substantial differences in results, a correct identification of P. stuartii by the miniaturized systems considered in our study is based mostly on fermentations of polyhydric alcohols (14). Of these carbohydrates, myo-inositol, adonitol, and trehalose allow a differential diagnosis between P. alcalifaciens and P. stuartii. API 20E includes only one of them, i.e., myo-inositol, whose results proved the least reliable (see above). This could explain why this system erroneously identified the highest number of P. stuartii strains as P. alcalifaciens, but, since myo-inositol fermentation is of little value in distinguishing P. stuartii from P. rettgeri, the failure to distinguish these two species must also have other causes. The carbohydrates that allowed differentiation between the two species and were included in the systems we considered are D-mannitol, adonitol, rhamnose, and trehalose. P. stuartii strains which are positive in the D-mannitol and adonitol tests can easily be misidentified as P. rettgeri, since the latter species shows a much higher percent positivity. Even a negative rhamnose fermentation test, which is almost mandatory for P. stuartii, cannot contribute very much to the ultimate percent identification, because the same reaction is shared by many P. rettgeri strains. Under these conditions and in the absence of a positive control, i.e., a test in which the percent positivity of P. stuartii is greatly superior to that of P. rettgeri, the matrix of probabilities could hardly offer a correct identification. By contrast, the only system including the trehalose fermentation test (TTE-RAS), which is positive for P. stuartii with a very good separation value, showed by far the best results, with a misidentification rate of only 1.3%.

In all the cases we have discussed so far, failure to identify P. stuartii was complete; i.e., this species was not included even among lower-confidence identifications. However, correct identification could not be obtained without supplementary tube tests in a much larger number of cases, ranging from 18.7% of overall Cobas-Bact identifications to 51.2% of those by Enterotube II. TTE-RAS again proved the most accurate system, requiring supplementary tests in two cases only (2.5%). In all these cases, the possible identification as P. stuartii often has a low probability, for substantially the same reasons as discussed above for cases of complete misidentification. Thus, this possibility could easily be overlooked by both laboratory and ward staff, mainly because awareness of the possible role of P. stuartii as a nosocomial pathogen is probably not yet sufficiently strong to prompt the use of identification techniques that are, as in the case of the individual tube tests, in sharp contrast to the present tendency to prefer rapid unitary procedures.

However, we think that at least the most frequent errors of the multitest systems should be eliminated by checking the isolates identified as Proteus penneri and ornithine-negative M. morganii and by confirming all diagnoses of mannotol-negative P. rettgeri and adonitol-negative P. alcalifaciens. Owing to the frequency of incorrect or low-confidence findings, it would be even better to carry out tube tests that have good separation values between species of the tribe Proteae whenever an isolate is thought to belong to this tribe. These tests, selected in accordance with those advocated by Farmer et al. (3), should be performed simultaneously with the tests already present in the miniaturized systems to avoid misidentification and a 24-h delay in obtaining high-confidence responses.

ACKNOWLEDGMENTS

We thank all those who sent us Providencia isolates, Giuseppe Satta for his helpful comments, and Anthony Steele for his help with the English version of this paper.

LITERATURE CITED


