Characterization of Indole-Positive *Proteus mirabilis*

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Thirteen indole-producing, swarming strains of *Proteus* were identified by additional biochemical testing as being *Proteus mirabilis*. These strains were characterized by 40 biochemical tests and by susceptibility testing to 11 antibiotics. All produced ornithine decarboxylase and were susceptible to members of the penicillin-cephalosporin groups of antibiotics. These indole-positive strains are similar to indole-negative *P. mirabilis* and are distinctly different from *P. vulgaris*. For greatest accuracy and to insure greatest clinical relevancy, *P. mirabilis* and *P. vulgaris* should be distinguished from one another in the laboratory by performing both the indole and ornithine decarboxylase tests.

*Proteus mirabilis*, the most frequently isolated member of its genus, is usually separated from other *Proteus* species in the clinical microbiology laboratory by its ability to swarm and its inability to produce indole. Correct identification of this organism is especially important because it is significantly more susceptible to the penicillin and cephalosporin antibiotics than the other three species of *Proteus*, which are characteristically indole positive. However, according to Ewing (2) some strains of *P. mirabilis* are indole positive whereas some strains of *P. vulgaris* are indole negative. Ewing also reports that almost all *P. mirabilis* strains produce ornithine decarboxylase but all strains of *P. vulgaris* are ornithine decarboxylase negative. The present study was carried out to see if ornithine decarboxylase production correlated better with the antibiotic susceptibility patterns of these two *Proteus* species than did indole production.

**MATERIALS AND METHODS**

The organisms studied were isolated from clinical specimens in the Diagnostic Microbiology Laboratory of the University of Minnesota Hospitals.

Thirty-four biochemical studies were performed as previously described (3). In addition, tests for tartrate and acetate utilization, production of corn oil lipase, hydrolysis of esculin, fermentation of erythritol, dulcitol, trehalose, and breakdown of alpha methyl glucoside were performed on the 13 indole-positive *P. mirabilis* strains by W. H. Ewing at the Center for Disease Control in Atlanta, Ga.

Antibiotic susceptibility testing was carried out by the high-content disc method of Bauer et al. (1) and also by the agar dilution method (4).

**RESULTS**

Swarming, H₂S-producing strains of *Proteus* which produced ornithine decarboxylase were designated as *P. mirabilis*, whereas those which were ornithine decarboxylase negative were called *P. vulgaris*. Thirteen indole-positive, ornithine decarboxylase-positive strains of *P. mirabilis* were found and confirmed as *P. mirabilis* by W. H. Ewing at the Center for Disease Control. The biochemical reactions of these organisms are shown in Table 1.

The antibiotic susceptibility of 13 strains each of indole-positive *P. mirabilis*, indole-negative *P. mirabilis, P. vulgaris, P. rettgeri*, and *P. morgani* to penicillin, ampicillin, cephalothin, and cephalexin is shown in Table 2. All strains of *P. mirabilis* were inhibited by 3.1 μg or less of ampicillin per ml, 12.5 μg or less of cephalothin per ml, 6.3 μg or less of cephaloridine per ml, and 12.5 units or less of penicillin per ml. The antibiotic susceptibility patterns of both indole-positive and indole-negative *P. mirabilis* were similar and distinctly different from the other *Proteus* species with respect to the penicillins and cephalosporins, as is evident in Table 2. The results of additional susceptibility testing of the two groups of *P. mirabilis* are shown in Table 3 and are obviously similar.
### TABLE 1. Forty biochemical reactions of 13 indole-positive Proteus mirabilis strains

| Test                              | No. positive |
|-----------------------------------|-------------|
| H₂S (TSI)                         | 13          |
| Oxidase                           | 0           |
| Nitrate                           | 13          |
| Methyl red (37 C)                  | 13          |
| (25 C)                            | 10          |
| Voges-Proskauer (37 C)            | 11          |
| (25 C)                            | 13          |
| Simmons citrate                   | 13          |
| Urease                            | 13          |
| Arginine dihydrolase              | 0           |
| Lysine decarboxylase              | 0           |
| Ornithine decarboxylase           | 13          |
| Phenylalanine deaminase           | 13          |
| Gelatin liquefaction              | 13          |
| Malonate                          | 0           |
| Mucate                            | 0           |
| Jordan’s tartrate                 | 13          |
| Corn oil lipase                   | 9           |
| Sodium acetate                    | 0           |
| Alpha methyl glucoside            | 0           |
| Esculin hydrolysis                | 0           |
| KCN                               | 13          |
| Adonitol                          | 0           |
| Arabinose                         | 0           |
| Cellobiose                        | 0           |
| Dulcitol                          | 0           |
| Glycerol                          | 13          |
| Glucose                           | 0           |
| Inositol                          | 0           |
| Lactose                           | 0           |
| Maltose                           | 0           |
| Mannitol                          | 0           |
| Raffinose                         | 7           |
| Rhamnose                          | 0           |
| Salicin                           | 0           |
| Sorbitol                          | 0           |
| Sucrose                           | 9           |
| Trehalose                         | 13          |
| Xylose                            | 13          |

* All strains were motile and swarming.

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**DISCUSSION**

The results of this study and the data of Ewing (2) indicate that *P. mirabilis* and *P. vulgaris* are more accurately differentiated by the use of the ornithine decarboxylase test than by a test for indole production. Ideally, both tests should be used. The value of species identification in this instance lies not only in the epidemiologic information obtained but primarily in the fact that *P. mirabilis* has a different pattern of susceptibility to the penicillins and cephalosporins. These antibiotics, because of their bactericidal action and low intrinsic toxicity, are usually the antibiotics of choice in *P. mirabilis* infections.

*P. mirabilis* is the most frequently isolated member of the *Proteus* genus and is also the *Proteus* species most often associated with infection. In our hospital, over an 8-year period, *P. mirabilis* was the organism isolated third most frequently from urinary tract infections, following *Escherichia coli* and *Klebsiella*, and comprised 10% of the organisms isolated in quantities of 100,000 colonies per ml or greater. The combined total of the three other *Proteus* species was only 2.7%. *P. mirabilis* was also the most common *Proteus* species isolated from blood culture specimens, again surpassing the total of the other three species. This information serves to emphasize the importance of *Proteus mirabilis* and to underline the desirability for its accurate identification.

In our work, we focused mainly on the differentiation of *P. mirabilis* from *P. vulgaris*, since these are the two swarming, H₂S-producing members of the genus. The identification of the small proportion of nonswarming, H₂S-negative strains of *P. mirabilis* is more difficult. These organisms could be confused with *P. morganii* (ornithine decarboxylase-positive) but would be differentiated by indole production. Moreover, the antibiotic susceptibility patterns of the *P. morganii* strains tested in this study were not consistent with *P. mirabilis*. A small number of strains of *P. rettgeri* (ornithine decarboxylase-negative) were sensitive to ampicillin, cephalothin, and cephaloridine.

In the literature, one frequently notes that authors have divided *Proteus* strains on the basis of indole production or nonproduction primarily because of the clinical significance of antibiotic susceptibility differences and because of the diagnostic accuracy for species identification. In view of the data presented here, this separation appears less than ideal. For greatest accuracy and to insure greatest clinical relevancy, *P. mirabilis* and *P. vulgaris* should be distinguished from one another in the laboratory by performing a minimum of two biochemical tests, namely the indole and ornithine decarboxylase tests.
TABLE 2. Agar dilution susceptibility results with Proteus species

| Antibiotic and organism | Minimum inhibitory concn* |
|-------------------------|---------------------------|
|                         | 0.8 | 1.6 | 3.1 | 6.3 | 12.5 | 25  | 50  | 100 | 200 |
| Penicillin              |     |     |     |     |      |     |     |     |     |
| *P. mirabilis* (indole +) | 1   | 10  | 2   |     |      |     |     |     |     |
| *P. mirabilis* (indole −) | 2   | 3   | 8   |     |      |     |     |     |     |
| *P. vulgaris*           |     |     |     |     |      |     |     |     |     |
| *P. rettgeri*           |     |     |     |     |      |     |     |     |     |
| *P. morganii*           |     |     |     |     |      |     |     |     |     |
| Ampicillin              |     |     |     |     |      |     |     |     |     |
| *P. mirabilis* (indole +) | 12  | 1   |     |     |      |     |     |     |     |
| *P. mirabilis* (indole −) | 2   | 4   | 7   |     |      |     |     |     |     |
| *P. vulgaris*           |     |     |     |     |      |     |     |     |     |
| *P. rettgeri*           |     |     |     |     |      |     |     |     |     |
| *P. morganii*           |     |     |     |     |      |     |     |     |     |
| Cephalothin             |     |     |     |     |      |     |     |     |     |
| *P. mirabilis* (indole +) | 2   | 11  |     |     |      |     |     |     |     |
| *P. mirabilis* (indole −) | 1   | 2   | 7   | 3   |      |     |     |     |     |
| *P. vulgaris*           |     |     |     |     |      |     |     |     |     |
| *P. rettgeri*           |     |     |     |     |      |     |     |     |     |
| *P. morganii*           |     |     |     |     |      |     |     |     |     |
| Cephaloridine           |     |     |     |     |      |     |     |     |     |
| *P. mirabilis* (indole +) | 13  |     |     |     |      |     |     |     |     |
| *P. mirabilis* (indole −) | 1   | 1   | 11  |     |      |     |     |     |     |
| *P. vulgaris*           |     |     |     |     |      |     |     |     |     |
| *P. rettgeri*           |     |     |     |     |      |     |     |     |     |
| *P. morganii*           |     |     |     |     |      |     |     |     |     |

* Strains (13) of each species were tested.

TABLE 3. Agar dilution susceptibility results with Proteus mirabilis

| Antibiotic* | Minimum inhibitory concn (µg/ml) |
|-------------|----------------------------------|
|             | 0.4  | 0.8  | 1.6  | 3.1  | 6.3  | 12.5 | 25  | 50  | 100 | 200 |
| Kanamycin   |      |      |      |      |      |      |     |     |     |     |
| I+ *P. mirabilis* | 12*  | 1    |      |      |      |      |     |     |     |     |
| I− *P. mirabilis* | 2    | 9    | 2    |      |      |      |     |     |     |     |
| Chloromycetin |      | 13   | 2    | 10   | 1    |      |     |     |     |     |
| Gentamicin  | I+   | 11   | 1    | 1    |      |      |     |     |     |     |
|             | I−   | 2    | 9    | 2    |      |      |     |     |     |     |
| Nitrofurantoin | I+  | 13   |      |      |      |      |     |     |     |     |
|             | I−   | 11   | 2    |      |      |      |     |     |     |     |
| Naladixic acid | I+  | 13   |      |      |      |      |     |     |     |     |
|             | I−   | 1    | 11   | 1    |      |      |     |     |     |     |
| Carbenicillin | I+  | 12   | 1    |      |      |      |     |     |     |     |
|             | I−   | 2    | 11   |      |      |      |     |     |     |     |
| Tetracycline | I+   | 1    | 12   |      |      |      |     |     |     |     |
|             | I−   | 2    | 10   | 1    |      |      |     |     |     |     |

* Strains (13) of each group were tested.

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