EDUCATIONAL COMMENTARY – PSEUDOMONAS AND PROTEUS IN URINE CULTURES

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

• Discuss the clinical significance of Pseudomonas and Proteus in urine cultures.
• Explain how to distinguish between Pseudomonas and Proteus.
• Explain how to differentiate swarming Proteus species.

Urinary tract infections (UTIs) are among the most common infections in humans, causing more than 6 million outpatient visits and 300,000 hospitalizations annually. In hospitalized patients, UTIs are a major source of gram-negative sepsis and nosocomial infections. Most UTIs are caused by Escherichia coli, but other bacteria are also frequently found, especially in complicated or hospital-acquired cases. Among these are Pseudomonas aeruginosa and 3 species of Proteus: P. mirabilis, P. vulgaris, and P. penneri.

P. aeruginosa and Proteus spp. all have virulence factors that enable them to cause significant disease in the urinary tract. P. aeruginosa produces exotoxin A, endotoxins, hemolysins, and proteolytic enzymes. It is resistant to many antibiotics. Proteus spp. easily adhere to kidney urothelium, which facilitates infection of the upper urinary tract. Proteus spp. also hydrolyze urea, which alters urine pH and stimulates production of kidney stones.

P. aeruginosa and the 3 Proteus species (P. mirabilis, P. vulgaris, and P. penneri) are all non-lactose fermenting gram-negative rods, but they can be quickly distinguished by colony morphology, characteristic odor, and the oxidase test (Table 1). The oxidase test is particularly useful in differentiating P. aeruginosa from Proteus spp. because P. aeruginosa is oxidase-positive, whereas Proteus spp. and other members of the family Enterobacteriaceae are oxidase-negative.
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Table 1. Distinguishing characteristics of *P. aeruginosa* and *Proteus* spp.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>P. aeruginosa</em></th>
<th><em>Proteus</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Usually flat and spreading with metallic sheen and serrated edges</td>
<td>Large, gray, and smooth; often displays swarming growth</td>
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<tr>
<td></td>
<td>Sometimes smooth, coliform, gelatinous, dwarf, or mucoid</td>
<td></td>
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<tr>
<td>Growth at 42°C</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Odor</td>
<td>Grape-like or corn tortilla-like</td>
<td>Foul; burned chocolate-like</td>
</tr>
<tr>
<td>Oxidase test</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>TSI agar reaction*</td>
<td>K/NC</td>
<td>A/A H₂S⁺ (P. vulgaris)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/A H₂S⁺/- (P. penneri)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K/A H₂S⁺ (P. mirabilis)</td>
</tr>
</tbody>
</table>

*K/NC = Alkaline slant over no change in the butt
A/A H₂S⁺ = Acid slant over acid butt with hydrogen sulfide production
A/A H₂S⁺/- = Acid slant over acid butt with variable hydrogen sulfide production
K/A H₂S⁺ = Alkaline slant over acid butt with hydrogen sulfide production

Identification of *Pseudomonas aeruginosa*

On blood agar, colonies of *P. aeruginosa* appear spreading and flat with serrated edges, a metallic pearlescent sheen, and confluent growth. They are often β-hemolytic, and they produce a characteristic grape-like or corn tortilla-like odor. Other colony forms also occur, including smooth, coliform, gelatinous, dwarf, and mucoid. Mucoid colonies are particularly prevalent in cystic fibrosis patients, but they are also seen in respiratory infections in patients with other chronic lung diseases and in UTIs in patients with indwelling catheters. Finally, since *P. aeruginosa* does not ferment lactose, it appears colorless on MacConkey agar.

Suspicious colonies may be identified as *P. aeruginosaby* commercial identification systems or by the following 4 tests:

- Oxidase-test positive
- Triple sugar iron (TSI) agar reaction of alkaline slant/no change in the butt
- Growth at 42°C
- Production of blue, blue-green, red, or brown pigment on Mueller-Hinton or trypticase soy agar
Identification of *Proteus* species

The 3 species of *Proteus* found in UTIs and other human infections—*P. mirabilis*, *P. vulgaris*, and (less commonly) *P. penneri*—are usually easily identified by characteristic colony morphology and 3 biochemical tests: oxidase, indole, and ornithine decarboxylase. Like *P. aeruginosa*, *Proteus* spp. do not ferment lactose, so colonies appear colorless on MacConkey agar. On blood agar, *Proteus* colonies appear large, gray, and smooth; and they produce a foul odor which some describe as “burned chocolate.” They often produce a highly characteristic thin film of growth over the agar surface, a phenomenon known as “swarming.” Swarming is a key identifying characteristic of these 3 species of *Proteus*.

The swarming growth seen with *Proteus* spp. may sometimes be confused with the spreading colony morphology typical of *P. aeruginosa*. The oxidase test will quickly distinguish these organisms, since *Proteus* spp. are oxidase-negative and *P. aeruginosa* is oxidase-positive.

*P. mirabilis*, *P. vulgaris*, and *P. penneri* can be differentiated by the spot indole and ornithine decarboxylase tests (Figure 1). *P. vulgaris* is indole-positive, whereas *P. mirabilis* and *P. penneri* are indole-negative. *P. mirabilis* is ornithine positive; *P. penneri* and *P. vulgaris* are ornithine-negative.

**Figure 1. Differentiation of swarming *Proteus* spp.**

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Spot indole positive?  yes  P. vulgaris

no

Ornithine decarboxylase positive?  yes  P. mirabilis

no

P. penneri
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Colonies which do not swarm must be identified by more extensive testing. This can be done with commercial systems (used by most laboratories) or by a series of biochemical tests based on the TSI agar reaction.

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Suggested Reading


