Pseudomonas aeruginosa

a microbiological aspect

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In clinical microbiology laboratories, most of the non-fermentative Gram-negative rods that are isolated from patients belong to the genus Pseudomonas. Of these, **P aeruginosa** accounts for about two thirds of these isolates.[1] Species other than **Pseudomonas aeruginosa** (**P aeruginosa**) that are primarily environmental organisms are sometimes called pseudomonads. This term has no taxonomic value.

The *Pseudomonaceae* are short, unicellular, straight or slightly curved, Gram-negative rods that measure 0.5 to 1 micrometer (µm) by 1.5 to 4 µm. They do not form spores. When motile, they have one or more polar flagella that are visible with the light microscope only when special stains are used; the flagella are unsheathed in most species (Figure 1). Isolates obtained from clinical specimens frequently contain pili that promote the attachment of the organism to the host cell. With light microscopy, the cells do not show any unusual features except for those species that accumulate poly-beta hydroxybutyrate in granules.[2] These granules can be seen with a phase microscope or by staining with Sudan black. With electron microscopy, it is evident that these bacteria have a typical multilayered cell wall of Gram-negative bacteria, similar in appearance to the cell wall of *Enterobacteriaceae*. Like the enteric bacteria, the *P aeruginosa* cell wall lipopolysaccharide (LPS) is composed of core polysaccharides that are common to all strains and side-chain polysaccharides that are strain specific.[3]

In addition, with proper fixation, an outer slime layer can be detected surrounding individual cells and sometimes encasing multiple cells, creating a microcolony. This layer has been referred to as the glycocalyx or mucoid substance layer and is composed of alginate, an anionic polymer of mannuronic acid and L-guluronic acid in which some of the mannuronate residues are mono-O-acetylated and others are di-O-acetylated.[4]

Clinical morphology varies so widely from species to species that, even within a single species, it is impossible to generalize about the appearance of *Pseudomonas* colonies on agar. All species of *Pseudomonas* grow well on simple nutrient agars and on common selective media, such as EMB and McConkey's agars. The colonial morphology of *P aeruginosa* is quite diverse. Phillips described six colony types on nutrient agar:[5] 1. flat irregular edges, a gray-green metal sheen, with or without a pocked surface; 2. raised, smooth, with entire edges resembling Enterobacteriaceae colonies; 3. raised, rough; 4. rugous; 5. dwarf; and 6. mucoid. Types one and two account for 90% of isolates. Mucoid strains do not usually maintain this form when passed in vitro and turn into type-one colonies when passed onto standard media.

Pyocyanin is a distinctive blue pigment produced by most strains of *P aeruginosa*. It is not fluorescent. Pyoverdin is a yellow, fluorescent pigment produced by most strains of *P aeruginosa*. Strains of *P aeruginosa* that make both pyoverdin and pyocyanin are common and stain the agar green. About 5% of strains of *P aeruginosa* make rust-red pigments (Figure 2).[5]

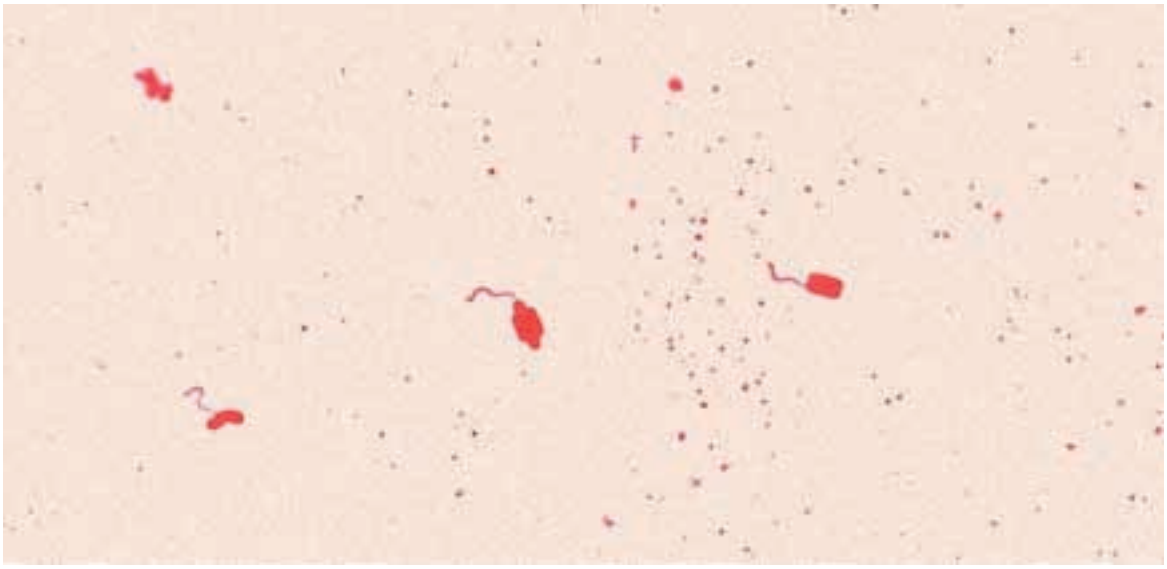

PHOTO: CDC's and the Public Health Image Library, available at phil.cdc.gov/Phil/default.asp

FIGURE 1

Pseudomonas mendocina with flagella

Metabolism

P aeruginosa can grow anaerobically utilizing nitrate as the terminal electron receptor in the presence of certain carbon compounds. To carry out nitrate respiration, nitrate and nitrate reductases are included in the presence of nitrate and the absence of oxygen.[6] The growth requirements for most species of *Pseudomonas* are extremely simple. They will grow in aqueous solutions of mineral salts with ammonium ions as the only nitrogen source and can use a variety of simple organic compounds as energy sources. They will utilize carbohydrates, alcohols, saturated and unsaturated fatty acids, amino acids, amines, and amides. Not all species can utilize all these different substrates. Differences in ability to utilize these organic compounds are useful taxonomic features.

P aeruginosa utilizes the Entner-Douderoff pathway to metabolize hexoses to intermediates that enter the tricarboxylic acid cycle (TCA). The TCA cycle is central to both catabolism and biosynthesis by *P aeruginosa.* Most species of *Pseudomonas* produce extracellular enzymes that degrade macromolecules, a process that is important to nutrition. *P aeruginosa* secretes several proteinases and phospholipase C (the hemolysin).

Pathogenic properties

P aeruginosa is not a successful parasite, but it is a virulent opportunistic pathogen. *Pseudomonas* infections occur almost exclusively in hospitalized patients with severely lowered host resistance. *P aeruginosa* produces infection by virtue of its ability to invade damaged natural barriers. Once having gained entrance into the tissue, the organism exhibits a peculiar tropism for blood vessels that it invades from the adventitial side, producing medical necrosis and thrombosis. This tropism has not been explained. *P aeruginosa* infections are characterized by fever and *Pseudomonas* bacteremia may be complicated by septic shock. It is likely that the organisms' LPS is responsible for these manifestations.

In addition to its potential endotoxin, *P aeruginosa* synthesizes a variety of exotoxins that also contribute to virulence. Exotoxin A is the best characterized of these toxins. It is a protoxin with two important subunits. One subunit is responsible for binding to the host cell membrane; the other enzymatically catalyzes the transfer of ADP-ribosyl from NAD to elongation factor 2.[7] Although exotoxin A and diphtheria toxin both produce the same metabolic effect, there are major differences between the infections. *P aeruginosa* is locally and systemically invasive, while *C diphtheria* alone results in neuritis or myocarditis. Unlike *diphtheria,* antibody to exotoxin A does not completely prevent disease. Other exoenzymes that may function as exotoxins are made by *P aeruginosa.* These

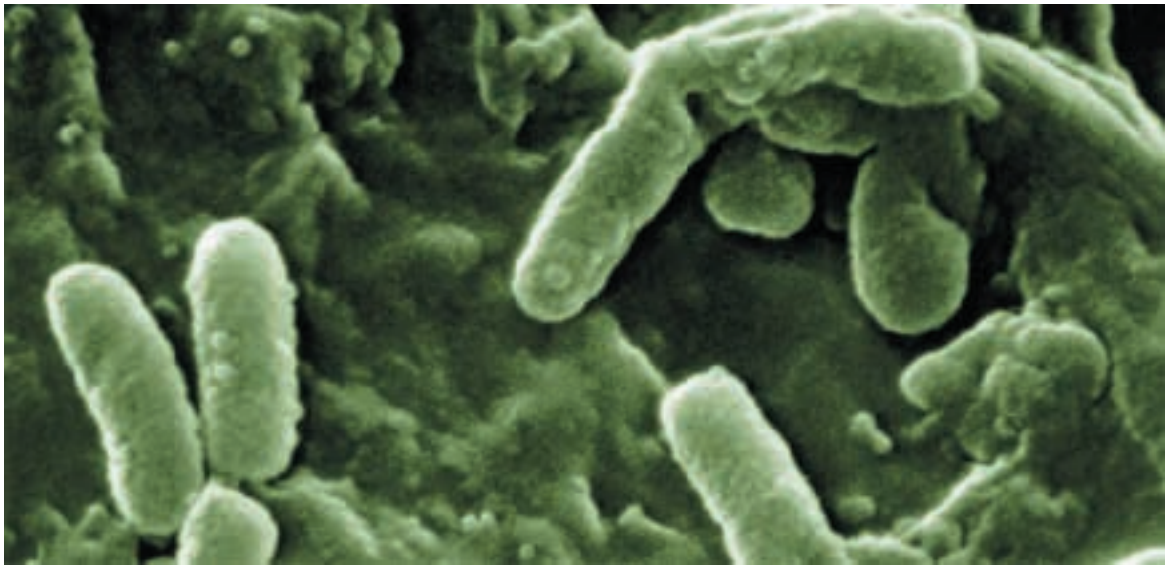

PHOTO: CDC's and the Public Health Image Library, available at *phil.cdc.gov/PHIL/default.asp*

FIGURE 2

Pseudomonas aeruginosa.

Pseudomonas Vocabulary

Acetyl — Acid molecule from which the hydroxyl group has been removed.

ADP — Adenosine 5c-diphosphate.

Alginate — Irreversible hydrocolloid consisting of salts of alginic acid, a colloidal acid polysaccharide obtained from seaweed and composed of mannuronic acid residues.

Antisera — Serum containing an antibody or antibodies specific for one or more antigens.

Arginine — Dibasic amino acid that occurs among the hydrolysis products of proteins.

Hydrolase — Hydrolyzing enzyme.

Bacteremia — Presence of viable bacteria in the circulating blood.

Catabolism — The breaking down in the body of complex chemical compounds into simpler ones (eg, glycogen to CO_2 and H_2O), often accompanied by the liberation of energy.

DNAse (Also DNAase and Dnase) — Abbreviations for deoxyribonuclease.

Elastase — A serine proteinase hydrolyzing elastin.

Elongation factor — Proteins that catalyze the elongation of peptide chains during protein biosynthesis.

Endotoxin — Bacterial toxin not freely liberated into the surrounding medium.

Entner-Douderoff pathway — A degradative pathway for carbohydrates in certain microorganisms (eg, *Pseudomonas* sp.) that lack hexokinase, phosphofructokinase, and glyceraldehyde-3-phosphate dehydrogenase.

Enzootic — Denoting a temporal pattern of disease occurrence in a population in which the disease occurs with predictable regularity with only relatively minor fluctuations in its frequency over time.

Glycocalyx — Filamentous coating on the apical surface of certain epithelial cells.

Hexoses — A simple carbohydrate (monosaccharide) group that includes glucose, fructose, and galactose.

Lysogeny — Triggering of the lytic cycle that may occur spontaneously or be caused by certain agents and results in production of the bacteriophage and lysis of the bacterial cell.

Maltose — Disaccharide consisting of two D-glucose residues and bound by the 1,4-)-glycoside link.

Mannuronic acid — Uronic acid derived from the oxidation of mannose, a component of alginic acid.

Nicotinamide adenine dinucleotide (NAD) — Ribosylnicotinamide 5c-phosphate (NMN) and adenosine 5c-phosphate (AMP) linked by phosphoanhydride linkage between the two phosphoric groups.

Phospholipase C — Enzyme that catalyzes the hydrolysis of phosphatidylcholine to produce choline phosphate and 1,2-diacylglycerol.

Pili — Bacterial hair that helps the organism move or attach to a host.

Polysaccharides — Often found in the bacterial wall, these carbohydrates act as an antigen.

Protease — Enzyme that breaks down peptide bonds in protein to transform it into amino acids.

Proteinase — Enzyme that encourages the breakdown of protein.

Pyocin — Bacteriocin produced by strains of *Pseudomonas*.

Ribosyl — Covalent attachment of one or more ribosyl groups to a molecule (usually a macromolecule), as in ADP ribosylation.

Schema — An outline or arrangement.

Tropism — Attraction (growth or movement toward a stimuli).

include phospholipase C, elastase, and two other broad-spectrum proteases. These enzymes may contribute to vascular necrosis, and local tissue destruction, especially ocular and pulmonary damage. When tested in a model of naturally occurring *P aeruginosa* infections in minks, immunization with a vaccine, made from toxoids of the elastase and protease, increased the survival of the immunized minks during an enzootic of pneumonia.[5]

Antigenic structure

Several sets of antisera have been developed for stereotyping *P aeruginosa*. The Fisher scheme is probably based on antibody to LPS and distinguishes seven stereotypes. The Homma scheme distinguishes 18 stereotypes. There is no correspondence between the two schemas and neither set of sera reacts with all clinical isolates. Pyocins produced by *P aeruginosa* are also antigenic and immune serum neutralizes the bactericidal effect of the pyocin.

Genetics

Gene transfer between *P aeruginosa* strains can occur through conjugation, transduction, and transformation.[2] Strain differences can be detected by serological typing of the O antigen, phage typing, and pyocin typing. Lysogeny is common, and most strains are lysogenic for at least one prophage. Comparisons of the genetic maps of pseudomonads to that of the enteric bacteria demonstrate fundamental differences in the arrangement of functionally related genes. The gene arrangement of the pseudomonads is not contiguous, whereas the enterics have a contiguous arrangement of similar genes.

Laboratory diagnosis

P aeruginosa is usually easily identified. Colonies have a characteristic fruity odor, colonial morphology, and extracellular pigment. Motile, nonfermentative, oxidase-positive bacteria that grow at 42° C and have a characteristic antibiotic susceptibility pattern can usually be identified as *P aeruginosa* if they produce an extra cellular pigment. Nonpigmented, polymyxin resistant strains may be harder to identify. They should be tested for gluconate and maltose oxidation, arginine dihydrolase and DNAse activity.

Conclusion

Pseudomonas aeruginosa is the epitome of an opportunistic human pathogen. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect, if the tissue defenses are compromised in some manner. *Pseudomonas aeruginosa* is a bacterial pathogen responsible for 20% of nosocomial infections. Individuals with severe burns, leukemia, and cystic fibrosis are at particularly high risk for *Pseudomonas* infection.

About the author

Jeffrey J Cortese, has been a certified surgical technologist for five years and worked at St Joseph Mercy of Macomb Hospital in Clinton Township, MI. He is also a student at Oakland University where he is working on a bachelor's degree in biology specializing in anatomy.

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