SIM tubes:
Organisms which are motile will exhibit diffuse growth that spreads out from the initial stab line, whereas organisms which are not motile will only grow where the agar was initially stabbed.

Dr. James Smith
USDA Emeritus Scientist

- James L. Smith received a BA (1952) and MA (1954) in Bacteriology, and a PhD in Microbiology (1962) from Indiana University, Bloomington, Indiana
- As a Medical Microbiologist at Fort Detrick, Maryland (1954-1959), he worked on the pathogenicity of the plague bacterium (*Pasteurella pestis*, now known as *Yersinia pestis*).
- He worked as a Microbiologist at the Agricultural Research Center in Beltsville, Maryland from 1963 to 1971
- He moved to Philadelphia and worked at the Eastern Regional Research Center where he studied the microbiology of meat fermentations and microbial food safety of meat products.
- In 1990, he retired and is now a USDA collaborating scientist at the Eastern Regional Research Center. He currently writes review articles and book chapters on microbial food safety subjects and has co-edited three books dealing with the pathogenicity of microorganisms. Dr. Smith also reviews manuscripts and acts as a mentor for many scientists at ERRC.

Dr. James Smith
“Smitty”
Born Dec. 23, 1928 and still going strong
The Genus *Pseudomonas*

- "*Pseudomonads*" are adaptable environmental opportunistic gram-negative bacilli
  - ubiquitous in soil, water, & organic matter as they can utilise organic compounds as sole source of carbon & energy
  - strict aerobes, oxidase +ve, catalase +ve, non-spore-forming, motile with polar flagella
- Most important species: *P. aeruginosa* (& also *P. fluorescens*)
Q. How do we identify the bug, *P. aeruginosa*?

- Blood agar: several colony types - small/rough; smooth/large, +/- hemolytic; gunmetal sheen; or very mucoid
- Grows on MacConkey agar as a nonlactose fermenter (NLF) = pale colourless colonies
- (like *Proteus* and *Salmonella*, but remember it is oxidase-positivel) Has “grape-like”/“fruity” odor or a corn taco odor
- Metallic sheen
- Produces pigments: pyocanin (blue), pyoverdin (yellow), pyorubin (red), pyomelanin (brown)
- Pyocanin causes “blue/green pus” seen in abscesses, wounds & wound dressings (bad prognosis)

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*P. aeruginosa*: a versatile opportunistic pathogen

“*The organism is the epitome of an opportunistic pathogen, because it rarely infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the host defenses are compromised*”

(Songer & Post text, page 155)

- **Not** commonly found as normal flora in mucosal membranes of healthy animals

- *P. aeruginosa* has talent, and is just waiting for a chance, but needs some help...
**Pseudomonas aeruginosa** - has lots of virulence factors

- Pili and flagella
- Capsule
- LPS
- Exotoxin A (a cytotoxin)
- Elastase & proteases, phospholipase C
- Iron-acquiring siderophores
- Ramnolipid - hemolysin and cytotoxin
- Type III secretory system - delivers four cytotoxic proteins (Exotoxins S,T,U,Y)

**P. aeruginosa has lots of virulence factors, yet it is not a facultative intracellular pathogen!**

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**Q.** - What conditions predispose to *P. aeruginosa* infections?

- *P. aeruginosa* needs help to breach host defenses.
- This occurs mainly by disruption of the protective normal flora, which allows *P. aeruginosa* to adhere, colonise, and invade

**Examples:**

- Systemic or topical treatment with antimicrobial drugs
- Use of topical disinfectants
- Skin trauma
- Burns
- Invasive procedures such as catheters, surgery
- Immunosuppression from corticosteroid treatment, chemotherapy (cystic fibrosis in humans)
Q. Why is *P. aeruginosa* also a nosocomial pathogen?

- A nosocomial pathogen, because:
  - it can survive in the hospital environment, and thrives in wet, poorly aerated environments
  - is multi-drug resistant: resistant to commonly used drugs, including beta-lactam drugs
  - forms biofilms on equipment (endoscopes) & medical devices (endotracheal tubes, catheters, hoses)
  - Survives in solutions - antimicrobial drugs and disinfectant solutions, mastitis preparations, irrigation fluids, hydrotherapy baths, semen extenders.....

*P. aeruginosa* - Quorum sensing by bacteria

- **Quorum sensing:**
  - bacterial cells talk to each other through chemical signals, (acyl homoserine lactones - AHLs)
  - coordinates the production of several virulence factors, important in acute infection,
  - (ex. *P. aeruginosa* Exotoxin A, rhamnolipid, elastase, proteases)
  - coordinates biofilm formation
**BIOFILMS - Life in Slime City**

Q. Why are biofilms so important?

- Bacterial growth on tissues or surfaces results in production of a slime-like mucoid polysaccharide called alginate, which is secreted into the extracellular environment - viscous gel that allows the safe growth of microcolonies = biofilms

- **Biofilms are complex bacterial communities**, adhering to a surface, such as rocks in streams, (ex. oil & water pipes), plastics, medical implant materials, & tissues such as in lungs of humans with cystic fibrosis and in the bladder of catheterized patients

- **Biofilms interfere with host defenses** by direct physical and chemical barrier against phagocytes: interfering with phagocytosis, stimulating production of inflammatory cytokines (IL-1 & 8, TNF)

- **Biofilms reduce susceptibility to antimicrobial agents:**
  - The matrix of the biofilm blocks penetration of antibiotics
  - Also cells within the biofilm have reduced metabolic activity, making them less susceptible to certain antibiotics

  “The rule of thumb is that 1,500 times more of an antimicrobial agent is needed to kill a biofilm than a planktonic bacteria.” —William Costerton

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**Cell to Cell Communication in a Biofilm.**

In the cartoon, various species of bacteria are represented by different colors. Bacteria can produce chemical signals (“talk”) and other bacteria can respond to them (“listen”) in a process commonly known as cell-cell communication or cell-cell signaling. This communication can result in coordinated behavior of microbial populations.

http://biofilmbook.hypertextbookshop.com/public_version/contents/chapters/chapter001/section004/green/page001.html

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**Environmental Health Perspectives**, Volume 106, Number 12, December 1998

Many bacteria can develop into sessile biofilms, consisting of numerous bacterial cells attached to a surface and embedded within a self-produced matrix material. Quorum sensing is involved during the maturation of *Pseudomonas aeruginosa* biofilms, which causes chronic infections in cystic fibrosis patients’ lungs.

P. aeruginosa
Can cause acute or chronic disease

- **Acute infection** with high production of extracellular virulence factors coordinated by quorum sensing
  - tissue damage, dissemination, systemic inflammation, endotoxic shock, organ failure, death

  **OR**

- **Chronic infection** with low production of extracellular virulence factors, production of alginate, biofilm mode of growth, less inflammation

- The factors needed for acute infections are generally well understood, whereas those needed for chronic infection are not.

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Q. Why does indiscriminate use of antimicrobial drugs select for P. aeruginosa infection?

- Antimicrobial treatment wipes out sensitive normal flora bacteria

- *P. aeruginosa* has **intrinsic resistance** to many antibiotics due to permeability barriers: capsule, LPS, cell wall "efflux pumps", alginate of biofilm

- **Resistant to**: penicillin, ampicillin, tetracycline, older cephalosporins, Convenia, macrolides & aminoglycosides (neomycin, streptomycin), chloramphenicol, trimethoprim-sulfa

- **Sensitive to**: aminoglycosides (amikacin, gentamicin), some newer cephalosporins (ceftazidime), fluoroquinolones, ticarcillin, polymyxin B

  (Note: *in vitro* prediction of susceptibility to topical antibiotics is unreliable)
Q. How can we prevent *P. aeruginosa* infections?

- By reducing its numbers in the relevant environment (animal holding areas, equipment, fluids, feed preparation areas).
- Limit use of antimicrobials & use wisely

Q. What diseases does this bug *P. aeruginosa* cause?

- *P. aeruginosa* causes disease in many animal species - wound & burn infections, urinary tract infections, septicemia, keratitis, abscesses, granulomas, botryomycosis (botryomycosis=granulomas)

(See the list of diseases in S&P, page 155)

Why are these animals at risk for opportunistic *P. aeruginosa* infections?

- Sheep in warm, rainy weather...
- Mink in mink farms...
- Horses racing on a muddy track...
- Water dogs...
- Burn wounds...
A few examples of *P. aeruginosa* diseases in animals

**Poultry** (chickens, turkeys) - embryo mortality

**Sheep** - pneumonia, mastitis, fleece rot = “green wool disease” a dermatitis with microabscesses, predisposed by wetting of fleece, high humidity, fleece becomes bluish green, causes downgrading of wool.

**Mink** - sporadic outbreaks of hemorrhagic pneumonia/ septicemia, high mortality

**Pet reptiles** - necrotic stomatitis = “mouth rot” /“canker mouth”

**Dog, cats** - chronic otitis externa, urinary tract infection (UTI), ulcerative keratitis, pneumonia

**Cattle** - herd mastitis problems from contaminated intramammary antibiotic infusions, teat-dipping solutions, udder wash water

**Horses** - metritis (from antibiotic infusions decreasing normal vaginal flora); abortion, ulcerative keratitis, botryomycosis

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**Pseudomonas aeruginosa: Diseases in humans**

- **Humans** - wounds, UTI, pneumonia, dermatitis, swimmer's ear, septicemia
- **A nosocomial pathogen**
  - intensive care, severe burns, leukemia or other cancers, cystic fibrosis, AIDS.
The genus *Burkholderia* (previously *Pseudomonas*)

- Aerobic, gram-negative rods, (MacConkey growth positive, except *B. mallei*) non-spore-forming, oxidase-positive (30+ species)
- Three species of interest to us as veterinary clinicians and diagnosticians:
  - *B. cepacia* - a multi-drug nosocomial opportunist
  - *B. mallei* and *B. pseudomallei* - both animal pathogens & cause serious infections in humans
  - *B. mallei* is nonmotile/doesn’t grow at 42°C, *B. pseudomallei* is motile/grows at 42°C.

*(Note: S&P text is wrong, page 161 - *B. mallei* doesn’t grow on MAC, but who cares?)*

*Burkholderia cepacia*:
Problems associated with preparing clinic disinfectant solutions

- *B. cepacia* is an opportunistic pathogen of immunocompromised humans (rarely animals)
- Tends to be highly resistant to antibiotics and can survive in disinfectants → nosocomial infections (just like *P. aeruginosa* and others...)

  **Case example:**
  *Burkholderia cepacia* was isolated in large numbers, pure culture from a skin punch biopsy of a dog with chronic dermatitis. The isolate was resistant to 11/13 antimicrobial tested.
  Follow-up testing of disinfectant solutions used in the veterinary hospital resulted in recovery of this same isolate from several preparations.
  Months later, another skin culture submission from the same hospital resulted in *B. cepacia* isolation.
  Follow-up discussion revealed that tap water was being used to prepare disinfectant solutions in the clinic.
  This tap water was most likely the source of the *B. cepacia*.

* A recent study: Murphy CP et al. The prevalence of bacterial contamination of surgical cold sterile solutions from community companion animal veterinary practices in southern Ontario. CVJ 2010;51:634-636.
The two IMPORTANT and EXTRAORDINARILY DANGEROUS
Burkholderia species - B. mallei and B. pseudomallei

- *B. mallei* is a host-adapted deletion clone of the versatile soil organism *B. pseudomallei*
- *B. mallei* has evolved as an obligate pathogen of horses, mules, and donkeys
- *B. mallei* became non-motile, while *B. pseudomallei* is motile
- Special pathogens – both have two circular chromosomes.

*B. mallei* and *B. pseudomallei* are biosafety level 3 organisms & Category B bioterrorism agents (aerosol infection and low infectious dose).

Q. What do we need to know about this bug *B. mallei* as veterinary clinicians and diagnosticians?

- *B. mallei* causes the disease “glanders”
- Glanders was once a widespread disease of equids (horses, mules, donkeys), one of the oldest disease known, described by Aristotle

Glanders has been eliminated from Canada & USA by government test-and-slaughter programs

- Is a reportable foreign animal disease in Canada and USA
- (Historical review article: Derbyshire, JB. The eradication of glanders in Canada. CVJ. 2002;43:722-726)

- BUT – it still occurs in Middle East, Philippines, S.E Europe, Central & S. America
- Can’t survive in environment (only on infected horse)
  - susceptible nonequids are infected by contact or consumption
  - cats, dogs, goats, camels, sheep, & humans (a zoonotic disease)
  - but not swine or cattle
- Recovered animals → latent infections, → source of infection for animals & humans
**B. mallei and glanders**

An INVASIVE Pathogen:
- Penetrates pharyngeal & intestinal mucosa ➔ lymphatics ➔ blood, localises in lymphatics of respiratory tract and skin

- **Nodular lesions ➔ pyogranulomas**
  - Nasal, pulmonary & cutaneous forms, can all occur simultaneously
  - Cutaneous form called "Farcy" nodular lesions ➔ superficial nodules ulcerate & discharge infectious exudate

- **Acute disease** (more common in mules/donkeys) - fatal in days

  - OR

- **Chronic disease** (particularly in horses) - purulent nasal discharge, fever, dyspnea, nodules on upper respiratory tract, submaxillary lymphadenopathy, nodules and ulcers along cutaneous lymphatics, fever, weight loss

- Human glanders: A NASTY potentially fatal acute localised infection, or septicemia, or pulmonary or chronic cutaneous infection, high mortality rate (50%) even with antibiotic treatment.

**B. mallei and glanders:**

Not in Canada or USA, but still occurs in other countries

- Glanders - purulent nasal discharge

- Glanders - "farcy" lesions ➔ chains of ulcerated lymphatic nodules
B. mallei as a biological warfare agent

- Glanders was one of the first agents to be used for biological warfare in the modern era - The American Civil War, WW I, WW II
- During World War I, German agents targeted horses and livestock in the United States, Romania, Spain, Norway, and Argentina for infection with glanders through inoculation and feed contamination. The infamous Japanese Unit 731 utilized glanders in its experiments during World War II, and the Soviet Union has been alleged to have employed glanders in its occupation of Afghanistan in the 1980s. Of note, the organism cannot persist in the environment outside its host (unlike B. pseudomallei—the agent of melioidosis), which potentially limits its usefulness as a bioweapon.

Q. What do clinicians need to know about glanders as a foreign animal disease?

- Importation of horses into Canada & USA requires official serodiagnostic testing
- Serology: Complement fixation test (cross reacts with P. pseudomallei)
- "Mallein test", lysate injected intrapalpebrally (standard field test)
  - Type IV cell mediated hypersensitivity (IgE/Mast Cells/ T cell) ➔ lid edema, lacrimation, photophobia, purulent discharge, pain
- Antibiotic treatment is inappropriate, equine import reactors destroyed; no vaccine
B. pseudomallei: Where does this bug live? In the mud!

- A saprophyte in soil/water (possibly able to live inside a free-living amoeba = endosymbiont)
- A sapronotic pathogen, not a zoonotic disease like glanders
- "Sapronoses" (saprozoanoses) = Greek "sapros" = decaying; "sapron" means a decaying organic substrate
- Sapronoses are human diseases transmissible from the abiotic (nonliving) environment (soil, water, decaying plants)
- Like B. mallei it has two chromosomes, one for housekeeping genes, one for adaptability/survival in different environments, for its saprophytic and parasitic lifestyle
- Occurs in tropics: South East Asia & Northern Australia
- Sporadic in other countries: USA (Georgia & Hawaii), France, China, Africa, India, Middle East, Caribbean, Central & South America.
- Not in Canada:
  - But is only an "Immediately Notifiable Disease" for laboratories
  - No control or eradication program.

Q. What do we need to know about melioidosis?

- Melioidosis is a NASTY potentially fatal pyogranulomatous disease resembling glanders
  - Like tuberculosis, it can remain dormant in host without symptoms for years, because it can survive in macrophages
  - Infection by ingestion of water, inhalation of dust, soil contamination of cuts or wounds (rice-paddies), carnivores eating infected carcasses, exposure to dead infected animals
  - Causes disease in horses, sheep*, goats*, pigs*, dogs, cats, rodents, humans and non-human primates, and a lot of other species. (Versus cattle, water buffalo, & crocodiles are considered resistant, although infection in cattle has been reported) (For a review on melioidosis in animals see Sprague LD and H Neubauer. J Vet Med 2004; B51:305-320)
  - Mainly causes chronic disease - pneumonia, arthritis, mastitis, CNS infection, epididymitis, dermal abscesses, abortion
  - Animals are not treated with antibiotics

Cattle, water buffalo, and crocodiles live in the mud, but are resistant to melioidosis.
Human infection with *B. pseudomallei*

**Human melioidosis** (Whitmore's disease):

- Predisposed by immunodeficiency, diabetes mellitus, renal failure, chronic lung disease
- High mortality rate, as high as 80%
- Often misdiagnosed, and is a chronic latent infection which reactivates into full-blown illness (one documented case of latent infection becoming clinical > 60 years from exposure during WW II)
- Resistant to many antibiotics, requires prolonged treatment, still can relapse.
- Therapy for culture-confirmed melioidosis consists of an intensive phase of intravenous antimicrobial agents (ceftazidime is drug of choice) that is required for a minimum of 10-14 days, followed by an eradication phase of oral antimicrobial agents required for a 12-20 week course of treatment, or longer if clinically indicated.

**Travel to endemic areas is a risk for *B. pseudomallei* infection!**

- Ex. military dogs during Vietnam war
- Still a problem in Vietnam veterans

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**B. pseudomallei - melioidosis in humans**

- The most severe clinical picture is melioidosis septic shock, which is often associated with bacterial dissemination to distant sites such as the lungs, liver and spleen. The lungs are the most commonly affected organ in adults, where there can be a localized or disseminated pulmonary infection, abscess formation or empyema. Chronic lung disease can also occur and can be difficult to distinguish from pulmonary tuberculosis. The clinical features of the disease in Thailand and Northern Australia (where most cases are reported) are largely shared, but there are some striking differences. Acute suppurative parotitis is the presenting feature in one-third of Thai paediatric cases but is uncommon in Australia; conversely, prostatic abscesses and brainstem encephalitis are more frequent in Australia.

- Pictures courtesy of Dr Wirongrong Chierakul, Wellcome Trust, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. CNS, central nervous system.
Melioidosis - the legacy of Vietnam