

# **Pseudomonas and clinically important aerobic non-fermenters**

Dr Nadisha Badanasinghe  
Dept. of Medical Microbiology

# Aerobic non fermenters

- *Pseudomonas aeruginosa*
- *Burkholderia spp.*
  - *Burkholderia cepacia*
  - *Burkholderia pseudomallei*
- *Stenotrophomonas maltophilia*
- *Acinetobacter baumannii*

# Aerobic non fermenters

- Oxidative gram-negative bacilli
- Produce acid from glucose or other carbohydrates only in the presence of oxygen (nonfermenters).
  - NOTE: Enterobacteriaceae, Aeromonas and Vibrio are fermentative and can utilize carbohydrates in the absence of oxygen.
- Ubiquitous
- Opportunistic pathogens
- Obligate aerobes
- Slow growers

# Pseudomonas spp.

- Belongs to the family Pseudomonadaceae
- Containing 211 validly described species
- **Motile** by means of one or more polar flagella
- Have a very **strict aerobic** respiratory metabolism with oxygen
- Most species are **oxidase positive** and catalase positive

# *Pseudomonas aeruginosa*

- Non fermenting, non capsulated, aerobic, motile, gram negative bacilli
- Meaning of “Pseudomonas”
  - Greek *pseudo* ('false') and *monas* ('a single unit').
- Meaning of Aeruginosa
  - Latin word for *copper rust*, as seen with the oxidized copper patina on the Statue of Liberty.
  - describes the blue-green bacterial pigment seen in laboratory cultures
- Grow in moist environments and hospital equipment, disinfectants and antiseptics



# Culture characteristics

- Grow in ordinary media
- **Non-lactose fermenter (NLF)** – on MacConkey
- **Positive oxidase test and catalase test**
- **Blue-green pigment in cultures/ pus samples**
  - combination of two metabolites, pyocyanin (blue- only in *P. aeruginosa*) and pyoverdine (yellow)
- **Sweet grape like smell in cultures**

# Other characteristics

- *Adaptability*
- *Innate resistance to antibiotics and antiseptics*
- *Virulent factors*
  - Increasingly recognized as an emerging opportunistic pathogen
  - antibiotic resistance is increasing in clinical isolates
  - One in ten hospital-acquired infections are from *Pseudomonas*

# Virulent factors

- Exotoxin A (=to diphtheria toxin)
- Pyocyanin
- Proteases, phospholypases
- Exopolysaccharides (slime layer)- biofilm formation
- Quorum sensing - cell-cell communication



# SDL

- Biofilms
- Quorum sensing

# Habitat

- Found in soil, water, most man-made environments, hospital environment and disinfectants
- Commensal in skin and GIT
- Can colonize many natural and artificial environments (hospital equipment)
- Infect damaged tissues or people with reduced immunity (opportunistic)
- Can survive in pharmaceuticals (antibiotics, ophthalmic solutions, creams ), hospital devices (catheters)

# Clinical presentations

- Respiratory tract-
  - Commonly cause pneumonia in patients on ventilators in the ICU-ventilator associated pneumonias (VAP)
  - pneumonia in cystic fibrosis patients
  - rarely cause community-acquired pneumonias
- Urinary tract
  - catheter related/ Hospital acquired
- Infection of burn wounds- commonest cause
- Otitis externa – commonest cause

# Lab diagnosis

- Specimen- mid stream urine/ catheter urine, blood, pus, sputum
- Gram stain- GNB with no particular arrangement
- Culture –
  - BA – flat colonies (pigment is poorly observed), with a metallic sheen
  - MacConkey - colorless colonies (non lactose fermentor)
  - Nutrient agar- Blue-green pigment
  - Has a characteristic fruity smell
  - KIA- no reaction
- Identification – oxidase+ , blue green pigment



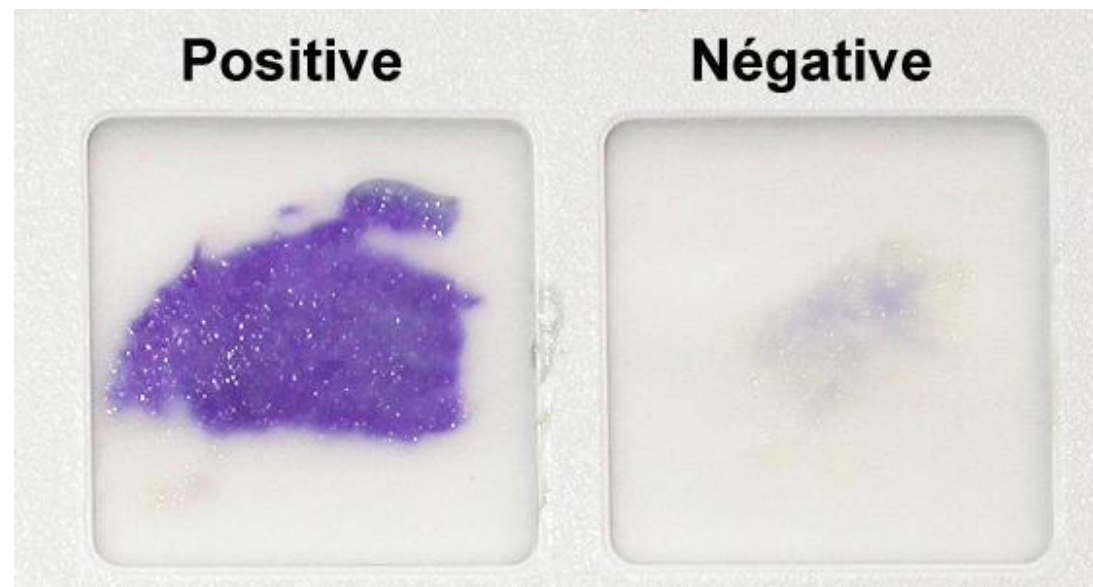
*Pseudomonas aeruginosa*



Photo by Tom Buchanan



Suryabrata Banerjee 2014



# Treatment

- Naturally resistant to a large range of antibiotics  
(Intrinsic resistance)
- Additional resistance after unsuccessful treatment  
(Acquired resistance)



# *Mechanisms of Antibiotic Resistance in Pseudomonas aeruginosa*

Antibiotic	Resistance Mechanisms
$\beta$ -lactams	$\beta$ -lactamase hydrolysis, decreased permeability, altered binding proteins
Aminoglycosides	Enzymatic hydrolysis by acetylation, adenylation, or phosphorylation; decreased permeability; altered ribosomal target
Chloramphenicol	Enzymatic hydrolysis by acetyltransferase; decreased permeability
Fluoroquinolones	Altered target (DNA gyrase); decreased permeability

# Anti-Pseudomonal antibiotics

- Aminoglycosides (gentamicin, amikacin)
- Quinolones (ciprofloxacin and levofloxacin but *not* moxifloxacin)
- Cephalosporins (ceftazidime, cefepime, cefpirome, but *not* cefuroxime, ceftriaxone, cefotaxime)
- Ureidopenicillins and carboxypenicillins (piperacillin, ticarcillin: *P. aeruginosa* is intrinsically resistant to all other penicillins)
- Carbapenems (meropenem, imipenem)
- Polymyxins (polymyxin B and colistin)
- Monobactams (aztreonam)



# *Burkholderia species*

- Currently 82 validly published species
- Medically important
  - *Burkholderia cepacia*
  - *Burkholderia pseudomallei*
- Aerobic gram negative non-fermenters
- Opportunistic pathogens
- Ubiquitous in the environment, hospital devices/ surfaces

# *Burkholderia cepacia*

- Causes
  - Pneumonia specially in cystic fibrosis pts
  - Rapidly fatal RTI and septicaemia in cystic fibrosis pts (capacia syndrome)
  - HAI (ICU)- antibiotic resistant
    - Pneumonia
    - Wound infection
- Intrinsically resistant to most antibiotics
  - Antibiotics – Co-trim, meropenem, doxycycline

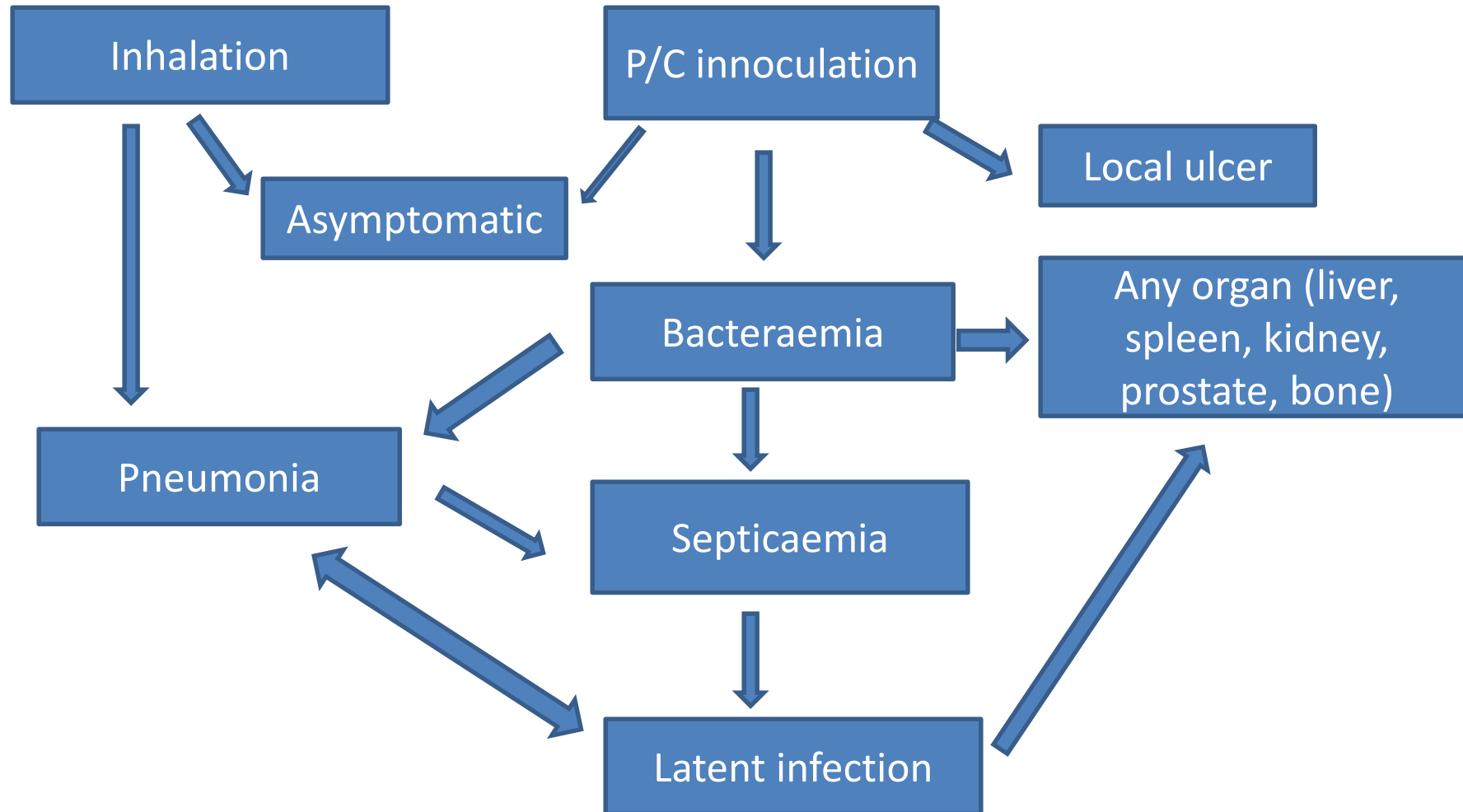
# *Burkholderia pseudomallei*

- Formerly known as *Pseudomonas pseudomallei*
- Saprophyte - widespread in soil and surface water in endemic areas
- Gram negative, non-fermentor, oxidase positive , motile (= to *Pseudomonas* spp.)
- Causative agent of melioidosis
- Almost universally resistant to Gentamicin and Colistin

# Epidemiology

- Endemic in South-East Asia (highest in Thailand) and Northern Australia – tropical/ subtropical areas
  - Thailand – 20% of CA-septicaemia and The third most frequent cause of death from infectious diseases
  - Northern Aus – commonest cause of fatal CAP
- Caribbean, the Middle East, South America
- Post –war (Vietnamese time bomb) / post Tsunami
- Associated with monsoonal rains and winds
  - 75% of cases presenting during the rainy season.
- Found in Rice paddies (mud), stagnant water
- Mortality - 30% to 47%. →90%
- SL –endemic

# Pathogenesis



# Presentations

- Pneumonia (acute/ sub-acute/ chronic)
- Fulminant septicaemia
- Deep organ abscesses
- Skin/ soft tissue abscesses, necrotizing fascitis
- GU – prostatic melioidosis
- Suppurative parotitis
- Neurological – brain stem enceph., CN palsy
- Orthopedic- Septic arthritis, osteomyelitis
- GI – mucosal ulcers/ LN, pancreatic & gall bladder
- Eye – orbital cellulitis, corneal ulcers, hypopyon
- Cardiac – pyopericardium, myocardial abscesses, endocarditis

2<sup>ry</sup> infective foci

# Risk factors

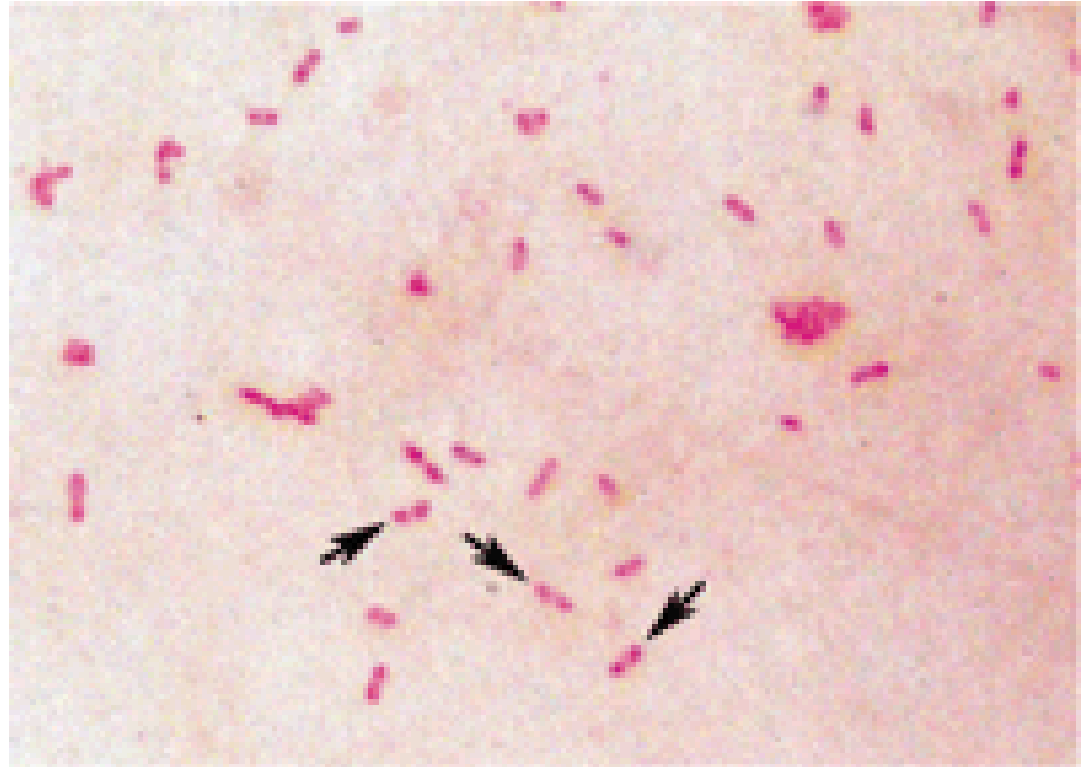
- Type II DM
- Renal disease
- Alcoholism
- Chronic lung disease (CF/ bronchiectasis)
- Thalasaemia
- CGD
- Steroid therapy
- Malignancy
- TB
- Iron overload
- Exposure to surface water /soil



# Diagnosis

- Culture - definitive diagnosis (Gold Standard)
  - Isolation and identification of the organism from blood, sputum, pus, urine, synovial fluid, peritoneal fluid and other sterile fluid
- Specimens
  - blood, urine, throat swab, respiratory secretions, pus, tissues and swabs from surface wounds
- Identification – difficult
  - Gram stain, colony characteristics, oxidase test, R to certain antibiotics
  - Commercial ID systems –API
  - Hazard group 3





# Diagnosis

- Serology (IHA, ELISA, IF, IB)

- high single titer in the presence of clinical signs
- 4 fold rise in paired sera



suggestive

- Cut-offs IHA titre

- 1:10–1:40 → indicate exposure
- 1:40–1:160 → indicate active disease

- Ag detection (ELISA, IF, LA)

- Genetic – PCR, restriction fragment length polymorphism, pulse-field gel electrophoresis, 16S rRNA sequencing, multilocus sequence typing (MLST)

- Haematological -WBC/DC, CRP, ESR, BP
- Radiological –X-ray, USSS, CTS
- Biochemical – liver/renal functions

# Treatment

- Phase 1 (I.V.) - initial treatment (in acute stage)
- Phase2 ( oral) – eradication therapy (to remove any residual infection)
- Need combination of antibiotics
- Need lengthy courses of treatment
- The combination, and duration depends on
  - Type
  - Severity
  - ABST

# *Stenotrophomonas maltophilia*

- Gram negative glucose non-fermenter
- Oxidase negative and motile
- may cause a wide range of infections - HAI
  - intravascular line associated bacteraemia
  - nosocomial pneumonia
  - Rarely meningitis, wound infection
  - in susceptible patients, notably those with an underlying haematological malignancy

# *Stenotrophomonas maltophilia*

## **EPIDEMIOLOGY**

- Hospital Epidemics from Contaminated Moist Reservoirs:
  - Disinfectant solutions
  - Respiratory equipment
  - Ice machines
  - Flower vases
- Risk Factors
  - Hospitalization
  - Impaired host defense mechanisms (e.g., highly immunocompromised)
  - Long-term broad-spectrum antibiotics (e.g., bone marrow transplant patients)

# Diagnosis

- Colonies may appear yellow or green on blood agar.
- Some strains may produce slight beta-haemolysis
- Resistance to imipenem in vitro is a useful indicator to suspect *S. maltophilia*.



# *Acinetobacter baumannii*

- Saprophyte
  - Moist environment
  - Hospital environment
- Strict aerobe
- Gram negative cocco-bacillus
- Source of infection
  - Hospital surfaces/ equipment/ disinfectants
  - Carriers – commensals in skin, oropharynx, GIT

# *Acinetobacter infections*

- HAI
  - HAP/ VAP
  - CLBSI
  - CA-UTI
  - Wound and soft tissue infections
  - Burn infections
- Outbreaks in ICU and wards
- Characterized by infections due to multi-resistant strains



# *Acinetobacter – laboratory diagnosis*

- Culture – grow in ordinary media
  - Non-lactose fermenter
  - Oxidase negative
  - Non motile
  - KIA- no reaction

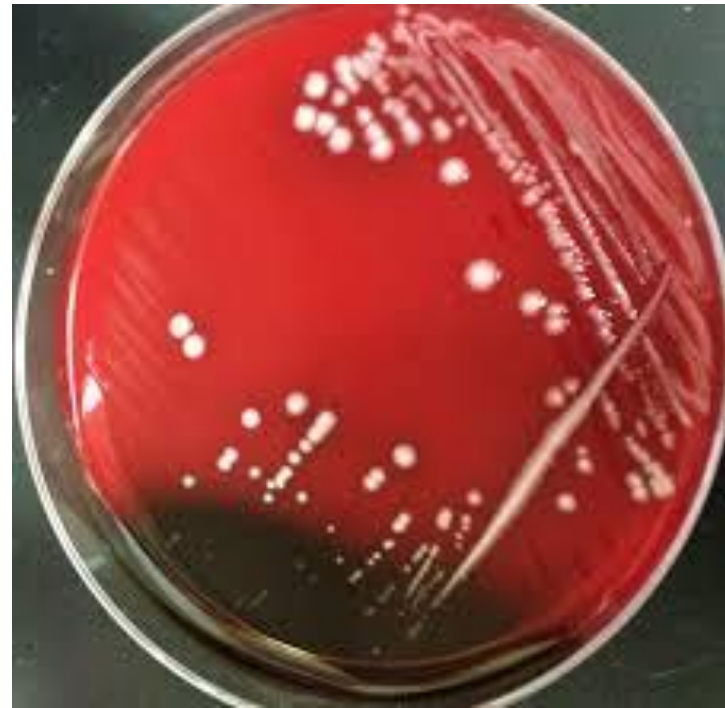


Photo by Tom Erdmann

# *Acinetobacter baumannii*

## **CLINICAL SYNDROMES**

### **Opportunistic Infections**

- Respiratory tract
- Urinary tract
- Wounds
- Septicemia

## **EPIDEMIOLOGY**

- Niches Include:
  - Natural environments
  - Moist surfaces in hospitals (e.g., respiratory therapy equipment)
  - Dry surfaces (e.g., human skin); rare for gram-negative bacilli
  - Occasionally normal flora in oropharynx

## **TREATMENT, PREVENTION & CONTROL**

- Antibiotic Resistance Common
- Empirical Treatment for Acute Infections:  $\beta$ -lactam + Aminoglycoside
- Specific Therapy According to Antibiotic Susceptibility

# Summary

- Clinical presentations, characteristics, lab diagnosis and treatment of
  - Pseudomonas
  - Burkholderia
  - Stenotrophomonas
  - Acinetobacter