Opportunistic Enterobacteriaceae and Pseudomonas

An opportunist does not cause disease in healthy persons, but can cause disease in those whose defenses are compromised.

Enterobacteriaceae

The family Enterobacteriaceae is the largest, most heterogeneous group of medically important Gram-negative bacilli. Members are found in water, soil, decaying organic matter and in human and animal intestines.

- >20 genera, 115 species, 9 "tribes"
- some are normal inhabitants of the human GIT: *facultative anaerobes (grow under many conditions)*
- Escherichia, Enterobacter, Klebsiella
- some are major human pathogens:
  - Escherichia, Shigella, Salmonella, Yersinia
- most are opportunists → will cause disease if conditions permit
- up to 90% of clinical isolates are E. coli, K. pneumoniae, or P. mirabilis
- traditionally associated with intestinal disorders but also:
  - urinary tract infections
  - extraintestinal enteric bacterioses:
    - colonization of prostheses
    - disease in patients with impaired immunity
    - protean manifestations
  - an important cause of nosocomial infections:
    - 5-10% of hospitalized patients → ~2 x 10^6/year
    - abscesses, wound infections, bacteremia, systemic disease
    - Gram-negative sepsis and endotoxic shock are important complications
  - multiple antibiotic resistance is common and increasing

<table>
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<tr>
<th>Tribe</th>
<th>Genera</th>
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<tbody>
<tr>
<td>Escherichiae</td>
<td>Escherichia</td>
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<tr>
<td>Enterobacteriaceae</td>
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<td>Salmonellae</td>
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<td>Citrobacteriaceae</td>
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<td>Klebsiellae</td>
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<td>Proteus</td>
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<td>Yersiniella</td>
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<td>Erwiniae</td>
<td>P. mirabilis</td>
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<tr>
<td>Miscellaneous genera (not yet assigned to a tribe)</td>
<td>Various &quot;enteric groups&quot; (not yet assigned to a genus)</td>
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</tbody>
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Translocate to deeper tissues with bacteremia *contain very toxic LPS*

Immune response leads to shock

*Share genes, even across spp. → virulence factor, etc.*
Small Gram-negative non-spore forming rods/cocccobacilli.
- cell envelope contains peptidoglycan, lipoprotein, phospholipid and lipopolysaccharide (LPS [O antigen]) → string of CHO on outside of LPS → used for *typing* of lact.
- if motile, have peritrichous flagella (H antigen)
- usually have fimbriae → attach to other bacteria, host cells or bacteriophage
- + capsule/slime layer (K antigen)
- Usually polysacchar. Unit some prob. Sub spp. dep on K Ag
- Metabolically diverse and quite active, characteristics used to identify clinical isolates.
  - facultative: vivo w/ & w/o O
  - ferment many carbohydrates → lactose and glucose
  - aerobically, use TCA and cytochromes → oxidase →
  - NO₃ → NO₂
  - many special media to expedite isolation and identification:
    - MacConkey agar
    - Hektoen agar
  - speciation based on patterns of CH₂O use, variations in endproducts, specialized biochemical tests, serologic typing (O, H, and K antigens), and genetic analysis.
- Virulence factors facilitate colonization and subsequent disease development. Not all species will have all of them.
  - cell surface components
  - exotoxins
  - iron acquisition systems

Conjugation and transduction are common within and between genera → transfer of antibiotic resistance and virulence factors.

The combination of common multiple antibiotic resistance and the availability of patients with underlying disease that predisposes them to infection make the opportunistic enterobacteriaceae important human pathogens.
- antibiotic resistance patterns must be known
- tx should be aggressive, especially in patients with septic shock

**Escherichia coli**

*E. coli* is the enteric bacterium most frequently isolated by clinical laboratories.
- inhabitant of the large intestine
- presence in food and water indicates fecal contamination
- identified biochemically and serologically based on O, H, and K antigens
- both an opportunist and a primary pathogen

*E. coli* can cause a spectrum of diseases.
- community acquired urinary tract infections (UTIs): localized & treatable w/ AB
- > 85% of community acquired urethritis, cystitis and polynephritis
- uropathic strains [UPEC] (limited O types) → pathogenicity islands (PAI):
  - P fimbriae (pili), hemolysin, aerobactin, toxins
  - females < 10 y.o. and 20-40 y.o. → anatomy and sexual activity
  - GC ratio: % guanine to cytosine → used to define an org.
- more aggressive, invasive & difficult to treat
- Sections of genome have GC ratio of strains of a given species, etc. [imp to acquire, interpret]
• seeded from colon
• predominantly ascending infections

• nosocomial/hospital acquired UTIs:
  • urinary flow obstruction
  • indwelling urinary catheters: → will form biofilms
  • ~50% will develop if catheterized > 5 days
  • may lead to septicemia
  • can also be descending → from blood to urinary tract or vice-versa

• bacteremia:
  • hospital acquired Gram − sepsis → often by uropathic strains
  • endotoxic shock, & multi-organ failure

• other diseases:
  • hospital acquired pneumonia → aspirate into lungs
  • neonatal meningitis → not a player, but in immunocompetent
  • diarrheal diseases → cause gastroenteral diarrhea

Uropathogenic E. coli (UPEC) virulence factors. → capsule, flagella

• adhesins:
  • Type I (common) pili:
    • bind to mannose containing host cell receptors → bladder colonization
    • binding stimulates uptake by uroepithelial cells
  • P fimbriae (P pili):
    • not inhibited by D-mannose
    • bind to P blood group antigen (globobiose) → bladder and kidney cells
    • some also synthesize afimbrial and Dr adhesins

• toxins:
  • lipopolysaccharide (O antigen, somatic antigen):
    • endotoxin
    • oligosaccharide-core polysaccharide-lipid A
    • induces fever, shock, WBC changes, host physiological changes
      • lipid A
      • ↓ complement mediated lysis, ↓ opsonization
      • ↑ mortality in patients with Gm − bacteremia → endotoxic shock
  • hemolysin/cytotoxin:
    • exotoxins
    • lyses host cells by creating pores → source of iron?; decreased phagocytosis
    • cytokines, superoxide are increased and ↓ ATP at lower toxin concentrations

• other virulence factors:
  • siderophores: & pull Fe from host muscles, even w/hemolysis
    • chelate iron for growth and reproduction → release from mammalian pools
    • enterobactin, aerobactin, yersiniabactin

• capsular (K) antigen:
  • usually polysaccharide but some are protein (K88, K99)
  • ↓ phagocytosis and alternative pathway complement activation

• flagellar (H) antigens:
  • protein → motility

• plasmids

This organism has acquired the ability new adherence factors for new host.
New, more robust, difficult to treat organism.
Susceptible to several antibiotics although resistance is a concern, especially in hospitals. Antibiotic therapy must be guided by in vitro susceptibility data and by clinical findings.

**Klebsiella**

The genus *Klebsiella* consists of three species. *K. pneumoniae*, *K. rhinoscleromatis*, and *K. oxytoca*
- common GIT inhabitants
- lactose fermented
- non-motile → no *flegella*

*K. pneumoniae* is the most common isolate; causes localized or systemic infection in debilitated persons; rarely in normal hosts.
- burn infections → associ. w/ wounds/burns → infect open tissue & get uniled
- UTIs (9%), bacteremia (14%), meningitis, wound infections
- most common loci of infection are:
  - urinary tract > lower respiratory tract > biliary tract > surgical wounds
  - invasive devices (e.g., catheters, endotracheal tubes) predispose to infection
  - other respiratory tract infections are predisposing factors
- associated with one important primary infection → lobar pneumonia → characteristic dz helps w/ differential dx
  - community acquired; opportunistic
  - alcoholism, diabetes, COPD
  - severe, acute onset → extensive lung destruction; abscess formation
  - 25 – 50% mortality despite aggressive treatment
  - other pulmonary sx are less dramatic; bronchopneumonia/bronchitis

**Primary virulence factor is an abundant mucoid polysaccharide capsule (K antigen).**
- ~70 serotypes
- also have endotoxin (O antigens) → lysis by MAC
  - mucoid biofilm w/ colonies → difficult to tx!!
- pili that increase adherence → act as ligands for rec.
- some produce heat stable and labile toxins similar to those of E. coli

**Isolates are often resistant to multiple antibiotics because of R plasmid transfer.**
- naturally resistant to ampicillin and carbenicillin
- β-lactamase producers → resist to penicillin
- third generation cephalosporins → ceftriaxone, cefotaxime are drugs of choice

**Enterobacter spp.**

Contains ~ 12 species; E. aerogenes, E. cloacae, and E. agglomerans are most commonly involved in human disease.
- ferment lactose
- motile
- capsule

Frequently found in hospitalized patients who:
- received antibiotic treatment
- have undergone an invasive procedure
- have indwelling catheters
- are diabetic or neutropenic

**Enterobacter cloacae is the most common hospital isolate.**
- associated with burn, wound, respiratory and urinary tract infections
- horizontal spread → poor aseptic technique by health care workers
- virulence factors:
  - O antigens that ↑ resistance to MAC induced lysis
  - polysaccharide capsule (K antigen) that ↑ phagocytosis efficiency
  - flagella (H antigen) → motility in aqueous environments
  - heat labile and heat stable toxins
  - many strains have an inducible β-lactamase → need new DOC
  - treatment with third generation cephalosporins/antipseudomonal penicillin + aminoglycoside

**Serratia spp.**

Contains nine species; most human disease is caused by Serratia marcescens.
- + lactose fermentation "dirt" bug he lives in ground. + causes human dz → others is thus or do not.
- O and H antigens:
  - ~ 120 serotypes
  - important epidemiological markers
- differentiated from other Enterobacteriaceae by:
  - extracellular DNase → degrades mammalian DNA
  - resistance to colistin and cephalothin
  - red pigment (prodigiosin) at room temperature → not definitive → hallmark
  - does not take in foreign DNA, able degrades it
Infections usually associated with underlying disease, immunosuppression or mechanical manipulations of the patient.

- hand to hand spread
- nosocomial infections very common → 75-90% hospital acquired:
  - catheters, urinary and respiratory tract instrumentation
  - UT and wound infections, pneumonia and septicemia → bacteremia → sepsis
- associated with infections in heroin addicts → endocarditis, osteomyelitis

Treatment is complicated by high incidence of multiple antibiotic resistance.

- β-lactamase
- amikacin ± antipseudomonal penicillin

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**Citrobacter spp.**

*C. amalonaticus, C. diversus*, and *C. freundii* have been implicated in human disease.

- citrate as sole carbon and energy source
- *C. freundii* is the most common isolate → H₂S from sodium thiosulfate

Diseases:

- urinary and respiratory tract infections in debilitated, hospitalized patients
- neonatal meningitis and brain abscesses → *C. diversus*

Virulence factors:

- possess O, H, and K antigens closely related to those of *Salmonella* and *E. coli*; some strains have Vi antigen like *S. typhi*
- more virulent strains have Omp not present on those that are less pathogenic
- enterotoxigenic strains make heat stable toxin (ST) → work on cells in GI tract

Treatment is based on antibiotic sensitivity patterns.

- most sensitive to aminoglycosides, tetracycline, or chloramphenicol
- resistance in isolates from hospitalized patients/those who have had previous antibiotic therapy → plasmid mediated β-lactamases
- resistance of *C. freundii > C. diversus*
Historically classified as *Proteus* but have been separated based on DNA relatedness.
- lactose not fermented
- motile
- phenylalanine deaminase +
- indole + except *P. mirabilis*

May account for ≤ 15% of nosocomial infections.

*Proteus mirabilis* is the most common clinical isolate.
- community acquired urinary tract infections:
  - functional or anatomic abnormalities including catheters
  - primarily upper urinary tract
- virulence factors that ↑ uropathogenicity:
  - fimbriae mediate colonization of uroepithelium: 
    - mannose resistant/*Proteus*-like (MR/P)
    - *Proteus mirabilis* fimbriae (PMF)
  - flagella facilitate bacterial spread
  - hemolysins
- urease catalyzes struvite/apatite stone formation, renal tubular toxicity → kidney stones
  - a nickel-metalloprotease
  - occasional bacteremia, wound infections and pneumonia

*Providencia* and *Morganella* are less common but important causes of nosocomial disease, especially in long-term health care institutions. → most debilitated of pts
- *P. stuartii* is increasingly isolated from catheterized nursing home patients:
  - a source of bacteremia
- *M. morganii* is an opportunistic secondary invader isolated from many sites

Treatment can be complicated because of antibiotic resistance. Culture and sensitivity are important.
- *P. mirabilis* remains sensitive to most common antibiotics except gentamicin and tobramycin
  - indole + isolates can be resistant to penicillins and aminoglycosides
- all three genera have inducible β-lactamases
- antibiotic susceptibility should be monitored
**Pseudomonas spp.**

Contains many species of Gram negative aerobic bacilli that naturally inhabit soil. Some are opportunists that infect medically compromised individuals. Infections are difficult to treat due to widespread antibiotic resistance and the debilitated host. **It can survive w/ low O2 tension, however.**

*Pseudomonas aeruginosa*, the most common human isolate; responsible for a broad spectrum of disease in a variety of clinical settings.

*Much more broad based predisposing factors & broad based type of infection.*

*Needs the RIGHT host w/ RIGHT condition → it is not aggressive.*

<table>
<thead>
<tr>
<th>Predisposing factor</th>
<th>Type of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local breach of the immune system</td>
<td>Pneumonia, chronic recurrent</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Osteomyelitis</td>
</tr>
<tr>
<td>Trauma</td>
<td>Endocarditis, septic arthritis, osteomyelitis</td>
</tr>
<tr>
<td>IV Drug abuse</td>
<td></td>
</tr>
<tr>
<td>Neurosurgical operations</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Surgical operations</td>
<td></td>
</tr>
<tr>
<td>Tracheostomy</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Intravenous lines</td>
<td>Cellulitis, supplicative thrombophlebitis</td>
</tr>
<tr>
<td>Corneal injury</td>
<td>Panophthalmitis</td>
</tr>
<tr>
<td>Kidney stones</td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>Catheterization</td>
<td>Urinary tract colonization and infection</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>Septicemia, pneumonia, abcesses</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Malignant otitis externa</td>
</tr>
<tr>
<td>Premature infants and neonates</td>
<td>Septicemia, meningitis, enteritis</td>
</tr>
<tr>
<td>Both systemic and local Burns</td>
<td>Burn wound infection, sepsisemia</td>
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</tbody>
</table>

**Microbiological characteristics of Pseudomonas aeruginosa.**

- a versatile bacterium:
  - a variety of nutritional and environmental conditions → pH, temp, etc.
  - luxuriant mucoid growth → pyocyanin + “fruity”/grape-like odor
  - biofilm formation
- an aerobe except with NO<sub>3</sub> → anaerobic respiration → in hypoxic environ
- oxidase +
- polar flagella
- resistant to most antibiotics except:
  - fluoroquinolones
  - aminoglycosides
  - β-lactams like imipenem → newer ones

**Widely distributed in the environment.**

- healthy human contact frequent and inconsequential
- common in moist hospital microenvironments; → nosocomial!!
- sinks, disinfection solutions, inhalation equipment, medicines, food
- presence of medically compromised patients increases infection probability
- second to E. coli as Gram(-) hospital isolate:
- 9% of nosocomial bacterial and fungal isolates
- second most common cause of hospital acquired pneumonia
- patients with cystic fibrosis, neoplastic diseases or severe burns are especially susceptible
- can cause a variety of infections:

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>PREDISPOSING CONDITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatitis</td>
<td>Hot tubs; loofah sponge use; mud wrestling</td>
</tr>
<tr>
<td>Burn infections</td>
<td>Third degree burns</td>
</tr>
<tr>
<td>Eye infections (ulcerative keratitis)</td>
<td>Long-wear soft contact lenses</td>
</tr>
<tr>
<td>Lung infections</td>
<td>Cystic fibrosis → most CF pts will get infected. Inhalation of hot air during a fire Intubated patients (ventilator associated pneumonia</td>
</tr>
<tr>
<td>Nosocomial septicemia</td>
<td>Surgical wounds</td>
</tr>
<tr>
<td>Nosocomial urinary tract infections</td>
<td>Infants in intensive care ward</td>
</tr>
</tbody>
</table>

Pathogenesis is complex and poorly understood.

1) Encounter from environmental source

| pili bind to asialoGM1, gangliosides on host cells → pili that act as ligand for Ps. aeruginosa
| LPS binds to cystic fibrosis transmembrane conductance regulator (CFTR)
| exoenzyme S (ExoS): disrupts target cell signal transduction; phagocytosis impaired, proinflammatory cytokines stimulated → extracellular. adds to undermining of host defenses
| elastase (Las A and B): proteinases that destroy elastin/other host proteins, may also ↓ innate immunity → undermining CT of lungs & ability of neutrophils
| phospholipase C/rhamnolipid: synergistic lung tissue destruction
| alginate capsule (mucoid exopolysaccharide [MEP]): adhesion, biofilm formation →↓ opsonization; ↓ antibiotic access
| pyocyanin: endothelial cell damage by OH− synthesis (O2 radicals)
| pyochelin (pyoverdin): a siderophore → compete for host Fe by binding very tightly
| ubiquitous; initial encounter may be from an environmental source:
| ingestion, inhalation
| poor adherence to normal intact epithelium → damage increases probability of colonization

- e.g., epithelium from cystic fibrosis patients binds more Ps. aeruginosa
- are typical extracellular parasites:
- multiplication and spread depends on evading ingestion by neutrophils
- neutrophils at risk
- bacterial virulence factors help secure nutrients for growth and reproduction
- host damage:
- hypotension and shock due to endotoxin and exotoxin A
- exotoxin induced local inflammation, tissue destruction and abscess formation
- permits bacterial survival and spread
- outcome dictated by:
  - nature and severity of infection
  - state of host defenses
  - treatment efficacy

Infection is a cause of repeated hospitalization, decline in pulmonary function, and early death in cystic fibrosis patients.
- cystic fibrosis is an autosomal recessive disease that occurs in about 1:3,300 live births.
- in part due to a plasma membrane protein gene mutation (cystic fibrosis transmembrane conductance regulator [CFTR]):
  - ATP-driven pump
    - chloride ion secretion → effect on volume of airway surface fluid → "clearing" of mucosal surfaces
  - receptor for P. aeruginosa → normally adheres poorly to respiratory epithelium
- because of the mutation, CF patients:
  - produce viscous pulmonary secretions → stimulated by LPS → PA takes advantage of this
  - have less nitric oxide in the lower bronchial epithelium
  - have more Ps. aeruginosa in lung → cannot eliminate from pulmonary airways:
    - subpopulation with affinity for respiratory tract epithelium
    - mucoid strains
    - bacteria in a biofilm; protected from mucociliary clearance, innate phagocytosis, complement, antibiotics, and disinfectants; also regulates virulence factor expression
  - localized pulmonary insufficiency
  - 80-90% develop chronic pulmonary infection:
    - cycles of bacterial growth, inflammation, disease, treatment
    - slow, progressive decline in pulmonary function
    - repeated treatment with antibiotics puts selective pressure on bacteria:
      - antibiotic resistance increases → decrease of Tx
      - respiratory failure and death

Resistant to most common antibiotics. Patient survival is predicated on preventing colonization, control of underlying disease, and an effective phagocytic system.
- antibiotic resistance mechanisms:
  - Δ ability of ABE to bind & enter cells
  - restricted entry into periplasmic space
  - drug efflux pumps → as soon as it enters
  - biofilm reduces effective concentration of ABE → ABE can't even contact the cell
  - enzymes like β-lactamases → break down ABE
- most are initially susceptible to aminoglycosides (e.g. tobramycin) and extended spectrum 4th generation penicillins (e.g. ticarcillin) → resistance if therapy prolonged; guided by antibiotic susceptibility patterns
- newer antimicrobial agents:
  - fourth generation cephalosporins → cefepime
  - new carbapenems → meropenem
  - broad spectrum fluoroquinones → levofloxacin and trovafloxacin

Note: The majority of this information was organized and provided by Dr. Thomas Lillich, former Associate Dean in the College of Dentistry