Gram Negative Bacteria in Clinical Medicine

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Gram Negative Bacteria in clinical medicine

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USAID PEER/Liberia ID Lecture Series
21 July 2020
Objectives

Define the molecular structure of Gram-negative organisms
Highlight differences between Gram-negatives and Gram-positives
Discuss the most important clinically-relevant Gram-negative bacteria
Review treatments for Gram-negatives
Illustrate clinical scenarios involving Gram-negative infection

(Won’t discuss much about laboratory classification, eg oxidase test, lactose fermentation, etc. If you run a lab, you will need to know this!)
You can approach thinking about causes of infections in two different ways.

One way is by organ system, e.g. pneumonia:

*Streptococcus pneumoniae, Klebsiella pneumoniae, Chlamydophila pneumoniae, Mycoplasma, Moraxella, E. coli, Staph aureus,* etc.

Advantage is that you can memorize various causes in a way that makes sense and not have to review every single organism in your head when you think about an infection, as there are dozens.

*But*...could you *miss* an organism this way?
You can approach thinking about causes of infections in two different ways

The second way is by organism, via categories, e.g.:

“Gut Gram-negatives, including *E. Coli, Klebsiella, Salmonella, Shigella, Enterobacter, Citrobacter, Serratia*”

Advantage here is that you can review in your head the major infectious organisms and *not miss an important microbe*

The point is you need to use *both* ways of thinking about infections in order to make sure your differential is complete

Only a computer can list all the manifestations of disease by organism, or all the infectious causes of a focal infection

But a human brain can do pretty well if you switch back and forth
The “ID Differential”

Non-infectious causes

- Opportunistic (i.e. HIV)

Infections

- “Routine”

- Unusual organisms (e.g. Mycoplasma, prions)

- Gram Positives
- Gram Negatives
- Anaerobes
- Viruses
- Fungi
- Parasites & Protozoa

Other bacteria:
-- Intracellular
-- Acid Fast/Modified
-- Spirochetes
What is a Gram stain?

Named after Dr. Gram (1884 paper) Some bacteria take up crystal violet dye; some don’t Some take up a counter-stain (safranin), *but some don’t* That is, not *all* bacteria can be seen by Gram stain (e.g. Mycobacteria)
Structural differences

The differences in the cell wall lead to differing pathogenesis, as well as different targets for antimicrobial (and other) therapy.

Crystal violet binds the peptidoglycan layer
**LPS (Lipopolysaccharide)**

- **Extracellular Space**
  - Polysaccharide "O-Antigen"
  - Core
  - Lipid A

- **Outer Membrane**
- **Periplasmic Space & Cell Wall**
- **Cytoplasmic Membrane**

**Bacterial Endoplasm**
Gram-negative rod

Gram-positive rod
Gram Negatives

- Gram Negative Cocci
  - Neisseria meningitidis
  - Neisseria gonorrhoeae
  - Moraxella

- Gram Negative Bacilli
  - Enterobacter*
  - Vibrios (cholera)
  - Campylobacter
  - Salmonella*
  - Shigella*
  - Yersinia*

- Pseudomonas
- Stenotrophomonas
- Burkholderia
- Acinetobacter
- Haemophilus

- Bartonella
- Francisella (tularemia)
- Pasturella
- Capnocytophaga
- Brucella
- Helicobacter pylori
- Legionella

*denotes members of Enterobacteriaceae family
Enterobacteriaceae: GI/GU organisms

- *Citrobacter*
- *Enterobacter*
- *Eschericia* (eg *E. coli*)
- *Klebsiella*
- *Morganella*
- *Proteus*
- *Providencia*
- *Salmonella*
- *Shigella*
- *Yersinia*

- Cholecystitis/cholangitis
- Diverticulitis
- Abdominal perforation
- Appendicitis (perforation)
- Gut translocation
- Diarrhea
- UTIs, esp *E. coli*, *Morganella*, *Proteus*, *Providencia*
- Bacteremia
Gram-negatives are exceptionally good at developing drug resistance.

- **Transformation**
  - Bacteria pick up DNA from the environment.
- **Transduction**
  - Bacteria pick up DNA from other bacteria.
- **Conjugation**
  - DNA is transferred from one bacterium to another through a pilus.

Additional mechanisms include:
- **Efflux pumps**
  - Bacteria pump out antibiotics.
- **Inactivating enzymes**
  - Bacteria produce enzymes that inactivate antibiotics.
- **Modified target**
  - Bacteria alter their targets to resist antibiotics.
- **Decreased uptake**
  - Bacteria reduce the uptake of antibiotics.
Options for treatment of Gram Negatives

PCNs (often not effective)
PCNs with beta-lactamases (Augmentin, ie amox-clav)
Piperacillin (ureidopenicillins)
3rd/4th gen cephalosporins (ceftriaxone, ceftazidime)
Carbapenems (meropenem)

Fluoroquinolones (ciprofloxacin)
Aminoglycosides (gentamicin)
Tetracyclines
Chloramphenicol
Sulfonamides (Septrin)
DIFFERENT CLASSES OF ANTIBIOTICS - AN OVERVIEW

**Key:**
- **β-Lactams**: Act as bacteriostatic agents, restricting growth & reproduction.
- **Aminoglycosides**: Commonly used as bactericidal agents, causing bacterial cell death.

### β-Lactams
- **Most widely used in the NHS**
- All contain a β-lactam ring
- **Examples**: Penicillins (shown) such as amoxicillin and flucloxacillin, cephalosporins such as cefalexin.
- **Mode of action**: Inhibit the synthesis of proteins by bacteria, leading to cell death.

### Aminoglycosides
- **Family of over 20 antibiotics**
- All contain aminoglycoside substructures
- **Examples**: Streptomycin (shown), neomycin, kanamycin, paromomycin.
- **Mode of action**: Inhibit synthesis of proteins by bacteria, preventing growth.

### Chloramphenicol
- **Commonly used in low income countries**
- Distinct individual compound
- **Mode of action**: Inhibit proteinsynthesis by bacteria, leading to cell death.

### Glycopeptides
- **Common 'drugs of last resort'**
- Consists of carbohydrates linked to a peptidoglycan from amino acids
- **Examples**: Vancomycin (shown), teicoplanin.
- **Mode of action**: Inhibit the synthesis of the cell wall, leading to cell death.

### Quinolones
- **Resistance evolves rapidly**
- All contain fused aromatic rings with a nitrogenous group attached
- **Examples**: Ciprofloxacin (shown), levofloxacin, trovafloxacin.
- **Mode of action**: Interfere with bacterial DNA replication and transcription.

### Oxazolidinones
- **Potent antibiotics commonly used as 'drugs of last resort'**
- All contain 2 oxazolidinones somewhere in their structure
- **Examples**: Linezolid (shown), dalbavancin, tezeprenoxil.
- **Mode of action**: Inhibit synthesis of proteins by bacteria, preventing growth.

### Sulfonamides
- **First commercial antibiotics were sulfonamides**
- All contain the sulfonamide group
- **Examples**: Prontosil, sulfaguanidine (shown), sulfadiazine, sulfacetamide.
- **Mode of action**: Do not kill bacteria but prevent their growth and multiplication.

### Tetracyclines
- **Becoming less popular due to development of resistance**
- All contain 4-membered cyclic hydrocarbon rings
- **Examples**: Tetracycline (shown), doxycycline, minocycline, oxytetracycline.
- **Mode of action**: Inhibit synthesis of proteins by bacteria, preventing growth.

### Macrolides
- **Second most prescribed antibiotics in the NHS**
- All contain a 13β, 15β, or 16-membered macrolide ring
- **Examples**: Erythromycin (shown), clarithromycin, azithromycin.
- **Mode of action**: Inhibit protein synthesis by bacteria, occasionally leading to cell death.

### Ansamycins
- **Can also demonstrate antiviral activity**
- All contain an aromatic ring bridged by an aliphatic chain
- **Examples**: Geodamesin (shown), rifamycins, niphosynmycin.
- **Mode of action**: Inhibit the synthesis of RNA by bacteria, leading to cell death.

### Streptogramins
- **Two groups of antibiotics that act synergistically**
- Combination of two structurally different compounds, from group A shown: A & B
- **Examples**: Pristinamycin II A (shown), Pristinamycin I A.
- **Mode of action**: Disrupt multiple cell membrane functions, leading to cell death.

### Lipopeptides
- **Instances of resistance rare**
- All contain a lipid bound to a peptide
- **Examples**: Daptomycin (shown), surbactam.
- **Mode of action**: Disrupt multiple cell membrane functions, leading to cell death.

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**Timeline**
- **Discovery**: 1930
- **1940**: Discovery of penicillin by Alexander Fleming
- **1950**: First sulfonamide (Prontosil) introduced
- **1960**: Tetracyclines become widely used
- **1970**: Discovery of macrolides
- **1980**: Macrolides become the second most prescribed antibiotics

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**Key**
- **Commonly used as bacteriostatic agents, restricting growth & reproduction**
- **Commonly used as bactericidal agents, causing bacterial cell death**
A 58 year-old man presents with acute cough, fever, and chills. The cough produces a thick, red sputum. Gram stain is shown.

Which is the most likely organism?
A. *Streptococcus pneumoniae*
B. *Providencia stuartii*
C. *Mycobacterium avium*
D. *Klebsiella pneumoniae*
E. *Mycoplasma pneumoniae*
Answer: *Klebsiella pneumoniae*

- Gram negative encapsulated organism
- “Currant jelly” sputum: localized tissue necrosis & tissue inflammation
- contrast with pneumococcus “rust colored” sputum
- ? May be more common in Africa than in Europe or US
CAP is most often *pneumococcus*, followed by everything else with lots of Gram-negatives (African epidemiology/distribution may be different)

https://www.ncbi.nlm.nih.gov/books/NBK519004/

A 22 year-old woman presents with dysuria, foul-smelling urine, chills, and pelvic pain x 48 hours.

Treated for UTIs at least six times over past 1-2 years. Can’t remember which abx given, been to different clinics & pharmacies.

Temp 100.7 F, HR 110, BP 120/78. Exam notable for mild/moderate tenderness on CVA percussion.

UA: 1.030, >100 WBCs, Leuk Est pos, nitrite pos, microscopy shown
Which of the following organisms are likely causes of her presentation?

A. *Staphylococcus saprophyticus*
B. *E. coli*
C. *Moraxella catarrhalis*
D. *Enterococcus faecium*
E. *Pasturella multocida*
Which of the following statements are true?

A. She requires short-course (ie, 3-day) treatment for UTI.
B. She is at low risk for sepsis secondary to bacteremia.
C. A fluoroquinolone such as cipro is the best choice to treat her.
D. Septrin would be contraindicated since it does not treat Gram-negatives.
E. This is a person for whom a urine culture with antibiotic susceptibilities would be very helpful in establishing proper treatment.
Recurrent UTIs

- *E. coli* accounts for ~80% of UTIs in women, ~70% in men
- Much more common in women however
- This patient has *pyelonephritis*, no cystitis (CVA tenderness)
- Will require prolonged abx as consequence
- At high risk of having drug resistance, most likely to FQs or Septrin since they are the most commonly prescribed UTI abx
- May require hospitalization
- *Minimum* five days therapy required, possibly longer

A 16 year-old male presents with sore L arm.

Onset early this morning. Woke out of sleep with pain; has gotten worse since then, now severe.

Noted playing with a dog who bit him yesterday.

Exam: HR 126, T 102.2, BP 110/68

Tremulous, diaphoretic, hand & forearm exquisitely tender, minimally swollen; streaking erythema across wrist & forearm
What is the next step in the patient’s management?

A. Consult surgery immediately.
B. Obtain echocardiogram to evaluate endocarditis.
C. Order CT of L upper extremity to evaluate for fluid collection.
D. Provide pain relief, await abx pending CBC.
E. Start empiric abx and admit for observation.
The following antibiotics are likely to be effective *except:*

A. Penicillin VK
B. Ampicillin
C. Clindamycin
D. Ciprofloxacin
E. Doxycycline
Animal bites and Gram-negatives

- Commonly associated with cat & dog bites
- *Pasturella multocida* seen in both
- Cat tooth = natural syringe
- *Bartonella* infections
- *Capnocytophaga* in dogs; encapsulated organism, issues with dogs & pts without spleens or partial spleens (eg Sickle Cell pts)

Bites are forms of injections—they move faster than “typical” cellulitis

*Pain out of proportion to exam* signals a surgical emergency
Immediate abx
PCNs most effective with *Pasturella*; most β-lactams
Clindamycin and erythromycin *not* likely to be effective
Don’t forget about rabies!
An 18 yo male presents with abdominal pain, fever and constipation x 72 hours

- Dry cough, non-productive
- Brought in by family
- Exam: HR 120, BP 116/68, T 103F
- Diffuse abd tenderness, mild, no rebound
- Neck supple

You consider typhoid fever in your differential. Which of the following is true?

A. A fluoroquinolone such as ciprofloxacin may be adequate tx
B. Ceftriaxone is always ineffective
C. Typhoid is unlikely because there is not a pulse-pressure dissociation
D. Hepatitis is not a complication
E. Typhoid is unlikely if there is not a “rose spot” rash
Typhoid fever

*Salmonella typhi* & *S. paratyphi*

Fecal-or oral spread infection

HIV pts at 20- to 100-fold higher risk (all *Salmonella* spp. incl non-typhoidal)

Extra-intestinal manifestations (CNS, hepato- & splenomegaly, bone/joint)

Widal test: good not great, have index of suspicion

Treat empirically while awaiting BCx

FQ resistance *may* be a problem in Liberia? (Research project!)

Cipro, chloramphenicol, ampicillin or amoxicillin, TMP/SMX, ceftriaxone, azithro all good empiric choices