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# Chapter 17. Gram-negative Bacilli Infections-II

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### CHAPTER PREVIEW

- Nonfermenter GNBs
- Fastidious GNBs
- Miscellaneous GNBs

This chapter deals with gram-negative bacilli (GNB) infections caused by the following organisms:

- **Nonfermenter GNBs:** Such as *Pseudomonas*, *Acinetobacter*, and *Burkholderia*
- **Fastidious GNBs:** Such as *Haemophilus*, *Brucella*, and *Bordetella*
- **Miscellaneous GNBs:** Such as *Campylobacter*, *Helicobacter pylori*, *Legionella*, *Gardnerella vaginalis*, and *Streptobacillus moniliformis*.

## NONFERMENTING GRAM-NEGATIVE BACILLI

Nonfermenters do not ferment any carbohydrates but utilize them oxidatively. Important human pathogens are discussed below.

### *Pseudomonas aeruginosa*

*P. aeruginosa* is a major pathogenic species, causing infections among hospitalized patients and in patients with cystic fibrosis.

## Pathogenesis

The pathogenesis is greatly attributed to its ability to develop widespread resistance to multiple antibiotics and disinfectants and produce several virulence factors.

- **Toxins**, e.g. exotoxin A. It acts by inhibiting protein synthesis
- **Enzymes**, e.g. phospholipases, elastases, etc.
- **Pigments:** *Pseudomonas* produces various pigments such as:
  - **Pyocyanin:** It is a blue-green pigment, produced only by *P. aeruginosa*
  - **Pyoverdin:** It is greenish-yellow pigment, produced by most species
  - **Pyorubin:** This pigment imparts red color.

## Clinical Manifestations

Most of the infections are encountered in hospitalized patients who get colonized with the organisms either from the heavily contaminated hospital environment or from the hospital staff (through contaminated hands). Colonized patients develop the disease in the presence of underlying risk factors such as burn wounds, patients with immunosuppression, and post surgeries. The manifestations are as follows:

- **Healthcare-associated infections** such as—(i) ventilator-associated pneumonia (VAP), (ii) central-line associated bloodstream infection (CLABSI), (iii) catheter-associated urinary tract infection (CAUTI), (iv) surgical site infection (SSI)
- **Chronic respiratory tract infections:** It occurs in patients with underlying conditions that cause airway damage such as cystic fibrosis, or bronchiectasis
- **Bacteremia** leading to sepsis and septic shock
- **Infective endocarditis (native valves):** It occurs among IV drug abusers
- **Ear infections:** The infections are either mild, such as *swimmer's ear* (among children), or serious necrotizing form designated as *malignant otitis externa* (in elderly diabetic patients)
- **Eye infections** such as corneal ulcers (in contact lens wearers) and endophthalmitis secondary to bacteremia
- **Shanghai fever:** It is a mild febrile illness resembling typhoid fever
- **Skin and soft tissue infections** such as burns wound infection, ecthyma gangrenosum, green nail syndrome, and cellulitis with blue-green pus
- **Other infections:** Bone and joint infections such as osteomyelitis and septic arthritis and meningitis (in postoperative or post-traumatic patients).

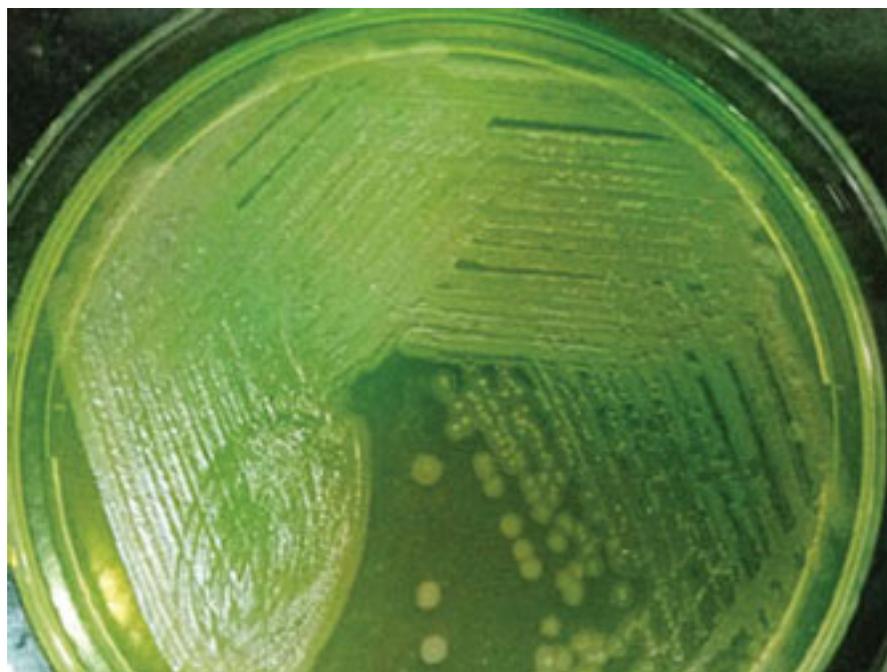
## Laboratory Diagnosis

Specimen collection depends upon the site of infection, such as—pus, blood, tracheal aspirate, sputum, wound swab, urine, etc.

- **Direct smear:** Reveals gram-negative bacilli, and pus cells
- **Culture:** Incubation at 37°C aerobically for 24 h yields the following growth

- Nutrient agar: Opaque, irregular colonies with metallic sheen (iridescence) and blue green diffusible pigments (Fig. 17.1)
- Blood agar:  $\beta$ -hemolytic gray moist colonies
- MacConkey agar: Non-lactose fermenting (NLF) colonies
- Selective media such as ceftrimide agar may be used.
- **Culture smear and motility:** Motile, gram-negative bacilli
- **Identification** from the colonies is made by automated ID systems such as MALDI-TOF or VITEK or by conventional biochemical tests such as—catalase (positive), oxidase (positive), indole, citrate, urease, TSI, etc.

**Fig. 17.1. *Pseudomonas aeruginosa* on nutrient agar showing: large irregular colonies with metallic sheen and green color pigmentation.**



Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

- **AST:** Antimicrobial susceptibility testing is performed by disk diffusion test or by automated MIC detection method (e.g. VITEK).

#### **TREATMENT**

*P. aeruginosa*

- *Pseudomonas aeruginosa* is intrinsically resistant to ceftriaxone, amoxicillin-clavulanate, ertapenem, tetracyclines, tigecycline, etc. Therefore, these drugs should not be used in the therapy
- Only limited agents have good anti-pseudomonal action such as ceftazidime, piperacillin-tazobactam, carbapenems, amikacin, quinolones (ciprofloxacin or levofloxacin), etc.

## Preventive Measures

Infection control measures (contact precaution) such as hand hygiene are crucial to limit the spread of *Pseudomonas* infection in the hospitals (see Chapter 38).

## Acinetobacter species

They are saprophytic bacilli, can cause widespread healthcare-associated infections, especially in patients with underlying diseases and immunosuppression.

- **Clinical manifestations:** *Acinetobacter baumannii* causes widespread healthcare associated infections such as:
  - Ventilator associated pneumonia
  - Central line associated bloodstream infection
  - Catheter- associated UTI
  - Wound and soft tissue infections
  - Infections in burn patients.
- **Laboratory diagnosis:** It is a nonfermenter, but differs from *P. aeruginosa*, by being nonmotile, oxidase negative, does not produce any pigment. Antimicrobial susceptibility testing is performed by disk diffusion test or by VITEK
- **Treatment** for *Acinetobacter* is similar to that of *Pseudomonas*, except that it responds to certain additional agents such as minocycline or tigecycline
- **Prevention:** Infection control measures such as improved hand hygiene are essential to prevent nosocomial infections due to *Acinetobacter* (refer contact precaution, Chapter 38).

## Burkholderia species

Important species that are pathogenic to man include *B. cepacia* complex and *B. pseudomallei*.

- **B. cepacia complex:** It inhabits a moist hospital environment and intravenous fluids; can cause fatal respiratory infections and septicemia in hospitalized patients with underlying diseases and immunosuppression
- **B. pseudomallei:** It is the causative agent of *melioidosis*; which presents in various clinical forms ranging from acute localized infection, subacute pulmonary infection, bloodstream infection, and chronic suppurative infection
  - **Diagnosis:** It shows bipolar staining in Gram-stained smear, intrinsically resistant to polymyxin B, and grows on selective media such as Ashdown's medium
  - **Treatment:** Compromises of—(i) intensive phase (2 weeks) with ceftazidime or meropenem, followed by (ii) maintenance phase (12 weeks) with oral cotrimoxazole.

## FASTIDIOUS GRAM-NEGATIVE BACILLI

Fastidious gram-negative bacilli include *Haemophilus*, *Bordetella*, and *Brucella*.

## Haemophilus species

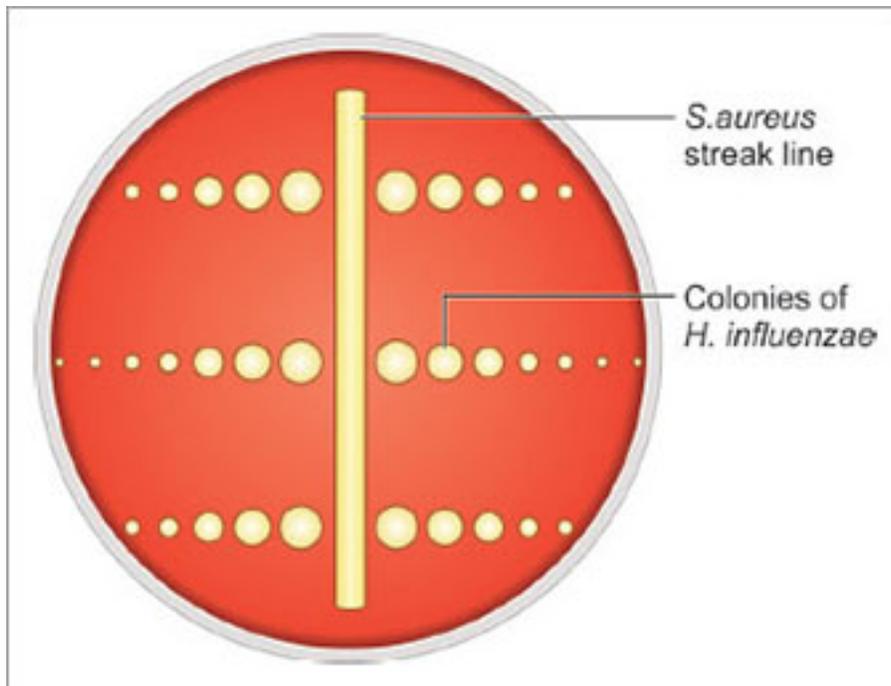
*Haemophilus* species are pleomorphic gram-negative bacilli that require special growth factors (such as X factor or V factor or both).

## Haemophilus influenzae

It is the most pathogenic species; causes pneumonia and meningitis in children. It requires both X and V factors for its growth.

- **Pathogenesis:** It is capsulated, which is the main virulent factor. Based on capsular polysaccharide antigen, it can be typed into 6 serotypes (a to f)—serotype b being the most pathogenic and invasive
- **Clinical manifestations:** The spectrum of illness can be divided into:
  - Invasive infections such as pneumonia, bacteremia, meningitis, and epiglottitis
  - Noninvasive infection such as otitis media, sinusitis, etc.
- **Laboratory diagnosis:** It is fastidious, grows in chocolate agar, not in blood agar
  - But it can grow on blood agar, adjacent to the *Staphylococcus aureus* streak line—a unique property described as *satellitism*. Factor X (hemin) is present in blood agar and factor V is released from *S. aureus*. Therefore larger colonies are formed adjacent to *S. aureus* streak line and size of the colonies decreases gradually away from the *S. aureus* streak line (Fig. 17.2)
  - Identification is confirmed by disk test for X and V requirements or automated ID systems such as MALDI-TOF.
- **Treatment:** Ceftriaxone is given for treatment
- **Vaccine:** Hib conjugate vaccine (*H. influenzae* type b) is available for children. Under the national immunization program, it is given as a part of the pentavalent vaccine at 6, 10, and 14 weeks.

**Fig. 17.2. Satellitism of *Haemophilus influenzae* (schematic diagram).**



## Haemophilus ducreyi

It is the causative agent of soft chancre (chancroid); a sexually transmitted infection characterized by painful genital ulcers and enlarged tender inguinal lymph nodes (bubo).

## HACEK Group

They represent a group of highly fastidious gram-negative bacilli, which are found as the normal commensal of the oral cavity but can cause serious infections such as endocarditis. They include—*Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*.

## Bordetella pertussis

*Bordetella pertussis* is the causative agent of *whooping cough*; a highly contagious toxin-mediated disease, characterized by paroxysmal cough ending in a high pitched inspiratory sound described as “whoop”.

- **Pathogenesis** is mediated by the expression of several virulence factors such as pertussis toxin, tracheal cytotoxin, adhesins, etc.
- **Laboratory diagnosis:** Nasopharyngeal aspirate collected by alginate swabs can be subjected to culture on special media such as Regan-Lowe or Bordet-Gengou media. It produces mercury drops or bisected pearls colony
- **Treatment:** Macrolide such as azithromycin is the drug of choice
- **Prevention:** Two vaccines are available— (1) whole-cell pertussis vaccine: which is given as a part of DPT under the national immunization schedule (see Chapter 13 for detail), and (2) acellular pertussis vaccine.

## Brucella species

Brucellosis is a highly contagious zoonotic febrile illness called undulant fever or Malta fever.

- **Agents:** *Brucella melitensis* is the most common species, affects sheep and goat. Other species are *B. abortus* (cattle), *B. canis* (dog), etc.
- **Transmission:** Transmitted from infected animals to man by various modes such as direct contact or by eating or drinking unpasteurized/raw dairy products
- **Clinical manifestations:** Overall brucellosis resembles typhoid-like illness. It manifests as a triad of fever, arthralgia, and hepatosplenomegaly
  - Fever is undulating in nature, i.e. afebrile period between febrile periods
  - Musculoskeletal involvement is common such as vertebral osteomyelitis or septic arthritis.
- **Laboratory diagnosis:** Specimen collected are blood or bone marrow
  - **Culture:** Blood culture or bone marrow culture is performed either by using (i) conventional blood culture bottles, or (ii) Castaneda's biphasic media (BHI broth/agar), or (iii) automated blood culture systems like BacT/ALERT
  - **Detection of antibodies** by serological tests such as standard agglutination test (SAT) or ELISA.
- **Treatment:** Comprises of doxycycline, in combination with rifampicin or streptomycin, given for a longer duration (6 weeks).

## MISCELLANEOUS GRAM-NEGATIVE BACILLI

### Campylobacter

*Campylobacter jejuni* is an important agent of inflammatory diarrhea or dysentery.

- **Transmission** is mainly by ingestion of raw or undercooked food products
- **Clinical manifestations:** Characterized by inflammatory diarrhea, abdominal pain, and fever. Extraintestinal complications can also be occasionally seen (mainly due to other species such as *C. fetus*) such as bacteremia, sepsis, meningitis, etc.
- **Diagnosis:** Stool culture can be done using selective media such as Skirrow's, Butzler's media and incubating in microaerophilic (5% O<sub>2</sub>) conditions. Gram stain reveals a curved gram-negative rod
- **Treatment:** Fluid and electrolyte replacement is the mainstay of treatment. Oral macrolides are the drug of choice (erythromycin or azithromycin).

## Helicobacter pylori

*Helicobacter pylori* is a curved gram-negative rod that colonizes the stomach.

- **Clinical manifestations:** *H. pylori* is associated with the pathogenesis of the following conditions:
  - Acute gastritis involving the antrum region
  - Peptic ulcer disease (duodenal and gastric ulcers): It presents with epigastric pain with a burning sensation; develops either following a meal (as in duodenal ulcer) or in an empty stomach (as in gastric ulcer)
  - Adenocarcinoma of stomach
- **Diagnosis:** Urea breath test and biopsy urease test are the preferred methods. Other diagnostic modalities include:
  - Fecal antigen (coproantigen) assay
  - Culture using Skirrow's media and chocolate agar and incubating the plates at 37°C under microaerophilic condition
- **Treatment** includes a triple-drug regimen, comprising omeprazole, clarithromycin, and metronidazole; given for 7–14 days.

## Legionella

*L. pneumophila* is a fastidious, pleomorphic gram-negative short rod, associated with two clinical syndromes—(i) Pontiac fever (an acute, milder flu-like self-limited illness), and (ii) Legionnaires' disease (a severe form of interstitial pneumonia)

- **Transmission:** Aspiration of the organism from oropharyngeal colonization or directly via the drinking of contaminated water is the most common mode. Aerosols from contaminated air conditioners, nebulizers, and humidifiers are another mode of transmission
- **Laboratory diagnosis** includes—(i) isolation of the organism in buffered charcoal, yeast extract (BCYE) agar, or (ii) urinary antigen detection
- **Treatment:** Macrolides (e.g. azithromycin) and respiratory quinolones are now the antibiotics of choice.

## Gardnerella vaginalis

It causes profuse watery vaginal discharge—a condition called bacterial vaginosis. It is diagnosed if any 3 of the following 4 findings are present (Amsel's criteria):

1. **Discharge:** Thin white homogeneous vaginal discharge uniformly coated on the vaginal wall

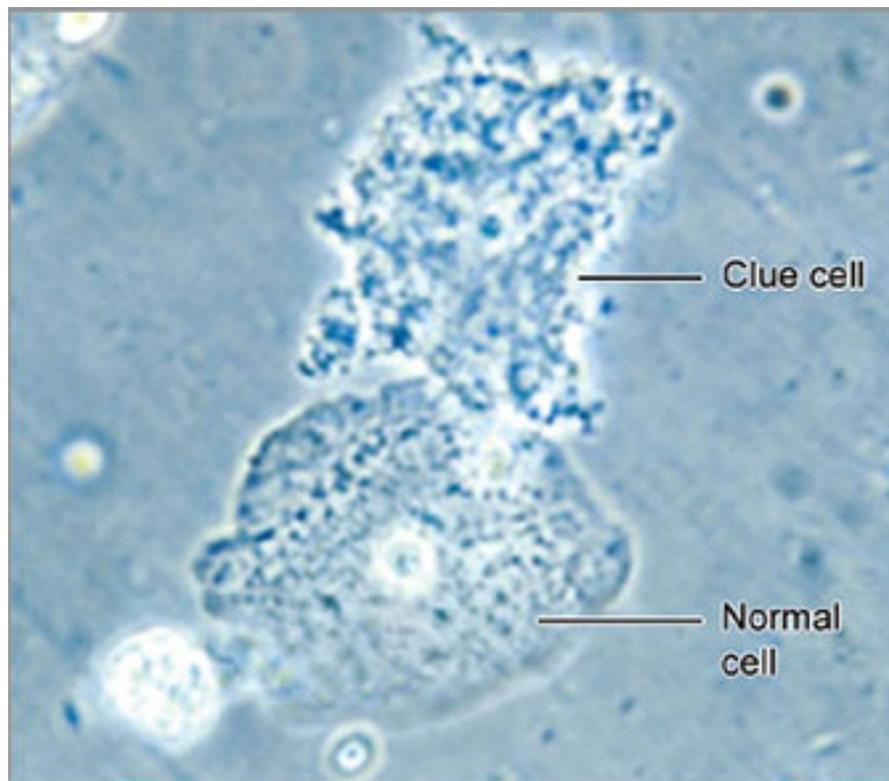
2. **pH** of vaginal discharge more than 4.5
3. **Whiff test:** Accentuation of a distinct fishy odor of vaginal secretions, when mixed with 10% solution of KOH
4. **Clue cells:** They are vaginal epithelial cells coated with coccobacilli, which have a granular appearance and indistinct borders observed on a wet mount (*Fig. 17.3*).

Treatment of bacterial vaginosis involves oral metronidazole, given twice daily for 7 days.

## Streptobacillus moniliformis

It causes a zoonotic systemic illness transmitted by rodents; called *rat-bite fever*. This condition is also caused by another gram-negative bacillus called *Spirillum minus*.

**Fig. 17.3. Wet mount of vaginal secretion depicting clue cell.**



*Source:* Public Health Image Library/ID#: 14574/ M. Rein/Centers for Disease Control and Prevention (CDC), Atlanta (*with permission*).

### EXPECTED QUESTIONS

1. **I. Write short notes on:**
  1. Infections caused by *Pseudomonas*.
  2. Laboratory diagnosis of *H. influenzae*.
  3. Melioidosis.
  4. Pertussis.

5. Infections caused by *Acinetobacter*.

**2. II. Multiple Choice Questions (MCQs):**

1. **Satellitism is observed in culture for \_\_\_?**

- a. *Bordetella*
- b. *H. influenzae*
- c. *Brucella*
- d. *Pseudomonas*

2. **Undulant fever is caused by \_\_\_\_\_?**

- a. *Burkholderia*
- b. *Helicobacter*
- c. *H. influenzae*
- d. *Brucella*

3. **Urea breath test is done \_\_\_\_\_?**

- a. *Campylobacter*
- b. *Burkholderia*
- c. *Helicobacter*
- d. *H. influenzae*

4. **Melioidosis is caused by \_\_\_\_\_?**

- a. *Burkholderia pseudomallei*
- b. *Burkholderia cepacia*
- c. *Burkholderia mallei*
- d. *Pseudomonas aeruginosa*

5. **Ecthyma gangrenosum is caused by:**

- a. *Pseudomonas*
- b. *Bordetella*
- c. *Brucella*
- d. *H. influenzae*

**Answers**

1. b	2. d	3. c	4. a	5. a
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