

NON-SPORING ANAEROBES

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Introduction

- **Anaerobes**

Microorganisms that grow only in complete or nearly complete absence of molecular oxygen.

Non-sporing Anaerobes – These do not form spores and are those which usually form the Normal flora Of Human beings and usually present in Skin, Oral cavity, GIT, Genitourinary tract and are opportunistic in nature.

HABITAT

- Normal flora of skin, mucosal surfaces, mouth, respiratory tract, GIT, genital tract
- Outnumber aerobes in many habitats
 - mouth and skin -10 to 30 times > aerobes
 - Intestines-1000 times >aerobes
- Estimated no of anaerobes in:
 - Saliva – 10^8 / ml
 - Small intestine – 10^5 / ml
 - Colon – 10^{11} / gm

- **Beneficial Role of Commensal non-sporing Anaerobes**
- **Part of normal flora, modulate physiological functions**
- **Compete with pathogenic bacteria**
- **Modulate host's intestinal innate immune response**

- **Production of vitamins like biotin, vit-B12 and K**
- **Polysaccharide A of *Bacteroides fragilis* influences the normal development and function of immune system and protects against inflammatory bowel disease.**
- **Lactobacilli maintain the vaginal acidic pH which prevents colonization of pathogens.**

Non-sporing Anaerobes Causing Disease

- **Anaerobic infections occur when the harmonious relationship between the host and the bacteria is disrupted**
- **Disruption of anatomical barrier (skin and mucosal barrier) by surgery, trauma, tumour, ischemia, or necrosis (all of which can reduce local tissue redox potentials) allow the penetration of many anaerobes, resulting in mixed infection**

Classification of non-sporing anaerobes

Gram-positive cocci

- **Peptostreptococcus**
- **Peptococcus**

Gram-negative cocci

- **Veillonella**

Gram-positive bacilli

- **Bifidobacterium**
- **Eubacterium**
- **Propionibacterium**
- **Lactobacillus**
- **Actinomyces**
- **Mobiluncus**

Gram-negative bacilli

- **Bacteroides**
 - **Prevotella**
 - **Porphyromonas**
 - **Fusobacterium**
 - **Leptotrichia**
- Spirochete**
- **Treponema, Borrelia**

Anaerobes as a part of normal flora

Anatomic Site	Total bacteria/ gm or ml	Anaerobic/Aerobic Ratio	Common anaerobic Normal flora
MOUTH			
Saliva	10^8-10^9	1:1	Anaerobic cocci Actinomyces Fusobacterium Bifidobacterium P.melaninogenica Spirochaetes
Tooth surface	$10^{10}-10^{11}$	1:1	
Gingiva	$10^{11}-10^{12}$	$10^3:1$	

Anaerobes as a part of normal flora - GIT

Anatomical Site	Total bacteria/ gm or ml	Anaerobic/ Aerobic Ratio	Common anaerobic Normal flora
Stomach	0–10⁵	1:1	Lactobacillus
Jejunum/ileum	10⁴–10⁷	1:1	Anaerobic cocci Bacteroides fragilis Fusobacterium Bifidobacterium P.melaninogenica
Terminal ileum & colon	10¹¹–10¹²	10³:1	Lactobacillus

Anaerobes as a part of normal flora

Anatomical Site	Total bacteria/ gm or ml	Anaerobic / Aerobic Ratio	Common anaerobic Normal flora
Skin		3:2	<i>Propionibacterium</i>
Vagina	10^7-10^9	10:1	Anaerobic cocci <i>Lactobacillus</i> <i>P.Melaninogenica</i> <i>Bifidobacterium</i>

Anaerobic cocci:

- 1. Peptococcus:** Small spherical, 0.5-1 μm in size, arranged singly, pairs, clumps. Coagulase negative.
 - Cause pyogenic infections of wound, puerperal sepsis and UTI
 - *P. niger* spp. Only
- 2. Peptostreptococcus and others:**
 - 0.3 to 1 0.5-1 μm in size, arranged in chains.
 - *P. anaerobius* most often cause puerperal sepsis
 - Cause suppurative lesions

Veillonella and others

- Small 0.3 to 2.5 μm in size.
- Gram negative cocci, pairs, chains or clumps
- Oxidase negative, occasionally cause blood stream infections

Anaerobic Gram positive bacilli

1. **Eubacterium**: strict anaerobe, Grow slowly
 - Normally present in mouth and intestine
 - *E.brachy*, *E.notatum* commonly associated with periodontitis
2. **Lactobacillus**: Bipolar and barred staining
 - Form lactic acid and grow best at Ph-5 or low
 - *L.acidophilus* synthesize biotin, vit B12, vit-K
 - Dental carries- Sucrose fermented into lactic acid which dissolve enamel and dentine

Doderlein bacilli

- Several spp. of lactobacilli in vagina collectively k/a doderlein bacilli
- Ferment glycogen deposited in vaginal epithelial cell and form lactic acid
- Which account highly acidic ph of vagina and they protect adult vagina from infections
- Best grow in media enriched with glucose, or blood in 5% co₂ and at ph 6
- Can cause ds in immunocompromised
- Advanced dental carries

Bifidobacterium

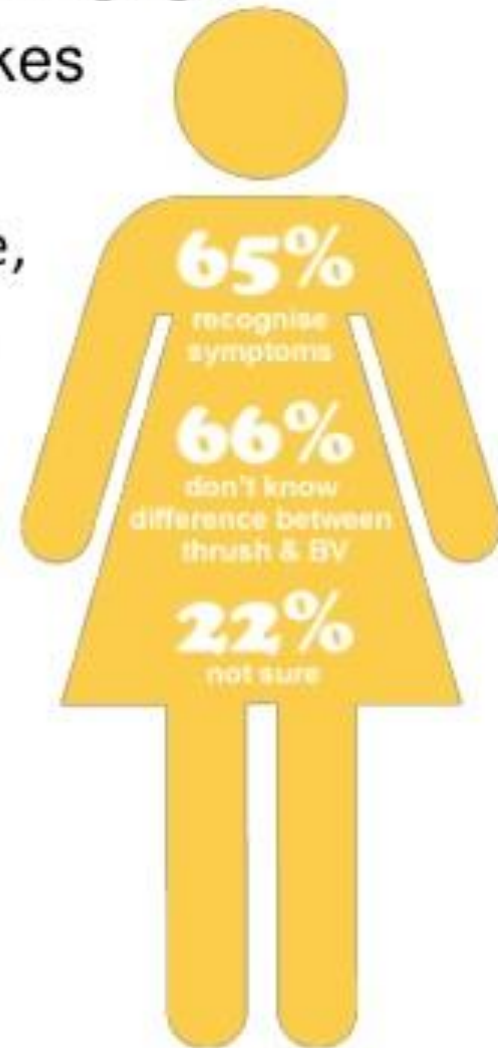
- GP, Non sporing, pleomorphic, non motile, bacilli
- Frequent Y shaped cells
- Normal flora of mouth and GIT
- Dental carries- *B.denticum*

Mobiluncus

- Generic name given by Spiegel and Robert (1984) to a group of bacteria isolated from human vagina with bacterial vaginosis
- Spp. *M. curtisii* short 1.7 x 0.5 μm and gram variable
- *M. mulieris*-long 2.9x0.5 μm and gram negative
- Motile, curved, gram variable,
- Bacterial vaginosis along with *Gardnerella vaginalis*

Bacterial vaginosis

- **BACTERIAL VAGINOSIS (BV)** is the most common cause of abnormal vaginal discharge in women of childbearing age.
- Condition first described by Gardner and Dukes in 1955.
- characterized by a foul smelling vaginal discharge, loss or reduction of the normal vaginal Lactobacilli, and overgrowth of other anaerobic bacteria.
- The causative organisms for this condition is *GARDNERELLA VAGINALIS*.



Bacterial vaginosis

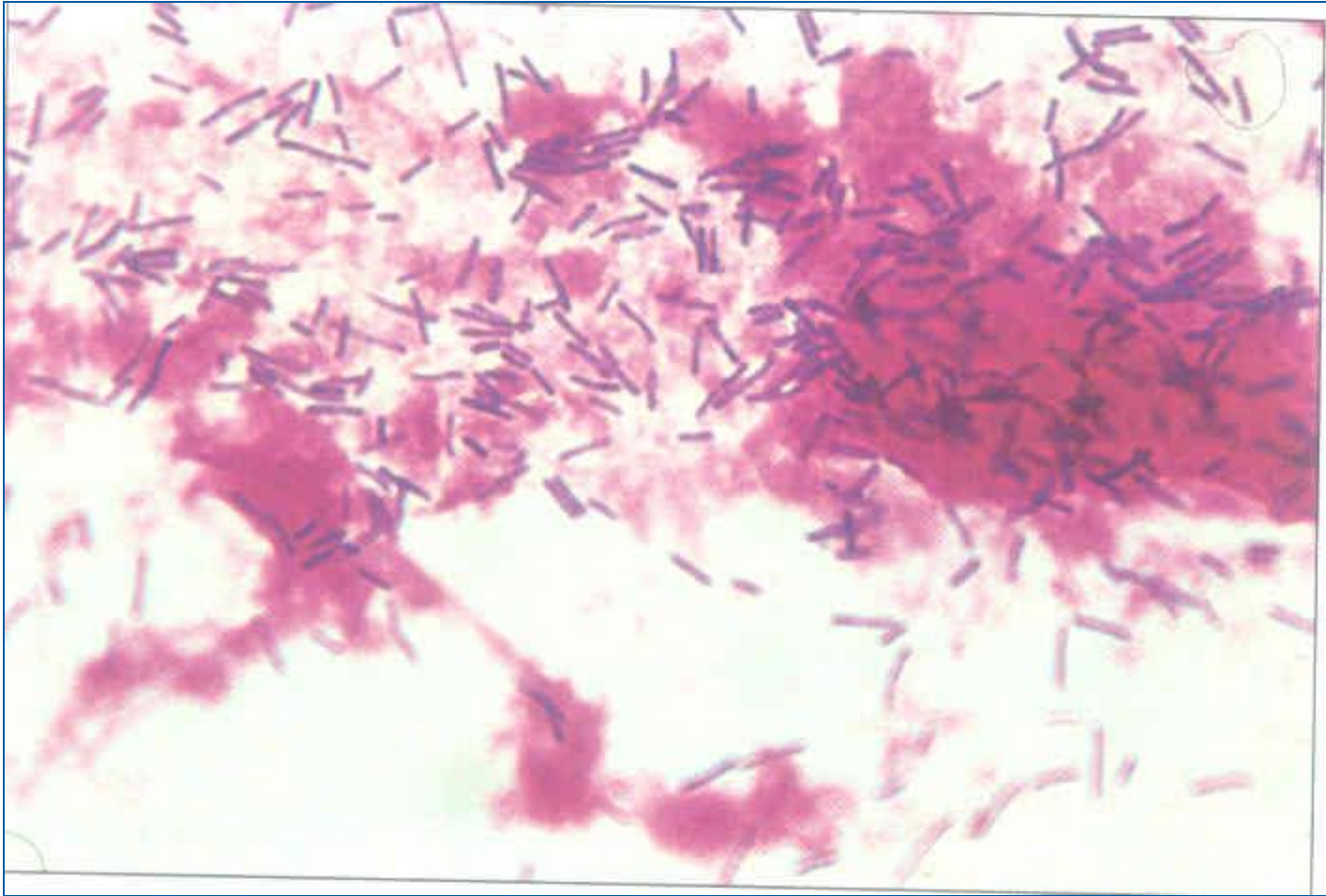
- In a patient suspected of BV, diagnosis can be made using

Amsel's criteria

(introduced 1984)

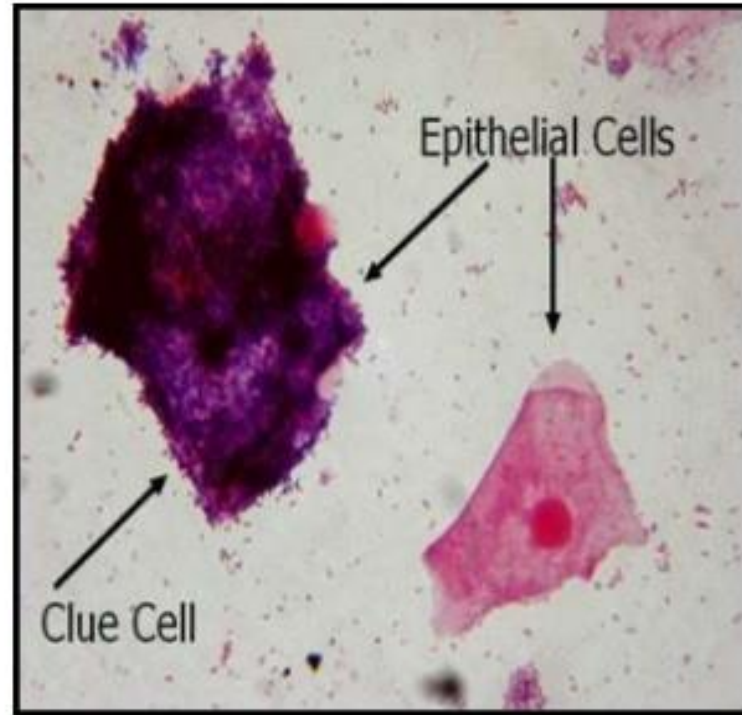
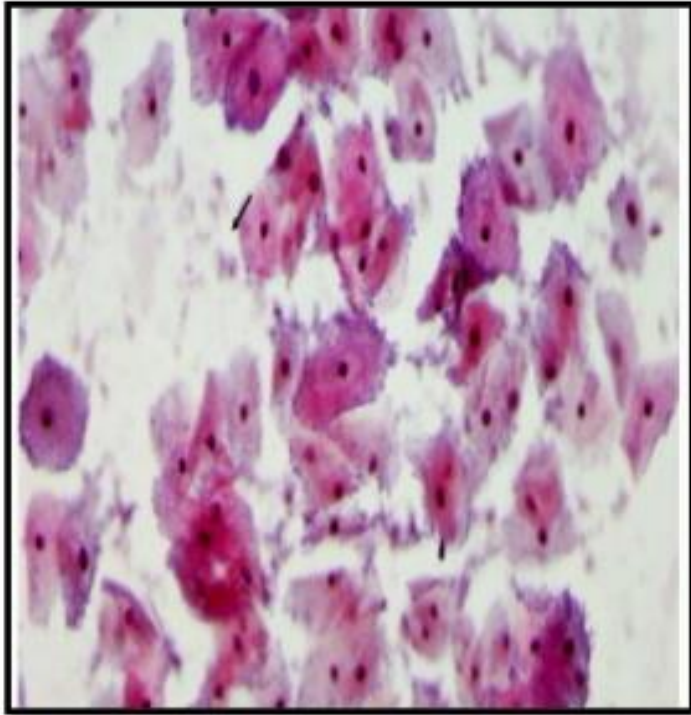
(3 out of 4 criteria below required to establish the diagnosis)

- Nonviscous homogenous white uniformly adherent vaginal discharge.
- High pH
- Clue cells –vaginal squamous cells covered by bacterial rods which blur the border of squamous cells
- Whiff test – adding 10% KOH to vaginal secretions produces an amine odour.



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Gram's stain of vaginal secretions showing clue cells



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Organism	VIRULENCE FACTORS
<p><i>Actinomyces species</i> Including <i>A.israeli</i>, <i>A.meyeri</i> <i>A.naeslundii</i> <i>A.odontolyticus</i></p>	<p>Not well characterised. Infections usually require disruptions of protective mucosal surface of the oral cavity, respiratory tract, GIT</p>
<p><i>Propionibacterium species</i> <i>Bifidobacterium species</i> <i>Eubacterium species</i> <i>Mobiluncus species</i></p>	<p>No definitive virulence factors are known</p>
<p><i>Bacteroides species</i> including <i>B.fragilis</i> <i>B.gracilis</i> <i>B.ureolyticus</i> <i>Prevotella species</i> <i>Porphyromonas species</i> <i>Fusobacterium nucleatum</i></p>	<p>These produce capsules and succinic acid, which inhibits phagocytosis and various enzymes that mediate cell damage</p>

PATHOGENESIS

ANAEROBIC COCCI

- *Peptococcus & Peptostreptococcus* – usually produce mixed infections along with Clostridia or anaerobic gram negative bacilli
 - Puerperal sepsis & other genital infections
 - Wound infections
 - Gangrenous appendicitis
 - UTI
 - Osteomyelitis
 - Abscesses in brain, lungs & other internal organs

PATHOGENICITY OF GRAM NEGATIVE ANAEROBIC BACILLI

<i>Bacteroides fragilis</i>	<i>Brain abscess, intra abdominal abscess, infections of female genitalia, cellulitis, diabetic ulcer, septicaemia</i>
<i>Prevotella melaninogenica</i>	Lung or liver abscess, empyema, pelvic infections in females, breast abscess, wound infections
<i>Porphyromonas</i>	Dental root canal infections, periodontal disease
<i>Fusobacterium necrophorum</i> <i>Fusobacterium nucleatum</i>	Aspiration pneumonia, lung/ liver abscess, oral infections, chronic sinusitis, abdominal infections

Sequence of the events

Trauma to the sites of protective barriers allow anaerobes of indigenous flora to gain access to deeper tissues.



Vascular stasis



Growth & multiplication of anaerobes



Anaerobic infection

Polymicrobial nature of the anaerobic infection

- Contamination of the tissue by the normal flora of the mucosa of the mouth, pharynx, GIT or genital tract.
- Multiple species are present including other anaerobes and facultative anaerobes.
- Aerobic bacteria may also be present.

CHARACTERISTICS SUGGESTING ANAEROBIC INFECTIONS

- ➔ Foul smelling discharge
- ➔ Infection in close proximity to mucosal surface.
- ➔ Tendency to form closed space infections either as discrete abscess (lung, brain, pleura) or by burrowing through tissue layers
- ➔ Polymicrobial nature.
- ➔ Infection associated with necrotic tissue (poor blood supply).

- ➔ Gas formation in tissues
- ➔ Failure to isolate organisms from pus (sterile pus) & negative aerobic cultures.
- ➔ Lack of response to usual antibiotic therapy.
- ➔ Infection of human or animal bite wounds.
- ➔ Detection of sulphur granules in pus .
- ➔ Gram negative bacteraemia is more common.

Infections of Non-sporing Anaerobes – Mouth, Head & Neck

Dental caries	Lactobacilli
Periodontal disease (gingivitis and periodontitis)	Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola
Dental root canal infections.	Porphyromonas endodontalis
Necrotizing ulcerative gingivitis (Vincent's angina, trench mouth)	Leptotrichia buccalis
Parapharyngeal space infections – quinsy, Ludwig's angina	Mixed flora containing anaerobes and aerobes
Cervicofacial actinomycosis	Actinomyces
Sinusitis	Prevotella, Fusobacterium, Peptostreptococcus
Chronic suppurative otitis media	Bacteroides fragilis

Infections of Non-sporing Anaerobes

CNS infections

Brain abscess, epidural abscess, and subdural empyema.

Peptostreptococcus, Fusobacterium, Prevotella, Propionibacterium, Eubacterium, Veillonella, Actinomyces

Pleuropulmonary infections

Aspiration pneumonitis, Necrotizing pneumonitis, Anaerobic lung abscesses, Empyema

Non-pigmented Prevotella, Peptostreptococcus, Bacteroides, Fusobacterium

Infections of Non-sporing Anaerobes

Pelvic and genitourinary infections	
Bartholin gland abscess, salpingitis, tubo-ovarian abscess, septic abortion, pyometra, endometritis and postoperative wound infection	Bacteroides fragilis, Prevotella (pigmented), Anaerobic cocci, Clostridium species
Puerperal sepsis	Anaerobic cocci
Bacterial vaginosis (also by Gardnerella vaginalis)	Mobiluncus, Prevotella, Peptostreptococcus

Infections of Non-sporing Anaerobes

Abdominal infections	
Peritonitis and abscesses	Mixed colonic flora Most common-Bacteroides fragilis
Diarrhoea	Enterotoxigenic Bacteroides fragilis
Bone and Joint infections	
Anaerobic infections of bone	Actinomyces, Fusobacterium Peptostreptococcus, Bacteroides,
Anaerobic septic arthritis	Fusobacterium
Cervical venous thrombophlebitis (Lemierre's syndrome)	Fusobacterium necrophorum

Infections of Non-sporing Anaerobes

Skin and Soft Tissue infections	
Crepitant cellulitis, Skin abscess, Foot ulcers of diabetic patients	Bacteroides, Peptostreptococcus, Clostridium
Anaerobic bacterial synergistic gangrene (Meleney's gangrene), a rare infection of superficial fascia (also due to Staphylococcus aureus)	Peptostreptococcus
Necrotizing fasciitis (also due to Streptococcus pyogenes)	Peptostreptococcus, Bacteroides
Fournier gangrene (consists of cellulitis involving the scrotum, perineum and abdominal wall)	Mixed anaerobic organisms

Infections of Non-sporing Anaerobes

Abdominal infections

**Peritonitis and abscesses
(following a breach in the
intestinal mucosa)**

**Mixed colonic flora
Most common-Bacteroides
fragilis**

Diarrhoea

**Enterotoxigenic Bacteroides
fragilis**

CLINICAL PRESENTATION OF ANAEROBIC INFECTIONS

- Infections adjacent to mucosal surfaces that bear anaerobic flora
- **Predisposing factors:**
 - Ischemia, Tumor
 - Penetrating trauma, foreign body, or perforated viscus
- Spreading gangrene involving skin, subcutaneous tissue, fascia, and muscle
- Foul smelling putrid pus
- Abscess formation

CLINICAL PRESENTATION ...

- Septic thrombophlebitis □
- Toxemia and fever not marked
- Failure to respond to antibiotics not with anaerobic activity
- Organisms seen in Gram stain, fail to grow in routine aerobic culture
- **Special features:** Gas in specimen (gas gangrene)
- Black pigment that fluoresce (*P. Melaninogenica*)

Laboratory Diagnosis

Suitable specimens

1. Head and neck

- Tissue fluid aspirate ,cerebrospinal fluid .

2. Respiratory tract

- pleural fluid,broncho alveolar lavage fluid.

3. Abdomen

- Peritoneal (ascitic fluid) abscess aspirate.

4. Urinary tract

- Suprapubic aspirate .

5. Bone and joint

- Bone marrow ,synovial fluid.

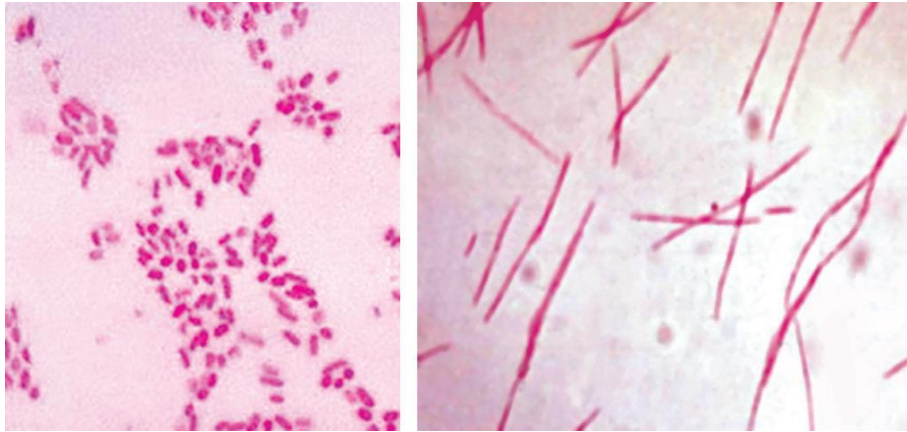
6. Genital tract

- Endoscopy specimen,endometrial aspirate.

LABORATORY DIAGNOSIS

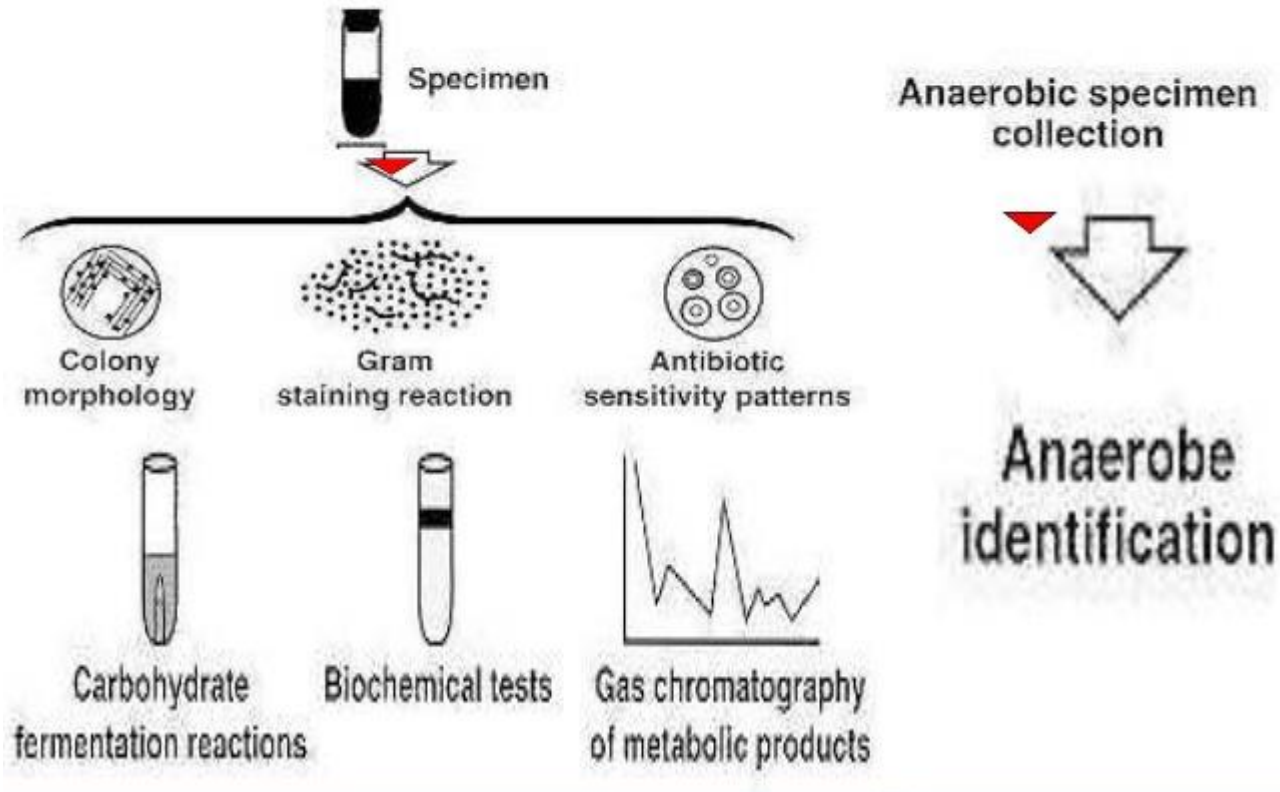
- **Specimens**
- All clinical specimens must be handled meticulously as brief exposure to oxygen may kill obligate anaerobes and result in failure to isolate them in the laboratory.
- **Accepted specimens:** Tissue bits, necrotic materials, aspirated body fluids or pus in syringes
- **Unacceptable specimens:** All swabs, sputum or voided urine
- Immediately put into RCM broth or other anaerobic transport media and brought to the laboratory as soon as possible

- **Microscopy**
- All clinical specimens from suspected anaerobic infections should be Gram stained and examined for characteristic morphology



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Methods of Diagnosis



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- **Microscopy** – Gram stain: different micro-organisms
- **Culture media**
 - Brucella blood agar (BRBA)
 - Phenyl ethyl alcohol agar (PEA)
 - Kanamycin-vancomycin-laked blood agar(KVLBA)
 - Bacteroides bile esculin agar(BEA)
 - Neomycin blood agar(NBA)
- **Culture methods** – Anaerobic jar, Gaspak

GRAM STAINING MORPHOLOGY

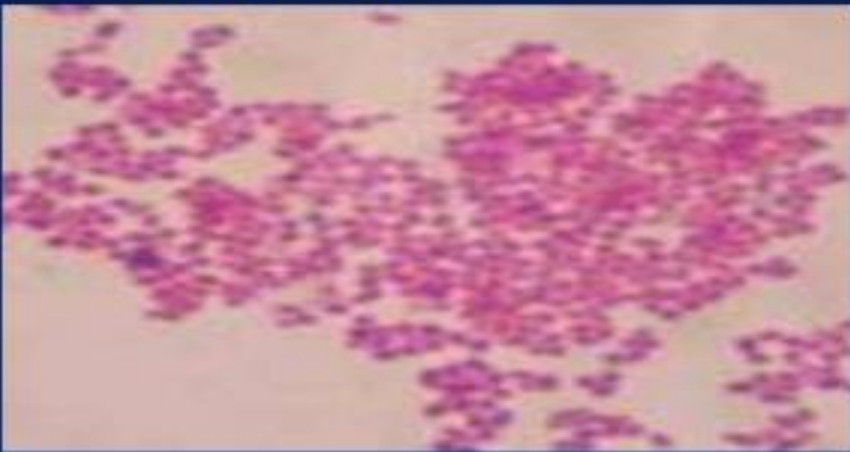
ACTINOMYCES



FUSOBACTERIUM NUCLEATUM



VELLIONELLA



BACTEROIDES FRAGILIS



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Processing of samples

Gross examination

- Blood
- Purulence
- Necrotic tissue
- Foul odor
- Sulphur granules



- **Colony morphology**

- Agar pitting
- Black or tan pigmentation
- Brick red fluorescence
- Fried egg

- Greening of medium

- Molar tooth

- Speckled or bread-crumb

Identification

Bacteroides ureolyticus

Porphyromonas

Prevotella

Fusobacterium necrophorum

Fusobacterium varium

Actinomyces

Fusobacterium nucleatum

Pigment production

- *Porphyromonas, prevotella* on anaerobic media
- Dark brown or black pigment.



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Treatment Anaerobic infections

- **Common antibiotics used:**
 - Metronidazole
 - Carbapenems (imipenem)
 - β -lactam/ β -lactamase inhibitor combination (ampicillin/sulbactam)
 - Chloramphenicol
- **Choice of antibiotics depends on** the site of infection, type of anaerobe involved and susceptibility to antibiotics
- **Antimicrobial resistance** in anaerobic bacteria is an increasing problem.

Treatment

- **Surgical** –
 - Drainage of pus from abscess
 - Wound debridement
 - Curettage & removal of necrotic tissues
- **Antibiotics** –
 - Metronidazole
 - Penicillin
 - Clindamycin
 - Cephalosporins
 - chloramphenicol

Antibiotic resistance

- Most of *bacteroides fragilis*, *prevotella* and *porphyromonas* species are resistant to penicillins and to many cephalosporins due to production of beta-lactamase.
- The resistance is overcome by treating with high concentration of piperacillin, imipenem along with beta-lactamase inhibitors.
- *Bacteroides* show plasmid mediated resistance to clindamycin.

HAVE A NICE DAY

