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## NON-SPORING ANAEROBES

### 36.1 INTRODUCTION

Anaerobic bacteria are widespread and very important. They do not require oxygen for growth, which is often toxic for them. They lack the enzymes superoxide dismutase, peroxidase and/or catalase, which makes them susceptible to oxygen derived free radicals. These organisms obtain energy from fermentation process. These bacteria form the commensal flora of mouth and oropharynx, gastrointestinal tract and genitourinary tract.



### OBJECTIVES

After reading this lesson, you will be able to:

- classify the non-sporing anaerobes.
- describe their pathogenic potential.
- explain the laboratory diagnosis of the important pathogenic species.

### 36.2 NON-SPORING ANAEROBES

The anaerobic bacteria can be sporogenous (eg. *Clostridium species*) or non-sporing (eg *Bacteroides species*). Non-sporing anaerobes constitute an important cause of human infections. Even in seemingly anaerobic conditions as the mouth and the skin, anaerobic bacteria are ten to thirty times more frequent than aerobes.

These bacteria differ widely in the degree of anaerobiosis required for their growth.

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### Non-Sporing Anaerobes

- (a) **Facultative anaerobes** - Can grow in the presence or absence of oxygen. Obtain energy by both respiration and fermentation. Oxygen not toxic, some use nitrate ( $\text{NO}_3^-$ ) or sulphate ( $\text{SO}_4^{2-}$ ) as a terminal electron acceptor under anaerobic conditions. E.g. *Peptostreptococcus*.
- (b) **Obligate (strict) anaerobes** - Oxygen is toxic to these organisms, do not use oxygen as terminal electron acceptor. E.g. *Bacteriodes*.
- (c) **Microaerophilic organisms** - require low levels of oxygen for growth, but cannot tolerate the levels present in the atmosphere. E.g. *Spirochetes*
- (d) **Aerotolerant anaerobes**: Metabolism is anaerobic but they are unaffected by the presence of oxygen. E.g. *Propionibacterium*.

### 36.3 CLASSIFICATION OF NON-SPORING ANAEROBES

On the basis of morphology and staining characters, non-sporing anaerobes are classified as under:

#### Gram -ve bacilli

*Bacteroides*  
*Prevotella*  
*Porphyromonas*  
*Fusobacterium*  
*Leptotrichia*

#### Cocci

*Peptococci*  
*Peptostreptococci*  
*Veillonella (gram -ve)*

#### Gram +ve bacilli

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

#### Spirochetes

*Treponema*  
*Borrelia*

### 36.4 ANAEROBIC COCCI

The anaerobic cocci are commonly found as commensals on the human skin, in the female genital tract, in the oropharynx, and in the gastrointestinal tract. Many of them are aero-tolerant and grow well under 10%  $\text{CO}_2$  in an aerobic or microaerophilic atmosphere. The important pathogenic species of anaerobic gram positive cocci include: *Peptostreptococcus anaerobius*, *Pst magnus*, *Pst asacchrolyticus*. Most of the peptococci have now been reclassified as peptostreptococci. *Peptococcus niger* is the only surviving member of the genus peptococcus. They may cause several clinical infections such as:

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- Puerperal sepsis
- Other genital infections
- Wound infections
- Gangrenous appendicitis
- Urinary tract infections
- Osteomyelitis
- Brain, lung, hepatic and other abscess
- Intra-abdominal infections
- Empyema and aspiration pneumonias

Anaerobic gram negative cocci include *Veillonella parvula*. These are obligate anaerobes and are usually non-pathogenic but may occasionally invade blood stream.

The anaerobic cocci are generally sensitive to metronidazole and penicillin and a wide range of other antibiotics like tetracycline, erythromycin, clindamycin and cephalosporins. These are however, resistant to streptomycin and gentamycin.



### INTEXT QUESTIONS 36.1

1. Anaerobic bacteria do not require ..... for growth
2. Anaerobic bacteria form the commensal flora of ..... & ..... tract of human
3. Facultative anaerobes obtain energy by ..... & .....
4. Organisms that are unaffected by the presence of oxygen are caused by .....

### 36.5 ANAEROBIC GRAM POSITIVE BACILLI

The medically important genera of this group are *Eubacterium*, *Propionibacterium*, *Lactobacillus*, *Mobiluncus* and *Bifidobacterium*.

**Eubacterium:** These are strictly anaerobic and form the normal flora of mouth and intestine.

**Propionibacterium:** Pleomorphic, gram positive, non-motile rods. They are aerotolerant.

*P. acnes* is constantly present on skin and is a common contaminant of blood and CSF cultures. These are not normally regarded as pathogens but are found in acne, in some cases of infective endocarditis and in infections associated with implanted prosthesis.

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**Lactobacillus:** Straight or curved gram positive rods. They are present in the mouth, intestines and adult vagina (Doderlein’s bacilli). They are generally non-pathogenic, but some species have been incriminated in the pathogenesis of dental caries and bronchopulmonary infections.

**Mobiluncus:** These are motile, curved, anaerobic bacilli that appear as gram variable rods. *Mobiluncus mulieris* and *M curtisii* leads to bacterial vaginosis along with other pathogens like *Gardnerella vaginalis*, *Mycoplasma hominis* and *Bacteroides species*. Bacterial vaginosis is a polymicrobial infection characterized by a thin foul smelling vaginal discharge. Its smell is accentuated when mixed with a drop of KOH solution. The vaginal pH is more than 4.5. Clue cells are seen in films.

**Bifidobacterium:** These are pleomorphic rods that show true and false branching. The name is derived from the frequent bifid ‘Y’ shaped cells. Most species are obligate anaerobes and are present in large numbers in the intestines and in the mouth.

### 36.6 ANAEROBIC GRAM NEGATIVE BACILLI

The anaerobic, gram negative, nonsporng and non-motile bacilli, ranging from short gram-negative rods to filamentous and fusiform shapes, belong to the family *Bacteroidaceae*. This family includes three genera: (1) *Bacteroides*, (2) *Fusobacterium* (3) *Leptotricha*. (4) *Porphyromonas* (5) *Prevotella*

(1) *Bacteroides*: These are the most common anaerobes isolated from clinical specimens. They grow well in an anaerobic atmosphere containing 10% CO<sub>2</sub>. They possess capsular polysaccharides which appear to be virulence factors.

Table 36.1

Degree of saccharolysis	Genus	Species	Pigment	Pathogenesis
Saccharolytic group	<i>Bacteroides</i>	<i>B fragilis</i>	nil	Meningitis, brain abscess, Pleural, peritoneal infections Wound, urogenital infections
Moderately saccharolytic group	<i>Prevotella</i>	<i>P melaninogenica</i>	Brick red (under UV light)	Lung, liver abscess Mastoiditis, intestinal lesions Lesions of mouth and gums
Asaccharolytic group	<i>Porphyromonas</i>	<i>P gingivalis</i> <i>P endodontalis</i>	black	Periodontal disease Root canal infections

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2. *Fusobacterium*: These are gram negative, strict anaerobic, long, thin or spindle shaped bacilli with pointed ends. They are commensals in the mouth and also cause infections of this and related sites. *F. nucleatum* and *F. necroforum* are the most commonly isolated species of this group. It may cause infections of head and neck regions including dental and periodontal infections and cerebral abscess.
3. *Leptotricha*: This genus contains only one species, *L. buccalis*. These are gram negative rods with tapering ends. They are regarded as essentially commensal in human mouth. These tend to occur in mixed infections such as putrefactive necrotic fusospirochaetal conditions (vincent's angina).

Most of the anaerobic gram negative bacilli are susceptible to metronidazole, which is the drug of choice.

## 36.7 LABORATORY DIAGNOSIS OF ANAEROBIC INFECTIONS

- (a) Specimen collection and transport: Minimise the contact with air.

Aspirate the specimen in an airtight syringe, plunge the needle into a rubber cork to seal it. Pus and other fluids may be collected in small bottles with air tight caps. Swabs should not be used when anaerobic infection is suspected. Pre-reduced anaerobically sterilized (PRAS) transport media or gassed out vials or hungate's tubes, Robertson's cooked meat medium (RCM) or Thioglycollate broth (TGB) can be used for transportation of specimens for anaerobic cultures.

- (b) Direct examination of the specimen:

- In the laboratory exposure should be limited to minimum.
- Presence of foul smell is indicative of anaerobic infections.
- Examination of the specimen under ultraviolet light may show the brick red fluorescence of *P. melaninogenica*.
- On gram's staining presence of abundant pus cells along with a mixed bacterial flora is suggestive of anaerobic infections, since most of the times anaerobic infections are polymicrobial.
- Gas liquid chromatography of the specimen may yield the presumptive information on the types of anaerobes present.

- (c) Culture: Media that can be used include blood agar, brain heart infusion agar, phenyl ethyl alcohol agar (PEA), kanamycin / vancomycin BA, bacteroides bile aesculin agar, thioglycollate broth, robertson's cooked meat

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broth (RCM). Culture can be done in anaerobic jars, Gaspak system or in anaerobic chambers. All cultures should be incubated for minimum 72 hours as most of the anaerobes are slow growing.



#### INTEXT QUESTION 36.2

1. P.acnes is commensal of .....
2. P.acnes are common contaminant of ..... & ..... culturally
3. ...., ..... & ..... tubes can be used for transportation of specimen
4. Presence of ..... in the specimens is indicative of anaerobic infections



#### WHAT YOU HAVE LEARNT

- Many anaerobic bacteria are pathogenic for human beings, and they outnumber aerobes in many habitats. They vary widely in the degree of anaerobiosis required for growth. They are classified on the basis of morphology and gram staining. *Bacteroides species* are the most commonly isolated pathogens amongst all the non-sporing anaerobes. Diagnosis of anaerobic infections is difficult. Most of the times such infections are associated with a putrid smell. Presence of pus cells along with a polymicrobial flora on microscopy is indicative of anaerobic infections. Culture is difficult. Exposure to oxygen should be prevented and longer incubation periods are required. Almost all the anaerobes are susceptible to metronidazole.



#### TERMINAL QUESTIONS

1. Classify anaerobic bacteria on the basis of the degree of anaerobiosis required for growth.
2. Classify non-sporing anaerobes.
3. Write short note on anaerobic gram positive cocci.
4. Discuss anaerobic gram negative rods.
5. Write briefly on the *Bacteroides species*.



## ANSWERS TO INTEXT QUESTIONS

### 36.1

1. Oxygen
2. Gastro-intestinal & Genito urinary
3. Respiration & fermentation
4. Aerotolerant anaerobes

### 36.2

1. Skin
2. Blood & CSF
3. Pre-reduced transportation media, gassed out violes & hungates
4. Foul smell

