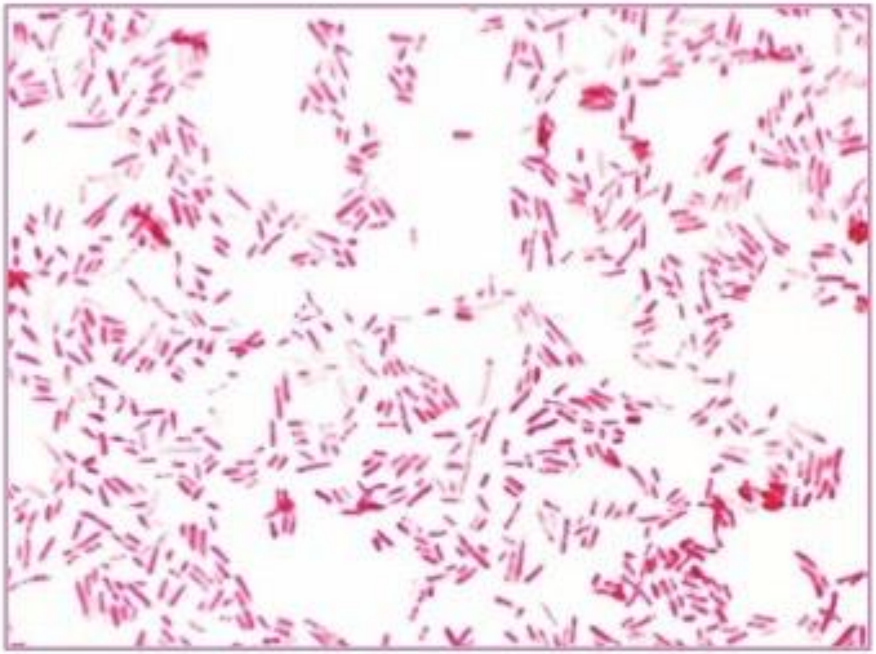
Chapter 14

Overview of major pathogens; anaerobes

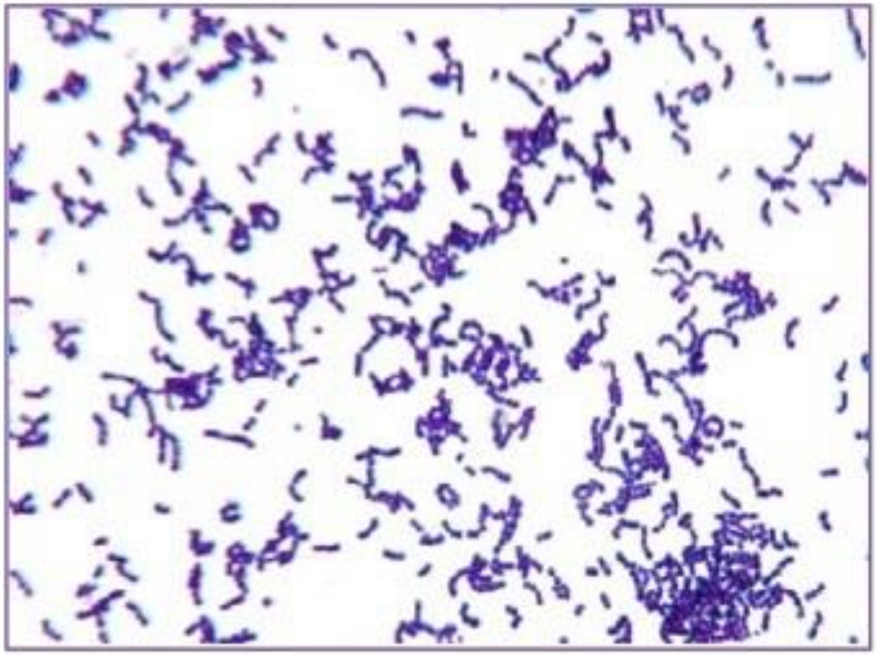
Overview of major pathogens

Most of bacteria are readily stained by Gram stain but some are difficult to stain.

Those which are easily stained can be **Gram positive** (cocci and rods) or **Gram negative** (cocci and rods).



Gram-Negative Bacteria



Gram-Positive Bacteria

Gram negative rods are further subdivided into:

1. Associated with **enteric tract**
2. Associated with **respiratory tract**
3. From animal sources (**zoonotic** bacteria)

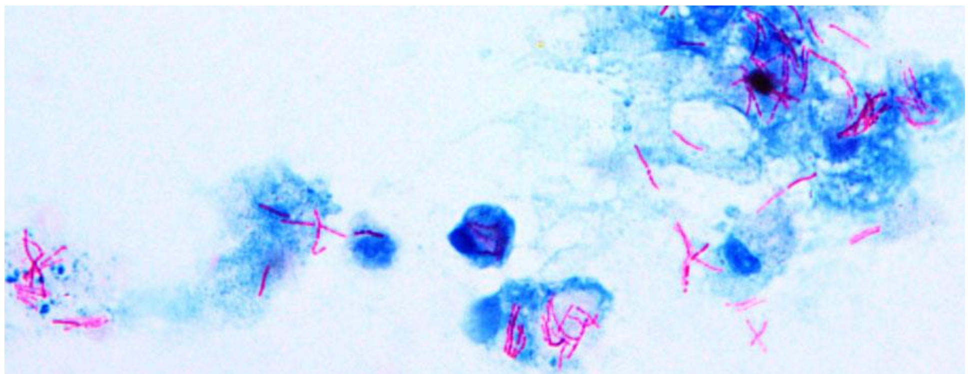
Enteric tract associated pathogens can further be subdivided into:

1. Pathogens present both **inside and outside** the enteric tract
2. Pathogens **inside** the enteric tract
3. Pathogens **outside** the enteric tract

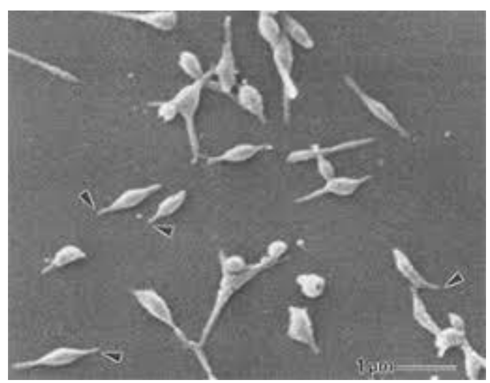
Some pathogens are difficult to put exclusively in one group, e.g., ***Campylobacter*** is an enteric pathogen but frequently has an animal source.

Those pathogens which are **not readily stained** by **Gram's method** fall in to four categories:

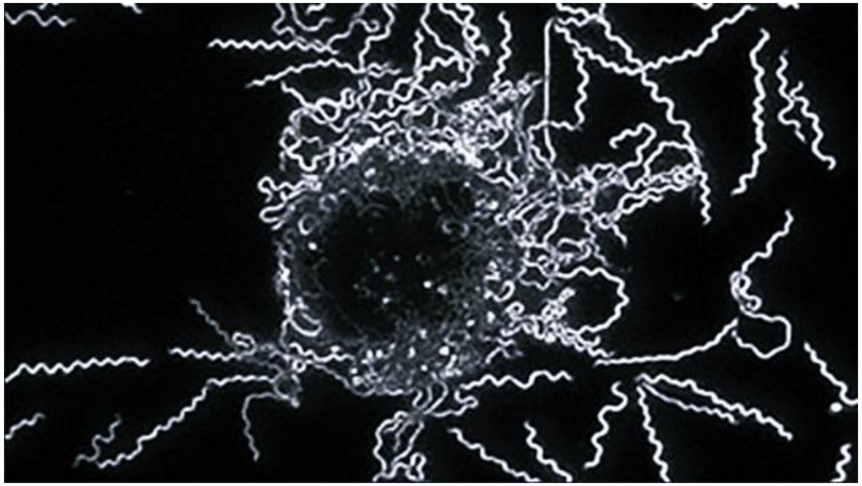
1. ***Mycobacterium*** **species** which are **acid fast** rods



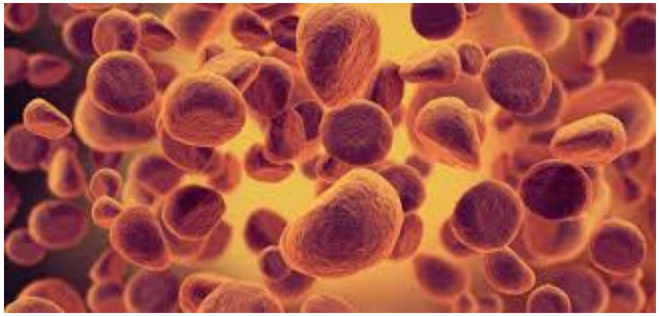
2. ***Mycoplasma*** **species** which **do not** have a cell wall

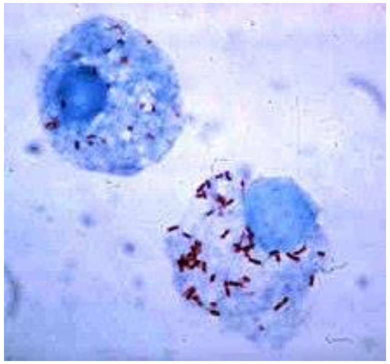


3. Spirochetes which are too thin to be **seen directly**



4. *Chlamydia* and *Rickettsia* (obligate parasites) which stain well with Giemsa stain but **poorly** with Gram stain





Introduction to anaerobic bacteria

Important properties

-- **Anaerobes** can grow only in atmosphere with **less than 20% oxygen**.

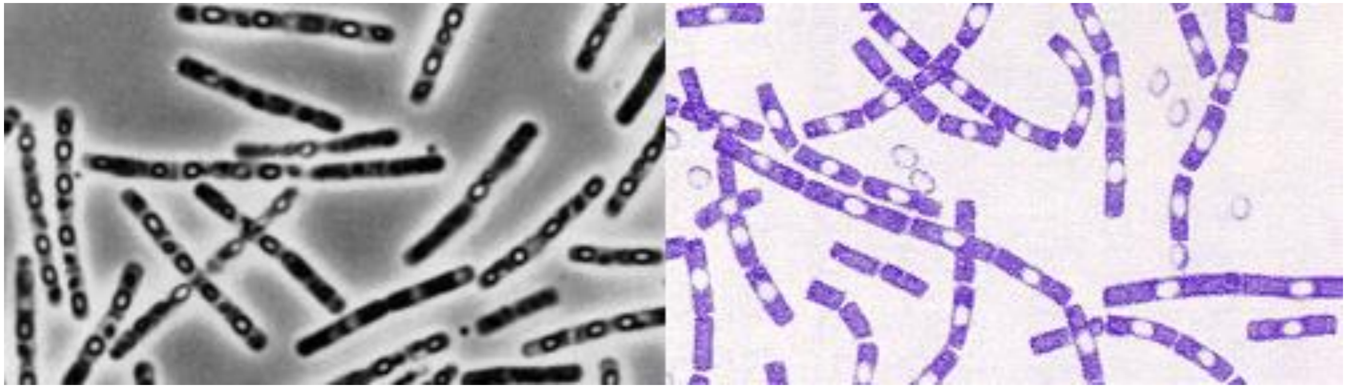
-- Some can grow **only in up to 0.02% oxygen**, e.g., ***Bacteriodes fragilis*** and ***Clostridium perfringens***. ***Campylobacter jejuni*** requires almost 5% oxygen.

-- Main reason why they cannot grow in excess of oxygen is the **reduced amount or absence of catalase and superoxide dismutase** which are necessary to remove toxic hydrogen peroxide and superoxide.

-- In addition to oxygen concentration, the **oxidation reduction potential (E_h)** in a tissue is an important determinant of the growth of anaerobes.

-- Areas with **low E_h** such as **periodontal pocket**, **dental plaque** and **colon** support the growth of anaerobes. **Crushing injuries** resulting in impaired blood supply also provide low E_h.

--   **Pathogenic anaerobes** can be Gram positive or Gram negative, cocci or rods. They can also be either spore forming (e.g., ***Clostridium***) or non spore formers (e.g. ***Bacteroides***).



Clinical infections

-- Many anaerobic pathogens are **part of normal flora** and cause disease **only when they leave their site**.

-- However, ***Cl. botulinum*** and ***Cl. tetani*** are **soil organisms**, where as ***Cl. perfringens*** is found both in **colon and soil**.

-- Diseases caused by **anaerobic normal flora** at other sites are usually in the form of **abscesses** which are most frequently located in the **brain, lungs, female genital tract, biliary tract** and other **intra-abdominal** sites.

-- Most of these abscesses contain **more than one organism**, either multiple anaerobes or a mixture of **anaerobes** and **facultative anaerobes** which consume sufficient oxygen to let anaerobes grow.

Three findings on physical examination are indicative of **anaerobic infection**:

1. **Foul smelling** discharge
2. **Gas** in the tissue
3. **Necrotic** tissue

In addition **location** is also important.

Infections in the setting of **pulmonary aspiration, bowel surgery, abortion, cancer, or human and animal bites** frequently involve anaerobes.

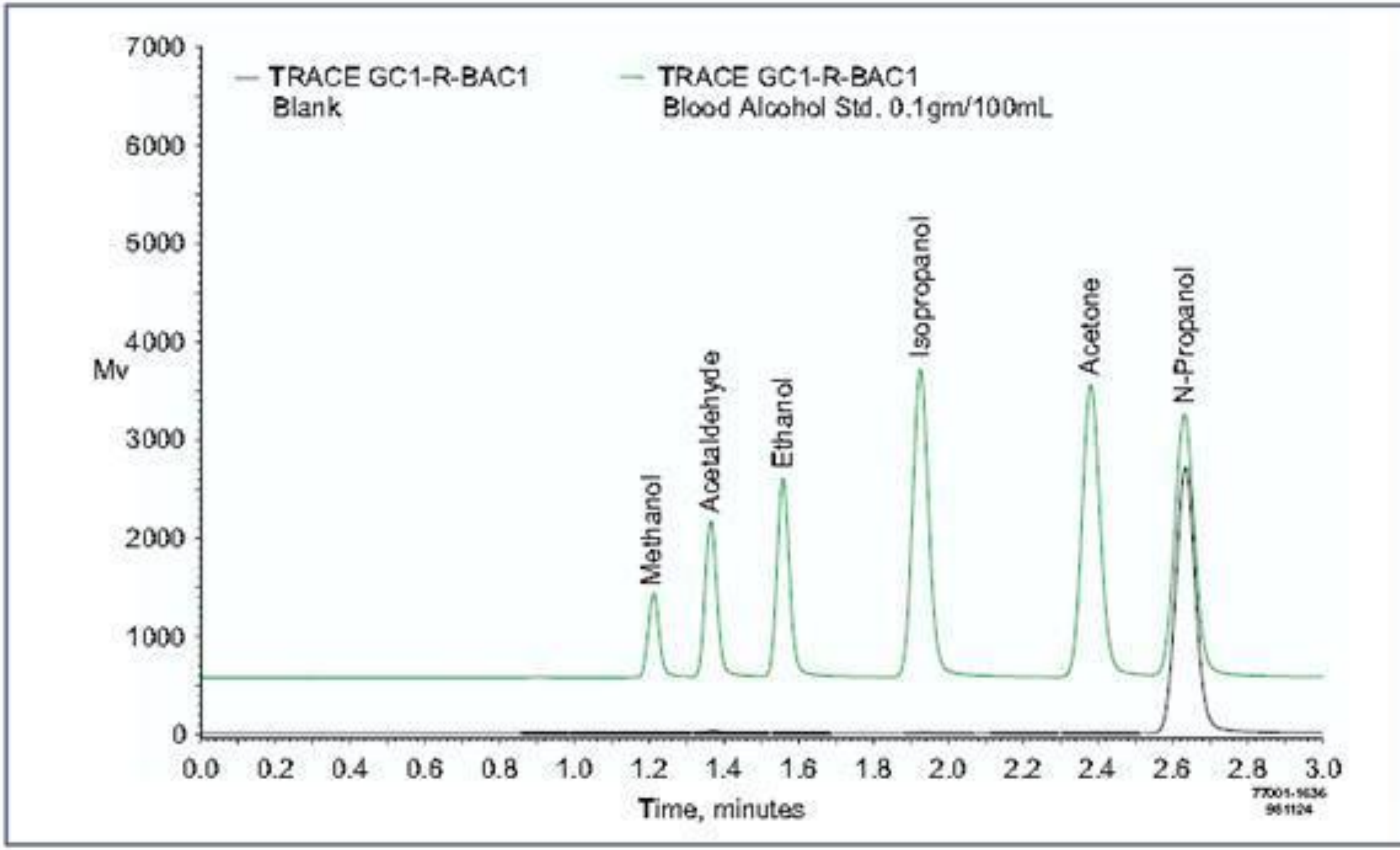
Laboratory diagnosis

Two aspects of microbiological diagnosis of an anaerobic infection are important even before the specimen is cultured:

1. **Obtaining the appropriate sample**, i.e, free of normal flora so sputum and feces are not appropriate.
2. **Rapid transport to laboratory under anaerobic conditions**

In the laboratory cultures are handled and incubated under anaerobic conditions.

Beside conventional methods, **gas chromatography** can be of great help. In this procedure, organic acids such as formic, acetic, and propionic acid are measured.



TRACE GC1-R-BAC1
Blank
TRACE GC1-R-BAC1
Blood Alcohol Std. 0.1gm/100mL
7000
6000
5000
4000
Mv
3000
2000
1000
0
Methanol
Acetaldehyde
Ethanol
Isopropanol
Acetone
N-Propanol
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
Time, minutes

Treatment

-- In general, **surgical drainage of the abscess** plus administration of **antimicrobial drugs** are indicated.

-- Drugs commonly used are **penicillin G, cefoxitin, chloramphenicol**, **clindamycin**, and **metronidazole**.

-- Many isolates of ***B. fragilis*** produce **α-lactamase** and are thus resistant to penicillin.

Chapter 15

Gram positive cocci

Staphylococcus

Gram positive cocci

Introduction

There are two important genera of Gram positive cocci: ***Staphylococcus*** and ***Streptococcus***. Both are **non-motile** and **do not form spores**.

Microscopically **staphylococci** appear in **grapelike clusters** and **streptococci** in **chains**.

Biochemically **staphylococci** produce **catalase** which degrades microbicidal H_2O_2 whereas **streptococci** do not.

Their most important representatives are ***Staphylococcus aureus*** and ***Streptococcus pyogenes***.

Staphylococcus

Diseases

-- ***Staphylococcus aureus*** causes a variety of **suppurative** (pus-forming) infections and **toxinoses** in humans.

-- It causes superficial skin lesions such as **boils**, **styes** and **furuncules**; more serious infections such as **pneumonia**, **mastitis**, **phlebitis**, **meningitis**, and **urinary tract infections.**

-- It also causes deep-seated infections, such as **osteomyelitis** and **endocarditis**.

-- ***S. aureus*** is a major cause of **hospital acquired (nosocomial) infection** of surgical wounds and infections associated with indwelling medical devices.

-- ***S. aureus*** causes **food poisoning** by releasing **enterotoxins** into food, and **toxic shock syndrome** by release of **superantigens** into the blood stream.

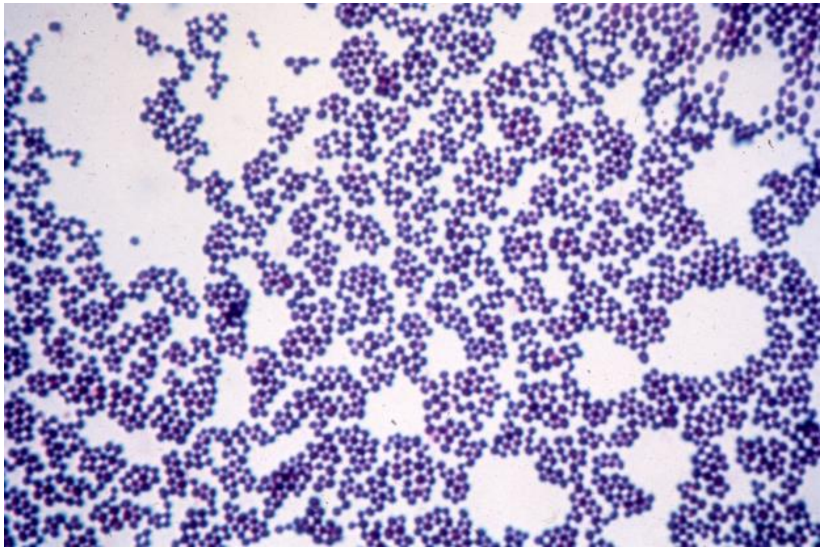
-- **Methicillin-resistant *Staph aureus* (MRSA)** have been entrenched in hospital settings for several decades.

-- **MRSA** strains have recently emerged outside the hospital becoming known as **community associated- MRSA (CA-MRSA)** or **superbug** strains of the organism.

-- These account for the majority of staphylococcal infections seen in clinics.

Important properties

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes.



-- Bacteriological culture of the **nose** and **skin** of normal humans invariably yields **staphylococci**.

-- Based on colony appearance, historically there are two species: ***Staphylococcus aureus*** (yellow) and ***Staphylococcus albus*** (white), now called ***Staphylococcus epidermidis***.

-- Another species ***Staphylococcus saprophyticus*** is involved in urinary infections.

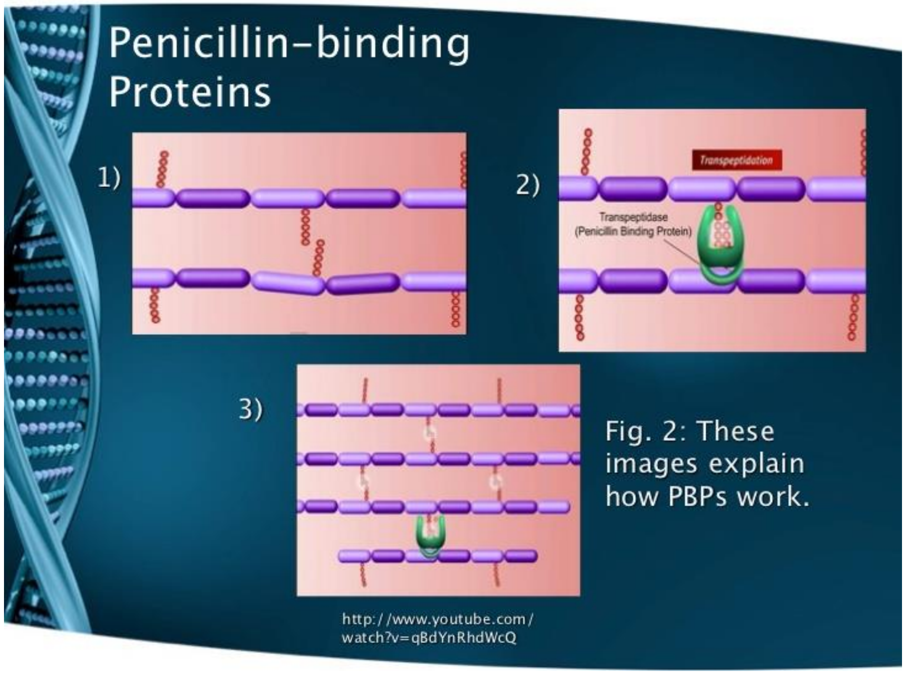
-- *Staphylococcus aureus* produce **yellow** colonies due to a pigment called **staphyloxanthin**.

-- It also **inactivates toxic oxygen species**.

-- *Staphylococcus aureus* usually **ferments mannitol** and **hemolyzes RBCs** whereas other two species do not. Hemolysis provides **iron** for growth.

-- More than **90%** ***Staphylococcus aureus*** strains contain **plasmids** that **encode β-lactamases**.

-- Some strains are even resistant to β-lactamase resistant penicillins such as **methicillin** and **nafcillin** by making changes in PBPs.



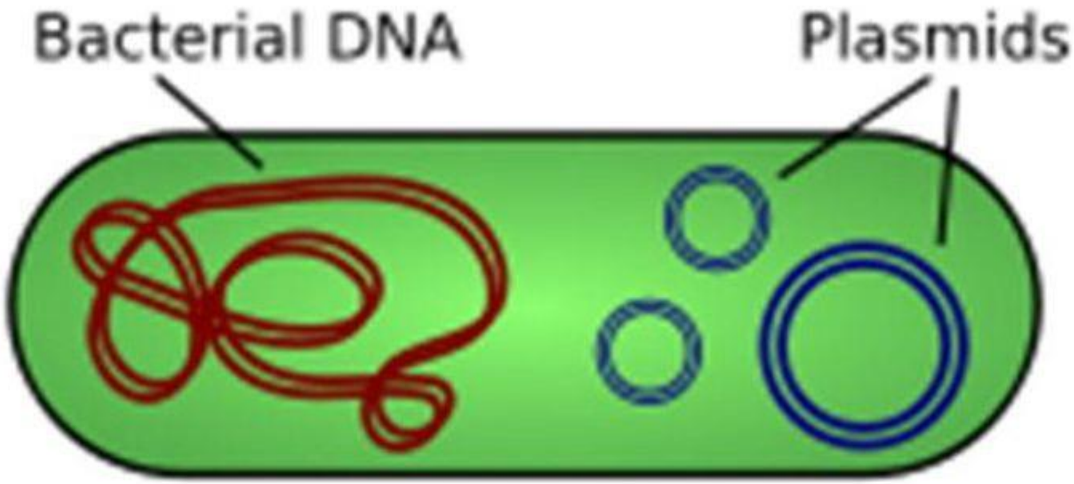
-- Relevant gene, ***mecA***, is located on chromosome.

- -- These strains are called **methicillin** or **nafcillin** resistant ***Staphylococcus aureus*** (**MRSA** and **NRSA**) and constitute for 50% of ***Staphylococcus aureus*** causing hospital infections in USA.

- -- Strains with intermediate and full resistance to vancomycin (**VISA** and **VRSA**) have been detected.

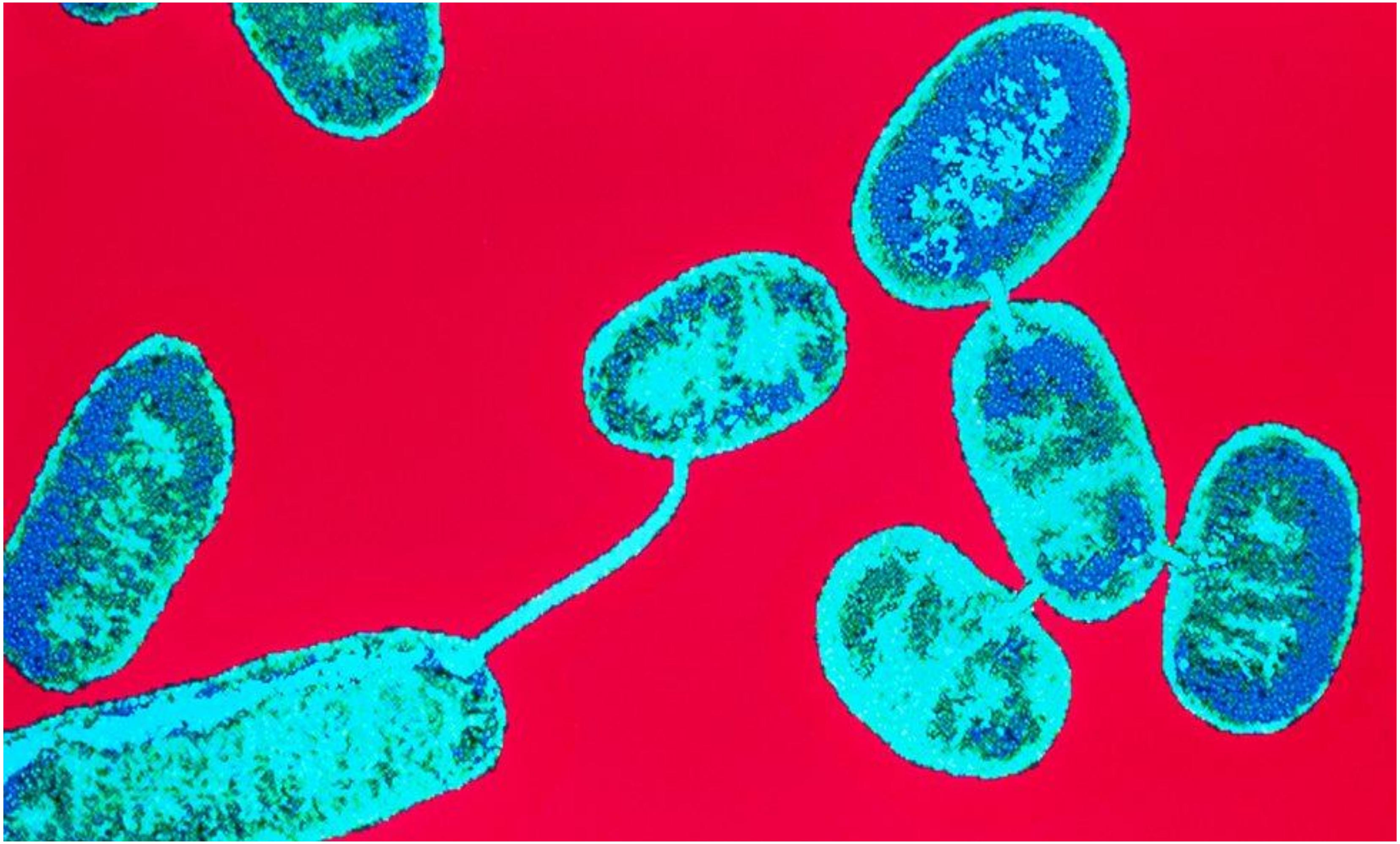
- -- The relevant genes are located on a **transposon** and encode enzymes that substitute **D-lactate** for **D-alanine**.

What are Plasmids?



- Plasmids are circular pieces of bacterial DNA that often contain genes not related to basic life functions
- Often contain antibiotic resistance genes
- Humans often cut open plasmids…attach a desired gene…reinsert the plasmid to the bacteria
- When complete, the bacteria will contain a new gene (instructions) to create a desired protein such as insulin.

CONJUGATION



Dr Linda Stannard, UCT/SPL 26th Sept 2014 iWonder BBC News

Transposon mediated resistance

-- Transposons are self directed elements that code for **transposases**.

-- **Transposases** are enzymes that bind to the ends of a transposon and **catalyze its movement** to another part of the genome by a **cut and paste mechanism** or a **replicative transposition mechanism**.

-- A typical drug resistance transposon is composed of **three genes flanked on both sides by shorter DNA segments**, usually a series of **inverted repeats** that mediate the interaction of the transposon and larger DNA.

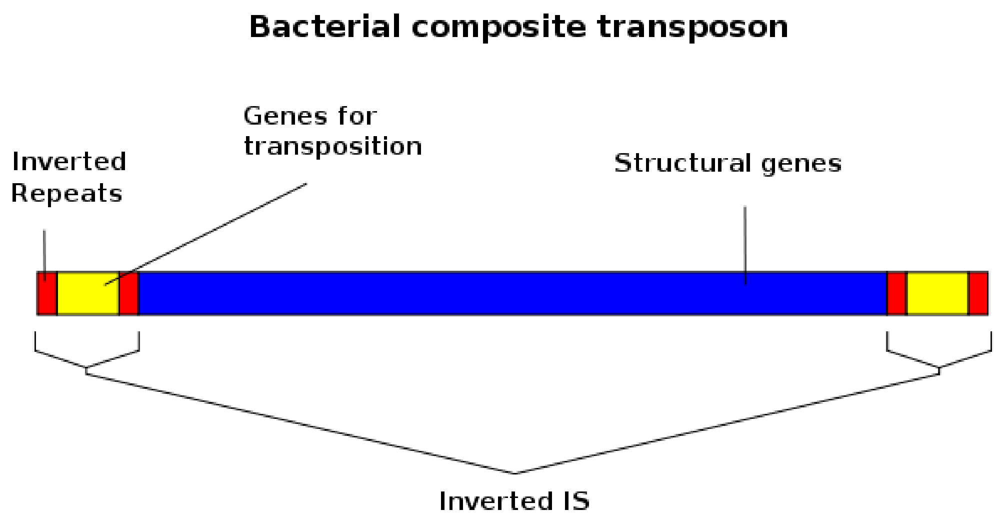
Transposon

The three genes code for :

1. **transposase** which controls excision and reintegration

2. **repressor** that controls synthesis of transposase and

3. **drug resistance** genes.




Thank You!