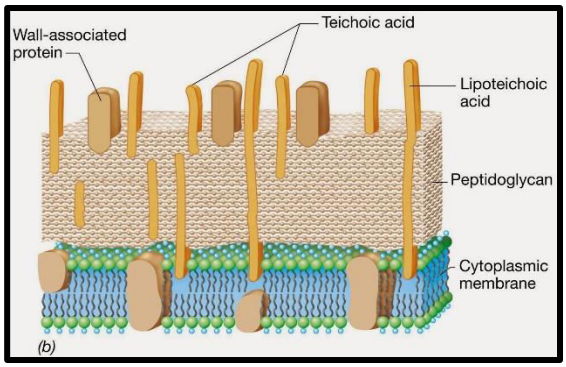
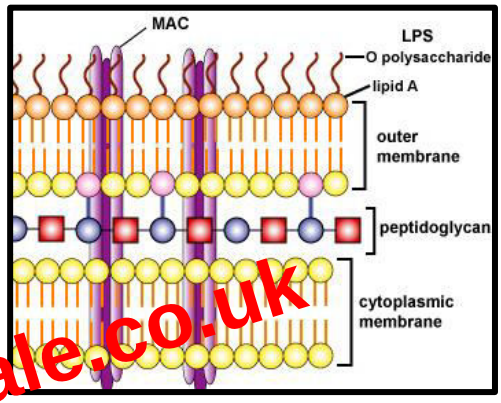
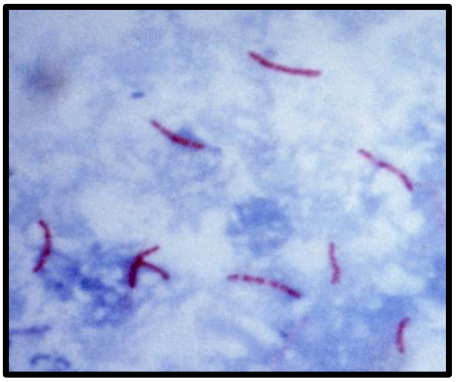
- The Gram stain works the way it does due to the differences in the structure of the bacterial cell wall
  - This cell wall is composed of sugars and amino acids – called **peptidoglycan**
    - Its role is to protect the cell from lysis and so give the cell shape
    - Peptidoglycan is a polymer of **N-acetyl glucosamine and N-acetyl muramic with side chains of alternating D and L amino acids**
      - **These D amino acids are useful targets for antibiotics**
    - Peptidoglycan is highly crosslinked meaning it will make the cell rigid



  - Gram positive cells contain large amount of another polymer named **teichoic acid**
    - The right image is of a Gram positive cell wall
  - Gram negative cell walls contain a **thin layer of peptidoglycan**; this is acting as a permeability barrier
    - The wall is also not highly cross-linked
    - Once the bacteria are stained with crystal violet followed by iodine, an iodine-crystal violet complex is formed – it is thought that the addition of alcohol to **decolourise results in pores of peptidoglycan to shrink and therefore remain a purple colour**
    - Gram negative cells also have **lipopolysaccharides** on the outer membrane of the cell
    - The right image is of a gram negative cell wall/membrane:



- The **Ziehl–Neelsen** stain, also known as the acid-fast stain is another staining technique used to identify **acid-fast organisms** such as *Mycobacterium tuberculosis*
  - These acid-fast organisms have a **waxy lipid** in their cell walls, therefore they do not stain readily with the Gram stain
  - The right image is an example of the Ziehl-Neelsen stain at 1000x magnification



- The Auramine-Phenol stain is another stain used to identify acid-fast bacteria
  - Although it is not as specific as the Ziehl-Neelsen, is **more affordable and so is used more often as a screening tool**
  - The microbes fluoresce under a light microscope with this stain
  - The image below shows the Auramine-Phenol stain at 40x magnification: