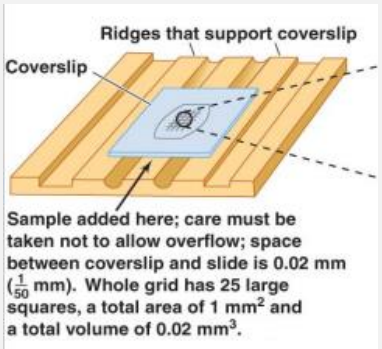
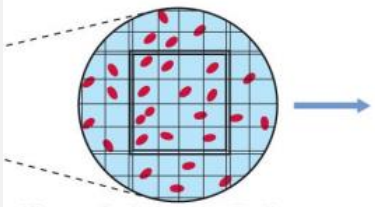
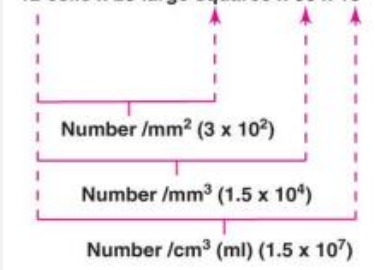
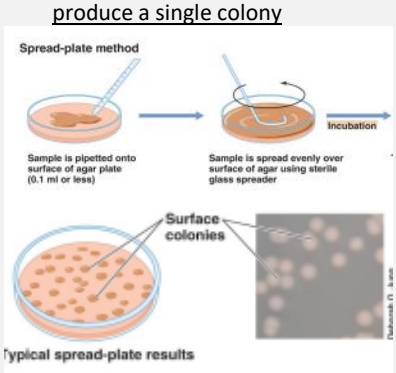
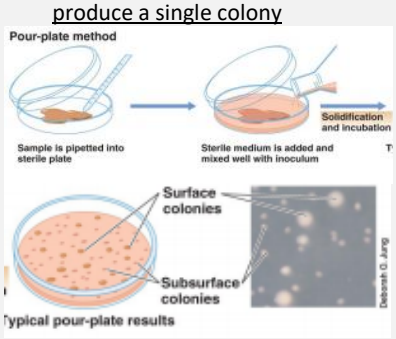
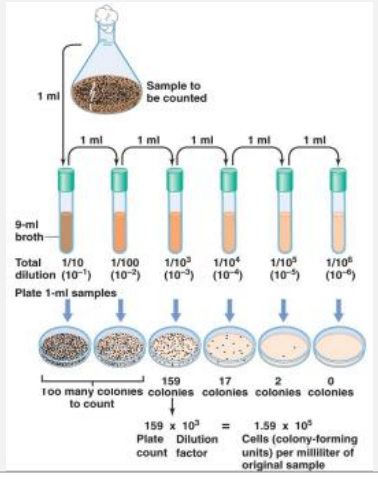
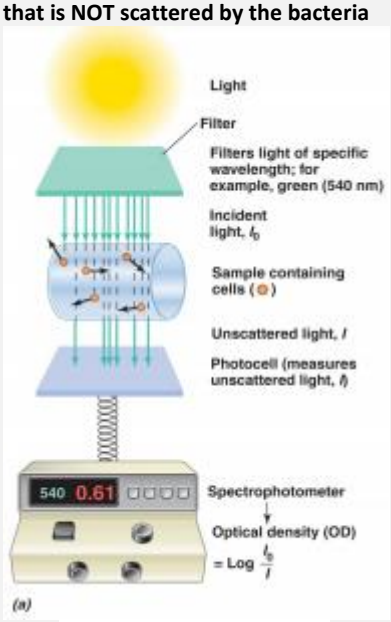
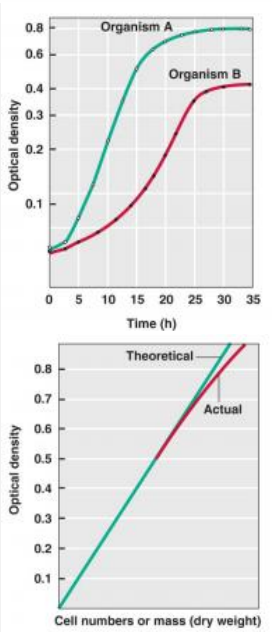
Contents

How to measure growth

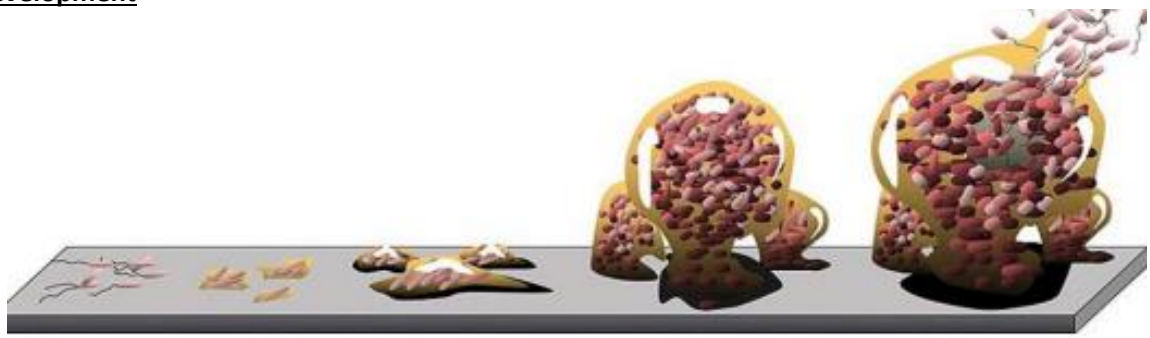
1. **Total cell count**
2. **Viable count**
3. **Turbidimetric measurements**

	Total cell count	Viable count	Turbidimetric
Advantages	1. Rapid way of estimating cells number	1. Highly sensitive 2. Samples containing few cells can be counted 3. Use of selective media allows counting specific organisms in mixed media	1. Rapid measurements can be made without disturbing or destroying a culture 2. Highly accurate
Disadvantages	1. Dead cell not distinguished from living cells 2. Small cells difficult to see under microscope 3. Precision sometimes difficult to achieve 4. Phase contrast is required when sample is not stained 5. Method is only suitable for bacteria at a density >10^6/mL	1. Number of colonies often dependent on conditions (temp, incubation time, medium) 2. Small colonies may be overlooked when counting 3. Replicate plates needed 4. Cell clumping may reduce counts	1. At high cell densities, backscattering leads to a deviation from linearity
Method	**Direct counting of cells under a microscope in a chamber of known volume**  Sample added here; care must be taken not to allow overflow; space between coverslip and slide is 0.02 mm ($\frac{1}{50}$ mm). Whole grid has 25 large squares, a total area of 1 mm^2 and a total volume of 0.02 mm^3.  Microscopic observation; all cells are counted in large square (16 small squares): 12 cells (in practice, several large squares are counted and the numbers averaged.) To calculate number per milliliter of sample: 12 cells x 25 large squares x 50 x 10^3 	**1. Spread plate method** ASSUMPTION: each viable cell will produce a single colony  **2. Pour plate method** ASSUMPTION: each viable cell will produce a single colony  **3. Serial dilution method** 	**Rapid method that uses spectrophotometer: measures light that is NOT scattered by the bacteria**  

BIOFILMS

Biofilm = a structured community of bacterial cells enclosed in a self-produced matric and adhered to an inert of living surface

Biofilm development



Reversible attachment	Irreversible attachment	Microcolony formation	Expansion	Dispersal
Flagella	fimbriae Adhesions	exo-polysaccharide extra-cellular DNA Quorum sensing Motility and chemotaxis Metabolic interactions		flagella enzymes

Why do bacteria form biofilms?

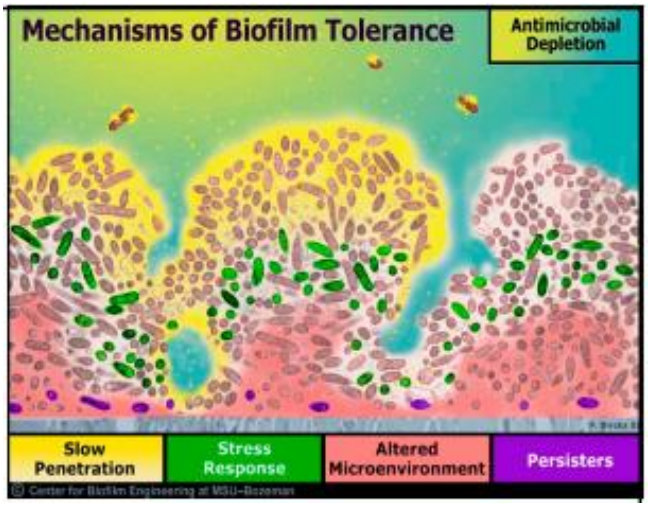
1. Self-defence – resist physical forces, phagocytosis, antibiotics
2. Allows cells to colonize favourable niches – attach to nutrient-rich surfaces or in locations where nutrients are continuously replenished
3. Enable bacteria to live together – permits cell-to-cell signalling, genetic exchange
4. Survival strategy – may be default mode of growth in natural environments where nutrients are limited

Examples of biofilms

Living tissues	Medical devices
• Tooth enamel • Heart valves • Lung • Middle ear • Bladder	• Urinary catheters • Central venous catheters • Contact lenses • Heart valves • Prosthetic joints

Biofilms & infection

- Biofilm bacteria can cause persistent (chronic) infections
- >60% of microbial infections involved biofilms
- Bacteria can withstand action of host defence system as well as the highest deliverable doses of antibiotics
- **How are biofilms so resistant to antimicrobials?**
  1. Slow penetration – outer layers absorb damage
  2. Stress response – inner layers have more time to adapt
  3. Altered microenvironment – nutrient depletion creates zones of altered activity
  4. Persister cells – tolerant to antibiotics due to a state of dormancy
  5. Antimicrobial depletion – absorption of antibiotics = lack of penetration

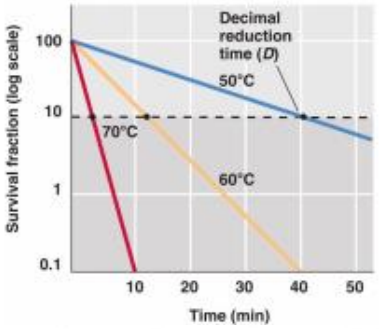


MICROBIAL GROWTH CONTROL

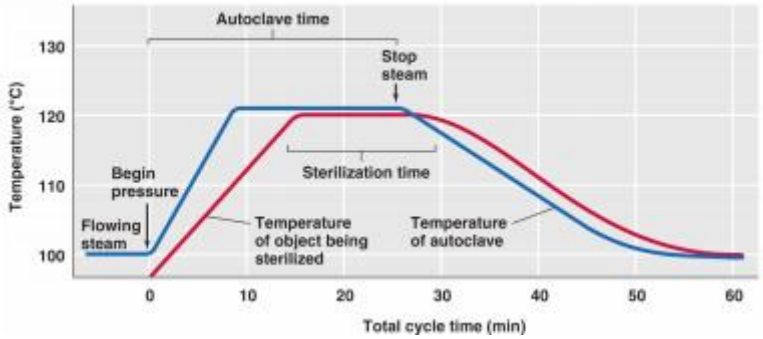
Definitions

- Sterilisation = the killing or removing of all viable organisms within a growth medium
- Inhibition = effectively limiting microbial growth
- Decontamination = the treatment of an object to make it safe to handle
- Disinfection = directly targets the removal of all pathogens, not necessarily all microbes

HEAT STERILISATION



- Most widely used method of microbial growth control
- High temperatures denature macromolecules
- Endospores – can survive heat the would rapidly kill vegetative cells
- Decimal reduction time – the time required for a 10-fold reduction in viability
- Thermal death time – the time it takes to kill all cells at a given temperature
- Autoclave – a sealed heating device that uses steam under pressure (moist heat sterilization)
    - temp of water gets above 100 degrees
    - 121 degrees for 10-15min is standard

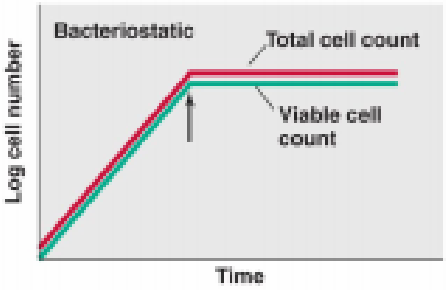


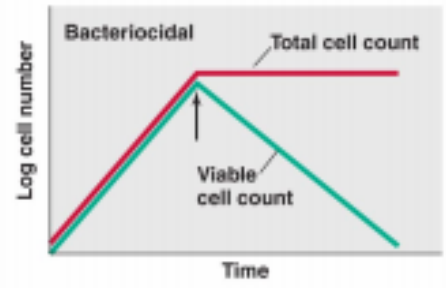
PASTEURIZATION

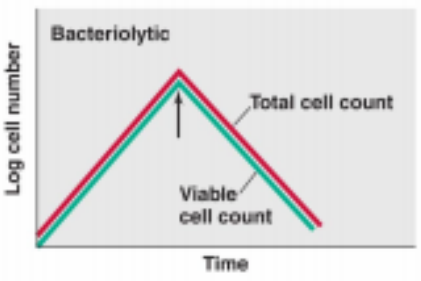
- the process of using precisely controlled heat to reduce the microbial load in heat-sensitive liquids (different to sterilisation, does not kill all organisms)

RADIATION STERILIZATION

- microwaves, UV, X-rays, gamma rays, electrons
- UV has sufficient energy to cause modifications and breaks in DNA
    - Decontamination of surfaces
    - Cannot penetrate solid surfaces (limitation)
- Ionizing radiation
    - Generates electrons, hydroxyl radicals, hydride radicals
    - Sources: cathode ray tubes, X-rays, radioactive nuclides
    - Used in medical and food industry; approved by WHO
- Some microbes are more resistant to radiation than others







FILTER STERILIZATION

- Avoids the use of heat for sterilization of sensitive liquids and gasses
    - Pores of filter too small for organisms to pass through
    - Pores large enough for liquid
- Depth filters – HEPA (high efficiency particulate air) filters
- Membrane filters – like a sieve

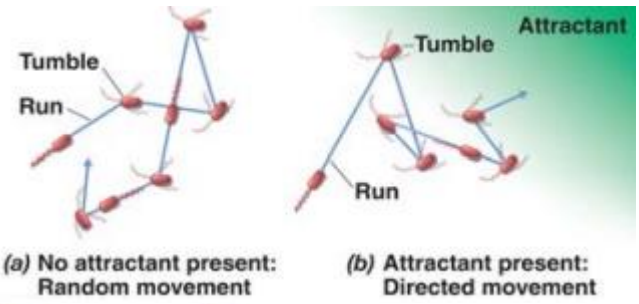
3 types of antimicrobial agents

1. **Bacteriostatic**
2. **Bacteriocidal**
3. **Bacteriolytic**

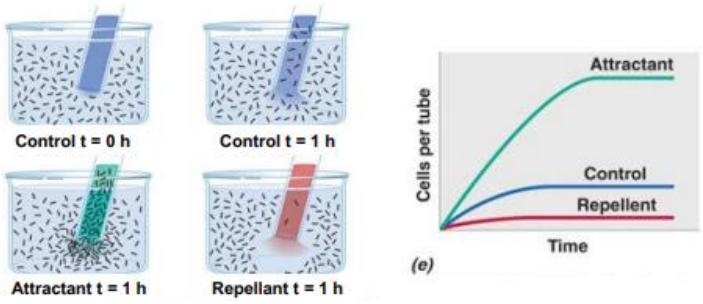
MICROBIAL TAXIS

- **Taxis** = directed movement in response to chemical or physical gradients
- **Chemotaxis** = a response to chemicals
- **Phototaxis** = a response to light
- **Aerotaxis** = a response to oxygen
- **Osmotaxis** = a response to ionic strength
- **Hydrotaxis** = a response to water

Chemotaxis example – *E. coli* peritrichous flagellated cells
  - "Run and tumble"
  - Attractants sensed by chemoreceptors
  - Increased tumbling as attractant concentration decreases and vice versa

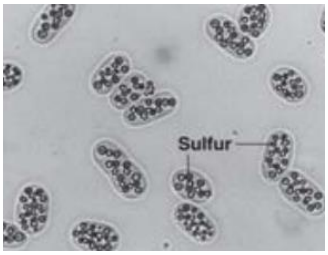


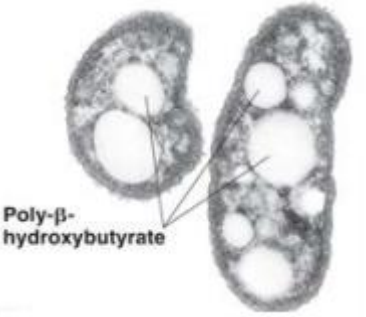
Measuring chemotaxis
Capillary tube assay →



CELL INCLUSIONS

- Synthesis of carbon and energy storage compounds
  - E.g. poly-beta-hydroxybutyric acid (lipid)
  - E.g. glycogen
- Some bacteria can accumulate inorganic phosphate (PO_4^{3-}) for nucleic acid, phospholipid and ATP synthesis
  - Stored polyphosphate is accumulated in phosphate-rich environments, and used when limiting
- Some bacteria can oxidise H_2S → to produce energy or as part of a CO_2 fixation process
  - Elemental sulphur is stored in sulphur globules in the periplasm





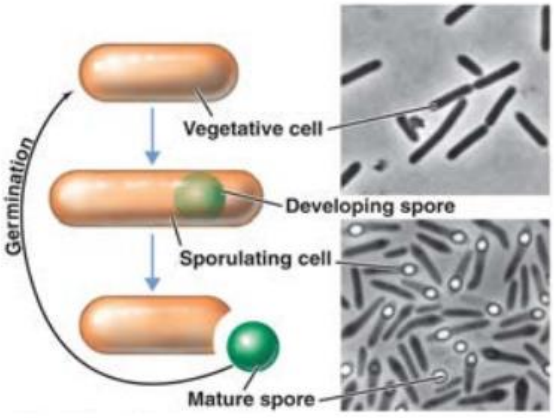
- Magnetosomes → intracellular particles of magnetite (Fe_3O_4) that enable bacteria to orient themselves in a specific arrangement within a magnetic field
  - Function unknown
  - Aquatic bacteria → orientation in a water column
  - E.g. *Magnetospirillum mangetotacticum*

GAS VESICLES

- = confer buoyancy in planktonic cells
- = Spindle-shaped gas-filled structures made of protein
- Membrane impermeable to water
- Allows photosynthetic bacteria (e.g. cyanobacteria) to position themselves in the water column

ENDOSPORES

- = highly differentiated cells; resistant to desiccation, heat, harsh chemicals, radiation
- Dormant stage of life cycle
- Ideal for dispersal via wind, water, animal gut
- Only present in some gram-positive bacteria e.g. *Bacillus* & *Clostridium*
- Structure differs remarkably from vegetative cell



pH and microbial growth

1. Acidophiles = grow optimally at low pH <6
2. Alkaliphiles = grow optimally at high pH >8
3. Neutrophiles = grow optimally pH 6-8

Salinity and microbial growth

1. Halophiles = grow optimally 1-15% NaCl
2. Extreme halophiles = grow optimally 15-30% NaCl
3. Halotolerant = can tolerate some NaCl but grow best with none

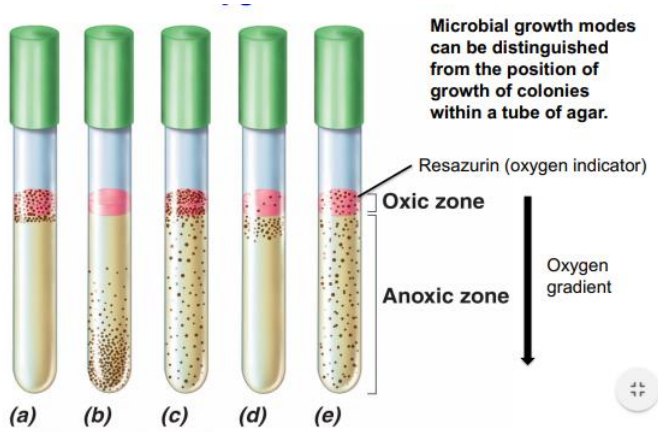
Note – seawater is ~3% NaCl

Pressure and microbial growth

1. Barophile (Piezophile) = thrives at high pressure e.g. Marianna Trench 10,897m (most also UV-sensitive)
2. Barotolerant = can survive high pressures but can exist in less extreme environments too
3. Obligate barophiles = cannot survive outside of high pressure

Oxygen and microbial growth

1. Aerobes = require oxygen; need 21% or higher
2. Anaerobes = do not need oxygen (sometime killed by exposure to oxygen)
3. Facultative organisms = can live with or without (but usually better with)
4. Aerotolerant anaerobes = can tolerate oxygen even though they cannot use it
5. Microaerophiles = can use oxygen only when it is present at level lower than that in air



Obligate anaerobes are oxygen sensitive because they cannot detoxify reactive oxygen species (ROS) with catalase ROS examples: superoxide (O_2^-) hydrogen peroxide (H_2O_2) hydroxyl radical ($OH\cdot$)

Examples:

Moritella yayanosii requires pressure of 700atm and temp of 2°C (Marianna Trench) → psychrophile & obligate barophile

Sulfolobus acidocaldarius grows at pH 2 and temp 75°C (Acidic mud in Yellowstone National Park) → thermophile & acidophile

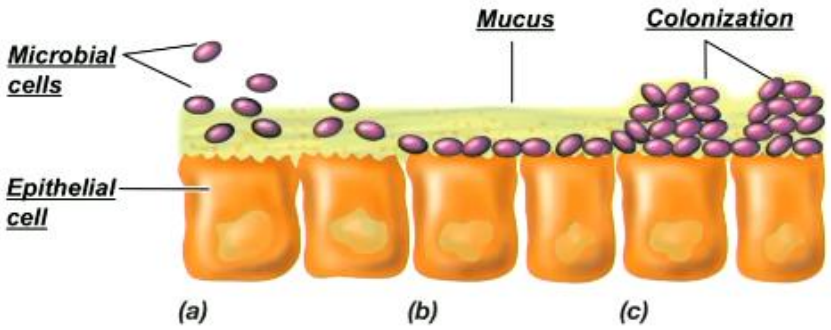
How do microbes adapt to extreme environments?

- Unsaturated and saturated fatty acid content
- Unsaturated fatty acids → increase fluidity; looser packing of unsaturated hydrocarbon chains
- Saturated fatty acids → decrease fluidity; tightly packed
- **Psychrophiles:** higher content of unsaturated fatty acids → semifluid membrane
- **Thermophiles:**
  - **Bacteria –** lipids rich in saturated fatty acids
  - **Archaea –** lipid monolayer rather than bilayer
- **Barophiles:** higher proportion of unsaturated fatty acids → keeps membrane from gelling at high pressure

MICROBIAL INTERACTIONS WITH HUMANS

Skin	Moist areas (e.g. sweat glands) readily colonised by gram-positive bacteria
Mouth	Complex, heterogenous microbial habitat. Bacteria colonise tooth surfaces by first attaching to acidic glycoproteins deposited there by saliva
Respiratory tract	Upper respiratory tract
Gastrointestinal tract	Contain 10^{13} to 10^{14} microbial cells. Microbial populations in distinct anatomical areas of GI tract influenced by diet and physical conditions
Urogenital tract	Urethra, vagina. The bladder is typically sterile in both males and females

Mechanisms by which the normal flora competes with invading pathogens

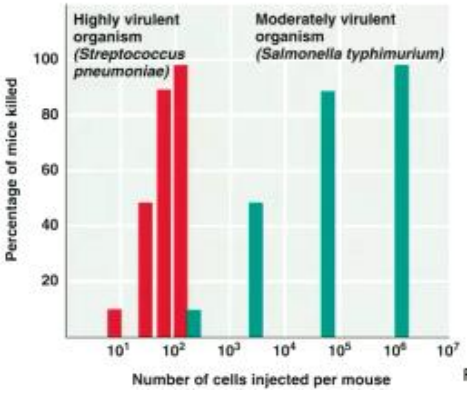


Staphylococcus aureus

- Most notorious accumulators of virulence factor (every category)
- Gram +ve cocci
- Part of normal skin flora; 40% of people are asymptomatic carriers
- Commonly known as "golden staph" because of its yellow colour when grown on culture plates
- MRSA = methicillin resistant *S. aureus*
  - Resistant to broad range of penicillin-analogues
  - Especially common in hospitals
  - Treat by running antibiotic sensitivity testing first to determine optimal therapy options
  - BUT instead of this, doctors over-prescribe Vancomycin (second-line antibiotic) → VRSA

Virulence factors

- = a bacterial product or strategy that contributes to virulence or pathogenicity
  1. Factors which aid in colonisation of the host
     e.g. adhesins such as pili (fimbriae), iron binding proteins, invasins
  2. Factors that evade the host immune system
     e.g. surface polysaccharides – capsule, lipopolysaccharide (LPS)
  3. Virulence factors that damage the host
     e.g. exotoxins



How do we measure virulence?

- Estimated from experimental studies → LD_{50} (lethal dose 50): the does of an agent that kills 50% of the animals in a test group
- Highly virulent pathogens show little difference in the number of cells required to kill 100% of the population compared to 50% of the population