Microbial Spoilage, Infection Risk & Contamination Control

Hugo and Russell's Pharmaceutical Microbiology, 8th Edition

Chapter 17 – Page 273

Mahmoud Alkawareek, PhD

Introduction

- In addition to the API, pharmaceutical products also contain a variety of other ingredients (i.e. excipients) which make the **formulation**:
  - Easy to manufacture
  - Stable
  - Safe
  - Effective
  - Convenient to the patient
- Products made in the pharmaceutical industry must meet high **microbiological specs**,

  i.e. if they are not **sterile**, they should have no more than a **minimal microbial population** at the time of product release.

Mahmoud Alkawareek, PhD

Introduction

- Occasionally some product batches with **unacceptable contamination** (level or type of organism) escape QA net. The **consequences** will be:
  - Product **spoilage** rendering it unsuitable for use
  - Potential **health hazard** to patients perhaps resulting in outbreaks of **medicament-related infections**.
  - **Financial loss**: **Direct**: wastage of individual batches

    Indirect: damaging publicity through product recall
- **Most commonly**, contamination occurs with **opportunistic m.o.** (e.g. *Pseudomonsa* spp) which has resulted in the spread of nosocomial infections in **compromised patients**.
- **Less common** is the contamination with **pathogenic m.o.** (e.g. *Salmonella)* & products contaminated with **toxic microbial metabolites**.

Mahmoud Alkawareek, PhD

Spoilage-- Chemical & Physicochemical Deterioration Of Pharmaceuticals

- As major contributors to the **natural recycling process**, m.o. have the **ability to degrade** a wide range of compounds; such degradation usually occurs at relatively mild physicochemical conditions.
- **Mixed microbial communities** are **more effective biodeteriogens** than individual species alone, why?

  Because the initial attack of complex substrates by one group of m.o. renders the them susceptible to further deterioration by secondary m.o.

Mahmoud Alkawareek, PhD

Spoilage-- Chemical & Physicochemical Deterioration Of Pharmaceuticals

- Novel pathways to attack **synthetic chemicals** may also emerge under environmental **selection pressures**!

  But the degradation **rate** of these 'xenobiotics' can **vary greatly**:

  $t_{0.5}$ (phenol)≈ few hours, while $t_{0.5}$ (halogenated pesticides)≈ several years!

- The overall **rate of deterioration** of a chemical depends on:
  - Molecular/chemical **structure** of the compound
  - The **physicochemical properties** of a particular environment
  - **Type & quantity** of microbes present
  - Whether the metabolites produced can serve as **source of usable energy & precursors** for biosynthesis, and hence the **multiplication of m.o.**

Pharmaceutical Ingredients Susceptible to Microbial Attack

❖Therapeutic Agents

- Active drug constituents may be degraded to **less potent, inactive or toxic forms**.
- Some drugs may be metabolized by m.o. and serve as substrates **supporting the growth** of more m.o., examples on these drugs are
  - Alkaloids (morphine, atropine), analgesics (aspirin & paracetamol), barbiturates and steroid esters
- Although reports of drug destruction by m.o. are **not that frequent**, there are some notable **exceptions** such as:
  - Inactivation of penicillin injection by b-lactamase producing bacteria,
  - Steroid metabolism in damp tablets & creams by fungi
  - Microbial hydrolysis of aspirin suspension

Pharmaceutical Ingredients Susceptible to Microbial Attack

❖Surface Active Agents

- Anionic Surfactants
  - Alkali metals & amine **soaps** of fatty acids are considered **stable** due to slight **alkaline pH** of the formulations, but once **diluted** into sewage are **readily degraded**
  - **Alkyl/alkylbenzene sulphonates & sulphate esters** are metabolized by multi-step oxidation followed by 'ring fission'.
  - Ease of degradation decreases with increasing **chain length** & complexity of **branching** of the alkyl chain

Pharmaceutical Ingredients Susceptible to Microbial Attack - Surface Active Agents

- Non-ionic Surfactants
  - Examples: alkyl polyoxyethylene alcohol emulsifiers, sorbitan esters (**Spans**) and polysorbates (**Tweens**)
  - **Readily metabolized** by wide variety of m.o.
  - Again, increasing chain length & branching decrease ease of attack
  - **Lipolytic cleavage** of fatty acids from sorbitan esters, polysorbates & sucrose esters is followed by **degradation of the cyclic nuclei**, producing small molecules readily utilizable by m.o

Pharmaceutical Ingredients Susceptible to Microbial Attack - Surface Active Agents

- Ampholytic Surfactants
  - Examples: surfactants based on **phosphatides, betaines** & **alkylamino substituted amino acids**
  - **Reasonably biodegradable**
- Cationic Surfactants
  - Examples: cetrimide and benzalkonium chloride (both are **QACs**)
  - Usually used as **antiseptics** and **preservatives**
  - Only **slowly degraded** at high dilutions (i.e. in sewage)

Pharmaceutical Ingredients Susceptible to Microbial Attack - Surface Active Agents

- Relative Ease of Degradation?! (generally speaking)

Non-ionic > Ampholytic > Anionic > Cationic

Pharmaceutical Ingredients Susceptible to Microbial Attack

❖ Organic Polymers

- Thickening & Suspending Agents
  - Many of these agents are subject to **microbial depolymerization** by specific **extracellular enzymes** yielding nutritive monomers & fragments
    - ✓ Examples: amylases (starch), pectinases (pectin), cellulases (carboxymethylcellulose), proteases (proteins)
    - ✓ An **exception** is **agar** (a complex polysaccharide) which is relatively **inert** and hence is used as a support for solidifying culture media.
- Synthetic Packaging Polymers
  - Examples: nylon, polystyrene & polyester
  - Extremely **resistant** to microbial attack.

Pharmaceutical Ingredients Susceptible to Microbial Attack

❖ Humectants

- Low molecular weight hygroscopic materials like **glycerol & sorbitol**
- Included in some formulations to **reduce water loss**
- **Readily metabolized unless present in high concentrations**

Pharmaceutical Ingredients Susceptible to Microbial Attack

❖ Fats and Oils

- **Extensively attacked** when **dispersed** in aqueous formulations (e.g. oil-in-water emulsions) aided by high solubility of oxygen in oils.
- Fungal attack can occur in condensed **moisture films** on the surface of oils in bulk or in water droplets contaminating the oil phase.
- **Lipolytic rupture** of triglycerides liberates glycerol & FAs, where FAs then undergo **β-oxidation** of the alkyl chain producing odiferous ketones

Pharmaceutical Ingredients Susceptible to Microbial Attack

❖ Sweetening, Flavouring & Colouring Agents

- Sugars & sweetening agents can be readily used as **substrates** for microbes, but if used at **high conc**., they inhibit microbial attack by **reducing water activity**
- In the past a variety of **colouring agents** (tartrazine & amaranth) & **flavouring agents** (peppermint water) were kept as stock solutions to be dispensed & diluted when required.
  - But many times they got contaminated by, and even supported the growth of, *Pseudomonas* spp.
  - Nowadays such stock solutions **should be preserved**.

Pharmaceutical Ingredients Susceptible to Microbial Attack

❖Preservatives & Disinfectants

- Many preservatives & disinfectants can be metabolized by **G-ve bacteria**, mostly when used **below their effective 'use' level**
  - ***Pseudomonas* spp** can grow in solutions of **QAC antiseptics & chlorhexidine** which has resulted in infection of patients.
  - *Pseudomonas* spp have also metabolized **parabens** in eye drops & caused serious eye infections.

Observable Effects of Microbial Attack on Pharmaceutical Products

- Microbial contaminants attack ingredients of a medicine and create substrates necessary for biosynthesis and energy production before they can replicate to levels where obvious spoilage becomes apparent.
- **Early indications** of spoilage are **organoleptic** (e.g. smell & taste)
  - "Sour" F.A. taste
  - Earthy taste
  - Bitter taste
  - "Fishy" amine smell
  - "Bad eggs" smell
  - Discoloration by microbial pigments

Observable Effects of Microbial Attack on Pharmaceutical Products

- **Loss of viscosity** due to **depolymerization of thickening & suspending** agents like acacia, CMC
  - This usually results in **sedimentation** of suspended ingredients
- Microbial **polymerization of sugars and surfactant molecules**
  - Production of **slimy, viscous masses** in syrups, shampoos and creams,
  - **'Gritty' texture** in creams by fungal growth.

Mahmoud Alkawareek, PhD

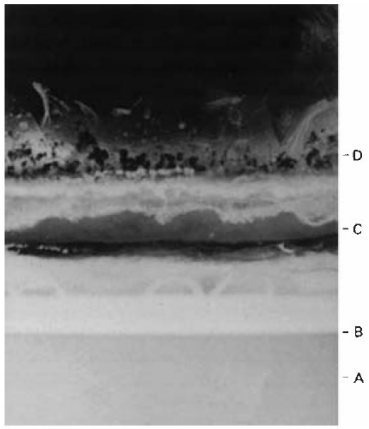
Observable Effects of Microbial Attack on Pharmaceutical Products

- Change of product **pH** by acidic or basic microbial metabolites, which may result in
  - Product instability and **deterioration**
  - **Enhancement of microbial growth** which was inhibited by the initial product pH (i.e. **secondary attack**)
- Production of **gaseous metabolites**
  - Seen as **trapped bubbles** within viscous formulations.

Mahmoud Alkawareek, PhD

Observable Effects of Microbial Attack on Pharmaceutical Products - Example

- Inadequately preserved o/w emulsion:
  - Pigments may **discolour** the product (A) – aqueous phase
  - **Metabolism of surfactants** reduces stability and accelerates '**creaming**' of oil globules (B)
  - Lipolytic release of fatty acids from oils **lowers pH** and encourages **coalescence** of oil globules (C) → cracked emulsion!
  - Also observed is a **fungal mycelial growth** on surface (D)



Mahmoud Alkawareek, PhD

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Types and Size of Contaminant Inoculum
  - Before doing a formula, the formulator should consider the **environment and usage** to which the product is likely to be subjected during its life and the **history of similar medicines**, why?

    to build as much protection as possible against **non-standard encounters**, such as additional preservation for a syrup if osmotolerant yeast contamination is particularly likely
  - If microbial **failure** occurs, **identification of the contaminant(s)** & knowledge of **microbial ecology** are very useful in tracking down the defective steps in the design or production process

Mahmoud Alkawareek, PhD

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Types and Size of Contaminant Inoculum
  - Very low levels of contaminants which are unable to replicate in a product might not cause appreciable spoilage but, if a **surge** in contaminant bio-burden occur, the built-in protection could be insufficient and spoilage occurs
  - This surge might arise if:
    1. **Raw materials** were unusually contaminated;
    2. A problem of the plant **cleaning** protocol occurred;
    3. **Biofilm** detached itself from within supplying pipework;
    4. There was demolition or **maintenance work** in the vicinity of the manufacturing site (i.e. dust)
    5. **Gross misuse** of the product during administration.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Types and Size of Contaminant Inoculum
  - Inoculum size **alone** is **not** always a reliable indicator of spoilage potential.

    e.g. a very low level of, aggressive *Pseudomonads* in a weakly preserved solution may suggest a greater risk than tablets containing fairly high numbers of fungal and bacterial spores.

    When an aggressive contaminant enters a medicine, there may be an appreciable **lag period** before significant spoilage begins, the duration of which decreases disproportionately with increasing contaminant loading. However, since there is usually a long delay between manufacture and administration of factory-made medicines, growth and attack could start during this period unless additional steps are taken to prevent it.

Mahmoud Alkawareek, PhD

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Types and Size of Contaminant Inoculum
  - The isolation of a particular m.o. from spoiled product does **not necessarily** mean that it was the **initiator of the attack**. It could be a **secondary opportunistic contaminant** which had overgrown the primary spoilage organism once the physicochemical properties had been favourably modified by primary spoiler.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Nutritional Factors
  - Many common spoilage microorganisms (i.e. **opportunistic m.o.**) have **simple nutritional requirements** and metabolic **adaptability**

    This enables them to **utilize many of the components** of medicines as substrates for biosynthesis and growth, even including trace materials contained in them.

    Even demineralized water prepared by good ion-exchange methods normally contains sufficient nutrients to allow significant growth of many water-borne Gram-negative bacteria such as *Pseudomonas* spp.

    That's why when such contaminants fail to grow in a medicine it is **unlikely** to be as a result of **nutrient limitation** but due to other, non-supportive, physicochemical or toxic properties.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Nutritional Factors
  - Most **acute pathogens** require **specific growth factors** normally associated with the tissues they infect but which are often absent in pharmaceutical formulations.

    Therefore, they will unlikely multiply in them, **but** they may **remain viable and infective** for an appreciable time in some dry products where the **conditions are protective**.
  - The use of crude **vegetable or animal products** in a formulation provides an additionally nutritious environment.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Moisture Content: Water Activity (Aw)
  - Microorganisms require readily **accessible water** in appreciable quantities for growth.
  - Although some solute-rich medicines such as syrups appear to be 'wet', microbial growth in them may be difficult, why?

    Since the microbes have to **compete** for water molecules with the large numbers of sugar and other molecules of the formulation which also interact with water via hydrogen bonding.
  - An estimate of the **proportion of the unbound water** in a formulation available to equilibrate with any microbial contaminants and facilitate growth can be obtained by measuring its **water activity** (Aw).

$$Aw = \frac{\textbf{vapour pressure of formulation}}{\textbf{vapour pressure of water}}$$ …(under similar conditions)

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Moisture Content: Water Activity (Aw), examples:
  1. Sucrose Solutions:

Sucrose (g)	Water (g)	% Sucrose	A_w
0	100	0	1.00
20	100	16.7	0.998
40	100	28.6	0.969
60	100	37.5	0.955
80	100	44.4	0.941
100	100	50.0	0.927
120	100	54.5	0.913
140	100	58.3	0.900
160	100	61.5	0.888
180	100	64.3	0.876
200	100	66.7	0.860

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Moisture Content: Water Activity (Aw), examples:
  2. NaCl Solutions:

NaCl (g)	Water (g)	% NaCl	A_w
0.9	99.1	0.9	0.995
1.7	98.3	1.7	0.99
3.5	96.5	3.5	0.98
7.0	93.0	7.0	0.96
10.0	90.0	10.0	0.94
13.0	87.0	13.0	0.92
16.0	84.0	16.0	0.90
22.0	78.0	22.0	0.86

- The scale is different for the %'s of different solutes
- **The greater the (same) solute concentration, the lower is the water activity.**

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Moisture Content: Water Activity (Aw)
  - With the exception of halophilic bacteria, most m.o. grow best in dilute solutions (high Aw) and, as solute conc. rises (lower Aw), growth rates decline until a minimal, **growth-inhibitory Aw** is reached.
  - Limiting Aw values are of the order of:

    Gram-negative rods: 0.95

    Staphylococci, Micrococci and Lactobacilli: 0.9

    Most yeasts: 0.88

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Moisture Content: Water Activity (Aw)
  - The Aw of aqueous formulations can be lowered to increase resistance to microbial attack by the addition of high concentrations of **sugars** or **polyethylene glycols**.
  - Since there are trends to eliminate sucrose from medicines, alternative solutes are used, such as **sorbitol** and **fructose**, which are not thought to encourage dental caries.
  - **But**, syrup-fermenting **osmo-tolerant yeasts** were found to spoil products with Aw levels as low as **0.73**, while some filamentous fungi can grow at even lower values, such as *Aspergillus glaucus* (0.61)

    That's why even Syrup BP (67% sucrose; Aw= 0.86) has been reported to occasionally fail to inhibit osmo-tolerant yeasts and hence additional preservation may be necessary

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Moisture Content: Water Activity (Aw)
  - Aw can also be reduced by **drying**, although the dry, often hygroscopic, medicines (tablets, capsules, powders) will require **suitable packaging** to **prevent reabsorption of water** and consequent microbial growth.
  - Some **tablet film coatings** are now available which greatly reduce water vapour uptake during storage. These might contribute to increased microbial stability during storage in particularly humid climates, although suitable **foil strip packing** may be more effective, but also more expensive.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Moisture Content: Water Activity (Aw)
  - Without proper packaging, **condensed water films** can accumulate on the surface of 'dry' products such as tablets or bulk oils following storage in damp atmospheres with fluctuating temperatures, resulting in **high localized Aw** which can initiate **fungal growth**.
  - **Dilute aqueous films** similarly formed on the surface of **viscous products** such as syrups and creams, or exuded by **syneresis** from hydrogels, reach sufficiently high Aw to permit surface yeast and fungal spoilage.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Redox Potential
  - The ability of microbes to grow in an environment is influenced by its **oxidation-reduction balance** (redox potential) since they require compatible terminal **electron acceptors** to permit function of their respiratory pathways.
  - The redox potential even in fairly viscous emulsion may be quite high due to the high solubility of oxygen in most fats & oils.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Storage Temperature
  - Spoilage of pharmaceuticals could occur over the range of about **-20° to 60°C**, although much less likely at the extremes.
  - The actual **storage temperature** may determine the spoilage by particular **types of microorganisms**.
  - Storage in a deep freeze at **-20°C or lower** is used for long-term storage of foodstuffs and some pharmaceutical **raw materials.**
  - Dispensed **total parenteral nutrition** (TPN) feeds have also been stored in hospitals for short periods at **-20°C** to even further minimize the risk of growth of any contaminants which might have been introduced during their aseptic compounding.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Storage Temperature
  - Reconstituted **suspensions** and **multi-dose eye drop packs** are sometimes dispensed with the instruction to store them in domestic **fridge (2°-8°C),** partly to **reduce the risk of in-use contamination**.
  - On the other hand, '**water for injection**' is usually held at **80°C or above** after distillation (prior to packing and sterilization) to **prevent potential regrowth of Gram-negative bacteria**, and the release of **endotoxins**

Factors Affecting Microbial Spoilage of Pharmaceuticals

- pH
  - **Extremes** of pH **prevent microbial attack**.
  - **Around neutrality** bacterial spoilage is **more likely**, with reports of *Pseudomonads* and related Gram-negative bacteria growing in antacid mixtures, flavoured mouth washes and in distilled or demineralized water.
  - **Above pH 8**, e.g. with soap-based emulsions, **spoilage is rare**.
  - For products with **low pH** levels such as the fruit juice-flavoured syrups (ca. pH 3-4) **mould or yeast attack is more likely**. Yeasts can metabolize organic acids and **raise the pH** to levels where **secondary bacterial growth** can occur.
  - In **food industry** low pH adjustment can be made to preserve foodstuffs (pickling, yoghurt), but this is **not practical for medicines** (why???).

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Packaging Design
  - Packaging should be made in a way to **control the entry of contaminants** during both **storage and use**.
  - The most important dosage form to be protected are the **parenteral drugs** because of the high risks of infection by this route.
  - **Self-sealing rubber closures** must be used to prevent microbial entry into **multi-dose injection** containers following drug withdrawals.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Packaging Design
  - **Wide-mouthed cream jars** will allow the entry of fingers with their high bioburden of contamination. Thus, it is better to replace them with **narrow nozzle** and flexible screw capped tubes.
  - For medicines which rely on their **low Aw** to prevent spoilage, packaging such as **strip foils** must be of **water vapour-proof** materials with fully efficient seals.
  - **Cardboard** outer packaging and labels themselves can become **substrates for microbial attack** under humid conditions; therefore **preservatives** are often included to reduce their risk of damage.

Factors Affecting Microbial Spoilage of Pharmaceuticals

- Protection of m.o. within Pharmaceutical Products
  - The survival of microorganisms in particular environments is influenced by the presence of various relatively **inert materials**.
  - Microbes can be **more resistant to heat or desiccation** in the presence of some **polymers** such as starch, acacia or gelatin.
  - Adsorption onto naturally occurring **particulate material** may aid establishment and survival in some environments.
  - The presence of some **surfactants, suspending agents and proteins** can increase the **resistance** of microorganisms to **preservatives**, over and above their direct inactivating effect on the agents.

Hazard to Health

- Contaminated pharmaceutical products may present a potential health hazard to the patient.
- Contamination with **pathogenic bacteria** (e.g. *Salmonella* spp) forms a **special risk** since they can cause infections in a **wide range of patients**.
- The presence of **opportunists** with limited pathogenicity also present significant challenge to **compromised patients**.

Hazard to Health

- The outcome of using contaminated products vary from **patient to patient** depending on the type & degree of contamination & how the product to be used
  - The most serious effects are expected to be with contaminated **injections** as generalized bacteraemic shock & sometimes death is reported
  - Wound or sore in broken skin may become locally infected which may extend the hospital bed occupancy
- Most medicament related infections are **difficult to be recognized** by health practitioners which causes the spread of infection **over several months**

Hazard to Health - Examples

- Gram -ve bacteria:
  - G-ve bacteria were responsible for numerous outbreaks.
  - ***Pseudomonas* spp** have **simple nutritional requirements** & multiply significantly in aqueous products
    - **Cornea** when scratched or damaged by irritant chemicals offers little resistance to *Pseudomonas* and hence **contaminated ophthalmic solutions** have resulted in frequent cases of infections; some leading to **loss of sight**.
    - *Pseudomonas* contaminating **antiseptic solutions** caused skin infections in burnt patients resulting in failure of skin grafts & death.
    - Infections of eczematous skin & respiratory infections in neonates were caused by contaminated ointments & creams.

Hazard to Health - Examples

- Gram -ve bacteria:
  - Infections by ***Salmonella*** **spp** were reported & the m.o. was isolated from products contaminated with it (tablets, **pancreatin, thyroid extracts**).
  - Oral mixtures & antacid suspensions can support the growth of Gram -ve bacteria & resulted in serious effects in immunocompromised patients (e.g. as a result of antineoplastic chemotherapy)
  - Bladder wash out solutions contaminated with Gram -ve bacteria caused painful infections

Hazard to Health - Examples

- Other cases:
  - **HIV** contaminated **factor VIII products** made from pooled human blood was reported
  - **Creutzfeldt-Jackob disease** infection from contaminated injections of h**uman growth hormone** derived from human pituitary

Hazard to Health

- Microbial toxins:
  - G-ve bacteria contain **endotoxins** (LPS) which can remain active in products even after cell death & some can **survive moist heat sterilization**
  - Endotoxins are **inactive via oral route** but if they enter **blood stream** via contaminated **infusion fluids** (even in ng level) or via diffusion across membranes from contaminated **haemodialysis** solution they can induce serious physiological effects.
  - Endotoxins cause **fever**, activation of the **cytokine system**, endothelial cell damage & these all lead to septic & often **febrile shock**.

Factors Determining the Outcome of a Medicament Borne Infection

- Clinical rxns may **range** from **local infections** of wounds or broken skin (contaminated topical prep) to GI infections (contaminated oral products) to serious widespread infections such as bacteraemia or septicemia possibly resulting in **death** (contaminated infusion)
- Clinical rxns resulting from the use of contaminated medicament may be evident in one patient but not in another one depending on **many factors**, among which are:
  1. Type & Degree of Microbial Contamination
  2. Route of Administration
  3. Resistance of the Patient to Microbial Infections

Factors Determining the Outcome of a Medicament Borne Infection

1. Type & Degree of Microbial Contamination
   - Microorganisms contaminating medicaments are classified into **true pathogens** or **opportunistic pathogens**.
   - Pathogens **rarely** occur in products but if present they **cause serious problems**, examples:
     - *Clostridium tetani*: caused wound infections & cases of neonatal death resulted from use of contaminated talcum powder
     - *Salmonella* spp: caused outbreaks of salmonellosis due to ingestion of contaminated thyroid & pancreatic powders

Factors Determining the Outcome of a Medicament Borne Infection

1. Type & Degree of Microbial Contamination
   - **Opportunists** like *P. aeruginosa*, *Klebsiella* spp. and *Serratia* spp are **more frequently** isolated from medicinal products.
     - The main concern with these organism is that their simple nutritional requirements enable them to survive in a wide range of pharmaceuticals, thus they present in high numbres 10^6-10^7 CFU/g or ml, although the product itself may not show visible sign of contamination.
     - **Compromised patients** are considered at risk from infection with these m.o.
   - The **critical dose of m.o.** that will initiate an infection is highly **variable** (i.e. varies between spp & within spp.)

Factors Determining the Outcome of a Medicament Borne Infection

2. Route of Administration
   - Ophthalmic & parenteral route:
     - Contaminated products **injected** directly into blood stream or instilled into the **eye** cause the most **serious problems**.
     - Injectable & ophthalmic solutions are often simple & provide sufficient nutrient for G-ve opportunists.

       If contaminated; the product may end up with a bioburden as high as 10^6 CFU/ml in addition to the potential production of endotoxins
     - **Total parenteral nutrition fluids** provide even more **nutritional support** for contaminants
     - **Intrathecal & epidural injections** are potentially hazardous procedures, and thus in practice they are given through **bacterial filters**.
     - *P. aeruginosa* (contaminant of eye drops) has caused serious ophthalmic infections, including **loss of sight**. The problem is compounded when the eye is damaged by improper use of contact lenses or scratched by fingernails or cosmetic applicators.

Mahmoud Alkawareek, PhD

Factors Determining the Outcome of a Medicament Borne Infection

2. Route of Administration
   - Contaminated **orally** ingested products have different fate. This depends on whether the drug was taken on **full or empty stomach** as stomach acidity provides a barrier.
   - Contaminants in **topical** products may cause **little harm** if applied on **intact skin**, why?
     - because intact skin provides a mechanical barrier & normal flora competes with the few contaminants.
     - however, **damaged skin** (sores, burns, surgery, wounds) may be rapidly colonized & infected by opportunists potentially causing **serious problems**

Mahmoud Alkawareek, PhD

Factors Determining the Outcome of a Medicament Borne Infection

3. Resistance of the Patient to Microbial Infections
   - Very important in determining the outcome of a medicament borne infection
   - **Hospitalized patients** are more exposed and susceptible to infection than those treated in the general community
   - **Neonates, the elderly, diabetics and traumatized patients** (by surgery, accidents…) are at **special risk** because they may have impaired defence mechanisms
   - **Immunocompromised people** (patients with leukaemia, HIV or treated with immunosuppressants) are **most prone** to infections.
     - ➢ That's why it is better to provide them with all medicines in a sterile form!

Preservation of Medicines Using Antimicrobial Agents

- Why to add a preservative:
  - To kill any anticipated low levels of contaminants, where from?
    a. Remaining in a non-sterile medicine after manufacturing
    b. Entering during storage
    c. Introduced during usage especially the repeated withdrawal of doses from a multi-dose container
  - To further **reduce the risk of spoilage and health hazard**

Preservation of Medicines Using Antimicrobial Agents

- If a medicine is **unlikely to encourage growth** or survival of contaminants and the infective risk is low, then a **preservative** might be **unnecessary**
  - Examples: tablets, capsules and dry powders
- Preservatives should **not** be added to deal with **failures** in poorly controlled manufacturing processes.

Preservation of Medicines Using Antimicrobial Agents

- Properties of an ideal properties:
  - **Broad spectrum** and **rapid** activity

    to rapidly kill all microbial contaminants as they enter the medicine
  - **Non-irritant** and **non-toxic** to the patient
  - **Selective in reacting with contaminants** and not the formulation ingredients
  - **Stable and effective** throughout the life of the product

Preservation of Medicines Using Antimicrobial Agents

- But:
  - The most active antimicrobial agents are generally non-selective
  - The remaining preservatives have only modest antimicrobial efficacy
  - There are no preservatives considered sufficiently non-toxic for use in highly sensitive areas such as CNS and within the eye
  - Rapid killing of all contaminants may only be possible for relatively simple aqueous solutions, whereas for physicochemically complex systems only inhibition of growth and slow rate of killing may be realistically achieved.

Factors Influencing Antimicrobial Activity Within Medicines

- Effect of preservative concentration, temperature and size of inoculum:
  - Changes in the efficacy of preservatives vary exponentially with changes in concentration depending on the type of preservative

    Remember the concentration exponent (η)!
  - Changes in product temperature will alter efficacy in proportions, related to different types of preservative

    Q_{10}!
  - If concurrent change in temp & conc → more complex scenario

    Example: if a 0.1% chlorocresol (η = 6, Q10 = 5) solution completely killed a suspension of *E. coli* at 30°C in 10 minutes, it would require around 90 minutes to achieve a similar effect if the temperature was lowered to 20°C and slight overheating during production had resulted in a 10% loss by vaporization in the chlorocresol concentration (other factors remaining constant)

Factors Influencing Antimicrobial Activity Within Medicines

- Effect of preservative concentration, temperature and size of inoculum:
  - Preservative molecules are used up as they inactivate microorganisms and as they interact non-specifically with the significant quantities of contaminant 'dirt' introduced during use.

    This will result in a progressive and exponential decline in the efficiency of the remaining preservative.
  - **Preservative 'capacity'** : describes the cumulative level of contamination that a preserved formulation can cope with before the preservative becomes ineffective.

    This will vary with preservative type and complexity of the formulation.

Factors Influencing Antimicrobial Activity Within Medicines

- Most preservatives interact in solution with ingredients of pharmaceutical formulations to varying extents via a number of weak bonding attractions as well as with any microorganisms present.
  - This can result in unstable equilibrium in which only a small proportion of the total preservative present is **'available**' to inactivate m.o., and the resultant rate of killing may be less than anticipated from the performance of simple aqueous solutions.
  - However, the **'unavailable' preservative** may still, contribute to the **irritancy and toxicity** of the product.
- When solute concentrations are very high and Aw is appreciably reduced, the efficiency of preservatives is often reduced and may be even **inactive at very low Aw**.
  - That's why it is pointless to include preservatives in very low Aw products such as tablets and capsules.

Factors Affecting the 'Availability' of Preservatives

1. Effect of product **pH**
   - In the weakly acidic preservatives, the unionized molecules are the active ones & they have significant efficacy at pHs where ionization is low.
     - Benzoic and sorbic acids (pKa = 4.2 and 4.75, respectively) have limited preservative usefulness above pH 5.
     - 4(p)-hydroxybenzoate esters with their non-ionizable ester group and poorly ionizable hydroxyl substituent (pKa ca. 8.5) have moderate protective effect even at neutral pH levels.
     - The activity of quaternary ammonium preservatives and chlorhexidine resides with their cations and are effective in products of neutral pH.
   - Formulation pH can also directly influence the sensitivity of microorganisms to preservatives

Factors Affecting the 'Availability' of Preservatives

2. Efficiency in multiphase systems
   - In a **multiphase formulation**, such as an oil-in-water emulsion, preservative molecules distribute themselves in an unstable equilibrium between the bulk aqueous phase and
     - the oil phase by **partition**
     - the surfactant micelles by **solubilisation**
     - **polymeric** suspending agents and other solutes by competitive displacement of water of solvation
     - particulate and container surfaces by **adsorption**
     - any **microorganisms** present

Factors Affecting the 'Availability' of Preservatives

2. Efficiency in multiphase systems
   - Generally, the overall preservative efficiency can be related to the small proportion of preservative molecules remaining **unbound in the bulk aqueous phase**

     Although as this becomes depleted some slow re-equilibration between the components can be anticipated
   - The loss of neutral molecules into oil and micellar phases may be favoured over ionized species, although considerable variation in distribution is found between different systems.

Factors Affecting the 'Availability' of Preservatives

3. Effect of container or packaging
   - Preservative availability may be reduced by interaction with **packaging materials**.
   - Examples:
     - The **permeation** of phenolic preservatives into the rubber closure of multi-dose injection or eye-drop containers and their interaction with flexible nylon tubes for creams.
     - Quaternary ammonium preservative levels in formulations have been significantly reduced by **adsorption** onto the surfaces of plastic and glass containers.
     - **Volatile** preservatives such as chloroform are lost by the routine opening and closing of containers

Quality Assurance & the Control of Microbial Risk in Medicines

Mahmoud Alkawareek, PhD

Quality Assurance & the Control of Microbial Risk

- QA forms a scheme of management which includes all the procedures necessary to **provide a high probability that a medicine will conform consistently to a specified description of quality**, it includes measures taken during:
  - Formulation design & development (R&D)
  - Good pharmaceutical manufacturing practice (GPMP)
  - Quality control (QC)
  - Post marketing surveillance

Mahmoud Alkawareek, PhD

Quality Assurance & the Control of Microbial Risk

- **Risk assessment** for each product should be made starting from raw materials, to administration.
  - Risk assessments are complicated due to uncertainties about the exact contaminant & spoilage expected.
  - Usually the manufacturers make the **worst-case scenario** & design strategies to cover it fully, so that lesser problems are also included.
- Also, it must be assumed that people administering the medicament are not highly skilled & aware of contamination control so that additional detailed **information on administration** & even training must be provided.

Formulation Design & Development

- The best way to eliminate the risk of contamination & spoilage is by **sterilization**, but!
  - it incurs **high cost** so sterile products are kept to be used for situations were there is high risk of consequent infection from contamination.
- In **parenteral** products, **high risk of infection** by contamination & presence of concerns of systemic **toxicity of preservatives** resulted in the production of **sterile single-dosage units**.
- However, with **eye-drops** the risk is lesser & **sterile multi-dose products** with **preservatives** to protect against in-use contamination is accepted.
  - Sterile ophthalmic single-dose units are more common in hospitals where there is increased risk of infection.

Formulation Design & Development

- **Oral & topical** routes of admin present **low risk of infection** & the emphasis is on the **control of microbial content** during manufacturing & subsequent protection of the formulation from spoilage.
- In the design process it is necessary to include features in the formulation & delivery **system** that provide protection against contamination because preservative use should be **only** considered when there is **clear benefit** due to toxicity & irritancy problems.
- Among these features:
  - **Manipulation of physicochemical properties** like Aw
  - Elimination of certain **ingredient**
  - Selection of container & **preservative** individually and collectively contribute to the microbial stability of the product.

Formulation Design & Development

- Laboratory tests (**preservative effectiveness tests**) were designed to **challenge the product** with artificial bioburden. It should be part of the formulation development & stability trials to ensure that activity is likely to remain throughout shelf life.
- Preservative efficacy tests could be **single challenge** test where large inoculum of each m.o. is introduced into the product & rate of inactivation is determined by VC at different time intervals, or **multiple challenge** test, where the product is exposed to repeated inoculation at set intervals & the efficiency of inactivation is monitored until the system fails.
  - **BP, USP & EP** describe **single challenge** test.
- Problems with these tests:
  - Does the performance of these tests gives reliable prediction of real in-use efficiency?
  - Repeated cultivation on microbiological media results in reduced aggressiveness of strains

Good Pharmaceutical Manufacturing Practice (GPMP)

- GPMP is concerned with **control of ingredients, plant construction, process validation, production & cleaning**.
- QC is part of GMP that deals with testing, specs, documentation & assessing conformance to specs.
- Relying on finished product testing may result in **financial loss** if non-compliance was detected at this late stage, besides microbiological test methods have **poor precision & accuracy**, so that end product testing may not detect failures.

Good Pharmaceutical Manufacturing Practice (GPMP)

- Assurance of overall product quality can only come from **detailed specs, control & monitoring of all stages of manufacturing process** not just from testing finished product.

  e.g. real estimate of product microbial quality comes from knowledge of bioburden of starting raw material, temp record of the granules, moisture level of the granules, validation record of the machine cleaning, foil strip packaging & testing of finished tablets.
- Each batch should meet all its specification, but this does not necessary means that all tests should be performed on finished product. The manufacturer can carry out **parametric release**.
- Parametric release: is to **provide assurance** that the product is of **stipulated quality** based on evidence of successful **validation** of the manufacturing process & review of the **documentation** on process monitoring carried out during manufacturing to provide the desired assurance of product quality.

Good Pharmaceutical Manufacturing Practice (GPMP)

- Points to be considered to assure finished product microbial quality:
  - Raw materials:

    Raw materials to be free from pathogenic microorganisms & with low bioburden. For ingredients from bovine source, exclude suppliers of materials were BSE is endemic
  - Manufacturing plant:

    Identify areas where m.o. can thrive & colonize to treat & clean them. The design & construction should allow for thorough cleaning. (machines are made from materials that can be cleaned & disinfected, some machines are provided with clean-in place systems)
  - Manufacturing process:

    Some products may require addition of some steps to reduce bioburden or improve lethal sterilization (e.g. include ultra filtration step rather than conventional sterilization cylcle)
  - Validation to the cleaning system:

    To challenge the ability of cleaning system to remove deliberately introduced contaminant

Quality control (QC)

- The current methods for counting & detecting some microbes in non sterile products have **poor accuracy & precision**, examples:
  - low no. m.o. sometimes damage the product but can't be isolated and hence products with active spoilage yields very low microbial count
  - m.o. in high no. but it is not the primary spoilage agent nor pathogenic
- Uneven distribution of m.o present serious **sampling problems**

Quality control (QC)

- Pharmacopeias (Ph Eur, BP & USP) have included **quantitative & qualitative standards** for **non-sterile products**.
  - There are maximum **total microbial levels** & **exclusion of specific m.o.** depending on route of administration.
  - Example: for non-aqueous oral preparations, the count per ml or gram should be:
    - no more than 10^3 total aerobic microbial count (**TAMC**)
    - no more than 10^2 total yeast and mold (fungi) count (**TYMC**)
    - no *E. coli*
  - **Higher levels** are permissible if the product contains raw material of **natural origin**.

Table 5.1.4.-1. – *Acceptance criteria for microbiological quality of non-sterile dosage forms*

Route of administration	TAMC (CFU/g or CFU/ml)	TYMC (CFU/g or CFU/ml)	Specified micro-organisms
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 ml)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 ml)
Rectal use	10^3	10^2	-
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Candida albicans* (1 g or 1 ml)
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (special requirements apply to liquid preparations for nebulisation)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) Absence of bile-tolerant gram-negative bacteria (1 g or 1 ml)
Special Ph. Eur. provision for oral dosage forms containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 10^3 CFU per gram or per millilitre	10^4	10^2	Not more than 10^2 CFU of bile-tolerant gram-negative bacteria (1 g or 1 ml) Absence of *Salmonella* (10 g or 10 ml) Absence of *Escherichia coli* (1 g or 1 ml) Absence of *Staphylococcus aureus* (1 g or 1 ml)
Special Ph. Eur. provision for herbal medicinal products consisting solely of one or more herbal drugs (whole, reduced or powdered): – herbal medicinal products to which boiling water is added before use – herbal medicinal products to which boiling water is not added before use	10^7 10^5	10^5 10^4	Not more than 10^2 CFU of *Escherichia coli* (1 g or 1 ml) Not more than 10^3 CFU of bile-tolerant gram-negative bacteria (1 g or 1 ml) Absence of *Escherichia coli* (1 g or 1 ml) Absence of *Salmonella* (10 g or 10 ml)

Quality control (QC)

- Endotoxin Test:
  - Endotoxin (pyrogen) levels in **parenterals** must be very low to **prevent endotoxic shock**.
  - Formerly this was tested by injecting **rabbits** & noting any **febrile response**.
  - Nowadays the test is performed using ***Limulus* amoebocyte lysate (LAL) test** where an amoebocyte lysate from the **horseshoe crab** *Limulus polyphemus* reacts **specifically with microbial lipopolysaccharides** to give a gel or opacity (turbidity) even at very high dilutions.
  - **Tissue culture** test are **under development** where the ability of endotoxins to induce **cytokine release** is measured directly

Post market surveillance

- It is impossible to guarantee that a medicine will never fail under real life conditions, but a proper quality assurance system must include procedures to **monitor in use performance** & **respond to customer complaints** in order to **re-evaluate** the constructed & implemented **schemes** for product safety & check whether they need **modification**.