Microbial Quality Control of Pharmaceuticals

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Subjects:

1. Analytical Microbiology
2. Microbial limit tests and quality control of nonsterile pharmaceuticals
3. Antimicrobial Preservatives
4. Sterility and Sterility Assurance

References:

1. Hugo, W.B. and Russell, A.D., Pharmaceutical Microbiology, Blackwell, 2000.

2. Baird, R.M. and Bloomfield S.F., Microbial quality assurance in cosmetics, toiletries and non-sterile pharmaceuticals. 2nd ed., Taylor & Francis, London, 1996.
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6. British Pharmacopeia (BP) (latest edition).

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- 8. Block, S.S. (ed) **Disinfection, Sterilization and Preservation,** 5th ed. Lippincott Williams & Wilkins, 2001.

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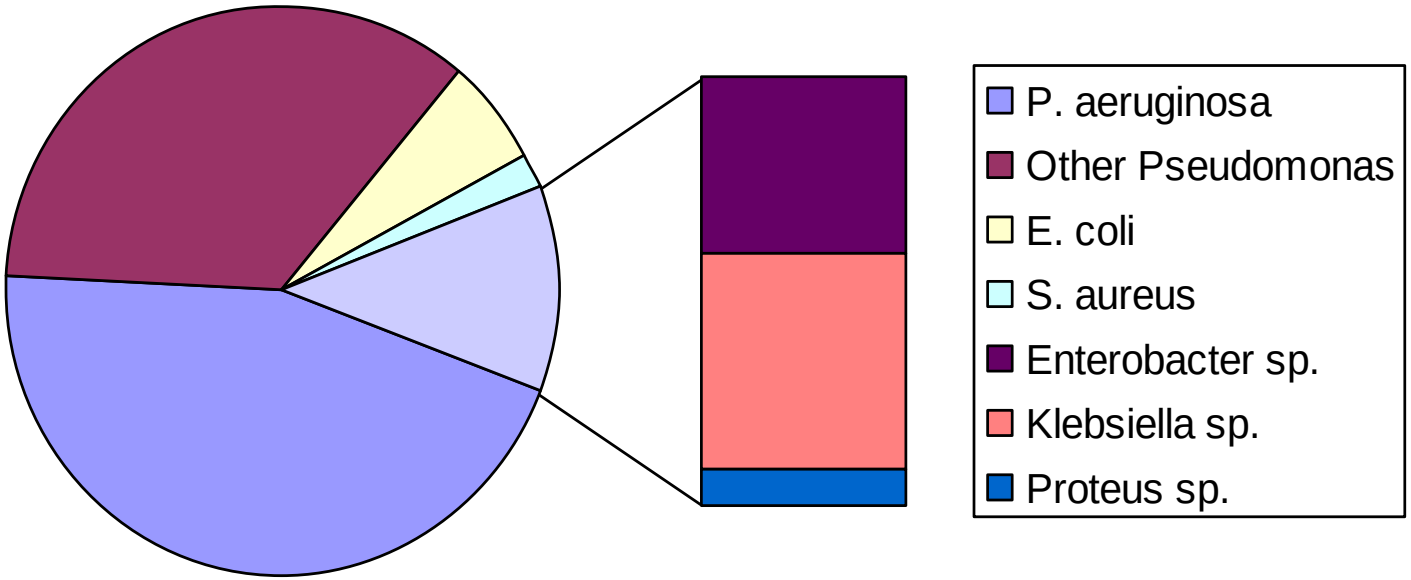
Introduction to:

Microbial contamination of Pharmaceuticals

Contamination rates for manufactured pharmaceutical products (1959-1979)

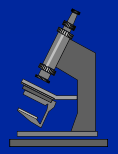
Product type	Total percentage contaminated	Percentage contaminated with>10^5/g or ml
Aqueous	**35**	**22**
Gels	**34**	**15**
Oily	**26**	**10**
Dry	**33**	**7**
Spirits	**3**	**3**
total	**32**	**18**

Contaminants isolated from pharmaceuticals, cosmetics and toiletries



Microbial contamination in pharmaceuticals is a potential hazard for two reasons:

- Represents a health hazard to the patient
- May cause product spoilage



Infection risk depends on four factors:

1. **Type of organisms:**

   pathogen or opportunistic

2. **Infective dose:**

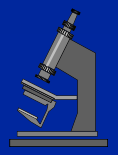
   S. aureus , intact skin 10^6

   injured skin 10^2

   E. coli, Salmonella , host resistance: 10^7-10^2

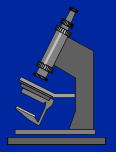
3. **Host resistance to infection**

4. **Route of administration**



Microbial contamination may result in:

- Degradation of active ingredients
- Degradation of Excipients (*polymers, emulsifiers*,…)
- Degradation of antimicrobial preservatives
- Fermentation & pH change
- Smell and color changes



Product spoilage

Is a dynamic process:

Product with low Wa (0.75-0.8)

↓

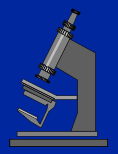
Osmophilic moulds

↓

Cocci

↓

Water born organisms (*Pseudomonas*)

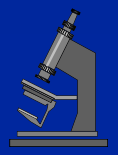


Contamination reports result in:

1. **Development and revision of microbial standards for non-sterile products**

   (microbial limit tests)

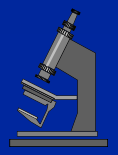
2. **Development of preservative systems**
3. **Environmental monitoring & control program**



Microbial standards
Non-sterile pharmaceuticals

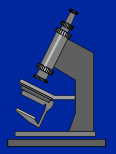
- Total viable counts (TBC+TYMC)
- Absence of specified microorganisms

  - Origin
    - Preparations with natural origins: *Salmonella sp*.
  - Rout of administration:
    - Oral suspensions & emulsions: *E. coli*
    - Topical preparations: *P. aeruginosa*, *S. aureus*
    - Vaginal & rectal preparations: mould & yeast
  - Population



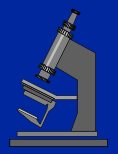
Indicator microorganisms (FDA):

- **Harmful:**
  - microorganism or its toxin causes infection (illness)
    - ≈ *Salmonella* sp., *Closteridia* sp.
- **Objectionable:**
  - Causes illness or product instability
    - ≈ *Pseudomonas putida*
- **Opportunistic:**
  - causes illness in patients, infants,…
    - ≈ Environmental microflora; G+ Cocci, G+ Bacilli, yeast, mould



Herbal medicines (BP-Category 4)

- **Herbal medicinal products to which boiling water is added before use:**
  - **TAVC: $< 10^7$ bacteria and $< 10^5$ fungi /g or ml**
  - **$< 10^2$ *E. coli* /g or ml**
- **Herbal medicinal products to which boiling water is not added before use:**
  - **TAVC: $< 10^5$ bacteria and $< 10^4$ fungi /g or ml**
  - **$< 10^3$ enterobacteria and certain other Gram-negative bacteria /g or ml**
  - **Absence of *E. coli*/g or ml**
  - **Absence of *Salmonella*/g or ml**

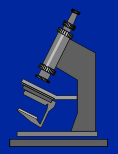


No.	Commercial name	TBC/g	TYMC/g	E. coli/g
1	Aliso tea	$3.4 * 10^6$	$3.6 * 10^2$	$>10^2$
2	Casia tea	$2.2 * 10^7$	$1.2 * 10^5$	Absent
3	Fumaria tea	$3.6 * 10^3$	<10	Absent
4	Hypericum tea	$7.6 *10^6$	$7.0 * 10^2$	Absent
5	Thymus tea	$2.9 * 10^7$	$8.7 * 10^5$	$>10^2$
6	Antidiabetic powder	$7.6 * 10^5$	$1.6 * 10^3$	Absent
7	Chahargol powder	$2.2 * 10^5$	$1.5 * 10^4$	Absent
8	Diuretic powder	$2.3 * 10^5$	$1.4 * 10^2$	Absent
9	Plantagel powder	$1.2 * 10^2$	<10	Absent

No.	Commercial name	TBC/g or ml	TYMC/g or ml	MPN coliform/g or ml
1	Alicum tablet	$9.0 * 10^4$	<10	<1
2	Menthazine tablet	$2.1 * 10^6$	<10	<1
3	Razine tablet	$1.3 * 10^5$	<10	<1
4	Samilax tablet	$6.4 * 10^2$	<10	<1
5	Sennamed tablet	$3.8 * 10^4$	<10	<1
6	Thymex tablet	$1.2 * 10^5$	<10	<1
7	Thymex syrup	$1.1 * 10^5$	<10	<1
8	Valiflore tablet	$1.8 * 10^5$	<10	>1 , <10
9	Ginco T D tablet	<10	<10	<1

Environmental monitoring program

1. Cleaning and sanitization of environment (surfaces, instruments, air,…)
2. Training of personell



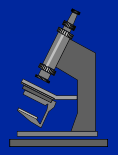
Microbial spoilage of pharmaceutical products:

- **Primary contamination (raw materials):**

1. Water, non-preserved solutions (peppermint water): ***G-negatives***

2. Organic solvents, alcohols: ***bacterial spores***

3. Dry powders & packaging materials: ***spores (G-positives & moulds)***



- **Production contamination:**

  Production facilities

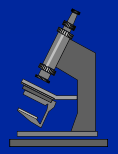
  Environment :

  - **Dry surfaces → G+ Bacilli, Cocci, spores**
  - **Wet surfaces → G- Bacilli**
  - **Air → loss of skin scales 10^4/min, Cocci, spores**

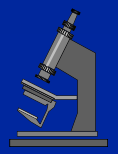
Bioburden of unused eucerin urea ointments

- All the samples examined immediately after purchase found to have total viable counts of lower than 10^2 cfu/g.
  - *Staphylococcus aureus (77%)*,
  - *Candida albicans (45.5%)*
  - *Escherichia coli (9.1%)*
  - *Pseudomonas aeruginos (4.5%)*

- After two weeks storage, contamination levels increased such that about **36.4%** of samples were found to have the total viable counts greater than 10^2 cfu/g
  - *Staphylococcus aureus (86%)*
  - *Candida albicans (59%)*
  - *Escherichia coli (18.2%)*
  - *Salmonella* sp. (9.1%)

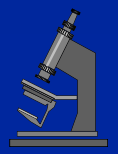


- **Secondary contamination**
  - **Contamination during use**
  - **Susceptible products are those with:**
    - **Multiple dose containers**
      - **High water activity**
      - **Sugars, vitamins, fats,…**



Development of a preservative system:

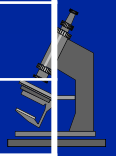
- An ideal preservative specification:
  - Spectrum of activity
  - Safety
  - Irritation, sensitization
  - Rate of kill
  - Cost
  - Environmental impact
  - Effect on the product
  - Functionality within product

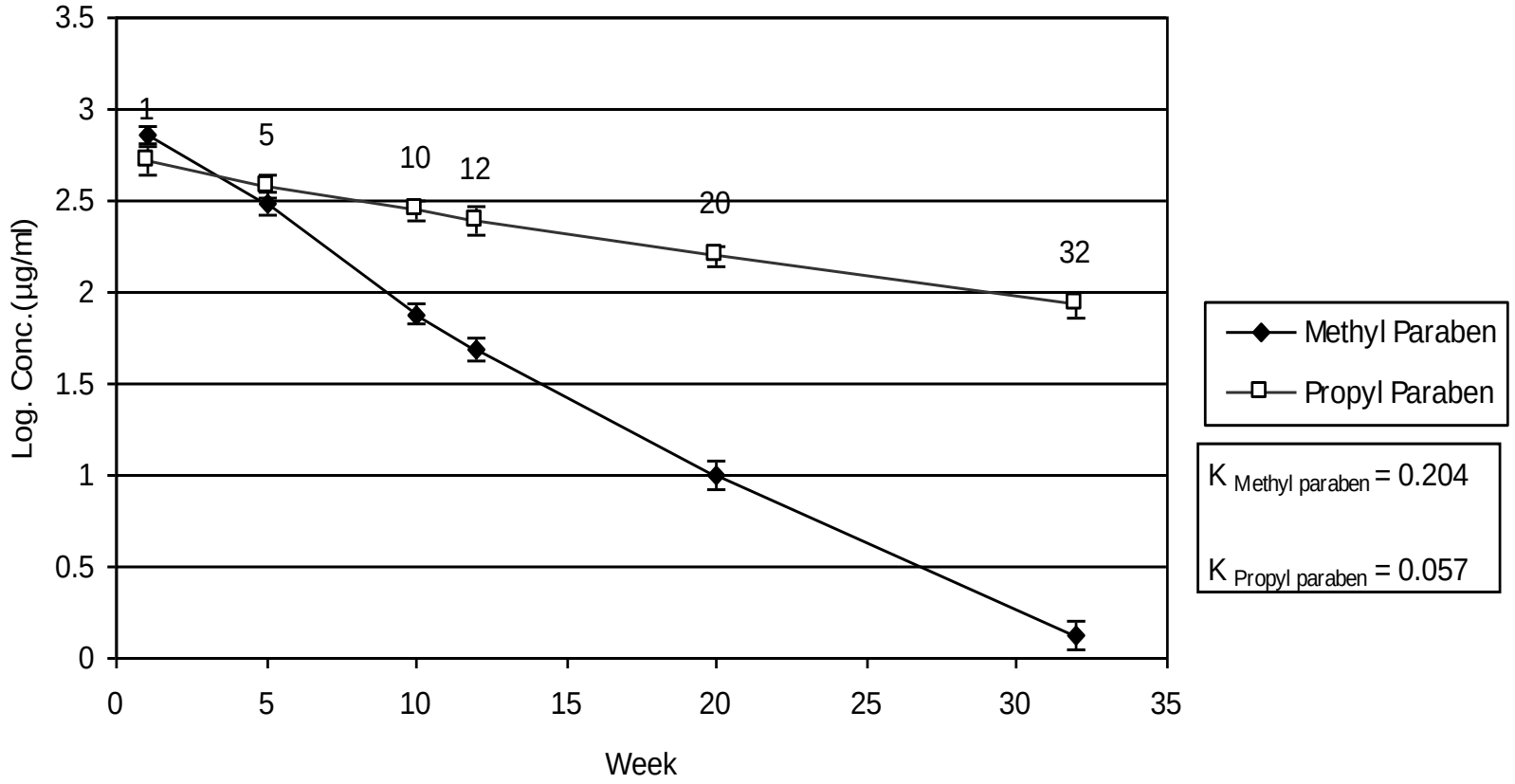


Class	Usual concentration (%)	Antimicrobial Spectrum
Acidic & Phenolics Benzoic Acid and Salts Sorbic Acid and Salts Boric Acid and Salts Phenol Cresol Chlorocresol Parabens	0.05-0.1 0.05-0.2 0.5-1.0 0.2-0.5 0.1-0.5 0.05-0.1 0.001-0.2	Antifungal Agent Broad Spectrum
Mercurials Thimerosal Phenyl Mercuric Acetate & Nitrate	0.001-0.1 0.002-0.005	Broad Spectrum and Synergist
Quaternary Ammonium Compounds Benzalkanium Chloride Cetylpyridinium Chloride Benzethonium Chloride	0.004-0.02 0.01-0.2 *	Broad Spectrum
Miscellaneous Alcohols Chlorobutanol Benzyl alcohol Phenoxy-2-ethanol Bromo-2-Nitroprpanediol-1,3	15.0-20.0 0.5 0.5-5.0 * *	Broad Spectrum

Effect of pH on Benzoic acid ionization, *pKa=4.19*

pH	% undissociated C_6H_5COOH	% dissociated $C_6H_5COO^-$
3.24	90	10
3.59	80	20
3.82	70	30
4.01	60	40
4.19	50	50
4.36	40	60
4.55	30	70
4.79	20	80
5.14	10	90

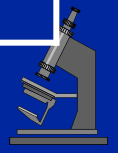




Hydrolysis of methyl paraben and propyl paraben in magnesium hydroxide suspension (25±2 ˚C, 60±5% RH)

Partition coefficient

preservative	Mineral oil K_w^o	Vegetable oil K_w^o
chlorcresol	1.5	117
Methyl paraben	0.02	7.5
Propyl paraben	0.5	80
Butyl paraben	3.0	280
CTAB	<1.0	<1.0



- Emulsifiers
- Suspensions
- Containers