

# Searching for microbes Part V.

## Microbes and outer influences Decontamination methods, and how to assess their effectivity

Ondřej Zahradníček

To practical for VLLM0421c

Contacts to me: 777 031 969

[zahradnicek@fnusa.cz](mailto:zahradnicek@fnusa.cz), ICQ 242-234-100

# Content of this slideshow

Microbes and outer influences

Decontamination methods: survey

Disinfection

Hand washing and disinfection

Higher level disinfection

Sterilization

Checking effectivity of decontamination

# What we know already

- We know how to use **three diagnostic methods**
  - **microscopy** as Wet mount, simple staining, Gram staining and capsular staining
  - **culture** of microbes on solid and liquid media (using solid media, we can get and **strain**)
  - **biochemical** (and other) **identification methods** for cultured strains
- Today we will fight microbes using decontamination methods.

# Tale

- There was a king with three sons. He had an awful **pyogenic ulcer of his leg**, and in the meantime he wanted to decide, who will be his follow him to the throne. So he decided, that the future king will be **the prince, that will definitively destroy his ulcer.**

## What the first two princes have done

- First prince sterilized the scalpel. He sterilized **enough time**, but at **too low temperature**. The scalpel was not sterile enough, and when the prince had cut the ulcer, it was reinfected soon.
- Second prince sterilized the scalpel, too. He used **sufficient temperature**, but **too short time**. So the result was the same as in the case of the first prince.

## What the third prince has done

- He sterilized the scalpel for **sufficient temperature and time**. So he cut his father's ulcer, it was OK, he became king, he married a sexy princess, and if he had not died, he lives there, having a lot of children.

# What to learn of it

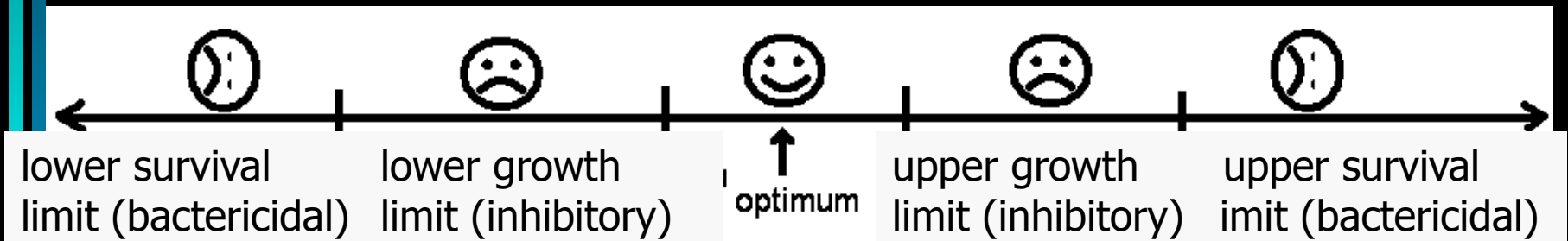
- When we try to kill microbes by **physical or chemical factors**, we have to **use correct parameters**.
- These parameters are **mutually related**, i. e. the necessary value of one changes in relation with the other
- **Time** uses to be **one of very important parameters**

# Microbes and outer influences



# Microbes and outer influences I

At decontamination methods, it is absolutely necessary to reach a value of the acting physical or chemical factor that is sufficient to **kill the microbe**.



Primarily, we are interested in survival limit (not growth limit, important for microbial cultivation).

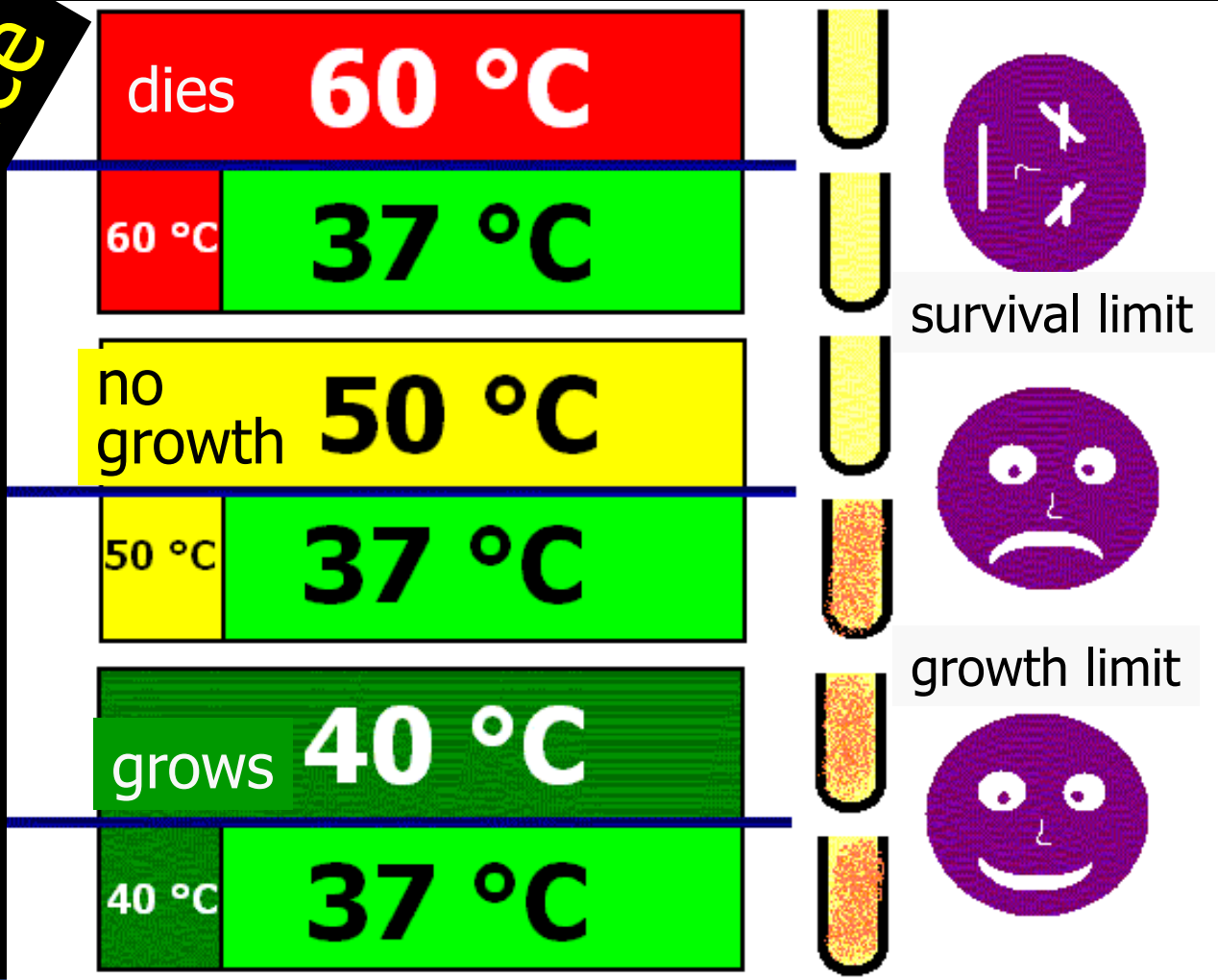
# Practical check of growth and survival limit

- If we want to find **survival limit** of bacteria, we have to remove the tested extreme parameters to the conditions and to let them then in optimal conditions for a sufficient time (to recover, if they are inhibited only).
- If we want to demonstrate **growth limit only**, we let them all the time in the extreme conditions (without chance to recover).

Above the line: **we act by a certain temperature, 24 h**

Below: **4 h only, then back to optimal temperature**

Methodological difference



# Microbes and outer influences II

Sometimes the action of factor **combines**

The factor allways important is the **time**

A resistant, spore forming bacterium	160 °C	170 °C	180 °C
20 min	survives	survives	dies
30 min	survives	dies	dies
60 min	dies	dies	dies

# A factor, allways combined with the other factors: TIME

- Remark: The lower the temperature is, the longer the time should be, to obtain a sufficient sterilization effect. This is also expressed in the intimation for sterilization saying what temperature and time should be used at steam sterilization and hot air sterilization.



Deconta-  
mination  
methods:  
survey

# Decontamination methods

- Those are **physical and chemical approaches of destroying microbes, insects and rodents** outside the organism.  
*(Some public health researchers put methods of destroying insects and rodents aside, and call them "assanation methods")*
- Destroying microbes in organism, although local (use of antiseptics), does not belong to decontamination methods.

*An antiseptic is a drug.*

*A disinfectant is not a drug*

# Survey of decontamination

methods (Czech intimation 195/2005)

<b>Sterilization</b>	Destroying of <b>all microbes</b> present in the environment
<b>Higher level disinfection</b>	Destroying of majority of microbes, some forms of life may survive (protozoan cysts etc.)
<b>Disinfection</b>	Destroying <b>harmful microbes</b> (depends on circumstances)
<i>Disinsection</i>	<i>Destroying harmful insects</i>
<i>Deratization</i>	<i>Destroying harmful rodents</i>



# Asepsis and antisepsis

- **Asepsis:** approaches passively defending an area against microbes
- **Antisepsis:** approaches, actively acting against microbes
- Basics of asepsis and antisepsis were introduced
  - to surgery by lord Joseph Lister
  - to obstetrics by Ignaz Semmelweis



# How to perform decontamination properly

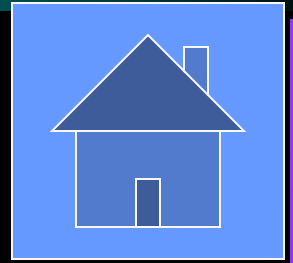
## (not regarding the type of method)

- To choose a proper sterilization/disinfection method/medium. „Proper“ means:
  - it should destroy (St) all organisms, or (Df) all pathogens that might be found in such an environment
  - it should not destroy disinfected or sterilized material
  - it should be practically applicable
- To use sufficient intensity of a factor (temperature, intensity, concentration)
- The factor should act enough time

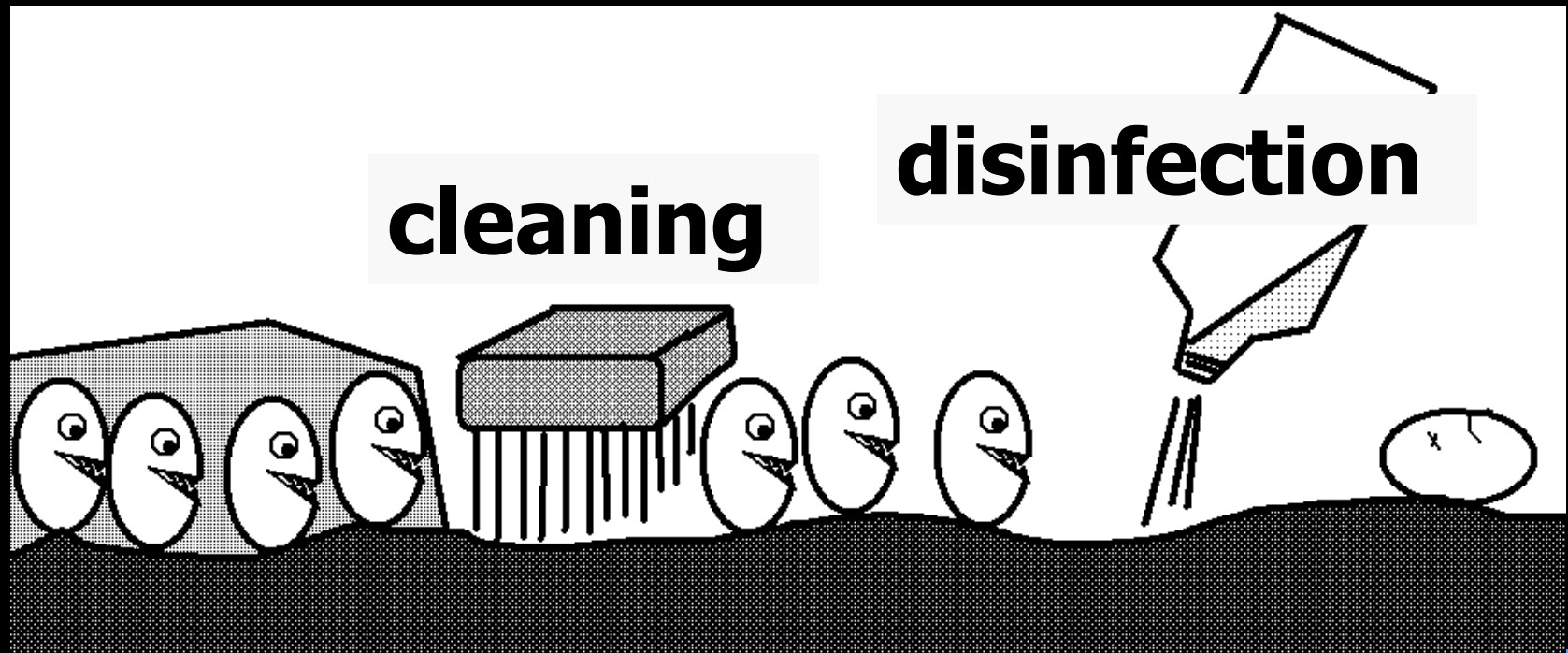
# Before and and after decontamination

- **Before decontamination** often preparation is necessary – mechanical cleaning, ensuring of a proper process. All the procedures take part in legal acts concerning decontamination methods
- **After decontamination** often some steps should be done (e. g. let the remaining gases to flow out). It is necessary to keep rules for storage of decontaminated objects.
- **Storage of decontaminated objects** (expiration) is described in intimations, too.

# Decontamination and cleaning



Tools, that are mechanically dirty, should be cleaned before decontamination. Without cleaning it would not be effective enough.



# Disinfection

# Disinfection – examples 1

- **A. PHYSICAL methods**
- 1. Boiling:
  - a) **at normal pressure** – in medicine at least 30 minut. (For coocing, less is enough, but the meal should be boiled profoundly!!!)
  - b) **in pressure vessels** – shorter time – even in this case it is NOT a sterilisation!
- 2. **Other physical methods** – filtration, sun rays, ultraviolet rays etc.

# Disinfection – examples 2

- **B. DISINFECTANTS**

- **Oxidation reagents**

- 3. Peroxides: **peracetic acid** ( $\text{CH}_3\text{COOOH}$ , in Czechia Persteril). For spores, fungi and tuberculosis; 0.5% solution = higher level disinfection. Disadvantage: aggressivity, decoloration of textiles, instability of solutions
- 4. **Hydrogen peroxide** ( $\text{H}_2\text{O}_2$ ) – similar, less aggressive, but also less effective





## Disinfection – examples 3

- 5. Halogen preparations – hypochloates:
  - chlorine bleach ( $\text{NaOCl}$ ), Savo in Czechia
  - calcium hypochlorite ( $\text{Ca(OCl)}_2$ )
- 6. Chloramin (Chloramin B in Czechia).
- 7. Iodine solutions (in Czechia mostly Jodonal B, Jodisol and Betadine). Unlike ancient iodine tincture, iodine is bound in a complex. At non-allergic patients iodine preparations should be used rather than detergents (Ajatin)



# Disinfection – examples 4

- 8. Kalium permanganate has no more use.
- 9. Formaldehyde – in mixtures
- 10. Kresol (lysol) is effective, but for aggressivity and bad smell not used at all
- 11. Ethylalcohol – in mixtures; alone not enough effectivity; the most effective is 70 % solution
- 12. Detergents: Orthosan BF 12
- 13. Ajatin – for skin disinfection
- 14. Septonex – rather an antiseptic.
- 15. Anorganic acids and hydroxydes, heavy metals
- 16. Combined agents, e. g. Incidur



# Effectivity of disinfection to various microorganisms

- In booklets and other materials, usually alphabetical system is used:
- A = effective to (vegetative forms of) bacteria and yeasts
- B = effective against viruses
- C = effective against bacterial endospores
- T = effective against TB mycobacteria
- M = effective against atypical mycobacteria
- V = effective against filamentous fungi

## Suitability to various purposes

Some disinfectants are only suitable for disinfection of surfaces, other are only for skin. Usually disinfectants are classified for

- disinfectants for tools
- disinfectants for surfaces
- disinfectants for hands (+ disinfection soaps)
- disinfectants for pipes in food industry
- disinfectants suitable for kitchens



# Hand washing and disinfection

# Washing and disinfection of hands

For hands, the rules are different than for tools of surfaces. Usually there is no thick layer of dirtiness on your hands

Recent legislative uses following terms:

- **Mechanic hand wash** (as a part of personal hygiene, or as a step preceding surgical hand rub)
- **Hygienic hand wash** (using antimicrobial soap; better than mechanic hand wash, but worse than hygienic hand rub)
- **Hygienic hand rub** (recommended for medicine)
- **Surgical hand rub** (before operations)



## Normal course of working day of a nurse or a medic

- **When comming at work:** washing one's hands and use alcohol disinfection
- **During working day:** use alcohol disinfection only, eventually hand care preparations
- **At finishing work:** wash one's hands again.

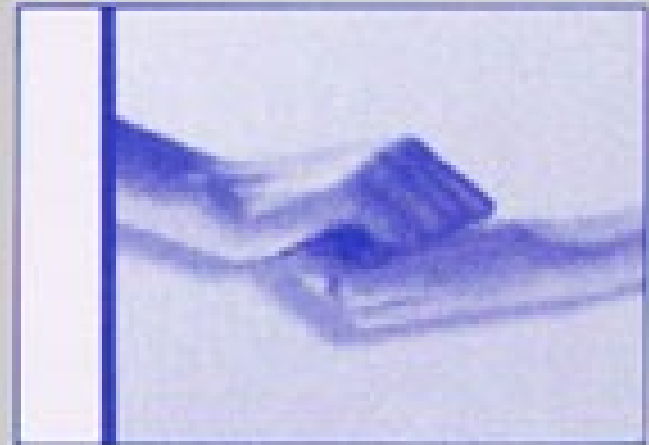
*During the working day, it is recommended rather to disinfect one's hands and not to wash them too often*



# Proper hand rub / wash technique

- **1<sup>st</sup> step:** Rub hands together palm to palm
- **2<sup>nd</sup> step:** Left palm over back of right hand and vice versa
- **3<sup>rd</sup> step:** Palm to palm, fingers interlaced
- **4<sup>th</sup> step:** Backs of fingers to opposing palms with fingers interlocked
- **5<sup>th</sup> step:** Clasp right thumb in left palm and vice versa
- **6<sup>th</sup> step:** Using a circular motion rub fingertips of right hand in left palm and vice versa
- (**7<sup>th</sup> step:** Wrist of one hand using the other hand)

# How to wash/rub your hands



1. Rub hands together palm to palm



2. Left palm  
over back of  
right hand  
and vice versa

3. Palm to  
palm, fingers  
interlaced

4. Backs of fingers to  
opposing palms with  
fingers interlocked





5. Very important:  
Clasp right thumb  
in left palm and  
vice versa



6. Using a circular motion rub fingertips of right hand in left palm and vice versa




(7. Wrist)

# What areas are the most frequently missed

The most commonly forgotten areas are light blue, dark blue are also frequently missed.

Handwashing poster showing areas frequently missed during handwashing.

Areas frequently missed during handwashing

-  Most frequently missed
-  Frequently missed
-  Less frequently missed



# A urban legend

~~„when using gloves,  
washing and disinfection  
is not necessary“~~

*very dangerous nonsense!*

## Why Wash Your Hands?

To remove or destroy potentially harmful microorganisms.

Areas frequently missed during handwashing

- Most frequently missed
- Frequently missed
- Less frequently missed



## When Must You Wash Your Hands?

Before...

- Handling high risk area clothing.
- Changing into high risk area clothing.
- Putting on gloves.
- Going into food handling areas.
- Handling ready to eat food.

After...

- Handling raw food.
- Handling waste.
- Using the toilet.
- Blowing your nose.
- Carrying out cleaning duties.

# HANDWASHING

## How To Wash Your Hands...



## When Using Gloves...

- 1 Wash and sanitise your hands as above.
- 2 Put on gloves, taking care not to tear them.
- 3 Remove gloves when leaving

- 4 Always change your gloves if there are any holes or tears, and report them to a line manager.

- 5 Dispose of gloves safely.
- 6 Thoroughly wash, dry and sanitise hands before re-applying a new pair of gloves.

Acknowledgement John Babb, Hospital Infection Research Laboratory, City Hospital NHS Trust, Birmingham, UK. ©Chilled Food Association 1999.

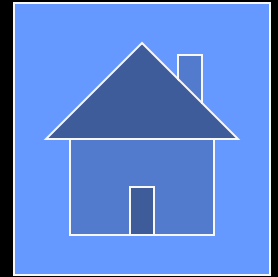


# There exist systems for disinfection checking using UV rays

<http://www.newhamuniversityhospital.nhs.uk/press.php?15a2e6d35a31864e72c9a857450ee983>



# Staff behaviour complexely



- **Important for all staff**

- Proper technique of **hand washing** is not as obvious as supposed, it should be trained

- **Use of gloves** and other barrier tools

- Proper **manipulation with needles** after use

- **Work organisation** (division of „clean“ and „dirty“ manipulation by place and/or time at all levels: dirty and clean trays, area for infusion preparation and a different one for biological material etc.)

Higer level  
disinfection



# Higher level disinfection



- „Something between sterilization and disinfection“

- It is not sterilization, because protozoan cysts and helminth eggs are not always destroyed.

- Glutaraldehyde, Secusept or Persteril

- Concentration always higher than for common disinfection

- For flexible endoscopes, where no sterilization methods can be used.

# Sterilization

# Sterilization – examples I

- **1. Hot steam sterilization under pressure.** Steam should be just saturated. It is suitable for objects made of glass, metal, ceramics, china, textile, rubber and some plastics. Temperatures 121–134 °C. (No more 115 °C!)
- **2. Hot air sterilization** (in apparatuses with artificial air circulation 180 °C 20 min or 170 °C 30 min or 160 °C an hour). For metal, glass, china.
- **3. Hot water sterilization under pressure** – not used anymore

# Steam sterilizer



## Sterilization – examples II

- 4. Gamma ray sterilization: in industrial production, e. g. for single use gloves.
- 5. Plasma sterilization in high-frequency electromagnetic field (a modern method)
- 6. Chemical sterilization by vapors of formaldehyde or by ethylenoxide (the procedure should be performed according to the schedule). In situations disabling use of physical methods.

# Formaldehyde sterilizer

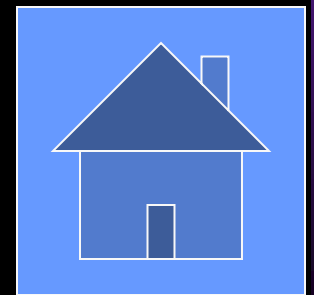


# Sterilization – examples III (not in health care)

- 7. **Fire sterilization** for microbiological loops only. Incineration for waste.
- 8. **Pascalisation** is a pressure sterilization, used in alimentary industry
- 9. **Other methods:** fractionated sterilization, solution filtration etc., used in research, not in practical medicine

# What method should be used?

- Suitability of methods is related with **material resistance** to various temperature, humidity, chemicals and other factors
- Do not forget, that parameters should be sufficient (**temperature in °C, pressure in kPa, radiation dose in Gy etc.**) and **time has to be sufficient, too!**



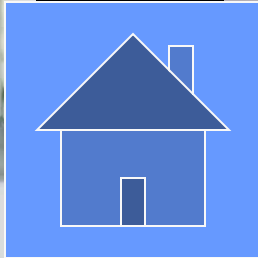


Checking  
effectivity of  
deconta-  
mination

# Checking up, whether sterilisation was done, and its quality assessment

- Orientation checking up – e. g. by typical smelling
- Assessment of real concentration of disinfectants (chemically)
- Chemical check up of sterilization uses indicators that change colour at a certain temperature
- Biological way uses resistant strains of *Bacillus* genus. These absolve the whole cycle and then their survival is assessed.

Photo O. Z.



The end



# Lord Joseph Lister

(5 April 1827 – 10 February 1912)

English surgeon

Lister became aware of a paper published by Louis Pasteur. Lister used carbolic acid (phenol) as a means of deodorizing sewage. He also made surgeons wear clean gloves and wash their hands before and after operations with 5% carbolic acid solutions. As the germ theory of disease became more widely accepted, it was realised that infection could be better avoided by preventing bacteria from getting into wounds in the first place. This led to the rise of sterile surgery. Some consider Lister "the father of modern antisepsis." In 1879 Listerine mouthwash was named after him for his work in antisepsis. Also named in his honour is the bacterial genus *Listeria*, typified by the food-borne pathogen *Listeria monocytogenes*.

[http://en.wikipedia.org/wiki/Joseph\\_Lister,\\_1st\\_Baron\\_Lister](http://en.wikipedia.org/wiki/Joseph_Lister,_1st_Baron_Lister)



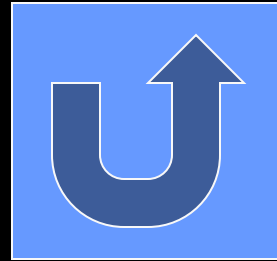
[http://cs.wikipedia.org/wiki/Soubor:Joseph\\_Lister.jpg](http://cs.wikipedia.org/wiki/Soubor:Joseph_Lister.jpg)



# Ignaz Fülöp Semmelweis

(1818 – 1865)

Hungarian physician.



He discovered that cases of puerperal fever, also known as childbed fever, could be cut drastically if doctors washed their hands in a chlorine solution before gynaecological examinations.

While employed at the Vienna General Hospital in Austria, Semmelweis introduced hand washing with chlorinated lime solutions for interns who had performed autopsies. This immediately reduced the incidence of fatal puerperal fever. Semmelweis' hypothesis, that there was only one cause, was largely ignored, rejected or ridiculed. He was dismissed from the hospital and harassed by the medical community in Vienna, which eventually forced him to move to Budapest. Semmelweis' practice only earned widespread acceptance years after his death, when Louis Pasteur developed the germ theory of disease. He is considered a pioneer of antiseptic procedures.

[http://en.wikipedia.org/wiki/Ignaz\\_Semmelweis](http://en.wikipedia.org/wiki/Ignaz_Semmelweis)



[www.answers.com/topic/ignaz-semmelweis](http://www.answers.com/topic/ignaz-semmelweis)

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