Biocidal Control of Mycoses and Mycotoxicoses

Elena Piecková
Assoc. Prof. in Public Health, Head of Mycology and Ntl Ref Ctr Disinf & Steriliz

Slovak Medical University, Bratislava, Slovakia
Agenda

- A sketch on mycotic health care-associated infections (HAI)
- Overview of fungicidal/-static biocides and disinfection procedures in health/social care - their efficacy and sensitivity vs resistance
- Laboratory testing of antifugal biocides
- Good cleaning/disinfection practice in health-care units
Clean Care is Safer Care
EC COUNCIL RECOMMENDATION (2009/C 151/01), 9 June, 2009

on patient safety, including the prevention and control of healthcare associated infections:

- in Member States between 8 % and 12 % of patients admitted to hospital suffer from adverse events whilst receiving healthcare

European Centre for Disease Prevention and Control (ECDC), 2012

- on average, healthcare associated infections occur in one hospitalised patient in 20, that is to say 4.1 million patients a year in the EU, and that 37 000 deaths are caused yearly
• over the past decade - an indispensable institution in the scope of European health policy

• a platform for discussion for the various stakeholders in the field of public health and health care

• 03-04/10/2013 - 6th EHFG: - an estimated total of 3.2 million patients (95% confidence interval: from 1.9 to 5.2 million) with a HAI in European hospitals each year
Ill health due to fungi

Mycotic Diseases

Allergies
Infections – Mycoses
Intoxications – Mycotoxicoses

HAI incidence – yeast 30/100,000 population
- mould 2/100,000 - - - IC patients’
mortality rate over 20 %
Prevalency of fungal nosocomial infections according to hospital departments

<table>
<thead>
<tr>
<th>Department</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>6.8</td>
</tr>
<tr>
<td>Psychiatry</td>
<td>0.4</td>
</tr>
<tr>
<td>Geriatrics</td>
<td>2.5</td>
</tr>
<tr>
<td>Gynaecology</td>
<td>1.2</td>
</tr>
<tr>
<td>IMU</td>
<td>12.4</td>
</tr>
<tr>
<td>Paediatrics</td>
<td>2.2</td>
</tr>
<tr>
<td>Internal Medicine</td>
<td>3.6</td>
</tr>
<tr>
<td>Surgery</td>
<td>3.6</td>
</tr>
</tbody>
</table>
The most frequent types of fungal nosocomial infections - EU

- Urinary tract infections: 12%
- Others (eye, ear, upper airways, skin/mucoses, GIT): 22%
- Pneumonia and other infections of lower airways: 26%
- Surgical site infections: 24%
- Blood stream infections/Sepsis: 16%
Yeast colonization of surfaces and its characterization
Aerosol

*Moulds* - conidia (6-12 x 3.5-4.0 μm)
Sexual developmental stage –

**cleistothecia** - 140 x 200 μm

**ascospores** - lemon-shaped, 1-celled, smooth-walled, yellowish/brownish (6-7 x 4.0-4.5 μm)

**Colonies** - hairy, cottony/lanose or moist

**Hyphae** - melanized, not when submerged (conidiogenesis also in submersion)
Fungal fragments (≤ 1.6μm optical size)

- aerosolized simultaneously with spores (probably liberated permanently)

- released in higher (10² x) numbers (10⁵ /cm²) - prediction based on the spore counts impossible
  --- fungus (dessication stress), dynamical conditions, adhesion (moisture of the material)

- also wide temporal variation of aerial concentrations

- fragments and spores share common antigens - fragments‘ activity higher!
Aerosols  g/l (m$^3$)

- fungal particles in drops  Φ ~10 μm (20 μm)
  (fine fog)

- sedimentation from 3 m ~ 19 min (5 min)
Mouldy smell – HVAC analysis
INHALING EXPOSURE

Dynamics ??
(„propagule burst“ vs. common air)

Deposition
- nasal breathing: 30-40 % particles in the nose, 30-40 % in the alveoli
- oral breathing: 70 % in the alveoli

conglomerates/aggregates/particles ??
- rhinopharynx, sinuses (conidia ??)

- trachea, bronchi (conidia, ascospores ??) - allergic bronchopulmonary mycosis

- alveoli (fragments, conidia ??)

Agent-release

-mucociliary effect ??
Secondary metabolites of indoor moulds - *in vitro* toxicity

- tracheal ciliary movement ceased in **24 h**

- lectin histochemistry – T II lung cells:

<table>
<thead>
<tr>
<th>Control</th>
<th>2.5%</th>
</tr>
</thead>
</table>

![Control Image](image1.png) | ![2.5% Image](image2.png)
## Microorganism Selection

<table>
<thead>
<tr>
<th>More Resistant</th>
<th>Microorganism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prions</td>
<td>Scrapie, Creutzfeld-Jacob disease, Chronic wasting disease</td>
<td></td>
</tr>
<tr>
<td>Bacterial Spores</td>
<td>Bacillus, Geobacillus, Clostridium</td>
<td></td>
</tr>
<tr>
<td>Protozoal Oocysts</td>
<td>Cryptosporidium</td>
<td></td>
</tr>
<tr>
<td>Helminth Eggs</td>
<td>Ascaris, Enterobius</td>
<td></td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>Mycobacterium tuberculosis, M. terrae, M. chelonae</td>
<td></td>
</tr>
<tr>
<td>Small, Non-Enveloped Viruses</td>
<td>Poliovirus, Paroviruses, Papilloma viruses</td>
<td></td>
</tr>
<tr>
<td>Protozoal Cysts</td>
<td>Giardia, Acanthamoeba</td>
<td></td>
</tr>
<tr>
<td>Fungal Spores</td>
<td>Aspergillus, Penicillium</td>
<td></td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td>Pseudomonas, Providencia, Escherichia</td>
<td></td>
</tr>
<tr>
<td>Vegetative Fungi and Algae</td>
<td>Aspergillus, Trichophyton, Candida, Chlamydomonas</td>
<td></td>
</tr>
<tr>
<td>Vegetative Helminths and Protozoa</td>
<td>Ascaris, Cryptosporidium, Giardia</td>
<td></td>
</tr>
<tr>
<td>Large, non-enveloped viruses</td>
<td>Adenoviruses, Rotaviruses</td>
<td></td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td>Staphylococcus, Streptococcus, Enterococcus</td>
<td></td>
</tr>
<tr>
<td>Enveloped viruses</td>
<td>HIV, Hepatitis B virus, Herpes Simplex virus</td>
<td></td>
</tr>
</tbody>
</table>

Less Resistant
Relationship between hand hygiene and the acquisition of health care-associated pathogens
Review of hand hygiene

- Water
- Plain (non-antimicrobial) soap
- Quaternary ammonium compounds
- Alcohols
- Chlorhexidine
- Chloroxylenol
- Hexachlorophene
- Iodine and iodophors
- Triclosan
- Other agents

How to wash your hands properly:
1. Wet your hands
2. Liquid soap
3. Lather and scrub - 20 sec
4. Rinse - 10 sec
5. Dry your hands
6. Turn off tap

DON'T FORGET TO WASH:
- between your fingers
- under your nails
- the tops of your hands
World Health Organization, 2009

- WHO Guidelines on Hand Hygiene in Health Care
- First Global Patient Safety Challenge
Biocidal products: **Regulation (EU) No 528/2012** published on 27 June, applies from 1 September 2013

- **BIOCIDAL PRODUCTS** - contain or generate active substances and
  - are used against harmful organisms (pests and bacteria)
  - both to protect human and animal health
  - include household products (disinfectants, rodenticides, repellents, insecticides) and for industrial applications as well
The 22 Product Types (‘PTs’) of the Biocidal Products Regulation (528/2012)

- **Group 1: Disinfectants** - employed in controlling hospital infection

**Human hygiene** – 1 Biocidal products used for human hygiene purposes, applied on or in contact with human skin or scalps for the primary purpose of disinfecting the skin or scalp.

**Disinfectants and algaecides** not intended for direct application to humans or animals.- used for the disinfection of surfaces, materials, equipment and furniture which are not used for direct contact with food or feeding stuffs.
Human hygiene products

• **Efficacy/efficaceous** - the (possible) effect of the application of a hand hygiene formulation when tested in laboratory or *in vivo* situations

• **Effectiveness/effective** - the clinical conditions under which a hand hygiene product has been tested for its potential to reduce the spread of pathogens, e.g. field trials

• **Surrogate microorganism** - a microorganism used to represent a given type or category of nosocomial pathogen when testing the antimicrobial activity of antiseptic
  
  - selected for their safety, ease of handling, and relative resistance to antimicrobials.
Evaluation methods

• of the antifungal efficacy of handrub and
• handwash agents and formulations for surgical hand preparation:

Current methods:
CEN standards EN 1499 and EN 1500 (Candida albicans, Trichophyton mentagrophytes; Aspergillus niger); ASTM E-1174202
EN 12791 (surgical hand preparation; ASTM E-1115 (surgical hand scrub)

Shortcomings of traditional test methods
The need for better methods, e.g. the protective effects of BIOFILMS - 3,000 x more resistant than plankton
Disinfectants’ efficacy against yeasts:

- pure cultures
- mixed cultures
- biofilm
Nosocomial oculomycosis

Ringer’s solution - - - *Purpureocillium lilacinum* (*Paecilomyces lilacinus*)

![Image of eye](image1.png)

![Image of fungus](image2.png)

![Image of mycelium](image3.png)

---

**Fungicide effectiveness of disinfectants against Purpureocillium lilacinum**

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>H₂O₂ (0.5%)</th>
<th>H₂O₂ (1%)</th>
<th>H₂O₂ (2%)</th>
<th>Glutaraldehyde (2%)</th>
<th>Chloramin (5%)</th>
<th>I₂ (3%)</th>
<th>Formaldehyde (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **eye**
- **Ringer’s solution**

Disinfectant
Hospital associated moulds, incl. heat-resistant ones

Disinfectant sensitivity of hospital associated fungi

Fungi
- Trichophyton mentagrophytes
- Geotrichum candidum
- Penicillium roqueforti
- Eupenicillium baarnense
- Talaromyces flavus
- Dichotomyces cejpii
- Talaromyces trachyspermus
- Neosartorya fischeri
- Talaromyces avellanues
- Byssochlamys nivea

Disinfectants:
- H2O2 (1%)
- H2O2 (0.5%)
- Chlorhexidin (1%)
- H2O2 (2%)
- QATs (3%)
- Chloramin (5%)
- Cl2 (5%)
- I2 (5%)

Time in minutes

0 20 40 60 80 100 120
Disinfectant Effectiveness Tests

- EN 1276 or 1040 (bacterial suspension test)
- 1650 (fungal suspension test)
- 13704 (sporicidal suspension test)
- 13697 (carrier test)
- 14476 (viral Testing)
- 14348 (TB Testing)

- AFNOR (France)
  - NFT 72-150 Suspension
  - NFT 72-190 Carrier Test

- DGHM (GER; Carrier & Suspension Tests)

TGA (Australia)
Common AOAC International Tests
(last rev. 2012)

• Use-Dilution Method Tests for Liquids
  – 955.14 *Salmonella enterica*
  – 955.15 *Staphylococcus aureus*
  – 964.02 *Pseudomonas aeruginosa*

• Germicidal Spray Products Test

• Confirmatory Tuberculocidal Activity Test

• **Fungicidal Activity of Test Substances**

• Sporicidal Activity of Disinfectants (966.04)

• EN-13697 offers valuable insight into quantitative surface testing
Examples of the protocols

- **Sterilant** (60 carriers each on two surfaces); spores of *Bacillus subtilis* ATCC 19659 and *Clostridium sporogenes* ATCC 3584; 3 lots (720 carriers)

- **Fungicide** (10 carriers rep. 2 lots killing all spores of *Trichophyton mentagrophytes* ATCC 9533)

- **Disinfectant** (60 carriers representing 3 lots) against 3 bacteria; *S. enterica* ATCC 10708, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15442

- **Tuberculocide** (2 lots killing all *Mycobacterium tuberculosis* var. bovis (BCG) on all carriers) or 4 LRV in quantitative test

- **Virucide** (2 lots at 4 replicates per each dilution showing inactivation at all dilutions if no cytotoxicity) – 4 LRV (3 LRV if cytotoxicity)

- **Sanitizer-N-FC** (3 LRV on surfaces within 5 min against *S. aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352 or *Enterobacter aerogenes* ATCC 13048)
Guidelines for GCP


- W.A. Rutala,1,2, D. J. Weber, 1,2, and the Healthcare Infection Control Practices Advisory Committee (HICPAC)

- 1Hospital Epidemiology University of North Carolina Health Care System Chapel Hill, NC 27514
- 2Division of Infectious Diseases University of North Carolina School of Medicine Chapel Hill, NC 27599-7030
• commercial formulations - unique products and must be registered (EPA, EC)
• given product - designed for a specific purpose and used in a certain manner - read labels carefully!
• disinfectants - not interchangeable!
• incorrect concentrations and inappropriate disinfectants - excessive costs
• occupational diseases among cleaning personnel (e.g., formaldehyde, glutaraldehyde, and chlorine) - precautions (e.g., gloves and proper ventilation) to minimize exposure!
Keys to a successful disinfection

- Antifungal agent
- Choosing the proper disinfectant for the job
- Testing protocol (practical, achievable & verifiable)
- Choose the method that best fits the situation
- Sanitization procedures
- Set up the proper rotation of disinfectants to control all organisms
- Change control
- Have all processes organized
Thank you for your attention!

All the citations are available upon request.