Bacterial Resistance to Microbicides in the Healthcare Environment

Dr. Jean-Yves Maillard, University of Cardiff

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A Webber Training Teleclass

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Bacterial resistance to microbicides in the healthcare environment

Dr Jean-Yves Maillard
Welsh School of Pharmacy
Cardiff University, Wales

DEFINITIONS

• Resistance / tolerance / insusceptibility??

• Resistance: surviving exposure to a biocide concentration that will kill the rest of the population

• Tolerance: inhibited but not killed
  Phenotypic tolerance: transient conditions (biofilm)
  Chapman. Int Biodeter Biodegrad 2003; 51: 133-8

• Insusceptibility: intrinsic property

OBJECTIVES

• To review the overall mechanisms of bacterial resistance to microbicides

• To discuss the factors affecting the antimicrobial efficacy of microbicides and their effects in helping microbial survival and emerging resistance

• To discuss the significance of emerging bacterial resistance in the healthcare environment

BIOCIDES USAGE

Disinfection - antisepsis - preservation

Disinfection
  Surface disinfection (non-/ semi-/ critical)
  High-level disinfection (AWDs)

Antisepsis
  Alcoholic rubs, etc.

Preservation
  low concentration (cosmetic)

Increased usage of microbicides in various products/surfaces

EVIDENCE OF RESISTANCE - in practice

• Surviving bacteria isolated following biocidal challenges

  Triclosan bath

  Triclosan handwash

  Chlorhexidine
  Nakahara & Kozukue. Sbl Bakt Hyg, I. Abt Orig A 1981; 251: 177-84

  QACs

  Glutaraldehyde
  Griffiths et al. J Appl Microbiol 1997; 82: 519-26

Incorporation of low concentration of microbicides into products, surfaces etc.

• Plastics
• Bed sheets - clothing
• Curtains
• Surfaces
• Door handles
• Shower rails
• Trolleys
• Laminate flooring - walls

Effect on microbial microflora in practice not yet determined

Incorporation of low concentration of microbicides into products, surfaces etc.
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EVIDENCE OF RESISTANCE – in practice

- Automated washer disinfectors (Martin & Maillard 2006)

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Location</th>
<th>Time (min) to achieve 5 Log₁₀ reduction</th>
<th>Chlorine dioxide* 2.25%</th>
<th>Hydrogen peroxide 7.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>* formulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis (veg)</td>
<td>Rinse water</td>
<td>&gt;60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>Rinse water</td>
<td>30</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Streptococcus sanguinis</td>
<td>Endoscope connectors</td>
<td>5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>Drain area</td>
<td>30</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus intermedius</td>
<td>Drain area</td>
<td>30</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

RESISTANCE MECHANISMS

(A) IMPERMEABILITY

- Intrinsic
  - spore coat and cortex
  - mycobacteria mycoyl-arabinogalactan GTA, QACs
  - outer envelope in Gram-negative QACs, biguanides, phenolics

- Acquired
  - change in lipopolysaccharides / membrane fatty acids
  - change in outer membrane protein (porins) QACs, biguanides
  - change in arabinogalactan composition

EVIDENCE OF RESISTANCE – in practice

- MRSA in ITUs – susceptibility to NaDCC (Williams & Maillard 2006)

<table>
<thead>
<tr>
<th>MIC (ppm)</th>
<th>CT (sec)</th>
<th>log₁₀ R (±SD)</th>
<th>MIC (ppm)</th>
<th>CT (sec)</th>
<th>log₁₀ R (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>325</td>
<td>30</td>
<td>3.85 (2.19)</td>
<td>49</td>
<td>400</td>
</tr>
<tr>
<td>14</td>
<td>300</td>
<td>30</td>
<td>2.01 (0.37)</td>
<td>52</td>
<td>400</td>
</tr>
<tr>
<td>51</td>
<td>325</td>
<td>60</td>
<td>6.16 (0.33)</td>
<td>60</td>
<td>6.38 (0.12)</td>
</tr>
<tr>
<td>47</td>
<td>300</td>
<td>55</td>
<td>2.45 (0.84)</td>
<td>30</td>
<td>5.93 (0.07)</td>
</tr>
<tr>
<td>Control</td>
<td>225</td>
<td>30</td>
<td>2.27 (1.74)</td>
<td>60</td>
<td>6.19 (0.11)</td>
</tr>
<tr>
<td>9518</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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  - change in arabinogalactan composition

RESISTANCE MECHANISMS

(A) SURFACE INTERACTIONS

Hydrophobicity QACs, CHX
Cell surface charge QACs


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RESISTANCE MECHANISMS

(B) EFFLUX (intrinsic or acquired)
- several families of efflux pumps identified

- MATE family
- MFS family
- SMR family
- RND family

- aminoglycosides, fluoroquinolones, cationics
- QACs, phenolics, CHX, metallic salts

- TolC

- Na^+ HH^++ ATP ADP + Pi

- acriflavine, BZC, cetrimide, CHX, pentamidine

- multiple drugs

From Piddock Clin Microbiol rev 2006; 19: 382-402

RESPONSE TO BIOCIDES EXPOSURE

- adaptation
- modification of targets
- overproduction of target enzymes
- stress response

- FMA

- QACs, phenolics, GTA, chlorine

- Gram-negative & -positive, mycobacteria

- examples of adaptation in situ

- Eliciting stress response
- induction of oxyR and soxRS as a result of hydrogen peroxide exposure
- followed by expression of efflux pump, reduction in OMP, changes in fatty acids (?)

- E. Coli ATCC 1053

- MIC (µg/ml)

- TCS alone TCS + CCCP TCS + OVA TCS + EDTA TCS + CCCP + OVA TCS + CCCP + EDTA TCS + EDTA

- Standard ND ND ND ND ND ND ND

- TM1 >1000 25 >1000 25 25 10-50 10-25

- TM2 >1000 50 >1000 25 25 10-50 10-25

- TM3 >1000 250 >1000 25 25 10-50 10-25

- TM4 >1000 25 >1000 25 25 10-25 10-25

Efflux pump “blockers”: CCCP (carbonyl cyanide m-chlorophenyl hydrazone), OVA (sodium orthovanadate)
Membrane permeabiliser: EDTA (ethylene diamine tetraacetic acid)

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RESPONSE TO BIOCIDES EXPOSURE
- Extracellular induction components (EICs)
  Acidification and heat response
- S. aureus pre-treatment with CHX – Low level resistance (3 fold increase) in unexposed cultures
- Quorum sensing (?)
  Quorum sensing governing specific gene expression
  Catalase and superoxide dismutase gene expression
  Hassett et al. Mol Microbiol 1999; 34: 1082-93

RESPONSE TO BIOCIDES EXPOSURE - POPULATION
- Selection
  - phenolics
  - QACs
  - CHX
  - GTA
  - chlorine

RESPONSE TO BIOCIDES EXPOSURE - POPULATION
- Selection
  - phenolics (triclosan, tea tree oil)
  - QACs
  - CHX
  - GTA
  - chlorine

RESPONSE TO BIOCIDES EXPOSURE - POPULATION
- Increasing transferable resistance (?)
- Effect of biocides on gene transfer

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Concentration</th>
<th>Conjugation</th>
<th>Transduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Povidone iodine</td>
<td>0.005%</td>
<td>Increased 2 folds</td>
<td>NT*</td>
</tr>
<tr>
<td></td>
<td>0.01%</td>
<td>NT</td>
<td>Reduced 10 folds</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>0.00005%</td>
<td>No effect</td>
<td>Reduced 10 folds</td>
</tr>
<tr>
<td>Cetrimide</td>
<td>0.0001%</td>
<td>Reduced 2 folds</td>
<td>Increased 1000 folds</td>
</tr>
</tbody>
</table>

RESPONSE TO BIOCIDES EXPOSURE - POPULATION
- Selection
  - phenolics (triclosan, tea tree oil)
  - QACs
  - CHX
  - GTA
  - chlorine

RESISTANCE MECHANISMS - Biofilms
- Selection
  - Biofilm number
  - biofilm phenotype
  - dormancy

Mycobacterium terrae

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RESISTANCE MECHANISMS - Biofilms
Establishing a concentration gradient
- Diffusion
- Interaction with cell constituents
- Lysed bacterial community
  (mechanistic inactivation/increased organic load)

Enhanced bacterial insusceptibility
- Degradation
- Efflux (more effective against reduced concentration)
- Early stress-response

Slow growth/metabolism
- Established a chemical gradient (reduced nutrients / O₂)

REDEFINING RESISTANCE- definitions
- Intrinsic and acquired resistance? The best definitions?
- Biofilm resistance
- Environmental resistance
  - growth conditions; nutrient limitation
  - cell uptake; lower amount taken by cell grown in broth
  - metabolic status
  - cell envelope plasticity
  (exacerbated in biofilms)

RESISTANCE MECHANISMS - Biofilms
Selection for increased resistance
- Formation of packets of surviving bacteria
- Dormant cells (might grow rapidly in the presence of exudate released from lysed community)

Acquisition of new resistant determinants
- Increased genetic exchange
- Intrinsic resistance
- Type of bacteria

RESISTANCE: A GENUINE CONCERN?
- High-concentration
  - emerging microbial resistance unlikely but NOT impossible
  - microbial contamination of undiluted formulations (e.g. QACs)
  - bacterial survival in glutaraldehyde (2% v/v), chlorine dioxide (2.25% v/v)

- Low-concentration
  - emerging microbial resistance?
  - interaction with the microbial cell?
  - eliciting stress response mechanisms?
  - selection of surviving clones?

REDEFINING RESISTANCE
- Evidence of microbial resistance in practice
  - inappropriate usage
    - use of weak solutions & 'topping-up' of containers
    - CHX used at a concentration of 1 in 5000 (200 µg/ml)
  - inactivation of QACs by the presence of cotton
  - inactivation by organic load – veterinary / environment
  - neutralization
    - hand creams containing anionic emulsifiers and cationic antiseptics
    - anionic surfactant with cationic disinfectant

- Emerging reports are rare (are incidents all reported?)
- No information on the effect of new biocide products/surfaces
  - to early / not studied

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RESISTANCE: A GENUINE CONCERN?

- Difficult to produce resistant mutants in vitro
  - well-documented (in vitro) studies on bacterial interaction with low-biocide concentration
  - selection
  - induction/expressions of resistant phenotype
  - stepwise training best method (unrealistic?)

The Next Few Teleclasses

- **April 25**
  - **Making Infection Control Really Work**
    - with Prof. Seto Wing Hong, University of Hong Kong
- **April 26**
  - **Environmental Surveillance for Infection Control**
    - with Andrew Strifel, University of Minnesota
- **May 8**
  - **Panton-Valentine Leucocidin Producing Staphylococcus aureus**
    - with Brenda Dale & Adam Brown, National Health Service, UK
- **May 10**
  - **Infection Control in the Dialysis Clinic**
    - with Dr. Charmaine Lok, University of Toronto
- **May 17**
  - **Ethics of Care During a Pandemic**
    - with Dr. Eric Wasylenko, Calgary Health Board

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RESISTANCE: A GENUINE CONCERN?

- Cross- and co-resistance
  - evidence in vitro only
  - no evidence in practice
    (not documented or reported)
  - no in situ evidence of microbicides selecting for antibiotic resistance at present
    (does not account for the increase usage of low concentrations of microbicides)

- surveillance programmes
  (ongoing)

Making predictions is difficult, Particularly about the future.

Sam Goldwyn