Chlorhexidine Use and Bacterial Resistance
Prof. Jean-Yves Maillard, Cardiff University, Wales
A Webber Training Teleclass

Chlorhexidine Use and Bacterial Resistance

Jean-Yves Maillard
Cardiff School of Pharmacy and Pharmaceutical Sciences
Cardiff University

Hosted by Dr. Lynne Sehulster

www.webbertraining.com September 27, 2018

OVERVIEW

- Background
- Bacterial responses to biocides
- Bacterial resistance to chlorhexidine in situ
- Bacterial resistance to chlorhexidine in vitro
- Reality check
- Conclusions

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BACKGROUND

BACKGROUND: context - biocide usage

DISINFECTION
Surface
Liquid
Materials (wipes)

ANTISEPSIS
Antimicrobial gel/liquid
dressings

DOMESTIC PRODUCTS
Washing liquid
Washing up liquid
Chopping board

‘ANTIMICROBIAL’ SURFACES
Environmental
Medical (Implant)

PRESERVATION
Wood
Plastic
textiles

PRESERVATION
Food
Pharmaceutical

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BACKGROUND: persistence

<table>
<thead>
<tr>
<th>Organism</th>
<th>Persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>3 days to 5 months</td>
</tr>
<tr>
<td><em>Clostridium difficile</em> (spores)</td>
<td>5 months</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp. including vancomycin-resistant enterococci</td>
<td>5 days to 4 months</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1.5 h to 16 months</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>2 h to &gt;30 months</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>1 day to 4 months</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6 h to 16 months</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>10 days to 4.2 years</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>2 days to 5 months</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em>, including MRSA</td>
<td>7 days to 7 months</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>12 days</td>
</tr>
</tbody>
</table>

BACKGROUND: interventions

- **Hand hygiene**: compliance 30-85%
- **Surface disinfection**: 32%

**Antimicrobial Surfaces**
- Hand hygiene compliance: 30-85%
- Surface disinfection: 32%
BACKGROUND: end of antibiotic era?

Deaths per annum worldwide


BACKGROUND: CHX RESISTANCE

Peer-reviewed articles / reviews since 1998
Title and abstract: chlorhexidine + resistance

Web of Science
Google Scholar
PubMed

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BACTERIAL RESPONSES TO BIOCIDES

Intrinsic resistance

Resistance to Biocides

- prions
- bacterial spores
- protozoal oocysts
- mycobacteria
- naked viruses
- protozoal cysts
- vegetative Gram-negative
- fungi
- protozoa
- vegetative Gram-positive
- enveloped viruses

Exceptions

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**BACTERIAL RESPONSES TO BIOCIDES**

**Bacteria – biocide interactions**

**DEGREE OF DAMAGE AND AUTOCIDAL ACTIVITY**

- Disruption of the transmembrane PMF leading to an uncoupling of oxidative phosphorylation and inhibition of active transport across the membrane
- Inhibition of respiration or catabolic/anabolic reactions
- Disruption of metabolic processes
- Disruption of replication
- Loss of membrane integrity resulting in leakage of essential intracellular constituents (K⁺, inorganic phosphate, pentoses, nucleotides and nucleosides, proteins)
- Coagulation of intracellular materials

**CONSEQUENCES**

- Short exposure
- Prolonged biocidal exposure
- Imbalance of pH
- Autocidal (commitment to a cell death pathway)
- Cell death

**LYSIS**

11

12

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BACTERIAL RESPONSES TO BIOCIDES

EXPRESSION OF SPECIFIC MECHANISMS

PHYSIOLOGICAL CHANGES

REPAIR

CROSS-RESISTANCE

CO-RESISTANCE

ACQUISITION OF GENETIC DETERMINANTS

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Ongoing use of chlorhexidine and related compounds has led to the emergence of bacterial resistance. Changes in bacterial membrane properties can result in reduced penetration of biocides. For example, Pseudomonas stutzeri with decreased MIC to chlorhexidine and CPC can show cross-resistance to polymyxin and gentamicin due to changes in LPS and reduction of porins.

### Bacterial Responses to Biocides

**Changes in membrane properties**

- **OMP profile**
  - LPS profile

- **LPS profile**
  - OMP profile

#### Reduction in Penetration

- Changes in membrane properties
  - Reduction in antimicrobial accumulation

**Outer membrane changes in Pseudomonas stutzeri resistant to chlorhexidine decylate and octylpyridinium chloride**

- U. Tettouw, J.-Y. Maillard, J.R. Farr, A.D. Read

**References**

- Nature Reviews Microbiology, 4, 629-636 (August 2006)
- Multidrug-resistance efflux pumps not just for resistance
  - A. J. V. Poole

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BACTERIAL RESPONSES TO BIOCIDES
Stress response and selective pressure

BIOCIDE

STRESS

SELECTIVE PRESSURE

GENERAL STRESS RESPONSE
SOS RESPONSE

Adaptive mutations

MUTATIONS

GLOBAL RESPONSE

NARROW RESPONSE

EFFLUX
MEMBRANE CHANGES
METABOLISM
REPAIR

BACTERIAL RESPONSES TO BIOCIDES

- Reports of bacterial resistance from 1958!

ALCOHOLS

BENZALKONIUM CHLORIDE

QACs

CHLORHEXIDINE

PHENOLICS

POVIDONE IODINE

GLUTARALDEHYDE

OXIDISING AGENTS

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BACTERIAL RESPONSES TO BIOCIDES

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

- Resistance in practice: Bacterial survival following biocide challenge at “in use”/“during use” concentration.

- Reduced susceptibility: increase in MBC comparing to the initial population or a reference strain
  - For data based on MIC changes: increase in MIC

- Tolerance: inhibited but not killed
  - Survival in a product (preservative system)

- Cross-resistance: Bacterial survival following biocide challenge at “in use”/“during use” concentration AND to unrelated antimicrobials; may include emerging clinical resistance to chemotherapeutic antibiotics

BACTERIAL RESPONSES TO BIOCIDES

Regulators

European Commission Opinions


- SCENIHR 2010: Research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides.
  http://ec.europa.eu/health/scientific_committees/emerging/docs/sccs_o_028.pdf

  http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/scss_o_023.pdf

- SCENIHR 2014: Nanosilver: safety, health and environmental effects and role in antimicrobial resistance.
  http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_038.pdf

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BACTERIAL RESPONSES TO BIOCIDES

Regulators

Biocide Products Regulation … and resistance (effective since 1/09/2013)

1-b(ii) … the biocidal product has no unacceptable effects on the target organisms, in particular unacceptable resistance or cross-resistance

3-b … the chemical diversity of the active substances is adequate to minimise the occurrence of resistance in the target harmful organism.

Effects on target organisms

75. Where the development of resistance or cross-resistance to the active substance in the biocidal product is likely, the evaluating body shall consider actions to minimise the consequences of this resistance. This may involve modification of the conditions under which an authorisation is given. However, where the development of resistance or cross-resistance cannot be reduced sufficiently, the evaluating authority shall conclude that the biocidal product does not satisfy criterion (ii) under point (b) of Article 19(1).

U.S. Food and Drug Administration

Press release 2nd September 2016

FDA issues final rule on safety and effectiveness of antibacterial soaps

The agency issued a proposed rule in 2013 after some data suggested that long-term exposure to certain active ingredients used in antibacterial products — for example, triclosan (liquid soaps) and triclocarban (bar soaps) — could pose health risks, such as bacterial resistance… This included data from clinical studies demonstrating that these products were superior to non-antibacterial washes in preventing human illness or reducing infection.

“…some data suggest that long-term exposure to certain active ingredients used in antibacterial products—for example, triclosan (liquid soaps) and triclocarban (bar soaps)—could pose health risks, such as bacterial resistance …”

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BACTERIAL RESISTANCE TO
CHLORHEXIDINE IN SITU

SKIN PREPARATIONS
• Skin care 2%
• Hand hygiene ± alcohol
• Patient preoperative scrub and showers (combined with alcohol)
• Vascular access site dressings (chlorhexidine sponge dressing and a chlorhexidine gel pad)
  - Vascular access - such as central venous catheters, skin preparation solutions and insertion site dressings are recommended as interventions that may prevent Central Line-Associated Bloodstream Infections (CLABSIs)
  - Vascular access catheters
  - Peripherally Inserted Central venous catheter

DEVICES
• Central Venous catheter – CHX impregnated catheters (intraluminally and extraluminally)
• Needleless IV connectors (combined chlorhexidine and silver)

SOLUTIONS
• Oral care mouthwash
• Urology – bladder irrigation 0.005%

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<table>
<thead>
<tr>
<th>Products</th>
<th>Concentration</th>
<th>Additional biocides</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical medicines (gel or liquid)</td>
<td>7.1%</td>
<td>None</td>
<td>Umbilical cord care to prevent cord infection and/or sepsis and reduce neonatal mortality.</td>
</tr>
<tr>
<td>Topical solution (liquid, cloth, sponge applicators, swab sticks)</td>
<td>2% or 3.15%, 4%, or 5%</td>
<td>Isopropyl alcohol</td>
<td>Skin preparation for surgery, invasive procedures, central lines to prevent hospital-acquired infections</td>
</tr>
</tbody>
</table>
| Scrub solution (liquid detergent) | 2% or 4%      | Isopropyl alcohol   | • Preoperative bathing, general skin cleansing to prevent hospital-acquired infection  
  • Preoperative hand scrub and hand disinfection to prevent the spread of microorganisms |
| Irrigation solution              | 0.015% or 0.05% | Cetrimide           | Irrigation of wounds to prevent infection                             |
| Topical cream                    | 0.1%          | Cetostearyl alcohol Cetrimide | Wound cleaning (over-the-counter first-aid cream) to prevent infection |
| Washcloth                        | 2%            | None                | Daily bathing in intensive care unit (ICU) patients to prevent hospital-acquired infection |
| Gauze dressing                   | 0.5%          | -                   | Wound or burn dressing to prevent infection                          |
| Catheter dressing                | 2%            | None                | Catheter dressings to prevent hospital- (gel pad, foam disk, semi-acquired infection permeable transparent dressing) |
| Hand rub (gel)                   | 0.5% or 1%    | Ethanol             | Hand sanitizing to prevent the spread of microorganisms               |
| Dental solution                  | 0.12% or 0.2% | Ethanol             | • Decontaminate oral cavity to prevent (oral rinse or spray)           
  • Periodontal disease and mucositis treatment |
| Concentrated stock solution      | 20%           | None                | Preparation of dilutions for skin cleansing and general disinfection   |

(accessed 19-09-2018)

BACTERIAL RESISTANCE TO CHX IN SITU

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BACTERIAL RESISTANCE TO CHX IN SITU

CHX contaminated products and infections

<table>
<thead>
<tr>
<th>Contaminant(s)</th>
<th>Site(s) of microbes</th>
<th>Mechanism of contamination/source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>Not stated</td>
<td>Refilling contaminated bottles; washing used bottles using cold tap water; contaminated washing apparatus; low concentration (0.05%)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp., <em>Serratia marcescens</em>, <em>Flavobacterium</em> sp.</td>
<td>Not stated</td>
<td>Not determined, but authors speculate due to over-dilution or refilling of contaminated bottles</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Wounds</td>
<td>Tap water used to dilute stock solutions, low concentration (0.05%)</td>
</tr>
<tr>
<td><em>Bulkingholderia cepacia</em></td>
<td>Blood, wounds, urine, mouth, vagina</td>
<td>Metal pipe and rubber tubing in pharmacy through which deionized water passed during dilution of chlorhexidine; low concentration</td>
</tr>
<tr>
<td><em>Ralstonia pickettii</em></td>
<td>Blood</td>
<td>Contaminated distillate water used to dilute chlorhexidine; low concentration (0.05%)</td>
</tr>
<tr>
<td><em>Ralstonia pickettii</em></td>
<td>Blood (pseudo-bacteremia)</td>
<td>Distilled water used to dilute chlorhexidine; low concentration (0.05%)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Blood, urine, wounds, sputum, others</td>
<td>Not determined, but use of nonsterile water for dilution to 2% and distribution in reusable nonsterile containers</td>
</tr>
<tr>
<td><em>Ralstonia pickettii</em></td>
<td>Blood (pseudo-bacteremia)</td>
<td>Distilled water used to dilute chlorhexidine; low concentration (0.05%)</td>
</tr>
<tr>
<td><em>Bulkingholderia cepacia</em></td>
<td>Blood</td>
<td>Intrinsic contamination. Contaminated 0.5% chlorhexidine</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Blood</td>
<td>Intrinsic contamination. 2% aqueous chlorhexidine antiseptic</td>
</tr>
</tbody>
</table>

BACTERIAL RESISTANCE TO CHX IN SITU

CHX contaminated products and infections

<table>
<thead>
<tr>
<th>Antiseptic</th>
<th>Contaminants</th>
<th>Mechanisms of contamination/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td><em>B. cereus</em>, <em>B. cepacia</em></td>
<td>Intrinsic contamination, contaminated tap water</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td><em>Pseudomonas</em> spp., <em>B. cepacia</em>, <em>Flavobacterium</em> spp., <em>Ralsonia pickettii</em>, <em>Achromobacter xylosoxidans</em>, <em>S. marcescens</em></td>
<td>Refilling contaminated bottle, contaminated washing apparatus (0.05%), Topping up stock solution (1:1000-1:5000), metal pipe (low concentration), contaminated water (0.05%), atomizer (0.06%)</td>
</tr>
<tr>
<td>Chlorhexidine + cetrimide</td>
<td><em>Ps. multivorans</em>, <em>St. maltophilia</em></td>
<td>Tap water (0.05% CHX &amp; 0.5% cetrimide), contaminated deionized water</td>
</tr>
</tbody>
</table>

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### Artificial Decrease in CHX Susceptibility

**Development of resistance to chlorhexidine diacetate in Pseudomonas aeruginosa**

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Original MIC (µg/mL) before multiple exposure to CHX (5 µg/mL)</th>
<th>MIC (µg/mL CHX) after 5 subcultures in CHX-free broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8-10</td>
<td>&gt;70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>&gt;40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>&gt;50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> standard parent strain
<sup>b</sup> cultures from step-wise training method
<sup>c</sup> these cultures were found stable after 15 subcultures in CHX-free broth
BACTERIAL RESISTANCE TO CHX \textit{IN VITRO}

Decreased susceptibility following short CHX exposure

\textit{Salmonella enterica} 1344 susceptibility following a 5 min exposure to CHG or BZC

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Baseline</th>
<th>0.0004 % CHG</th>
<th>0.0001 % CHG</th>
<th>0.00005 % CHG</th>
<th>0.0004 % BZC</th>
<th>0.0001 % BZC</th>
<th>0.00005 % BZC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHG</td>
<td>0.01</td>
<td>0.20 ± 0.00</td>
<td>0.20 ± 0.09</td>
<td>0.04 ± 0.00</td>
<td>0.30 ± 0.00</td>
<td>0.20 ± 0.00</td>
<td>0.20 ± 0.10</td>
</tr>
<tr>
<td>BZC</td>
<td>0.003</td>
<td>0.20 ± 0.00</td>
<td>0.05 ± 0.02</td>
<td>0.20 ± 0.20</td>
<td>0.80 ± 0.00</td>
<td>0.20 ± 0.00</td>
<td>0.30 ± 0.20</td>
</tr>
</tbody>
</table>

GREEN = increased MBC by 10-50 folds
RED = >50 folds

Reproducibility

CHG exposure: 0.0004 % for \textit{S. enterica} 1344 and 0.0001 % for \textit{S. enterica} 14028S

<table>
<thead>
<tr>
<th>Baseline MIC</th>
<th>CHG MIC 1</th>
<th>CHG MIC 2</th>
<th>CHG MIC 3</th>
<th>CHG MIC 4</th>
<th>Baseline MIC</th>
<th>CHG MIC 1</th>
<th>CHG MIC 2</th>
<th>CHG MIC 3</th>
<th>CHG MIC 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1344</td>
<td>0.003</td>
<td>0.08</td>
<td>0.06</td>
<td>0.06</td>
<td>0.067</td>
<td>0.01</td>
<td>0.20</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>14028S</td>
<td>0.003</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
<td>0.006</td>
<td>0.10</td>
<td>0.09</td>
<td>0.09</td>
</tr>
</tbody>
</table>

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### Bacterial Resistance to CHX In Vitro

#### Decreased susceptibility following short CHX exposure

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Number of passages</th>
<th>Baseline susceptibility</th>
<th>Without CHG</th>
<th>With CHG 0.004%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Burkholderia lata 383</strong></td>
<td>5 min CHG exp</td>
<td>1</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>CHG MBC (%)</td>
<td>0.01</td>
<td>0.5</td>
<td>0.008</td>
<td>0.009</td>
</tr>
<tr>
<td>BZC MBC (%)</td>
<td>0.003</td>
<td>0.15</td>
<td>0.004</td>
<td>0.006</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Number of passages</th>
<th>Baseline susceptibility</th>
<th>Without CHG</th>
<th>With CHG 0.004%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella enterica 14028S</strong></td>
<td>5 min CHG exp</td>
<td>1</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>CHG MBC (%)</td>
<td>0.006</td>
<td>0.5</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>BZC MBC (%)</td>
<td>0.008</td>
<td>0.3</td>
<td>0.008</td>
<td>0.007</td>
</tr>
</tbody>
</table>

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BACTERIAL RESISTANCE TO CHX IN VITRO
Cross-resistance between CHX and antibiotics

<table>
<thead>
<tr>
<th>Bactérie</th>
<th>Antibiotiques</th>
<th>Référence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Quinolones</td>
<td>Oggioni et al 2015</td>
</tr>
<tr>
<td></td>
<td>Beta-lactames</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrolides</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>Carapéne</td>
<td>Fernandez-Cuenca et al, 2015</td>
</tr>
<tr>
<td></td>
<td>Aminoglycoside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tétraçycline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norfloxacine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tobramycine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gentamicine</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Antibiotiques multiples</td>
<td>Sekiguchi et al, 2015</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Antibiotiques multiples</td>
<td>Nakahara &amp; Kosukoe 1981</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Antibiotiques multiples</td>
<td>Conceicao et al, 2015</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Oxaciline</td>
<td>Cook et al, 2007</td>
</tr>
<tr>
<td></td>
<td>Gentamicine</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus warneri</td>
<td>Rifampicine</td>
<td>Cook et al, 2007</td>
</tr>
</tbody>
</table>

CHX and carbapenem resistance

- 160 K. pneumoniae
- 50 E. coli
- 69 hospitals
- July 2010 to August 2015
- Rectal swabs, urine samples, faeces, blood cultures

Spearman’s r scores

<table>
<thead>
<tr>
<th>Strong</th>
<th>Moderate</th>
<th>Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>+0.7 to +0.9</td>
<td>0.4 to 0.6</td>
<td>+0.1 to +0.3</td>
</tr>
<tr>
<td>-0.7 to -0.9</td>
<td>-0.4 to -0.6</td>
<td>-0.1 to -0.3</td>
</tr>
</tbody>
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Cross-resistance between CHX and antibiotics

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<thead>
<tr>
<th>Bacteria Source of isolates</th>
<th>Biocide exposure</th>
<th>Resistance to unrelated biocides</th>
<th>Resistance to antibiotics</th>
<th>Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Burkholderia lata</em></td>
<td>CHG (0.005%)</td>
<td>No significant change in MIC or MBC to CHG or BZC</td>
<td>Decrease in susceptibility to CAZ, CIP, IMP</td>
<td>Upregulation of outer membrane protein and ABC transporter</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>TRI (0.0004%)</td>
<td>Increase in MIC and MBC to TRI</td>
<td>Resistance to CIP, AMP</td>
<td>ND</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>CHG (0.0004%)</td>
<td>No change in MIC or MBC to CHG</td>
<td>Resistance to TOB, TIC, AMP</td>
<td>ND</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>H₂O₂ (0.001%)</td>
<td>No change in MIC or MBC to H₂O₂</td>
<td>Resistance to CIP, AMP</td>
<td>ND</td>
</tr>
<tr>
<td>Clinical isolates of <em>S. aureus</em></td>
<td><em>In situ</em></td>
<td>High MIC to CHG</td>
<td>Resistance CEF, Rif, TSX, CHL</td>
<td>Efflux: qacAB</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>CHG (4%)</td>
<td>Increased MIC to CHG</td>
<td>Resistance to CIP, IMP, MEM, GEN, TOB, NEL, TET, DOX</td>
<td>Efflux: increased expression in adeβ, adeβS, amvA</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>BZC (0.1%)</td>
<td>Increased MIC to BZC</td>
<td>Resistance to CIP, GEN, NEL, TET, DOX</td>
<td>Efflux: increased expression in adeβ, adeβS, porins; decreased expression in ompA, carO</td>
</tr>
</tbody>
</table>

*J-Y Maillard – Teleclass, 2018*

**BACTERIAL RESISTANCE TO CHX IN VITRO**
Genetic basis for resistance – multiple mechanisms

- Genotypic, transcriptomic proteomic and phenotypic of *Salmonella enterica* serovar Typhimurium tolerant to chlorhexidine.

- Alteration of antibiotic susceptibility with clinical significance following exposure to CHX 1 µg/mL for 30 min (mid log phase culture)

- Implication of a defence network including multiple cellular targets associated with membrane synthesis, SOS response, virulence and metabolism

*J-Y Maillard – Teleclass, 2018*
### Bacterial Resistance to CHX *In Vitro*

**Carriage of Efflux Pump Genes in Healthcare Setting Isolates**

<table>
<thead>
<tr>
<th>Efflux gene (%) carriage in isolate</th>
<th>Bacteria (number of isolates)</th>
<th>Resistant to</th>
</tr>
</thead>
<tbody>
<tr>
<td>qacA/B (83.0%)</td>
<td>High-level mupirocin-resistant S. aureus (MRSA) (53)</td>
<td>Chlorhexidine</td>
</tr>
<tr>
<td>smr (77.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>norA (49.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>norB (28.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qacA/B (80%)</td>
<td>Staphylococcus epidermidis (25)</td>
<td>Chlorhexidine</td>
</tr>
<tr>
<td>sepA (95.3%)</td>
<td>MRSA (82), methicillin-sensitive S. aureus (MSSA) (219)</td>
<td>Chlorhexidine</td>
</tr>
<tr>
<td>mepA (89.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>norA (86.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lmR (60.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qacAB (40.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>smr (3.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qacA/B (83%) smr (1.6%)</td>
<td>MRSA (60)</td>
<td>Benzalkonium chloride, Benzethonium chloride, Chlorhexidine</td>
</tr>
</tbody>
</table>

| qacA (26% for HMRSA, 67% for VISA) | Hospital-acquired (HA)-MRSA (38), 25 Community-acquired (CA)-MRSA (25) | QAC, Chlorhexidine |
| qacC (5% for HMRSA, 4% MSSA, 17% VISA) | Vancomycin-insensitive S. aureus (VISA) (6), MSSA (25) |                |

39 Maillard J-Y. Bacterial Resistance to Biocides In Block’s Disinfection, Sterilization & Preservation. submitted

---

**Bacterial Resistance to CHX *In Vitro***

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---

53 high-level mupirocin resistant MRSA

- 83% CHX MIC > 4 µg/mL
### Bacterial Resistance to CHX In Vitro

Carriage of efflux pump genes in healthcare setting isolates

53 high-level mupirocin resistant MRSA

<table>
<thead>
<tr>
<th>Gene</th>
<th>% carriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>qacA/B</td>
<td>83</td>
</tr>
<tr>
<td>smr</td>
<td>77</td>
</tr>
<tr>
<td>qacH</td>
<td>13</td>
</tr>
<tr>
<td>norA</td>
<td>96</td>
</tr>
<tr>
<td>norB</td>
<td>98</td>
</tr>
<tr>
<td>norC</td>
<td>93</td>
</tr>
<tr>
<td>sepA</td>
<td>96</td>
</tr>
<tr>
<td>sdrM</td>
<td>91</td>
</tr>
<tr>
<td>mepA</td>
<td>91</td>
</tr>
<tr>
<td>mdeA</td>
<td>94</td>
</tr>
</tbody>
</table>

#### Multiple gene carriage

<table>
<thead>
<tr>
<th>Multiple gene carriage</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>qacA/B + smr</td>
<td>53</td>
</tr>
<tr>
<td>qacA/B + smr + qacH</td>
<td>11</td>
</tr>
<tr>
<td>norA + norB + norC + sepA + sdrM + mepA + mdeA</td>
<td>76</td>
</tr>
</tbody>
</table>

#### Overexpression

<table>
<thead>
<tr>
<th>Overexpression</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 1 Chromosome-mediated efflux gene</td>
<td>60</td>
</tr>
<tr>
<td>norA</td>
<td>49</td>
</tr>
<tr>
<td>NorB</td>
<td>29</td>
</tr>
<tr>
<td>norC</td>
<td>10</td>
</tr>
<tr>
<td>mepA</td>
<td>6</td>
</tr>
<tr>
<td>mdeA</td>
<td>8</td>
</tr>
<tr>
<td>sepA</td>
<td>4</td>
</tr>
<tr>
<td>sdrM</td>
<td>4</td>
</tr>
</tbody>
</table>

---

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www.webbertraining.com
### Microorganisms and MIC mg/L

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus spp</td>
<td>1 - 3</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>1.8 - 70</td>
</tr>
<tr>
<td>Corynebacterium spp</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>0.5 - 6</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>2000 - 5000</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>0.1 - 7</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2.5 - 7.5</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>1.5 - 12.5</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>3 - 100</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>3 - 60</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>3 - 75</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>1.6 - 5</td>
</tr>
<tr>
<td>Aspergillus spp</td>
<td>75 - 500</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>7 - 15</td>
</tr>
<tr>
<td>Microsporum spp</td>
<td>12 - 18</td>
</tr>
<tr>
<td>Penicillium spp</td>
<td>150 - 200</td>
</tr>
<tr>
<td>Saccharomyces spp</td>
<td>50 - 125</td>
</tr>
<tr>
<td>Trichophyton spp</td>
<td>2.5 - 14</td>
</tr>
</tbody>
</table>

### References

1. www.webbertraining.com

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### REALITY CHECK

#### CHX concentrations and applications

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC mg/L</th>
<th>Microorganisms</th>
<th>MIC mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus spp</td>
<td>1 - 3</td>
<td>Aspergillus spp</td>
<td>75 - 500</td>
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<tr>
<td>Clostridium spp</td>
<td>1.8 - 70</td>
<td>Candida albicans</td>
<td>7 - 15</td>
</tr>
<tr>
<td>Corynebacterium spp</td>
<td>5 - 10</td>
<td>Microsporum spp</td>
<td>12 - 18</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>0.5 - 6</td>
<td>Penicillium spp</td>
<td>150 - 200</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>2000 - 5000</td>
<td>Saccharomyces spp</td>
<td>50 - 125</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>0.1 - 7</td>
<td>Trichophyton spp</td>
<td>2.5 - 14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>2.5 - 7.5</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>1.5 - 12.5</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>3 - 100</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>3 - 60</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>3 - 75</td>
</tr>
<tr>
<td>Salmonella spp 45</td>
<td>1.6 - 5</td>
</tr>
</tbody>
</table>

#### Applications | Concentration (mg/L)
--- | ---
Eye drop | 20 - 60
Skin disinfection | 5,000
Surgical scrub | 20,000 - 40,000
Irrigation | 150 - 500
Topical cream | 1,000
Wash cloth | 2,000

---

### REALITY CHECK

#### Factors affecting CHX efficacy

Factors inherent to the product:
- concentration
- formulation
- water activity
- pH

CONCENTRATION EXPONENT = 2
PRECIPITATION

---

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Factors inherent to the product
- concentration
- formulation
- water activity
- pH

Factors inherent to the application
- surface
- organic load (soiling)
- temperature
- contact time
- humidity

INCOMPATIBILITIES
- Anionic and non-ionic surfactants
- Viscous materials such as acacia, sodium alginate, sodium carboxymethylcellulose, starch, and tragacanth
- Brilliant green, chloramphenicol, copper sulfate, fluorescein sodium, formaldehyde, silver nitrate, and zinc sulfate.
- Cork (container)

PRECIPITATION
In the presence of inorganic acids, certain organic acids, and salts, hard water
Solubility increases with cetrimide
Factors affecting CHX efficacy

Factors inherent to the product
- concentration
- formulation
- water activity
- pH

Factors inherent to the application
- surface
- organic load (soiling)
- temperature
- contact time
- humidity

Factors inherent to the use of the product
- Actual exposition time
- Residual concentration
- Frequency of applications
- Dilution during application
- Formulation delivery
Chlorhexidine Use and Bacterial Resistance
Prof. Jean-Yves Maillard, Cardiff University, Wales
A Webber Training Teleclass

REALITY CHECK
Predicting resistance and cross-resistance


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REALITY CHECK
Predicting resistance and cross-resistance

Bacterial resistance to biocides - *Salmonella enterica* exposure to CHG and BZC

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REALITY CHECK
Predicting resistance and cross-resistance


Bacterial resistance to biocides
Ps. aeruginosa exposure to a mouthwash
0.000125% chlorhexidine (1/40 in use dilution)

Ps. aeruginosa exposure to a shampoo
0.000015% benzalkonium chloride (1/100 in use dilution)

CONCLUSIONS
CONCLUSIONS
The obvious?

A DEAD BUG CANNOT BECOME RESISTANT

CONCLUSIONS
The obvious? Complex formulations

TOXICITY
Efficacy: Broad spectrum
Other performances (cleaning)
Organoleptic properties
Stability

BIOCIDAL PRODUCT

CLP classification
CONCLUSIONS

The obvious?

40%

Median hand hygiene compliance from 95 studies.


CONCLUSIONS

The obvious – product usage

Improving practices (product usage) and product efficacy are essential for a better control

Otter et al. JCAH 2011;32:687-99
Chlorhexidine Use and Bacterial Resistance
Prof. Jean-Yves Maillard, Cardiff University, Wales
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