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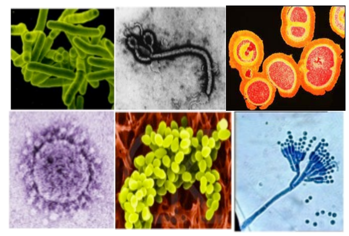
PRIFYSGOL CAERDYDD

Chlorhexidine Use and Bacterial Resistance



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

Hosted by Dr. Lynne Sehulster

CARDIFF UNIVERSITY PRIFYSGOL CAERDYDD
Cardiff Institute for Tissue Engineering and Repair (CITER)
Athrofa Adeiladu ac Atgyweirio Meinwe Caerdydd (CITER)

www.webbertraining.com September 27, 2018

OVERVIEW

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- 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

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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

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

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BACKGROUND: interventions

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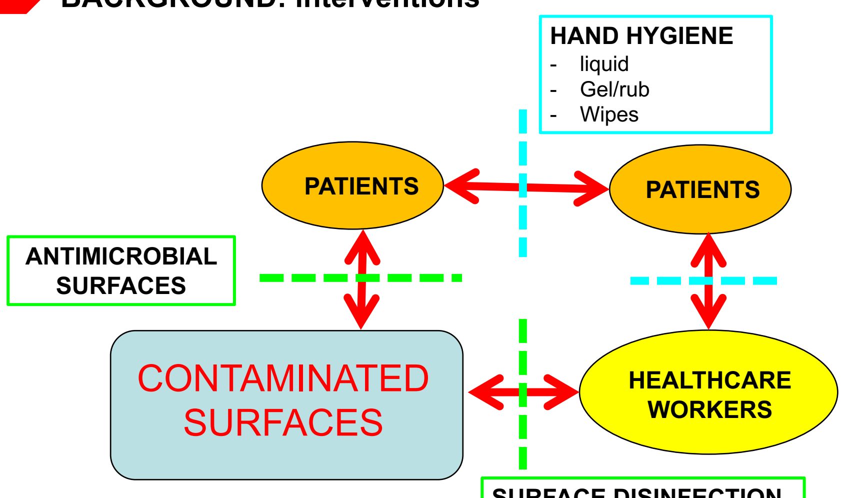

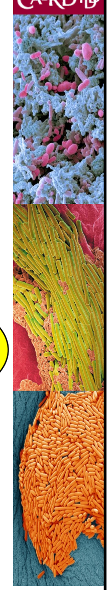

- Hand hygiene compliance: 30-85%
- Surface disinfection: 32%

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BACKGROUND: end of antibiotic era?

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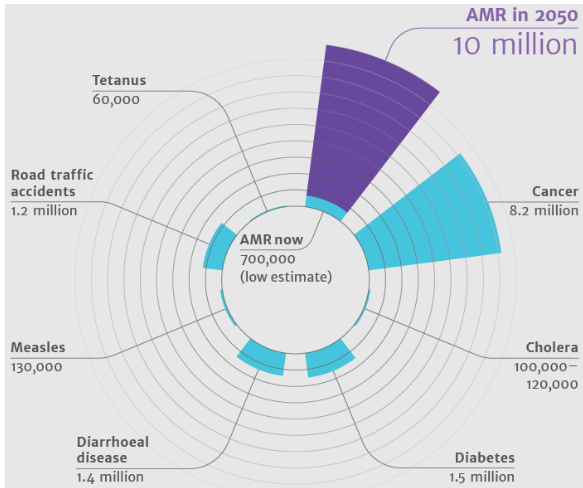
Deaths per annum worldwide



O'Neill. 2016. Tackling drug-resistant infections globally: Final report and recommendations. The Review Antimicrobial resistance. HM Government.

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BACKGROUND: CHX RESISTANCE

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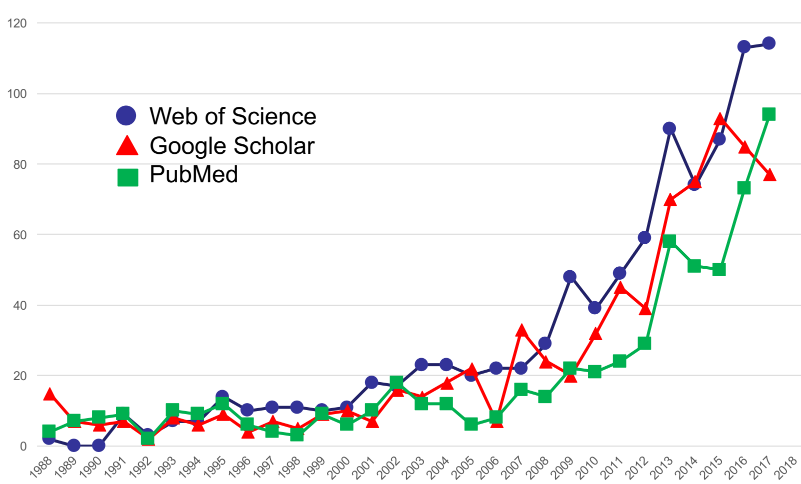
Peer-reviewed articles / reviews since 1998

Title and abstract: chlorhexidine + resistance



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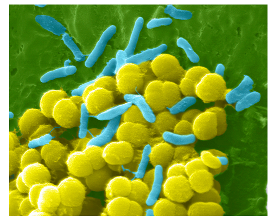
BACTERIAL RESPONSES TO BIOCIDES



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BACTERIAL RESPONSES TO BIOCIDES

Intrinsic resistance

Resistance to Biocides

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

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BACTERIAL RESPONSES TO BIOCIDES

Bacteria – biocide interactions

DEGREE OF DAMAGE AND AUTOCIDAL ACTIVITY	CONSEQUENCES	
➢ 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	Short exposure	Reversible events
➢ Disruption of metabolic processes ➢ Disruption of replication	Prolonged biocidal exposure	
➢ Loss of membrane integrity resulting in leakage of essential intracellular constituents ($K^{+,}$ inorganic phosphate, pentoses, nucleotides and nucleosides, proteins)	Imbalance of pHi	Irreversible events
➢ Coagulation of intracellular materials	Autocidal (commitment to a cell death pathway)	
➢ **LYSIS**	Cell death	

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BACTERIAL RESPONSES TO BIOCIDES

Bacteria – biocide interactions

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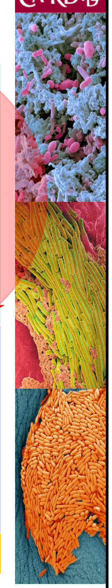

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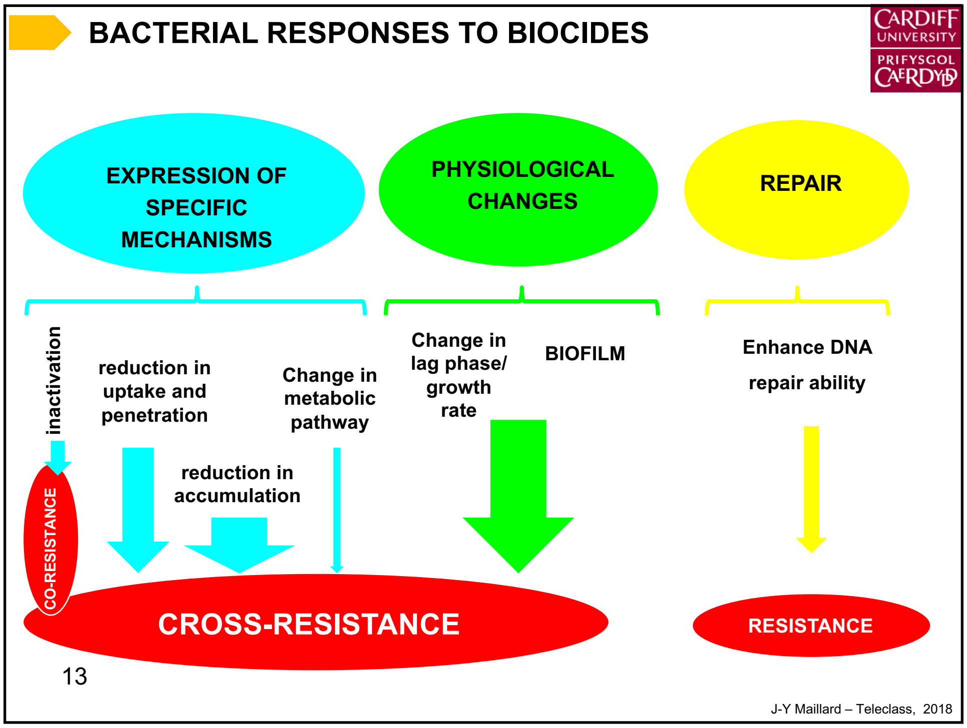
BACTERIAL RESPONSES TO BIOCIDES
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EXPRESSION OF SPECIFIC MECHANISMS
PHYSIOLOGICAL CHANGES
REPAIR
inactivation
reduction in uptake and penetration
Change in metabolic pathway
Change in lag phase/ growth rate
BIOFILM
Enhance DNA repair ability
reduction in accumulation
CO-RESISTANCE
CROSS-RESISTANCE
RESISTANCE
13
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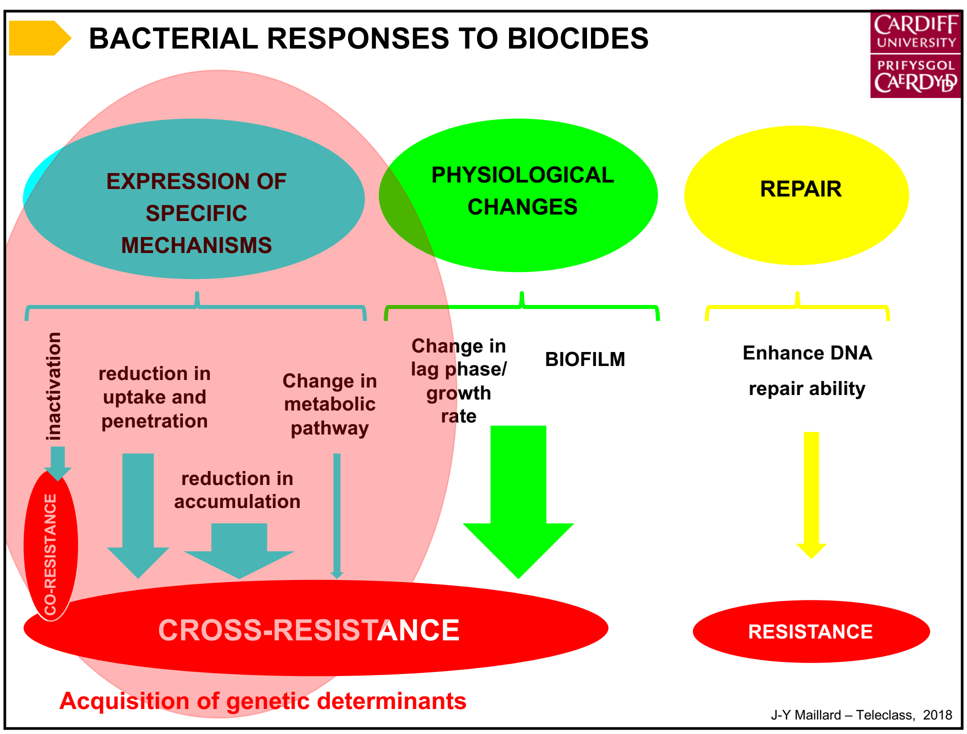
BACTERIAL RESPONSES TO BIOCIDES
CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD
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Enhance DNA repair ability
reduction in accumulation
CO-RESISTANCE
CROSS-RESISTANCE
RESISTANCE
Acquisition of genetic determinants
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BACTERIAL RESPONSES TO BIOCIDES

Changes in membrane properties

REDUCTION IN PENETRATION

Journal of Applied Microbiology 1999, 87, 323–331

Comparative responses of *Pseudomonas stutzeri* and *Pseudomonas aeruginosa* to antibacterial agents

U. Tattawasart, J.-Y. Maillard, J.R. Furr and A.D. Russell
Welsh School of Pharmacy, Cardiff University, Cardiff, UK

7133/03/99: received 31 March 1999, accepted 21 April 1999

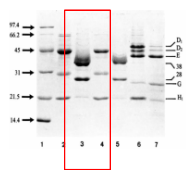
ELSEVIER International Journal of Antimicrobial Agents 16 (2000) 233–238 Antimicrobial Agents www.ischemo.org

Outer membrane changes in *Pseudomonas stutzeri* resistant to chlorhexidine diacetate and cetylpyridinium chloride

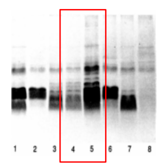
Unchalee Tattawasart, J.-Y. Maillard, J.R. Furr, A.D. Russell *

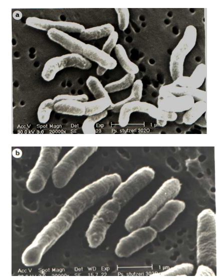
Change in LPS, reduction of porins

OMP profile



LPS profile





- *Pseudomonas stutzeri* with decreased MIC to chlorhexidine and CPC
- Cross-resistance to polymyxin and gentamicin

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BACTERIAL RESPONSES TO BIOCIDES

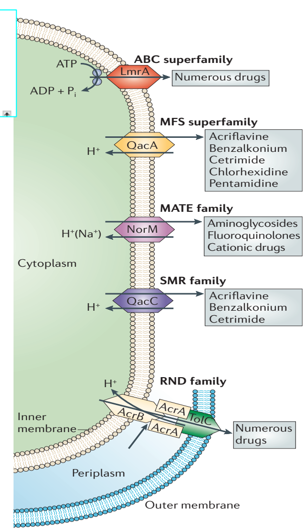
Reduction in antimicrobial accumulation

Nature Reviews Microbiology **4**, 629-636 (August 2006) | doi:10.1038/nrmicro1464

OPINION

Multidrug-resistance efflux pumps ? not just for resistance

Laura J. V. Piddock[1] About the author



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Nature Reviews | Microbiology

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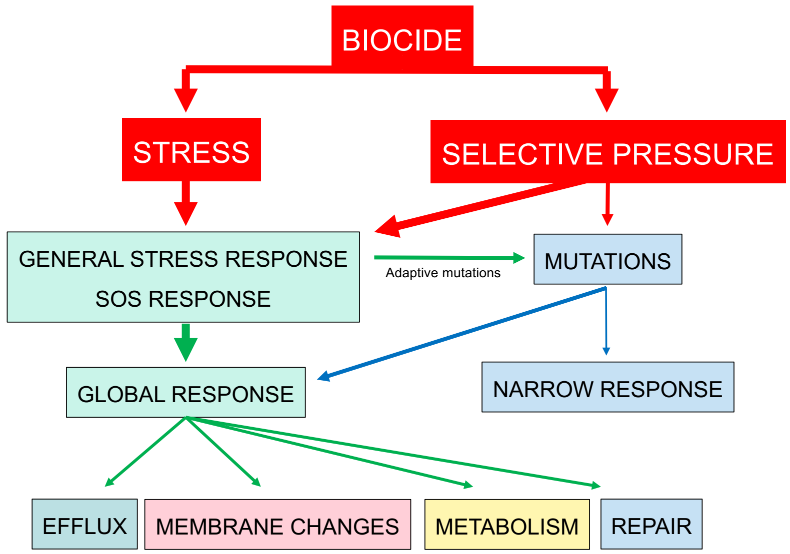
BACTERIAL RESPONSES TO BIOCIDES

Stress response and selective pressure



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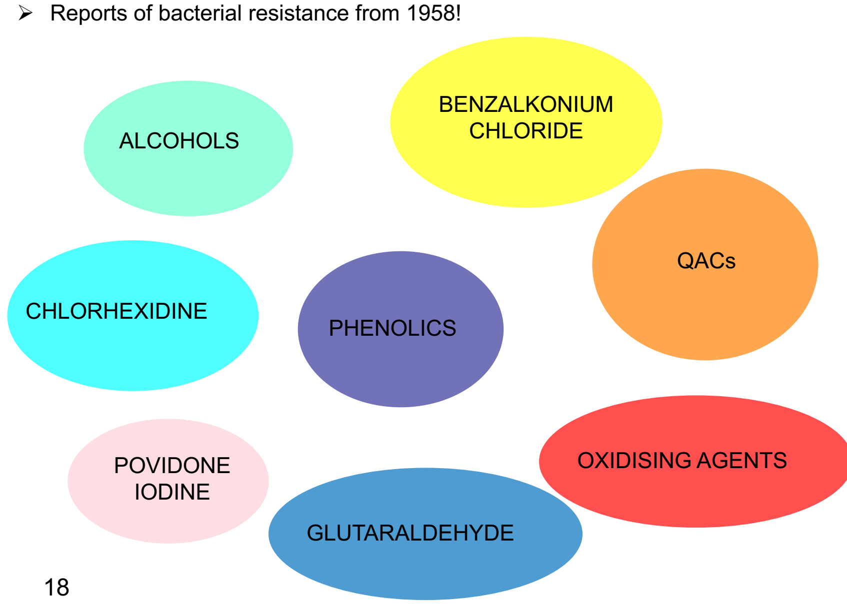
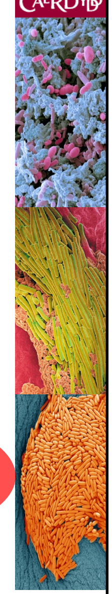
BACTERIAL RESPONSES TO BIOCIDES

- Reports of bacterial resistance from 1958!





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BACTERIAL RESPONSES TO BIOCIDES

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

Russell. *Lancet Infect Dis* 2003; 3: 794-803

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

Maillard & Denyer. Chem Oggi 2009; 27: 26-8.
Maillard et al. Micro Drug Resist 2013; 19:344-54.
Wesgate et al. AJIC 2016, 44, 458-464.

- 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

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BACTERIAL RESPONSES TO BIOCIDES

Regulators

European Commission Opinions

- SCENIHR 2009: Assessment of the Antibiotic Resistance Effects of Biocides.

  http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf

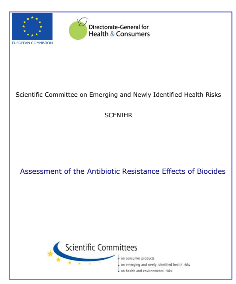
- SCENIHR 2010: Research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides.

  http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_028.pdf

- SCCS 2011: Opinion on Triclosan Antimicrobial Resistance.

  http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_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_039.pdf


Directorate-General for Health & Consumers

Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Assessment of the Antibiotic Resistance Effects of Biocides

Scientific Committees


Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

Opinion on triclosan

Antimicrobial Resistance

Scientific Committees

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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).

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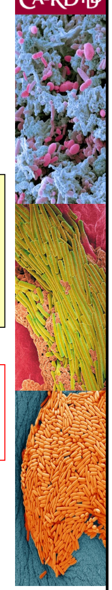
BACTERIAL RESPONSES TO BIOCIDES

Regulators



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 …"

http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm517478.htm (accessed 19/09/2018)

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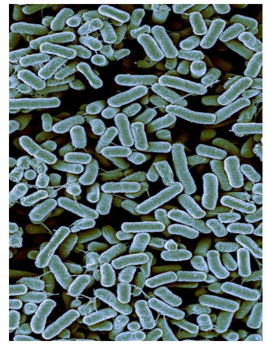
BACTERIAL RESISTANCE TO CHLORHEXIDINE *IN SITU*



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BACTERIAL RESISTANCE TO CHX *IN SITU*

CHX applications

SKIN PREPARATIONS

- Skin care 2%
- Hand hygiene ± alcohol

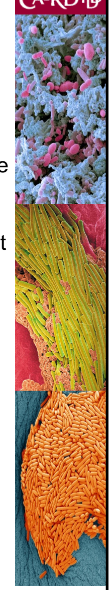
- Patient preoperative scrub and showers (combined with alcohol)
- Vascular access site dressings (chlorhexidine sponge dressing and a chlorhexidine gel pad)
  - Vascular access - such as central venous catheters, skin preparation solutions and insertion site dressings are recommended as interventions 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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BACTERIAL RESISTANCE TO CHX *IN SITU*

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CHX applications

Products	Concent-ration	Additional biocides	Uses
Topical medicines (gel or liquid)	7.1%	None	Umbilical cord care to prevent cord infection and/or sepsis and reduce neonatal mortality.
Topical solution (liquid, cloth, sponge applicators, swab sticks)	2% , 3.15%, 4%, or 5%	Isopropyl alcohol	Skin preparation for surgery, invasive procedures, central lines to prevent hospital- acquired infections
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

https://www.healthynewbornnetwork.org/hnn-content/uploads/CWG-Chlorhexidine-Applications-English_October_2015.pdf
(accessed 19-09-2018)

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BACTERIAL RESISTANCE TO CHX *IN SITU*

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BACTERIAL RESISTANCE TO CHX *IN SITU*

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CHX contaminated products and infections

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

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Maillard J-Y. Bacterial Resistance to biocides In *Block's Disinfection, Sterilization & Preservation.* submitted

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BACTERIAL RESISTANCE TO CHX *IN SITU*

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CHX contaminated products and infections

Antimicrobial Agents and Chemotherapy
AAC Article | Journal Info. | Authors | Reviewers | Permissions | Journals.ASM.org

Antimicrob Agents Chemother. 2007 Dec; 51(12): 4217–4224. PMCID: PMC2167968
Published online 2007 Oct 1. doi: 10.1128/AAC.00138-07

Outbreaks Associated with Contaminated Antiseptics and Disinfectants

David J. Weber,[1,2,*] William A. Rutala,[1,2] and Emily E. Sickbert-Bennett[1]

Antiseptic	Contaminants	Mechanisms of contamination/source
Alcohols	*B. cereus*, *B. cepacia*	Intrinsic contamination, contaminated tap water
Chlorhexidine	*Pseudomonas* spp., *B. cepacia*, *Flavobacetrium* spp., *Ralsonia pickettii*, *Achromobacter xylosoxidans*, *S. marcescens*	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%)
Chlorhexidine + cetrimide	*Ps. multivorans*, *St. maltophilia*	Tap water (0.05% CHX & 0.5% cetrimide), contaminated deionized water

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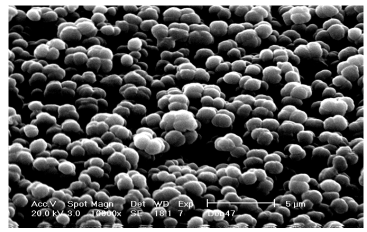
BACTERIAL RESISTANCE TO CHLORHEXIDINE *IN VITRO*



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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Artificial decrease in CHX susceptibility

Journal of Hospital Infection (2000) 46: 297–303
doi:10.1053/jhin.2000.0851, available online at http://www.idealibrary.com on IDEAL

Development of resistance to chlorhexidine diacetate in *Pseudomonas aeruginosa* and the effect of a 'residual' concentration

Louise Thomas, J.-Y. Maillard, R. J. W. Lambert* and A. D. Russell

*Pharmaceutical Microbiology Research, Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3XF and *Unilever Research Colworth, Sharnbrook, Bedfordshire, UK*

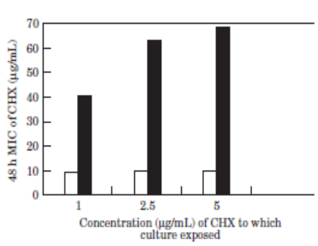

Figure 2 MICs in broth at 37°C of *P. aeruginosa* following single exposure to 'residual' concentrations of CHX of 1, 2.5 and 5 µg/mL. □, before exposure to CHX; ■, after exposure to CHX.

MICs of *Ps aeruginosa* cultures following repeated exposure to CHX (5 µg/mL)

Culture number	Original MIC (µg/mL) before multiple exposure to CHX (5 µg/mL)	MIC (µg/mL CHX) after 5 subcultures in CHX (µg/mL)
1[a]	8-10	>70[c]
2	28[b]	>70[c]
3	>40[b]	>70[c]
4	>50[b]	>70[c]
5	70[b]	>70[c]

[a]: standard parent strain
[b]: cultures from step-wise training method
[c]: these cultures were found stable after 15 subcultures in CHX-free broth

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Decreased susceptibility following short CHX exposure

Salmonella enterica 1344 susceptibility following a 5 min exposure to CHG or BZC

Mean MBC (%)

Biocide	Baseline	0.0004 % CHG	0.0001 % CHG	0.00005 % CHG	0.0004 % BZC	0.0001 % BZC	0.00005 % BZC
CHG	0.01	0.20 ± 0.00	0.20 ± 0.09	0.04 ± 0.00	0.30 ± 0.00	0.20 ± 0.00	0.20 ± 0.10
BZC	0.003	0.20 ± 0.00	0.05 ± 0.02	0.20 ± 0.20	0.80 ± 0.00	0.20 ± 0.00	0.30 ± 0.20

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

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Decreased susceptibility following short CHX exposure

Salmonella enterica 1344 susceptibility following a 5 min exposure to CHG or BZC

Mean MBC (%)

Biocide	Baseline	0.0004 % CHG	0.0001 % CHG	0.00005 % CHG	0.0004 % BZC	0.0001 % BZC	0.00005 % BZC
CHG	0.01	0.20 ± 0.00	0.20 ± 0.09	0.04 ± 0.00	0.30 ± 0.00	0.20 ± 0.00	0.20 ± 0.10
BZC	0.003	0.20 ± 0.00	0.05 ± 0.02	0.20 ± 0.20	0.80 ± 0.00	0.20 ± 0.00	0.30 ± 0.20

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

Reproducibility

CHG exposure: 0.0004 % for *S. enterica* 1344 and 0.0001 % for *S. enterica* 14028S

	Baseline MIC	CHG MIC 1	CHG MIC 2	CHG MIC 3	CHG MIC 4	Baseline MBC	CHG MBC 1	CHG MBC 2	CHG MBC 3	CHG MBC 4
1344	0.003	0.08	0.06	0.06	0.067	0.01	0.20	0.10	0.10	0.15
14028S	0.003	0.01	0.02	0.03	0.01	0.006	0.10	0.09	0.09	0.2

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Decreased susceptibility following short CHX exposure

Journal of Applied Microbiology
Journal of Applied Microbiology ISSN 1364-5072
ORIGINAL ARTICLE 2013
The effect of cationic microbicide exposure against *Burkholderia cepacia* complex (Bcc); the use of *Burkholderia lata* strain 383 as a model bacterium
L. Knapp[1], L. Rushton[2], H. Stapleton[3], A. Sass[4], S. Stewart[5], A. Amezquita[5], P. McClure[5], E. Mahenthiralingam[2] and J.-Y. Maillard[1]

Burkholderia lata 383		Number of passages						
			without CHG			With CHG 0.004%		
Baseline susceptibility		5 min CHG exp	1	5	10	1	5	10
CHG MBC (%)	0.01	0.5	0.008	0.009	0.006	0.15	0.1	0.01
BZC MBC (%)	0.003	0.15	0.004	0.006	0.006	0.019	0.05	0.006

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Decreased susceptibility following short CHX exposure

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Burkholderia lata 383		Number of passages						
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Baseline susceptibility		5 min CHG exp	1	5	10	1	5	10
CHG MBC (%)	0.01	0.5	0.008	0.009	0.006	0.15	0.1	0.01
BZC MBC (%)	0.003	0.15	0.004	0.006	0.006	0.019	0.05	0.006

Salmonella enterica 14028S		Number of passages						
			without CHG			With CHG 0.004%		
Baseline susceptibility		5 min CHG exp	1	5	10	1	5	10
CHG MBC (%)	0.006	0.5	0.001	0.006	0.009	0.08	0.08	0.006
BZC MBC (%)	0.008	0.3	0.006	0.007	0.006	0.019	0.02	0.008

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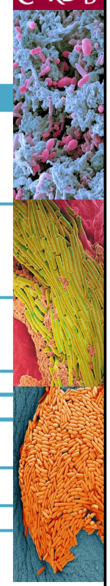
BACTERIAL RESISTANCE TO CHX *IN VITRO*

Cross-resistance between CHX and antibiotics

Bactérie	Antibiotiques	Référence
Staphylococcus aureus	Quinolones Beta-lactames Macrolides	Oggioni et al 2015
Acinetobacter baumannii	Carbapénème Aminoglycoside Tétracycline Ciprofloxacine	Fernandez-Cuenca *et al*, 2015
Pseudomonas spp.	Ciprofloxacine Norfloxacine Tobramycine Gentamicine	Gajadhar *et al*, 2003
Pseudomonas aeruginosa	Antibiotiques multiples	Sekiguchi *et al*, 2005
Escherichia coli	Antibiotiques multiples	Nakahara & Kosukoe 1981
Staphylococcus aureus	Antibiotiques multiples	Conceicao *et al*, 2015
Staphylococcus epidermidis	Oxacilline Gentamicine	Cook *et al*, 2007
Staphylococcus warneri	Rifampicine	Cook *et al*, 2007



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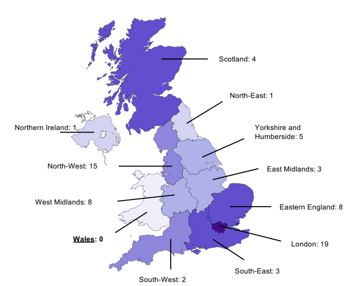
BACTERIAL RESISTANCE TO CHX *IN VITRO*

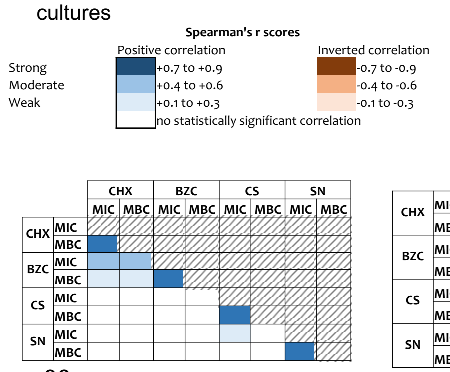
CHX and carbapenem resistance

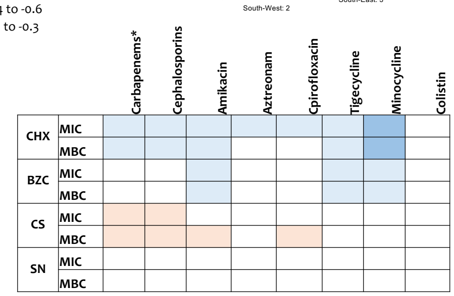
- 160 *K. pneumoniae*
- 50 *E. coli*

- 69 hospitals
- July 2010 to August 2015

- Rectal swabs, urine samples, faeces, blood cultures







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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Cross-resistance between CHX and antibiotics

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Bacteria Source of isolates	Biocide exposure	Resistance to unrelated biocides	Resistance to antibiotics	Mechanisms
Burkholderia lata	CHG (0.005%) BZC (0.005%)	No significant change in MIC or MBC to CHG or BZC	Decrease in susceptibility to CAZ, CIP, IMP	Upregulation of outer membrane protein and ABC transporter
S. aureus	TRI (0.0004%)	Increase in MIC and MBC to TRI	Resistance to CIP, AMP	ND
E. coli	CHG (0.0004%)	No change in MIC or MBC to CHG	Resistance to TOB, TIC, AMP	ND
S. aureus	H_2O_2 (0.001%)	No change in MIC or MBC to H_2O_2	Resistance to CIP, AMP	ND
Clinical isolates of *S. aureus*	*In situ*	High MIC to CHG	Resistance CEF, RIF, TSX, CHL	Efflux: *qacAB*
Acinetobacter baumannii	CHG (4%)	Increased MIC to CHG	Resistance to CIP, IMP, MEM, GEN, TOB, NEL, TET, DOX	Efflux: increased expression in *adeb, abeS, amvA* Porins: decreased expression in *ompA*
Acinetobacter baumannii	BZC (0.1%)	Increased MIC to BZC	Resistance to CIP, GEN, NEL, TET, DOX,	Efflux: increased expression in *adeb*, *abeS* Porins: decreased expression in *ompA*, *carO*

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Maillard J-Y. Bacterial Resistance to biocides In *Block's Disinfection, Sterilization &Preservation*. submitted

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Genetic basis for resistance – multiple mechanisms

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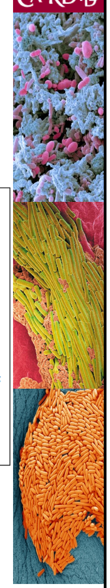

frontiers in MICROBIOLOGY — ORIGINAL RESEARCH ARTICLE, published: 01 August 2014, doi: 10.3389/fmicb.2014.00373

Comparative analysis of *Salmonella* susceptibility and tolerance to the biocide chlorhexidine identifies a complex cellular defense network

Orla Condell[1,2 †], Karen A. Power[1], Kristian Händler[3], Sarah Finn[1], Aine Sheridan[4], Kjell Sergeant[5], Jenny Renaut[5], Catherine M. Burgess[4], Jay C. D. Hinton[3,6], Jarlath E. Nally[7] and Séamus Fanning[1,8]*

- 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

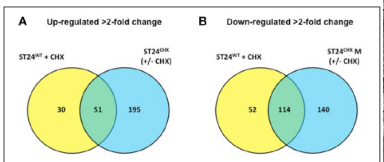

FIGURE 2 | Number and distribution of genes (A) up-regulated in the sensitive $ST24^{WT}$ following chlorhexidine exposure and/or the tolerant mutant $ST24^{CHX}$ relative to the reference strain ($ST24^{WT}$) without chlorhexidine exposure. (B) Down-regulated in the sensitive $ST24^{WT}$ following chlorhexidine exposure and/or the tolerant mutant $ST24^{CHX}$. The figure shows the differentially expressed genes relative to the reference strain ($ST24^{WT}$) without chlorhexidine exposure.

$ST24^{WT}$ CHX MIC: 1.96 µg/mL
$ST24^{CHX}$ CHX MIC: >50 µg/mL

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Carriage of efflux pump genes in healthcare setting isolates

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Efflux gene (% carriage in isolate)	Bacteria (number of isolates)	Resistant to
***qacA/B* (83.0%)** ***smr* (77.4%)** ***norA* (49.0%)** ***norB* (28.8%)**	High-level mupirocin-resistant -meticillin-resistant *S. aureus* (MRSA) (53)	Chlorhexidine
***qacA/B* (80%)**	*Staphylococcus epidermidis* (25)	Chlorhexidine
***sepA* (95.3%)** ***mepA* (89.4%)** ***norA* (86.4%)** ***lmrS* (60.8%)** ***qacAB* (40.5%)** ***smr* (3.7%).**	MRSA (82), methicillin –sensitive *S. aureus* (MSSA) (219)	Chlorhexidine
***qacA/B* (83%) *smr* (1.6%)**	MRSA (60)	Benzalkonium chloride Benzethonium chloride Chlorhexidine
***qacA* (26% for HMRSA, 67% for VISA)** ***qacC* (5% for HMRSA, 4%MSSA, 17%VISA)**	Hospital-acquired (HA)-MRSA (38), 25 Community-acquired (CA)-MRSA (25) Vancomycin insensitive *S. aureus* (VISA) (6) ; MSSA (25)	QAC Chlorhexidine

39 Maillard J-Y. Bacterial Resistance to biocides In *Block's Disinfection, Sterilization &Preservation.* submitted

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Carriage of efflux pump genes in healthcare setting isolates

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Diagnostic Microbiology and Infectious Disease 82 (2015) 278–283

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

ELSEVIER

Frequency of biocide-resistant genes and susceptibility to chlorhexidine in high-level mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MuH MRSA)

CrossMark

Qingzhong Liu [a,*], Huanqiang Zhao [a], Lizhong Han [b], Wen Shu [a], Qiong Wu [c], Yuxing Ni [b]

[a] Department of Clinical Laboratory, Shanghai First People's Hospital, Shanghai Jiaotong University, Shanghai, China
[b] Department of Clinical Microbiology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China
[c] Department of Clinical Laboratory, Shanghai Sixth People's Hospital, Shanghai Jiaotong University, Shanghai, China

53 high-level mupirocin resistant MRSA

- 83% CHX MIC > 4 µg/mL

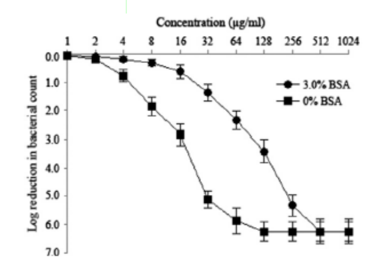

Fig. 1. The average of MBC values of chlorhexidine detected on the 53 MuH MRSA isolates. Results are presented as log10 reduction in cell counts compared with those of the control sample treated with sterile saline. The viable cell count before the exposure to chlorhexidine was $2.84 \pm 0.441 \times 10^7$ CFU/mL. Black quadrangles represent "clean" condition (0% BSA); black circles, "dirty" condition (3.0% BSA). Error bars represent SDs of results from 3 experiments. The MBC was defined as the lowest concentration that produced a 5 log10 reduction following incubation at 35 °C for 48 h after being exposed to chlorhexidine for 5 min at 20 ± 2 °C.

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Carriage of efflux pump genes in healthcare setting isolates

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53 high-level mupirocin resistant MRSA

Gene	% carriage
Plasmid-mediated	
qacA/B	83
smr	77
qacH	13
Chromosome-mediated	
norA	96
norB	98
norC	93
sepA	96
sdrM	91
mepA	91
mdeA	94

Mutliple gene carriage	%
qacA/B + smr	53
qacA/B + smr + qacH	11
norA + norB + norC + sep A + sdrM + mep A + mdeA	76

Overexpression	%
At least 1 Chromosome-mediated efflux gene	60
norA	49
NorB	29
norC	10
mepA	6
mdeA	8
sepA	4
sdrM	4

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BACTERIAL RESISTANCE TO CHX *IN VITRO*

Carriage of efflux pump genes in healthcare setting isolates

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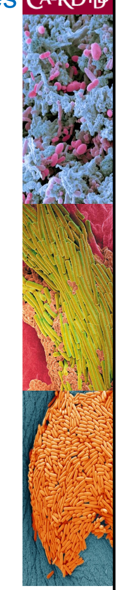
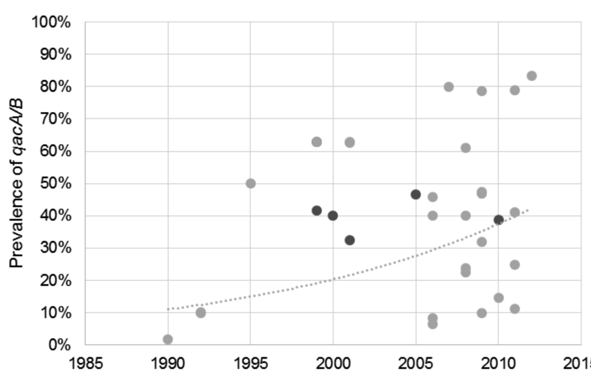


ACS Infectious Diseases 2015 Review pubs.acs.org/journal/aidcbc

Quaternary Ammonium Compounds: An Antimicrobial Mainstay and Platform for Innovation to Address Bacterial Resistance

Megan C. Jennings,[†] Kevin P. C. Minbiole,[‡] and William M. Wuest[*,†]
[†]Department of Chemistry, Temple University, Philadelphia, Pennsylvania 19122, United States
[‡]Department of Chemistry, Villanova University, Villanova, Pennsylvania 19085, United States



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Reported prevalence of QAC resistance genes in MRSA isolates

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REALITY CHECK

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REALITY CHECK

CHX concentrations and applications

Microorganisms	MIC mg/L
Bacillus spp	1 - 3
Clostridium spp	1.8 - 70
Corynebacterium spp	5 - 10
Staphylococcus spp	0.5 - 6
Streptococcus faecalis	2000 - 5000
Streptococcus spp	0.1-7

Microorganisms	MIC mg/L
Aspergillus spp	75 - 500
Candida albicans	7 - 15
Microsporum spp	12 - 18
Penicillium spp	150 - 200
Saccharomyces spp	50 - 125
Trichophyton spp	2.5 - 14

Microorganisms	MIC mg/L
Escherichia coli	2.5 - 7.5
Klebsiella spp	1.5 - 12.5
Proteus spp	3 - 100
Pseudomonas spp	3 - 60
Serratia marcescens	3 - 75
Salmonella spp	1.6 - 5

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REALITY CHECK

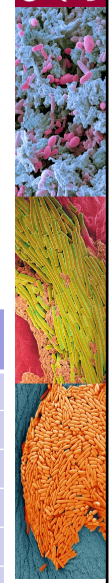
CHX concentrations and applications

Microorganisms	MIC mg/L
Bacillus spp	1 - 3
Clostridium spp	1.8 - 70
Corynebacterium spp	5 - 10
Staphylococcus spp	0.5 - 6
Streptococcus faecalis	2000 - 5000
Streptococcus spp	0.1-7

Microorganisms	MIC mg/L
Aspergillus spp	75 - 500
Candida albicans	7 - 15
Microsporum spp	12 - 18
Penicillium spp	150 - 200
Saccharomyces spp	50 - 125
Trichophyton spp	2.5 - 14

Microorganisms	MIC mg/L
Escherichia coli	2.5 - 7.5
Klebsiella spp	1.5 - 12.5
Proteus spp	3 - 100
Pseudomonas spp	3 - 60
Serratia marcescens	3 - 75
Salmonella spp	1.6 - 5

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



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REALITY CHECK

Factors affecting CHX efficacy

Factors inherent to the product

- **concentration** → CONCENTRATION EXPONENT = 2; PRECIPITATION
- formulation
- water activity
- pH

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REALITY CHECK

Factors affecting CHX efficacy

Factors inherent to the product

- concentration
- **formulation**
- water activity
- pH

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

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REALITY CHECK

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

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REALITY CHECK

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

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REALITY CHECK

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

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REALITY CHECK

Predicting resistance and cross-resistance

American Journal of Infection Control 44 (2016) 458-64
Contents lists available at ScienceDirect
American Journal of Infection Control
journal homepage: www.ajicjournal.org

Major article

Use of a predictive protocol to measure the antimicrobial resistance risks associated with biocidal product usage

Rebecca Wesgate BSc [a], Pierre Grasha PhD [b], Jean-Yves Maillard BSc, PhD [a,*]
[a] Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK
[b] Deb Group, Denby, UK

MICROBIAL DRUG RESISTANCE
Volume 00, Number 0, 2013
© Mary Ann Liebert, Inc.
DOI: 10.1089/mdr.2013.0039

MECHANISMS

Does Microbicide Use in Consumer Products Promote Antimicrobial Resistance? A Critical Review and Recommendations for a Cohesive Approach to Risk Assessment

Jean-Yves Maillard,[1] Sally Bloomfield,[2] Joana Rosado Coelho,[3] Phillip Collier,[4] Barry Cookson,[5] Séamus Fanning,[6] Andrew Hill,[7] Philippe Hartemann,[8] Andrew J. Mcbain,[9] Marco Oggioni,[10] Syed Sattar,[11] Herbert P. Schweizer,[12] and John Threlfall[13]

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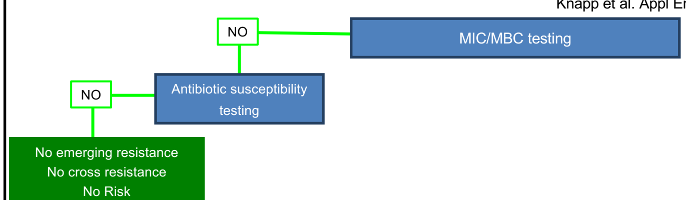
REALITY CHECK

Predicting resistance and cross-resistance

Knapp et al. Appl Environ Microbiol 2015; 81(8):2652-9.



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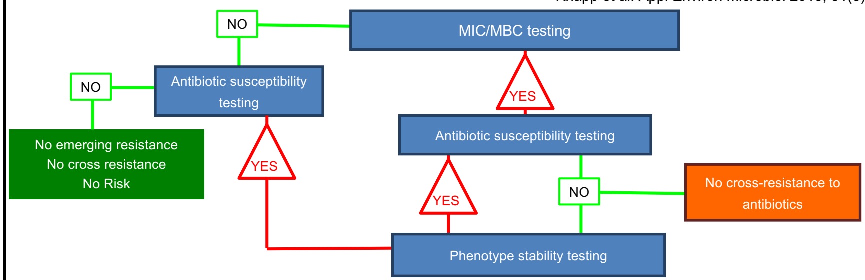
REALITY CHECK

Predicting resistance and cross-resistance

Knapp et al. Appl Environ Microbiol 2015; 81(8):2652-9.



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REALITY CHECK

Predicting resistance and cross-resistance

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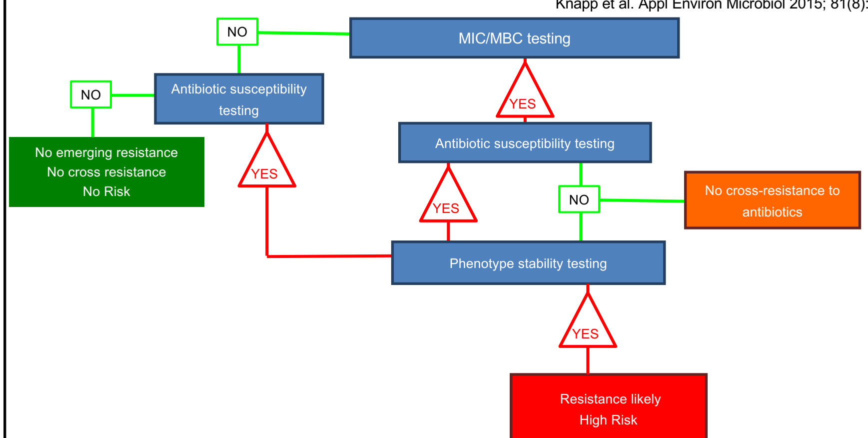

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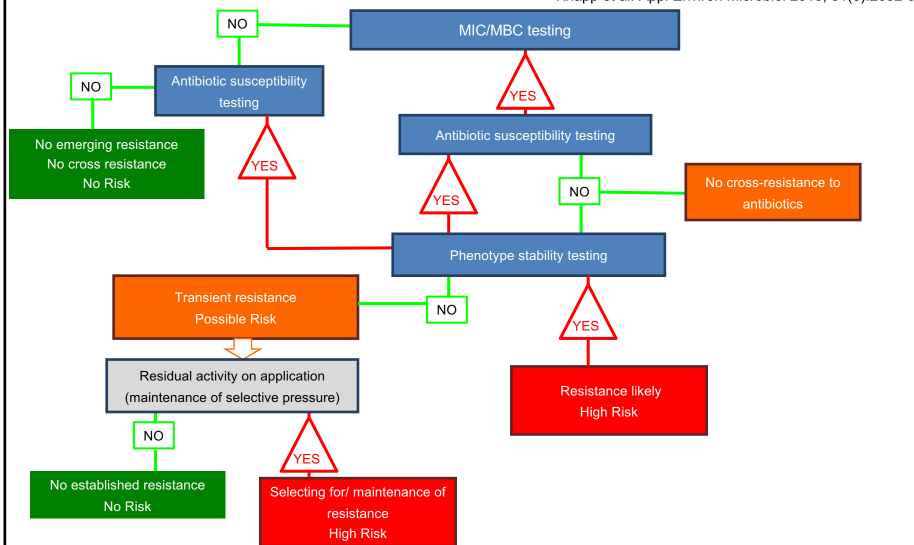
REALITY CHECK

Predicting resistance and cross-resistance

Knapp et al. Appl Environ Microbiol 2015; 81(8):2652-9.



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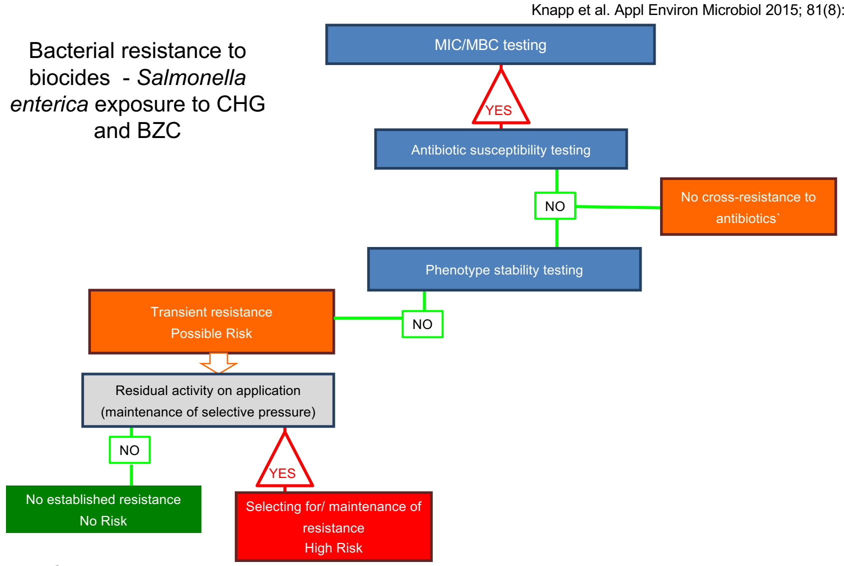
REALITY CHECK

Predicting resistance and cross-resistance

Knapp et al. Appl Environ Microbiol 2015; 81(8):2652-9.

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



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REALITY CHECK

Predicting resistance and cross-resistance

Knapp et al. Appl Environ Microbiol 2015; 81(8):2652-9.

MIC/MBC testing
NO
Antibiotic susceptibility testing
NO
No emerging resistance
No cross resistance
No Risk

- Imipenem (10 µg)
- Ceftazidime (30 µg)
- Meropenem (15 µg)
- Tobramycin (10 µg)
- Aztreonam (30 µg)

Bacterial resistance to biocides
Ps. aeruginosa exposure to a mouthwash
0.0000125% chlorhexidine (1/40 in use dilution)

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

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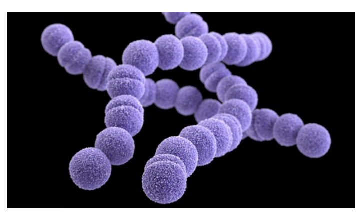
CONCLUSIONS



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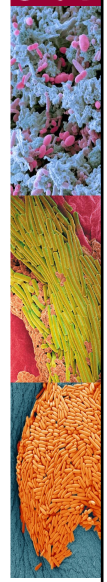
CONCLUSIONS

The obvious?

A DEAD BUG CANNOT BECOME RESISTANT



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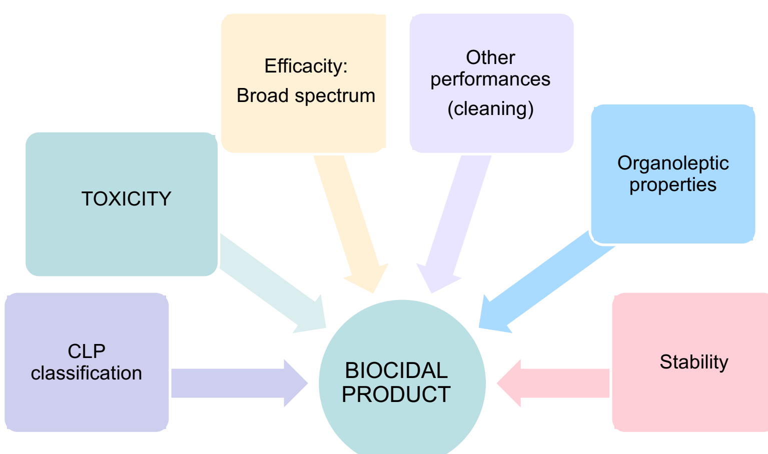
CONCLUSIONS

The obvious? Complex formulations



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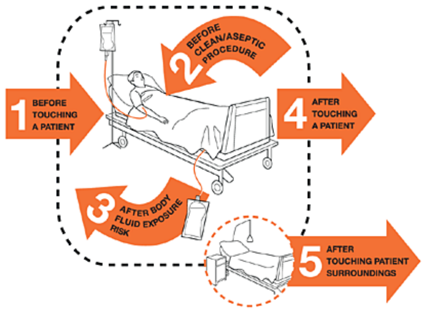
CONCLUSIONS

The obvious?



vs.

OVERUSE

40%

Median hand hygiene compliance from 95 studies.

Erasmus *et al. Infect Control Hosp Epidemiol* 2010;31:283-94.

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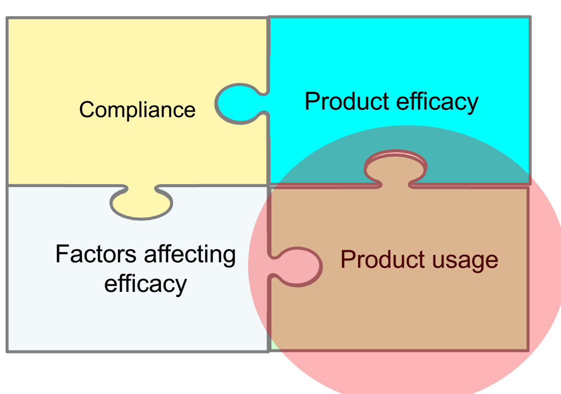
CONCLUSIONS

The obvious – product usage

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

Otter *et al. ICHE* 2011;32:687-99
Rutala & Weber. *J Hosp Infect* 2001;48:S64-8.
Boyce. *J Hops Infect* 2007;65:50-4.



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THANK YOU

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September 30, 2018	*(FREE European Teleclass - Broadcast live from the 2018 IPS conference)* **Cottrell Lecture ... SURVEILLANCE BY OBJECTIVES: USING MEASUREMENT IN THE PREVENTION OF HEALTHCARE ASSOCIATED INFECTIONS** Speaker: **Prof. Jennie Wilson**, University of West London
October 2, 2018	*(FREE European Teleclass - Broadcast live from the 2018 IPS conference)* **Ayliffe Lecture ...THE IMPACT OF DISINFECTANTS ON ANTIMICROBIAL RESISTANCE - AN AYLIFFE PREDICTION** Speaker: **Prof. Shaheen Mehtar**, Stellenbosch University, Cape Town, South Africa
October 11, 2018	*(FREE CBIC Teleclass)* **INFECTION CONTROL CHAMPIONS ARE MADE, NOT BORN** Speaker: **To be announced**
October 17, 2018	*(South Pacific Teleclass)* **BIOFILMS IN THE HOSPITAL ENVIRONMENT - INFECTION CONTROL IMPLICATIONS** Speaker: **Prof. Karen Vickery**, Macquarie University, Australia
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