Bacterial resistance to microbicides in the healthcare environment

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BIOCIDE USAGE

Disinfection - antisepsis - preservation

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

Rutala & Weber. Healthcare Epidemiol 2004; 39: 702-9
Rutala & Weber. Am J Infect Control 2004; 32: 226-31

Antisepsis
- Alcoholic rubs, etc.

Preservation
- low concentration (cosmetic)

Increased usage of microbicides in various products/surfaces

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

BIOCIDE USAGE

- 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

DEFINITIONS

- Resistance / tolerance / insusceptibility??
- Resistance: surviving exposure to a biocide concentration that will kill the rest of the population — Russell. Lancet Infect Dis 2003; 3: 794-803
- Tolerance: inhibited but not killed
  Phenotypic tolerance: transient conditions (biofilm)
  Chapman. Int Biodeter Biodegrad 2003; 51: 133-8
- Insusceptibility: intrinsic property
- Resistance in practice: bacterial survival following microbicide challenge at "in use" concentration.

EVIDENCE OF RESISTANCE - in practice

- Surviving bacteria isolated following biocidal challenges

Triclosan bath	Cookson *et al.* Lancet 1991; 337: 1548-9
Triclosan handwash	Webster *et al.* J Paediatr Child health 1994; 30: 59-64
Chlorhexidine	Nakahara & Kozukue. Sbl Bakt Hyg, I. Abt Orig A 1981; 251: 177-84
QACs	Geftic *et al.* Appl Environ Microbiol 1979; 39: 505-10
Glutaraldehyde	Griffiths *et al.* J Appl Microbiol 1997; 82: 519-26

EVIDENCE OF RESISTANCE – in practice

- **Automated washer disinfectors** (Martin & Maillard 2006)

Bacterial strains	Location	Time (min) to achieve 5 Log_{10} reduction	
		Chlorine dioxide* 2.25%	Hydrogen peroxide 7.5%
Bacillus subtilis (veg)	Rinse water	>60	60
Micrococcus luteus	Rinse water	30	0.5
Streptococcus sanguinis	Endoscope connectors	30	0.5
Streptococcus mutans	Drain area	5	0.5
Staphylococcus intermedius	Drain area	30	0.5

* formulation

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)

	MSSA				MRSA		
	MIC (ppm)	CT (sec)	log_{10} R (±SD)		MIC (ppm)	CT (sec)	log_{10} R (±SD)
13	325	30	3.85 (2.19)	49	400	30	5.81 (1.15)
		60	5.96 (0.36)			60	6.38 (0.12)
14	300	30	2.01 (0.37)	52	400	30	1.75 (1.76)
		60	6.16 (0.33)			60	6.14 (0.09)
51	325	30	2.76 (1.53)	17	400	60	3.46 (1.94)
		60	5.26 (2.05)			120	5.93 (0.07)
47	300	30	2.45 (0.84)	55	350	30	5.22 (1.66)
		60	6.46 (0.31)			60	6.41 (0.24)
Control	225	30	2.27 (1.74)				
9518		60	6.19 (0.11)				

RESISTANCE MECHANISMS

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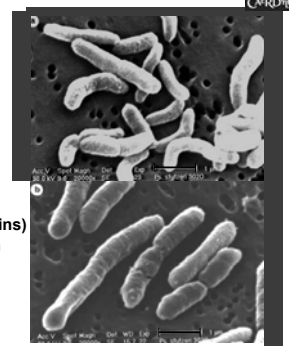


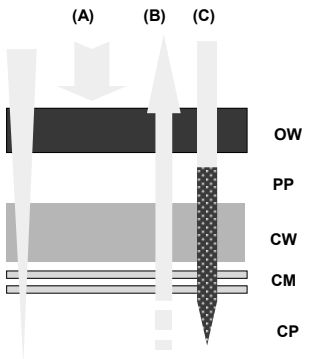
Tattwasart *et al.* J Hosp Infect 1999, 42: 219-29
Tattwasart *et al.* Int J Antimicrob Agent 2000, 16: 233-8

RESISTANCE MECHANISMS

REDUCTION OF UPTAKE AND PENETRATION
Reduction in concentration

REDUCTION OF ACCUMULATION
Reduction in concentration

INACTIVATION
Reduction in concentration



RESISTANCE MECHANISMS

(A) SURFACE INTERACTIONS

Hydrophobicity — QACs, CHX

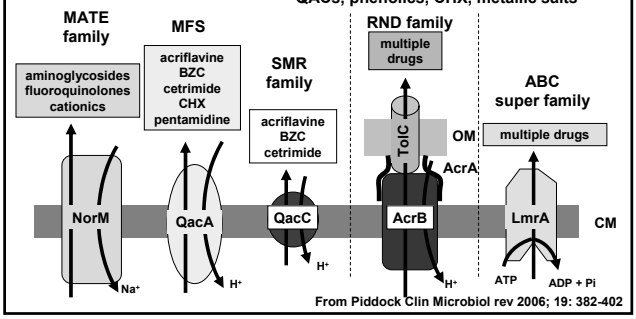
Cell surface charge — QACs

Bruinsma *et al.* J Antimicrob Chemother 2006, 57: 764-6

RESISTANCE MECHANISMS

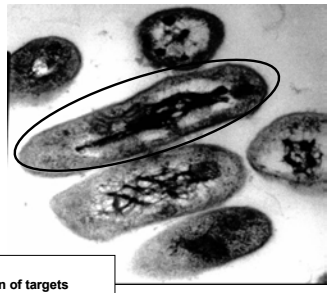
(B) EFFLUX (intrinsic or acquired)

- several families of efflux pumps identified



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

RESPONSE TO BIOCIDE EXPOSURE



Mycobacterium terrae

Adaptation
- modification of targets
- overproduction of targets
- stress response

RESISTANCE MECHANISMS

(C) DEGRADATION (intrinsic or acquired)

Phenolics, metallic salts, FMA

RESPONSE TO BIOCIDE EXPOSURE

- **Adaptation**
  - numerous examples of in vitro training
    QACs, CHX, phenolics, GTA, chlorine
    Gram-negative & -positive, mycobacteria
  - examples of adaptation *in situ*
- **Modification of target**
  - triclosan (enoyl acyl carrier reductase; *fabI* gene)
- **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 (?)

RESISTANCE MECHANISMS

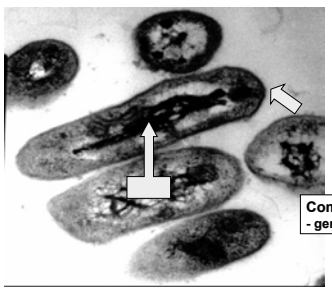
More than one mechanisms involved

E. Coli ATCC 1053	MIC (μg/ml)						
	TCS alone	TCS + CCCP	TCS + OVA	TCS + EDTA	TCS + CCCP +OVA	TCS + CCCP + EDTA	TCS + OVA + EDTA
Standard	0.1	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 (ethylenediamine tetraacetic acid)

RESPONSE TO BIOCIDE EXPOSURE



Communication
- gene transfer

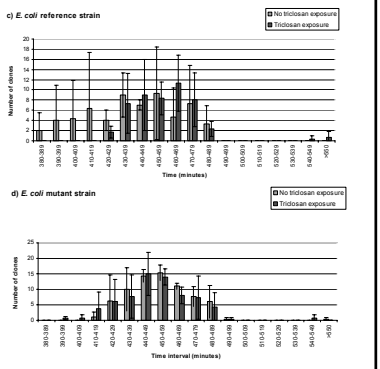
Mycobacterium terrae

RESPONSE TO BIOCIDE EXPOSURE

- **Extracellular induction components (EICs)**
  Acidification and heat response
  Rowburry. Adv Microbiol Physiol 2001; 44: 215-57
  ***S. aureus* pre-treatment with CHX – Low level resistance (3 fold increase) in unexposed cultures**
  Davies & Maillard. J Hosp Infect 2001; 49: 300-1
- **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 BIOCIDE EXPOSURE - POPULATION

- **Selection**
  - **phenolics**
    (triclosan, tea tree oil)
  - **QACs**
  - **CHX**
  - **GTA**
  - **chlorine**



RESPONSE TO BIOCIDE EXPOSURE

- **Increasing transferable resistance (?)**
- **Effect of biocides on gene transfer**

Pearce et al. J Hosp Infect 1999; 43: 101-7

Biocide	Concentration	Increase/decrease in gene transfer by	
		Conjugation	Transduction
Povidone iodine	0.005%	Increased 2 folds	NT*
	0.01%	NT	Reduced 10 folds
Chlorhexidine	0.00005%	No effect	Reduced 10 folds
Cetrimide	0.0001%	Reduced 2 folds	Increased 1000 folds

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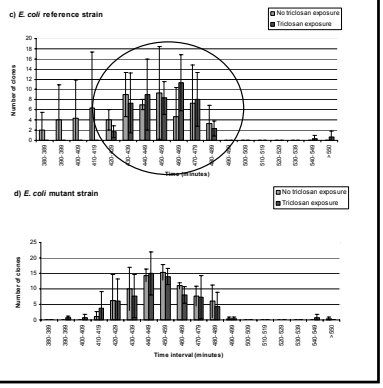
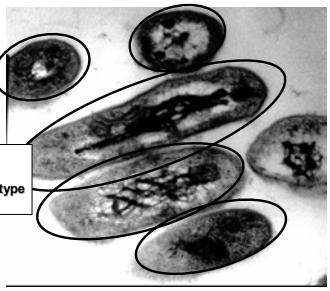


RESPONSE TO BIOCIDE EXPOSURE - POPULATION

Selection

Mycobacterium terrae

RESISTANCE MECHANISMS - Biofilms



Biofilm
-number
-biofilm phenotype
-dormancy

Mycobacterium terrae

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 / O_2)

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

Brill *et al.* Int J Hyg Environ Health 2006; 209: 89-95

  - 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)

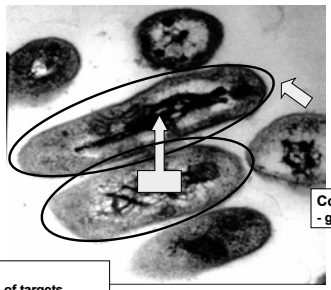
Acquisition of new resistant determinants
- Increased genetic exchange

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



Mycobacterium terrae

RESISTANCE: A GENUINE CONCERN?

- 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

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/expression of resistant phenotype
  - stepwise training best method (unrealistic?)

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