Slide 1

Disinfectants in Infection Control:
Can they Make Bacteria Resistant to Antibiotics

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Slide 2

Overview

• Bacteria and in their natural settings (exposed to biocide)
• Bacteria and biocidal treatments
• Biocide action-resistance mechanism
• Can misuse of biocides lead to biocide/antibiotic resistance?

Slide 3

Bacteria Grow as Biofilms

• Ubiquitous
• Functional consortia of microbial cells that form at surfaces
• Often enveloped with an extra-cellular matrix (Slime)
• Physiologically Distinct
• Monocultures in closed systems
• Phenotypic & Genotypic Heterogeneity
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Bacteria in their domestic setting

First thing in the morning

Slide 5

SKIN

Slide 6

Problematic
Pipe-work
Prepared foods
Biodeterioration
Biodegradation
Food poisoning
Infected Medical Implants
Catheters
Cross-infection
Spoilage
Disease
Biofouling

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Biofilm Bacteria and Biocides

Biofilm bacteria are 50-1000 x less susceptible to anti-bacterials and antibiotics than are planktonic bacteria.

What makes biofilms resistant?

• Restricted access of anti-microbial
• Physiological status of biofilm cells
• Phenotypic heterogeneity and Localised high cell density

Reaction-Diffusion Limitation

Penetration Failure
Binding
Chemical Reaction
Oxidisers
Halogens
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Both Factors Conspire to Make Out-lying Cells Die First
Can only delay DEATH

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Biocide treatment of mixed population biofilm
Time 0min 30min 60min 120min
Monochloramine But Typical
Huang et al.

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Phenotypic heterogeneity
Less susceptible clones?
Cloned efflux mutants?
Response to sub-effective biocide treatment?
What are these pockets of survivors?

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A Potential Cycle of Resistance Development
Associated with sub-effective dosing

Slide 17
Massive expansion in use of “Anti-bacterials”
Skin care, personal products, tooth-pastes, ceramics, plasctics, chopping boards, washing-up liquids, washing powders, fruit washes, soaps, cloth, dish-cloths, socks, under-pants, anti-bacterial sprays, shampoos, toys, carpets, condones, paints, coatings, wound dressings, sutures, skin care, personal products, tooth-pastes, ceramics, plastics, chopping boards, washing-up liquids, washing powders, fruit washes, soaps, cloth, dish-cloths, socks, under-pants, anti-bacterial sprays, shampoos, toys, carpets, condones, paints, coatings, wound dressings, sutures, skin care, personal products, tooth-pastes, ceramics, plastics, chopping boards, washing-up liquids, washing powders, fruit washes, soaps, cloth, dish-cloths, socks, under-pants, anti-bacterial sprays, shampoos, toys, carpets, condones, paints, coatings, wound dressings, sutures, skin care, personal products, tooth-pastes, ceramics, plastics, chopping boards, washing-up liquids, washing powders, fruit washes, soaps, cloth, dish-cloths, socks, under-pants, anti-bacterial sprays, shampoos, toys, carpets, condones, paints, coatings, wound dressings, sutures.

We are drowning in Anti-bacterials
Can misuse lead to resistance?

Slide 18
Can use and abuse of biocides lead to biocide resistance?

• Pre-date antibiotics
• Effective for more than a Century
  – Critical to effective hygiene
  – Major contributor to public health
• As yet - little or no evidence of loss in effectiveness in hygienic situations - Why
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**BIOCIDE MECHANISM**

- Multiplicity of target site
- Growth Inhibition
- Death by starvation
- Death by stabbing
- Death by hanging
- Death by poison

Increase in targets with increasing concentration

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**BIOCIDE RESISTANCE**

- Changes in susceptibility towards sub-effective levels may occur where only ONE target is affected (MIC)
- If use-concentration is c.MIC then possible failure of preservatives
- ESSENTIALLY modification of ALL targets with susceptibility below the in-use level is required for RESISTANCE

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**The Harbingers of Doom**

- Can biocide resistance parallel the development of antibiotic resistance?
- Could we be entering a Post-biocide Era?

NO

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**The Harbingers of Doom**

- Can changes in biocide susceptibility at sub-effective levels (i.e. affecting MIC) confer antibiotic resistance?
  - Possibly -

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**Dilution from Point of Use**

Reduction in number of target sites by dilution

- Nutrient
- Death by starvation
- Death by stabbing
- Death by hanging
- Death by poison

**Shared Target**

Dilution away from point of use

Slide 24

**Dilution from Point of Use**

Reduction in number of target sites by dilution

- Nutrient
- Death by starvation
- Death by stabbing
- Death by hanging
- Death by poison

**Shared Target**

Dissipation within a biofilm
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What are these pockets of survival?
What is the probability that antibiotics and biocides share common targets?

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**The Harbingers of Doom**

*Triclosan*

- Inhibits growth of E. coli through inhibition of an enoyl reductase enzyme
- Point mutation (Gly93-Val) in the enoyl reductase confers resistance to this enzyme and occurs with moderate frequency
- MIC towards mutant strains radically altered
- Mutants easily selected in laboratory monoculture

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**Triclosan**

- The *enoyl reductase* in *Mycobacteria* is the sole target for the anti-tubercular antibiotic Isoniazid
- The *enoyl reductase* is also the target for Hexachlorophane and the Diazaborines

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The Harbingers of Doom

- Isoniazid resistant tuberculosis retains susceptibility to triclosan
  - i.e. same target different sites
- FabI mutants not selected in panels of skin, drain and oral bacteria
- Triclosan-based molecules offer way forward for new class of anti-tubercular drugs

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Is Triclosan a One-off

Slide 30

Sub-lethal levels of isothiazolone biocides, and phenylethanol cause filamentation in bacteria and yeast. Highly reminiscent of filamentation induced by fluoroquinolones and beta lactams. CAUSE UNKNOWN but possible selection pressure on topoisomerases / PBP's

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![Image of Ciprofloxacin Susceptibility graph]

- **Ciprofloxacin Susceptibility**
  - n.b. NOT resistance

Passage Number

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**Cationic Biocides**

- Polyhexamethylene biguanides and bisbiguanides, and many quats, cross Gram-negative cell envelope by cation displacement and self-promotion
- Mechanism of cell entry shared by aminoglycosides

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**Yet More**

- MDR 2001, Genetic linkage between resistance to QAC and beta-lactam antibiotics in food related Staphylococcus spp
- AAC 2002, Cation efflux pump gene associated with chlorhexidine resistance in Klebsiella pneumoniae
- AEM 2002, Pine oil cleaner-resistant Staphylococcus aureus: reduced susceptibility to Vancomycin and Oxacillin: Involvement of SigB
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The effects of sub-effective dosing

Common mechanisms of resistance associated with agents that possess very different mechanisms

Efflux

What are these pockets of survivors?

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Bacterial Multi-Drug Efflux Pumps

the microbial vomit response

• All bacteria can respond to the presence of certain noxious materials by expressing efflux pumps (AcrAB through Mar)
• Expression has been shown to be induced by sub-lethal exposure to antibiotics (i.e. tetracycline) solvents and some biocides (i.e. quats, pine-oil) as well as by salicylic acid
• Triclosan is a substrate of AcrAB but not an inducer
• Exposure to triclosan will therefore select for efflux-on mutants (Bulimic bacteria)

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Bacterial Multi-Drug Efflux Pumps

the microbial vomit response

• Pump induction is sufficient to confer resistance to many chemically distinct antibiotics (i.e. multiple drug resistance)
• Efflux-ON mutants implicated in clinical antibiotic resistance
• Pump induction will alter susceptibility towards some biocides but will NOT confer resistance
Efflux Pump Induction

E. coli species
MarO⁻LacZ

More than half induced Mar

X-gal top-agar

Slide 37

Mustard

Chilli-paste

N. Broth

Salicylate

Slide 38

Multi-Drug Efflux Pumps
the microbial vomit response

BUT

- They have evolved to contend with natural anti-infectives (incl antibiotics), secondary metabolites etc.
- Also induced, up-regulated in response to exposure to many natural food preservatives
- Garlic, mustard, cheese-dip, chilli-pepper, Sunny Delight etc.

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The Real World

- MICRO COSMS
- Not Monocultures
- Fitness Cost Implications in Competition
- Degradation and loss of biocide

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Survey evidence fails to demonstrate that use of antibacterials has affected antibiotic efficacy in the real world

Fitness cost of resistance

- *Clinical Microbiology Reviews*
- July 2003 (pdf available)

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*In Vitro* Microcosm Studies

**Drains**
- Triclosan - *Applied & Environmental Microbiology* September 2003,
- QUAT - *Submitted AEM*

**Mouth**
- Chlorhexidine - *Applied & Environmental Microbiology* August 2003,
- Triclosan - *Antimicrobial Agents and Chemotherapy* November 2003

CONCLUSIONS SIMILAR IN ALL
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The constant-depth film fermenter (CDFF)

Slide 44
Kitchen drain microcosm biofilm

Slide 45
Clonal expansion of insusceptibles in Drain Microcosm after chronic Triclosan exposure

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Before Stress

After TRD Stress

Clonal Expansion

Slide 47

Triclosan-degrader
Clonally expanded

Triclosan-solubiliser
Bacterial rhamnolipid

Slide 48

Microcosm Conclusions

Resistance selection was not evident in lab microcosms
Fitness costs – “Super-bugs” rejected
Competition
Innate insusceptibility of much of the flora
Degradation ???

Sub-lethal Exposure does NOT select
for resistance development
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The Harbingers of Doom
• Is the 'uncontrolled' use of biocides in the environment a selection pressure towards antibiotic resistance?
• A probable NO

Slide 50

The Harbingers of Doom
• Has the indiscriminate use of 'anti-bacterial products' compromised therapeutic solutions to infection
• No in-vivo evidence of link
• No evidence from long-term laboratory microcosm studies

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Targeted Hygiene
ahead with caution
• Limit the use of "anti-bacterials" to those applications which have demonstrable benefit
• Do not abandon "good hygienic practices" where there is a proven benefit to product / system integrity and especially in limiting disease

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Targeted Hygiene

- Use “anti-bacterials” that lose effectiveness rapidly as they are diluted from the point of application, and leave no residual
- OXIDISERS / Bleach
- Reductions in GID and Cross-infection through hygiene will limit development of antibiotic resistance
- BEWARE
  The Harbingers of Doom