Disinfectants, Can They Make Bacteria Resistant to Antibiotics

Presented by Dr. Peter Gilbert

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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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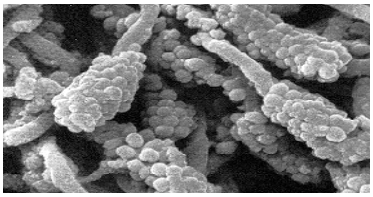
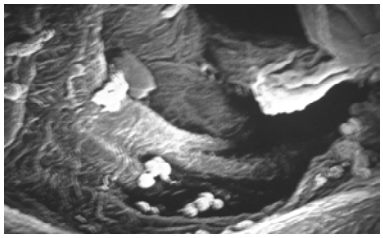
Bacteria in their domestic setting



First thing in the morning

Slide 5



SKIN

Slide 6

Washed Lettuce

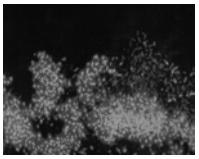
Problematic

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

Slide 7

Biofilm Bacteria and Biocides

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



RESISTANCE
Antibiotics, Anti-bacterials
Biocides, Preservatives

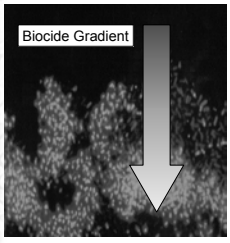
Slide 8

What makes biofilms resistant?

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

Slide 9

Reaction-Diffusion Limitation

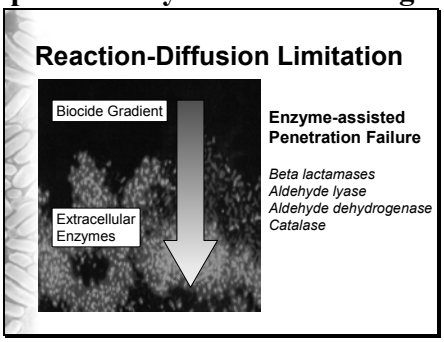


Penetration Failure

Binding
Cationics

Chemical Reaction
Oxidisers
Halogens

Slide 10



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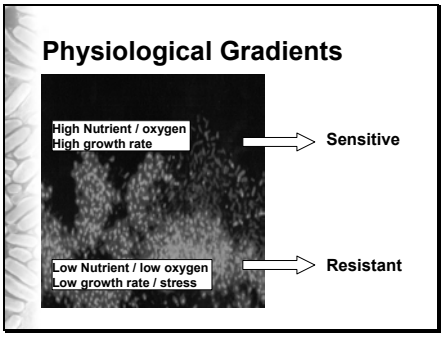

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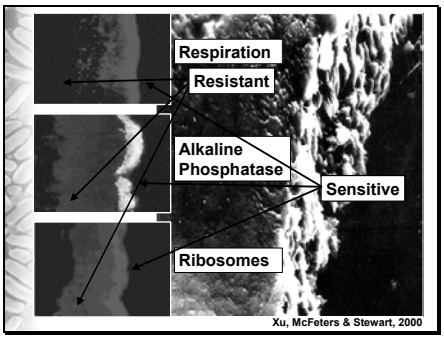

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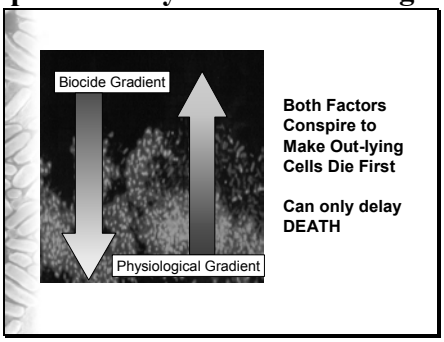

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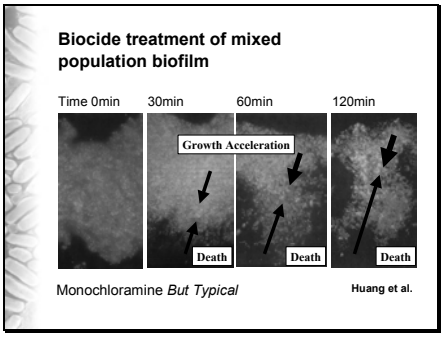

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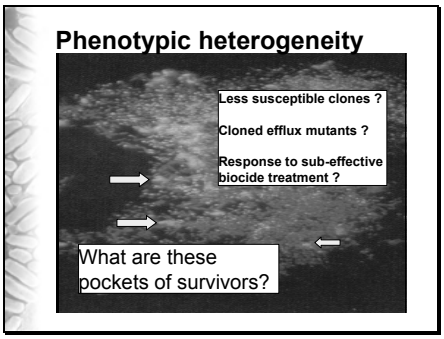

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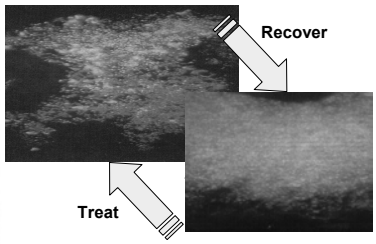
A Potential Cycle of Resistance Development Associated with sub-effective dosing



Slide 17

Massive expansion in use of *"Anti-bacterials"*

Skin care, personal products, tooth-pastes, ceramics, plastics, chopping boards, washing-up liquids, washing powders, fruit washes, soaps, cloth, dish-cloths, socks, under-pants, anti-bacterial sprays, shampoos, toys, carpets, condoms, 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, skin care, personal products, tooth-pastes, ceramics, plastics, chopping boards, washing-up liquids, washing powders, fruit washes, soaps, cloth, dish-cloths, socks, underpants, anti-bacterial sprays, shampoos ……etc. etc. etc.

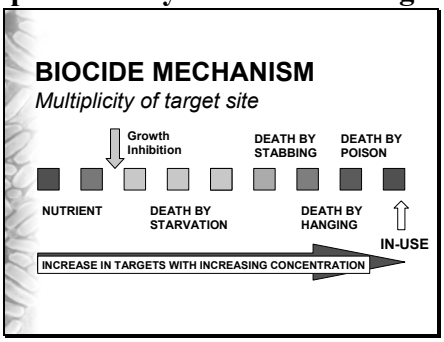
We are drowning in Anti-bacterials
Can misuse lead to resistance?

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

Slide 19



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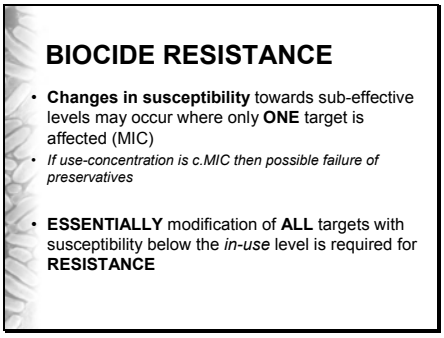

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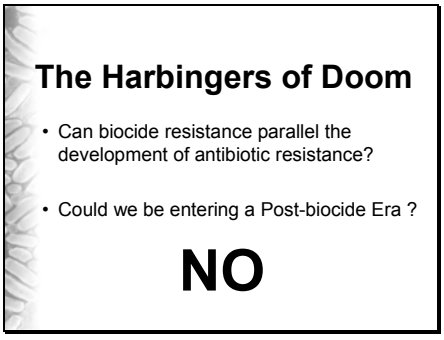
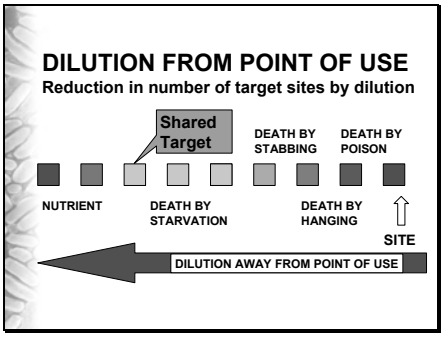


Slide 22

The Harbingers of Doom

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

Slide 23



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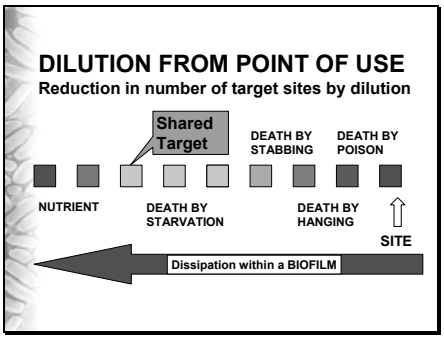
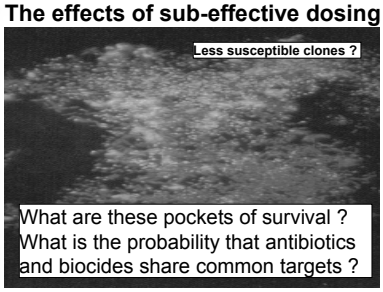


Slide 25

The effects of sub-effective dosing



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

Slide 26

The Harbingers of Doom
Triclosan

- Inhibits growth of *E. coli* through inhibition of an *enoyl reductase* enzyme
- Point mutation (*Gly*93-*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

Slide 27

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 Diazoborines

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The Harbingers of Doom

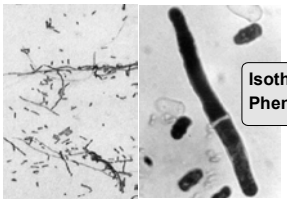
The Small Print

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

Slide 29

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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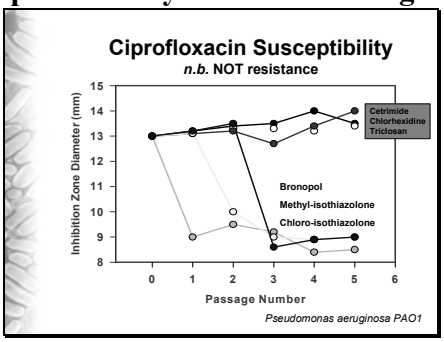

Slide 32

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

Slide 33

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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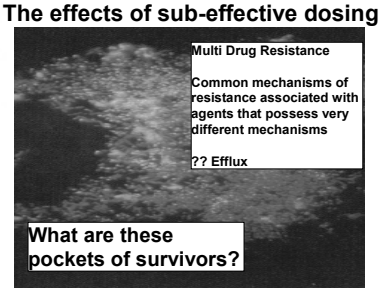
The effects of sub-effective dosing



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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 (*Acr*AB 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 *Acr*AB 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

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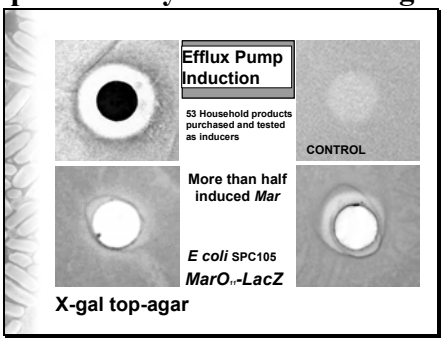

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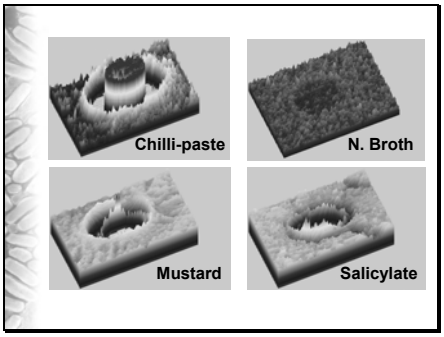

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Multi-Drug Efflux Pumps
the microbial vomit response
BUT

- 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.

Slide 40

The Real World

- MICROCOSMS
- Not Monocultures

- Fitness Cost Implications in Competition
- Degradation and loss of biocide

Slide 41

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

Fitness cost of resistance

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

Slide 42

***In Vitro* Microcosm Studies**

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

Mouth
Chlorhexidine - Applied & Environmental Microbiology August 2003,
Triclosan – Antimicrbial Agents and Chemotherapy November 2003

CONCLUSIONS SIMILAR IN ALL

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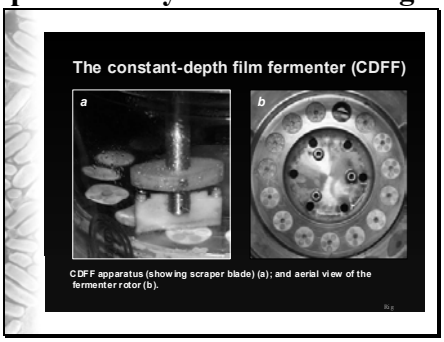

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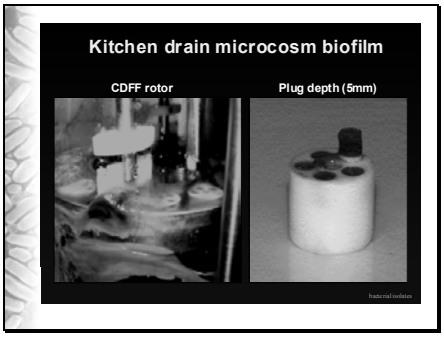

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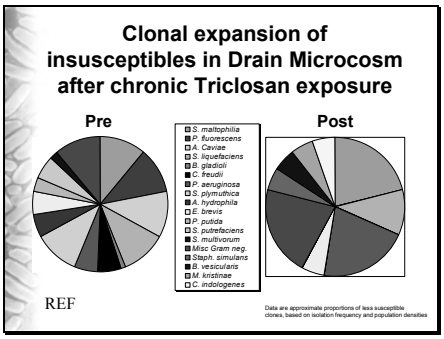

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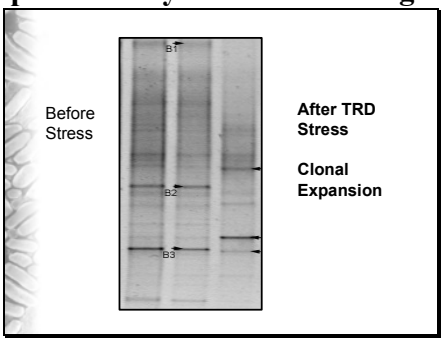

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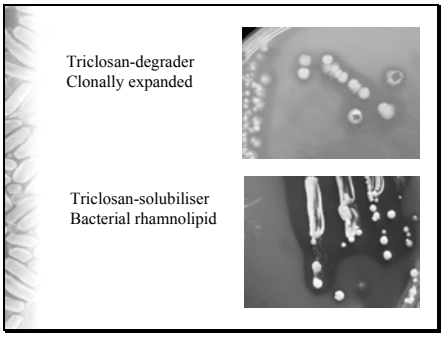

Slide 48

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

Slide 49

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

Slide 51

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