Antimicrobial Susceptibility and Biofilms

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Susceptibility

Wild-type MIC (Antibiotics)

Typical Use-levels (Disinfectants/Biocides)

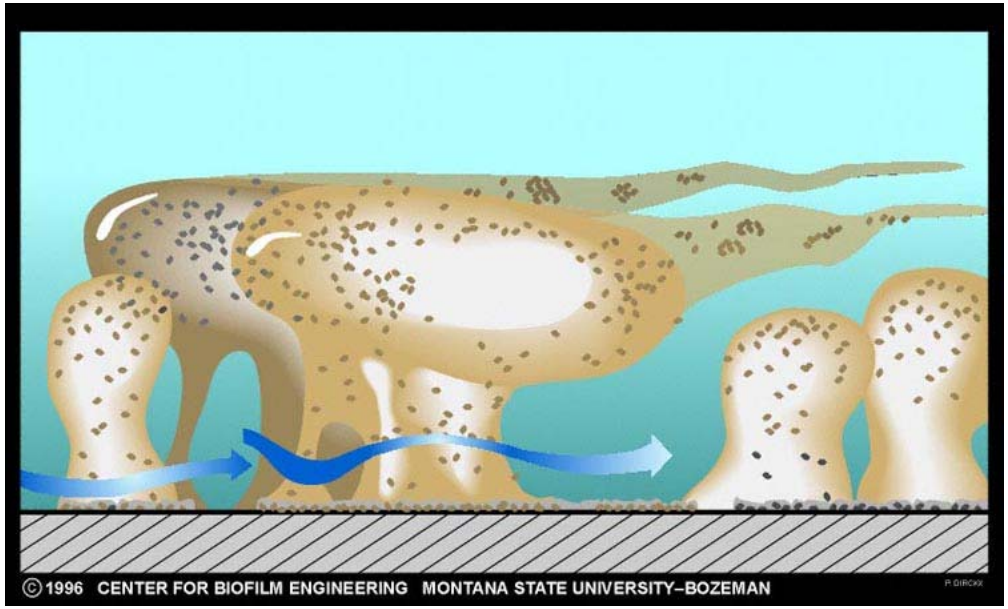
- Susceptibility is a continuum of responses to an antimicrobial agent.
- Sensitive and Resistant describe susceptibility in relation to a fixed value determined by the wild-type MIC.
- Susceptibility can change in response to physiological and environmental parameters.

Reduced Susceptibility of Biofilms

- Antibiotics, disinfectants, preservatives.
  - Susceptibility reductions of 2-1000x.
- Mechanisms
  - Reduced permeation
  - Altered physiology
  - Persistence
  - Mutation

Reduced Permeation of Antimicrobials

- The Biofilm Matrix
  - Highly hydrated extracellular polysaccharides & proteins
  - Anionic
  - Morphologically diverse and complex
- Chemistry of the Antimicrobial Agent
  - Charge
  - Hydrophobicity
  - Reactivity



Reduced Permeation of Antimicrobials

- Kinetics
  - Chlorine and chloramine penetration rates in biofilms impaired
    - But eventually reach equilibrium anyway (Stewart et al.)
- Increase in response time
  - Induction of damage response systems (SOS, rpoS, oxyR, etc.)

Reduced Permeation of Antimicrobials

- Increased possibility of sub-inhibitory dosing, consequences include:
  - Increased biofilm formation
  - Selection of resistant mutants
  - Horizontal gene transfer
  - Increased mutation rates

Lauretti et al. *Antibiotics* ***2013, 2, 100-114; doi:10.3390/antibiotics2010100***

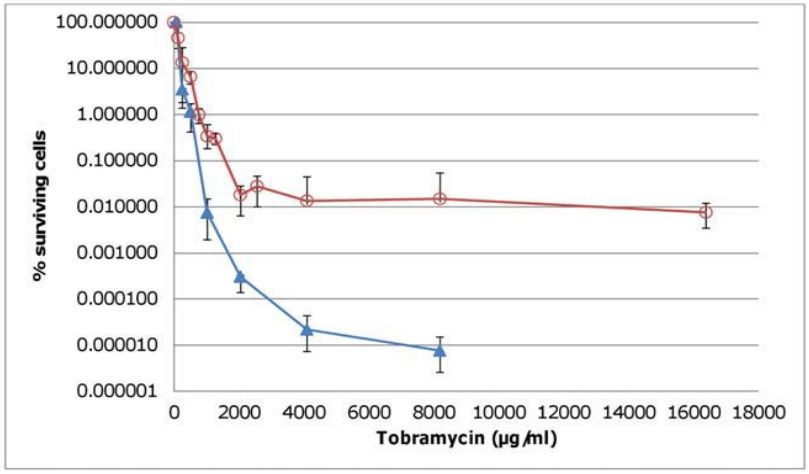
Altered Physiology

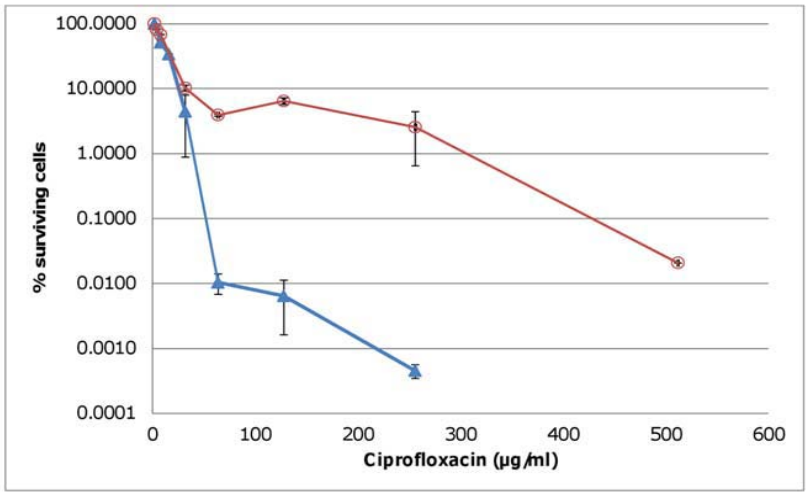
- Consequences of growth in a fixed location versus suspended growth
  - Gradients
    - Oxygen, nutrients, excretions, pH
    - Variations in growth rates, food sources, environmental conditions
  - Communication and Interaction
  - Widespread alterations in gene expression
    - Estimate for *Burkholderia*: >1000 genes with altered expression, up or down
    - Expression of new metabolic capabilities

Persistence

- Persistence: A physiological state of dormancy that renders cells *non-susceptible* to many drugs.
- Not resistance *per se*:
  - The regenerated population displays the same pattern of drug susceptibility as the original one.
- Significant in recurrent infections.
- Postulated to involve toxin-antitoxin modules which inhibit growth at the physiological level.
  - Some bacteria have multiple redundant TA modules.

Persistence





Van Acker et al. (2013) PLOS 8: e58943

Persisters occur at higher frequencies in biofilms than in planktonic populations.

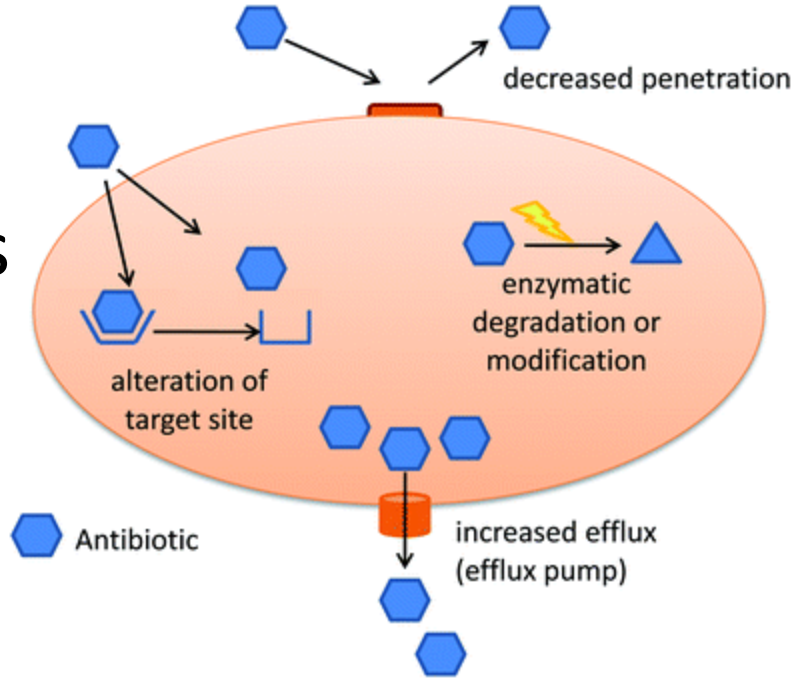
Spoerling & Lewis 2001: 100x
van Acker et al 2013: 1000-3000x more frequent.

Antimicrobial Resistance in Biofilms

- Resistance: stable, inheritable reduction in susceptibility.
  - Occurs by:
    - *de novo* alterations in DNA
    - Acquisition of new genetic material
- Resistance development is more frequent in biofilms.
  - Sub-lethal exposures to antimicrobials.
    - Horizontal gene transfer
    - Increased mutation rates due to damage responses
  - "Spontaneous rate" increase due to altered gene expression (eg. Repression of radical detox systems)

Resistance Mechanisms

- Target site alteration
- Reduction in target access
- Expulsion
- Inactivation



Abreu et al. Nat. Prod. Rep., 2012,29, 1007-1021

- Why the concern?
  - Inability to eradicate biofilms with one agent leads to ineffectiveness of other agents .
    - Cross-resistance of biocides and antibiotics.

Cross-resistance

- Expression of a resistance mechanism to once class of antimicrobial agents leads to resistance to a mechanistically unrelated agent.
  - Common with mutations that lead to reduced target access, eg., loss of outer membrane porins.
    - Fluoroquinolones, isothiazolones
  - Not so with target site alteration and inactivation mechanisms – mostly due to their enzymatic specificity.
    - triclosan resistance and isoniazid.

Co-resistance

- The co-location of two resistance determinants on a mobile genetic element.
  - Plasmids, integrons, insertion sequences, etc.
  - Horizontal gene transfer
- Example: Quaternary ammonium compounds and multiple antibiotics
  - Strains isolated from biofilms in healthcare and food preparation facilities.

Opportunities?

- Biofilm-specific antimicrobials
- Persister-specific antimicrobials
  - "re-awaking" persisters
  - Exploitation of the persister phenotype
- Biofilm disrupting or dispersing agents
  - Cell signal analogues
  - Chemical agents
- Biofilm-prevention strategies
  - Active or passive surfaces


ARE THERE
ANY QUESTIONS?
BIOFILMS

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