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# Antimicrobial Selection to Combat Resistance

## (Dead Bugs Don't Mutate!)

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Associate Professor CE Microbiology
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Ryan Veterinary Hospital
University of Pennsylvania

2003

PERSPECTIVES

# Dead Bugs Don't Mutate: Susceptibility Issues in the Emergence of Bacterial Resistance

**Charles W. Stratton**[*1]

The global emergence of antibacterial resistance among common and atypical respiratory pathogens in the last decade necessitates the strategic application of antibacterial agents. The use of bactericidal rather than bacteriostatic agents as first-line therapy is recommended because the eradication of microorganisms serves to curtail, although not avoid, the development of bacterial resistance. Bactericidal activity is achieved with specific classes of antimicrobial agents as well as by combination therapy. Newer classes of antibacterial agents, such as the fluoroquinolones and certain members of the macrolide/

and macrolides (the antibacterial agents used most frequently for pneumococcal infections) have become prevalent throughout the world. Indeed, rates of *S. pneumoniae* resistance to penicillin now exceed 40% in many regions, and a high proportion of these strains are also resistant to macrolides. Moreover, the trend is growing rapidly. Whereas 10.4% of all *S. pneumoniae* isolates were resistant to penicillin and 16.5% resistant to macrolides in 1996, these proportions rose to 14.1% and 21.9%, respectively, in 1997 (9). A more recent

# Charles W Stratton, EID 2003: 9: 10 -16

- The urgent need to curtail proliferation of antibacterial resistant bacteria **has refocused attention on the proper use** of antibacterial agents.
  - HAS IT?

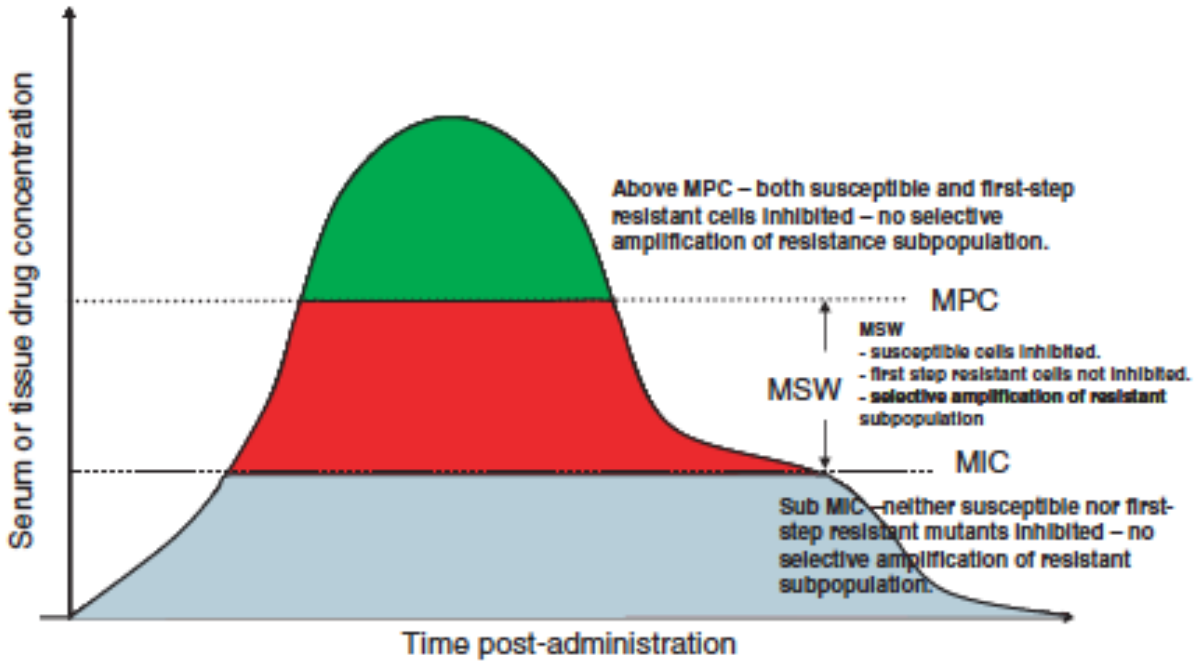
- The use of bactericidal rather than bacteriostatic agents as first line therapy **is recommended** because the eradication of microorganisms serves to curtail, although not avoid, the development of bacterial resistance.
  - IS IT?

# MPC

## Mutant Prevention Concentration

(above the MIC)

# Mutant Prevention Concentration




The MPC is the theoretical upper boundary of an antibiotic concentration window in which resistant mutants are selectively amplified

# Below the MPC…

- Fleming commented in 1945, '**…**But I would like to sound a note of warning**…**.it is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to euthanize them and the same thing has occasionally happened in the body.'

- Undoubtedly, Fleming was warning against exposing bacteria to insufficient concentrations of drug and that doing so would ultimately encourage resistance selection.

Blondeau, JM. Veterinary Dermatology, 2009: 20, 383–396.

# Mutant Selection Window Hypothesis

- Resistant mutants are selected *exclusively* within a concentration range (mutant selection window) that extends from the point where growth inhibition begins, approximated by the MIC, up to the MPC.

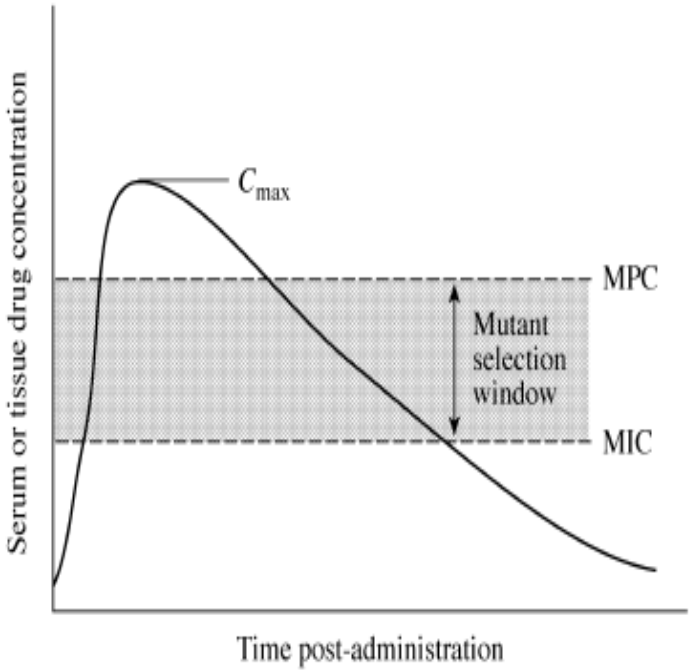
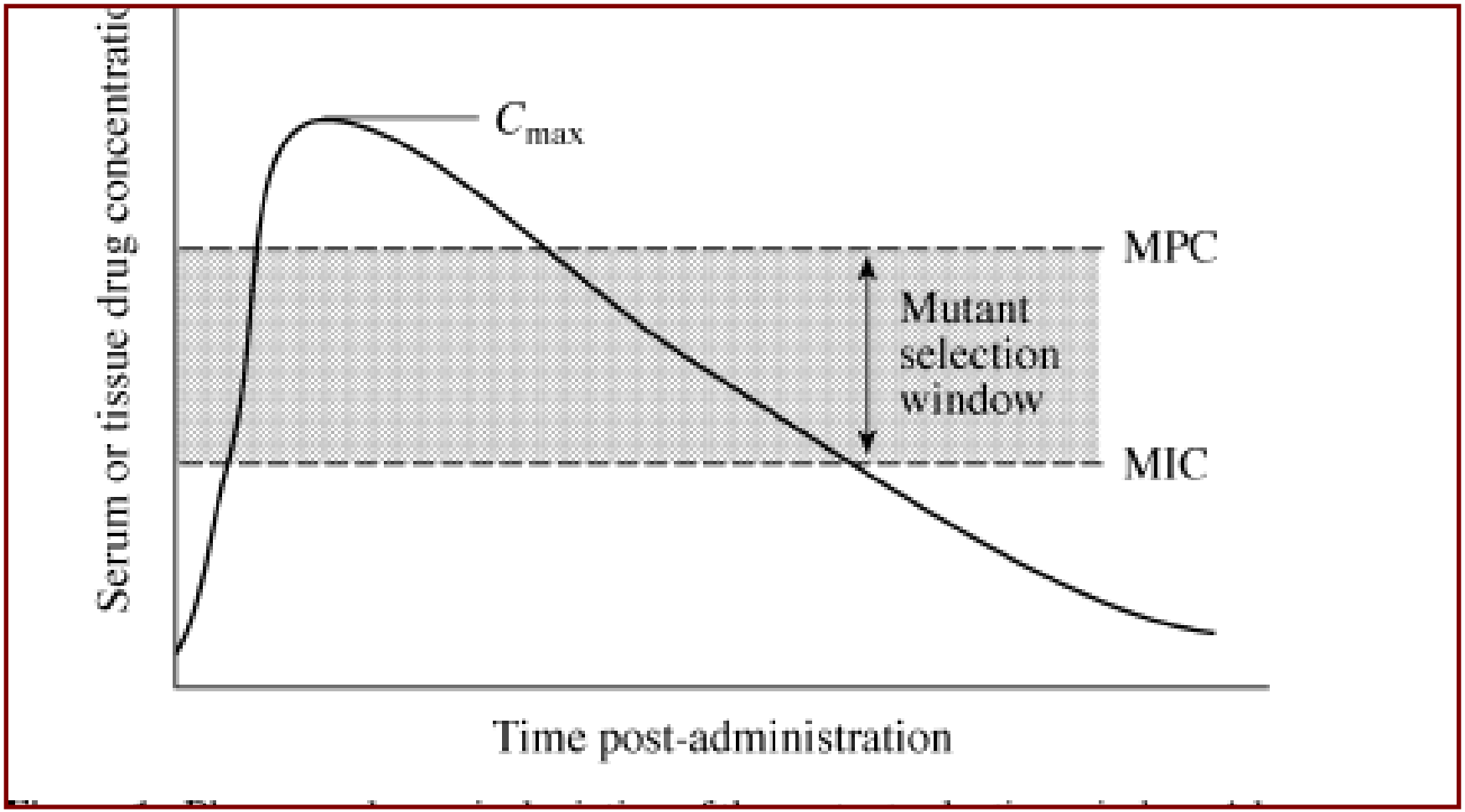
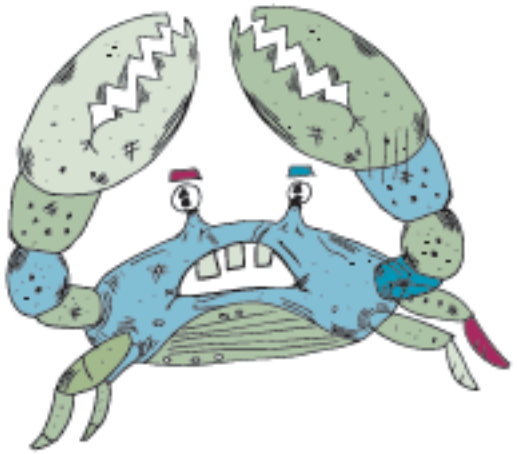



**Figure 1.** Pharmacodynamic depiction of the mutant selection window. A hypothetical pharmacokinetic profile is shown in which MIC and MPC are arbitrarily indicated. Double-headed arrow indicates the mutant selection window.

# But what happens below the MIC?



“bactericidal antibiotics can function as active mutagens and may have a role in the emergence of multidrug resistance.”



ANTIMICROBIALS

# Reactive resistance

- Treatment of bacteria with low concentrations of bactericidal antibiotics can generate multidrug resistance through an increase in the mutation rate that is driven by the formation of reactive oxygen species (ROS).

# And I be like…

Molecular Cell
Article

2010

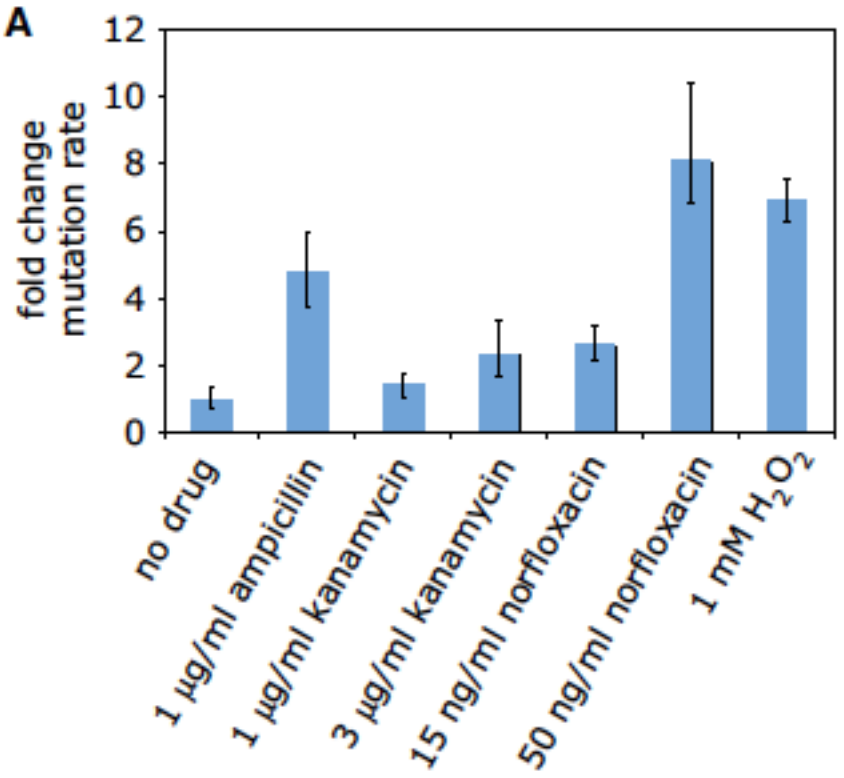
# Sublethal Antibiotic Treatment Leads to Multidrug Resistance via Radical-Induced Mutagenesis

Michael A. Kohanski,[1,2,3,4,5] Mark A. DePristo,[2,3,4,6] and James J. Collins[1,2,3,4,5,7,*]
[1]Howard Hughes Medical Institute

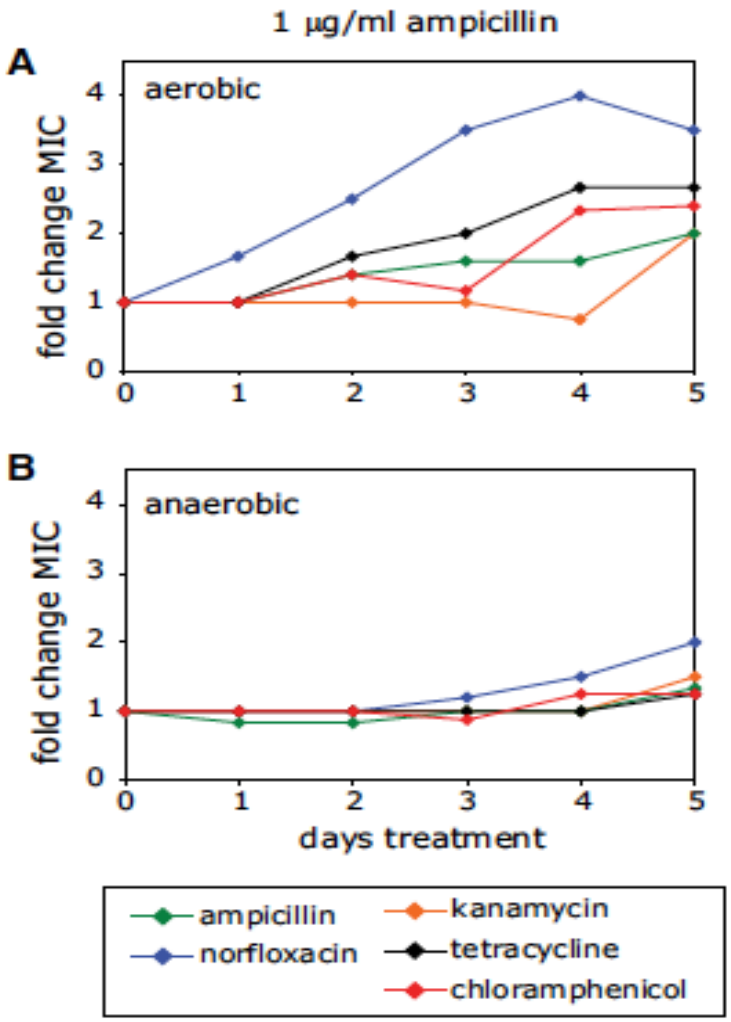
To test the hypothesis that ROS formation, due to treatment with low levels of bactericidal antibiotics, leads to an increase in mutation rates which can lead to drug resistance, mutation rates were examined in *E. coli* following treatment with low levels of norfloxacin, ampicillin and kanamycin.

# Low Levels of Bactericidal Antibiotics Increase Mutation Rate Due to Reactive Oxygen Species Formation

- Overnight treatment with low concentrations of antibiotics.
- All three treatment regimes led to a significant increase in the mutation rate (up to eightfold) compared with untreated



# *E. coli* - 5 days growth in 1 mg/ml ampicillin




Treatment of wild-type *E. coli* with 1 **u**g/ml ampicillin for 5 days led to an increase in the MIC for ampicillin **AND to increased MICs for the unrelated drugs** norfloxacin, kanamycin, tetracycline, and chloramphenicol

# Below the MIC – the fast track to MDR?

- While the majority of the multidrug cross-resistant strains exhibited resistance against the treatment drug, ampicillin, the results demonstrate that treatment with ampicillin can also generate mutants that are not resistant to ampicillin yet are resistant to other classes of antibiotics.

- Prolonged exposure to weakly inhibitory drug concentrations can springboard *E. coli* and *S. aureus* from drug-sensitive to MDR (Kohanski et al 2010)

Molecular Cell
Previews

# The Fast Track to Multidrug Resistance

Benjamin B. Kaufmann[1,2] and Deborah T. Hung[1,2,*]
[1]Infectious Disease Initiative, The Broad Institute, Cambridge, MA 02141, USA
[2]Massachusetts General Hospital, Boston, MA 02114, USA
*Correspondence: hung@molbio.mgh.harvard.edu
DOI 10.1016/j.molcel.2010.01.027

In this issue of *Molecular Cell*, Kohanski et al. (2010) demonstrate that even subinhibitory concentrations of bactericidal antibiotics result in the generation of reactive oxygen species, leading to an increase in mutation rate and the emergence of multidrug-resistant bacterial strains.

Many classes of bactericidal antibiotics have been shown to provoke bacteria to generate ROS.

Consequently these results carry a startling corollary: that any bactericidal drug in a therapeutic cocktail may assist bacteria in attaining resistance to the entire combination.

# Summary

- Low doses of bactericidal antibiotics cause mild and transient stress in bacteria and that allows ROS accumulate to a level that is sufficient for ROS to be beneficial mutagens and inducers of protective functions.

- From an evolutionary perspective – is the destructive role of ROS is merely collateral damage arising from protective activities or does self-destruction confer a selective advantage to bacterial populations?

Thank you!

Questions?

# 2010 - 2017

- In the past few years new research has shown some interesting new twists.
- Factors that interfere with antibiotic lethality can compromise efficacy and contribute to emergence of resistance. One of these is the consumption of antioxidant dietary supplements, since they interfere with antimicrobial lethality.

- ROS may also be clinically significant if ways are found to boost intracellular ROS production.
- Such work could lead to novel strategies to increase the lethal action of many antibiotics (at low doses?).

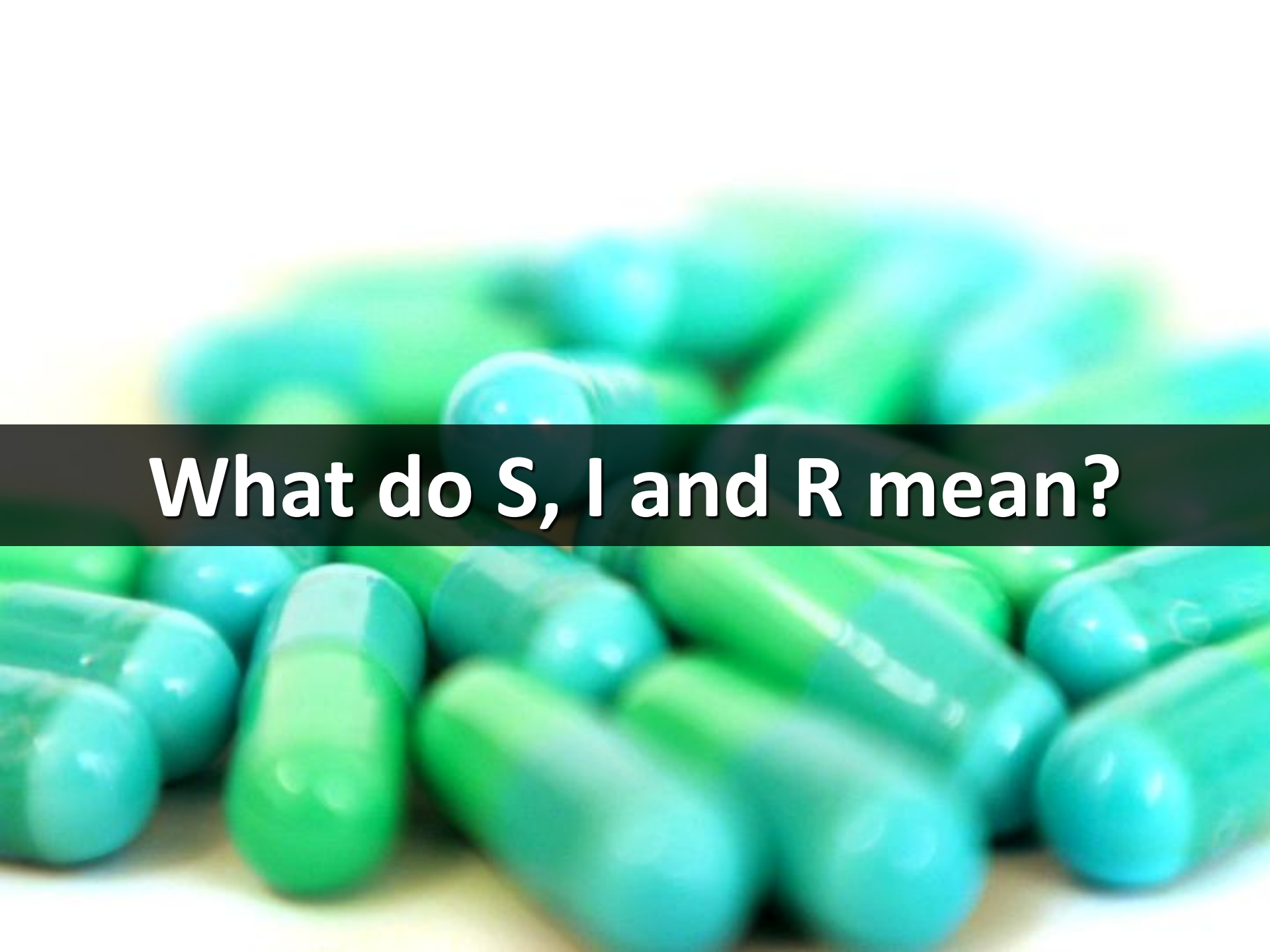
Cell Metabolism
Article

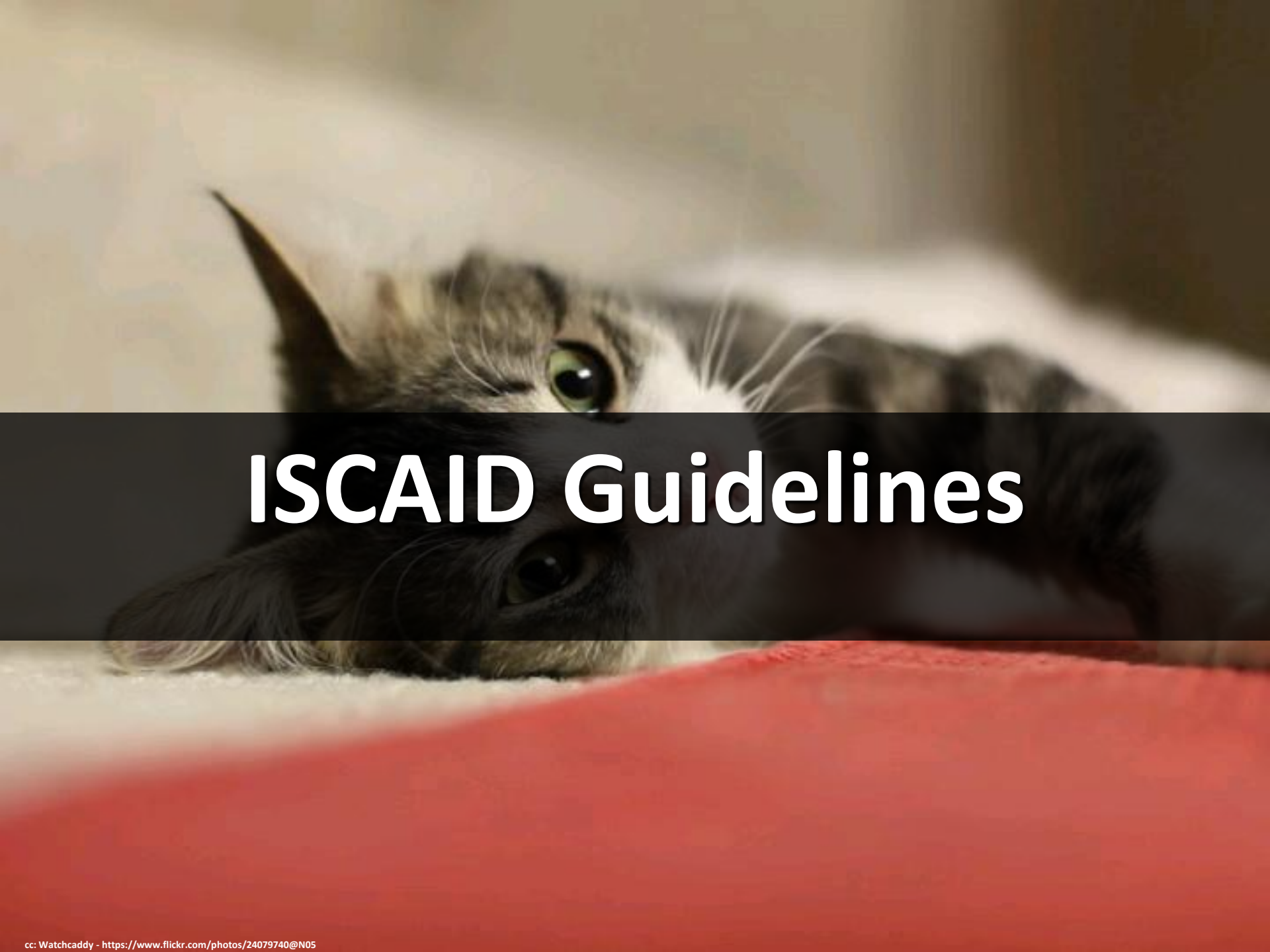
# Exogenous Alanine and/or Glucose plus Kanamycin Kills Antibiotic-Resistant Bacteria

Bo Peng,[1,2,3] Yu-bin Su,[1,3] Hui Li,[1,3] Yi Han,[1] Chang Guo,[1] Yao-mei Tian,[1] and Xuan-xian Peng[1,*]
[1]Center for Proteomics and Metabolomics, State Key Laboratory of Biocontrol, School of Life Sciences, MOE Key Lab Aquat Food Safety, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, People's Republic of China
[2]Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720-8197, USA
[3]Co-first author
*Correspondence: pxuanx@sysu.edu.cn
http://dx.doi.org/10.1016/j.cmet.2015.01.008

A strategy to overcome bacterial resistance to antibiotics is described that bypasses the need for discovery or design of novel drugs or reagents. This strategy uses non-toxic compounds to modulate the metabolome of antibiotic-resistant bacteria, promote the TCA cycle, increase PMF, and stimulate transport of extracellular antibiotics through the bacterial cell wall/membrane into the intracellular environment.


"This is one sick dog!"


What do S, I and R mean?



# SODAPOP: A Tool for Veterinary Antimicrobial Selection

Stephen Cole, VMD, MS

Shelley Rankin, PhD

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# Antimicrobial Stewardship

- Increasing antimicrobial resistance → need for veterinarians to practice antimicrobial stewardship

- Fajt et al. 2013 in a survey of veterinary schools determined that while a majority of schools teach antimicrobial stewardship, less than half use a case-based approach (mainly powerpoint).

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# Antimicrobial Stewardship

- PennVet's Clinical Microbiology team have developed a mnemonic device to implement in the 4th year clinical rotation.

- We have performed a study to evaluate effectiveness (which I'm not going to talk about in detail because it is currently undergoing peer review).

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# The SODAPOP Method

- Source
- Organism
- Decide to treat
- Antimicrobials
- Patient
- Options
- Plan

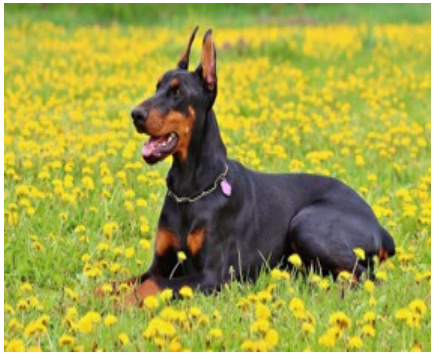
# Choosing an Antimicrobial

| | |
|---|---|
| S | Source |
| O | |
| D | |
| A | |
| P | |
| O | |
| P | |

Patient
Itchy (5 yo MC Doberman)
Source
Multiple intact dermal pustules

Final Result
Methicillin resistant Staphylococcus pseudintermedius

| Sensitivity Analysis | MRSP | |
|---|---|---|
| Amikacin | | S |
| Amoxicillin/K Clavulanate | <=4/2 | R |
| Ampicillin | >8 | BLAC |
| Cefazolin | 4 | R |
| Cefpodoxime | | R |
| Chloramphenicol | <=8 | S |
| Ciprofloxacin | >2 | R |
| Clindamycin | >2 | R |
| Enrofloxacin | | R |
| Erythromycin | >4 | R |
| Gentamicin | 8 | I |
| Imipenem | <=1 | R |
| Marbofloxacin | | R |
| Oxacillin | >2 | R |
| Penicillin | >8 | BLAC |
| Rifampin | >2 | R |
| Tetracycline | >8 | R |
| Trimethoprim/Sulfamethoxazole | >2/38 | S |
| Vancomycin | <=2 | S |



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# Choosing an Antimicrobial

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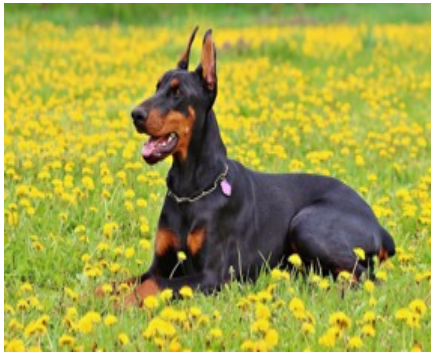

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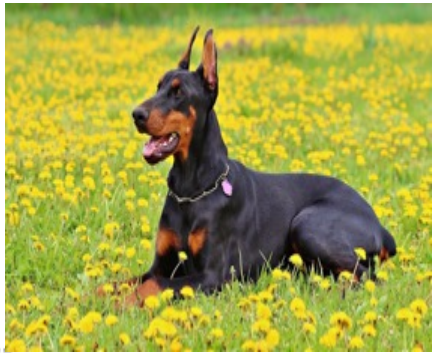
# Choosing an Antimicrobial

| | |
|---|---|
| S | Source |
| O | Organism |
| D | Decide to Treat |
| A | |
| P | |
| O | |
| P | |

YES

Patient
Itchy (5 yo MC Doberman)
Source
Multiple intact dermal pustules



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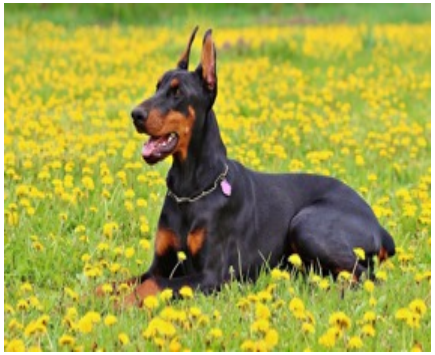

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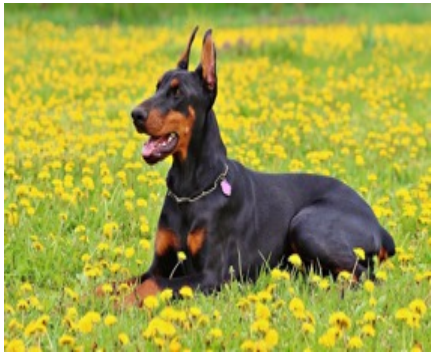
# Choosing an Antimicrobial



| | |
|---|---|
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| **O** | Organism |
| **D** | Decide to Treat |
| **A** | Antimicrobials |
| **P** | |
| **O** | |
| **P** | |

- ~~Amikacin~~
  - Injectable formulation only
  - High risks for nephrotoxicity
- Chloramphenicol
- TMS
- ~~Vancomycin~~
  - NOT a veterinary drug
  - Critical drug to human medicine

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# Choosing an Antimicrobial

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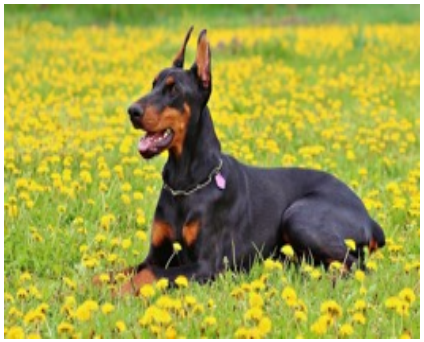

- Chloramphenicol
  - Contraindicated in dogs with liver disease
- ~~TMS~~
  - Contraindicated in dogs with hx of autoimmune disease
  - Contraindicated in "black-and-tans," samoyeds and miniature schnauzers

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# Choosing an Antimicrobial

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| **O** | Organism |
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| **A** | Antimicrobials |
| **P** | Patient |
| **O** | Options |
| **P** | |

- Topical Therapy?
  - Chlorohexidine? Benzoyl peroxide? Dilute bleach baths?

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| **O** | Options |
| **P** | Plan |

- Chloramphenicol
  - 50 mg/kg PO TID for 3 weeks.
  - Baseline CBC to monitor for bone marrow suppression.
  - Treat UNDERLYING FOOD ALLERGY with strict diet.
  - Monitor for resolution of clinical signs; describe timeline progression to client.

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# Evaluation of SODAPOP

- Students on 4th year Small Animal Diagnostic Services Rotation were asked to voluntarily participate in an intervention-based study.
- Participants completed a pre-survey (Qualtrics), watched an instructional video on SODAPOP and then completed a post-survey.
- Evaluated students' perceived challenges in antimicrobial selection, confidence in antimicrobial selection and ability (in mock scenarios).

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# Student Opinion of SODAPOP

- 56.7 % of students agreed or strongly agreed that SODAPOP made them consider factors they don't usually consider.
- 86.7 % of students agreed or strongly agreed that they would use SODAPOP in the future.
- 93.3 % of students agreed that SODAPOP is a useful tool.

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# Future Directions

- Use in different student population (e.g. large animal students)
- Use earlier in curriculum (i.e. 2nd year).

- Use to evaluate veterinarians at various stages following graduation.
- This is a work in progress but early results are promising.

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