CHI's 5th annual Drug Discovery Chemistry

NVC-422, a Novel *N*-Chlorotaurine Derivative as Topical Antimicrobial

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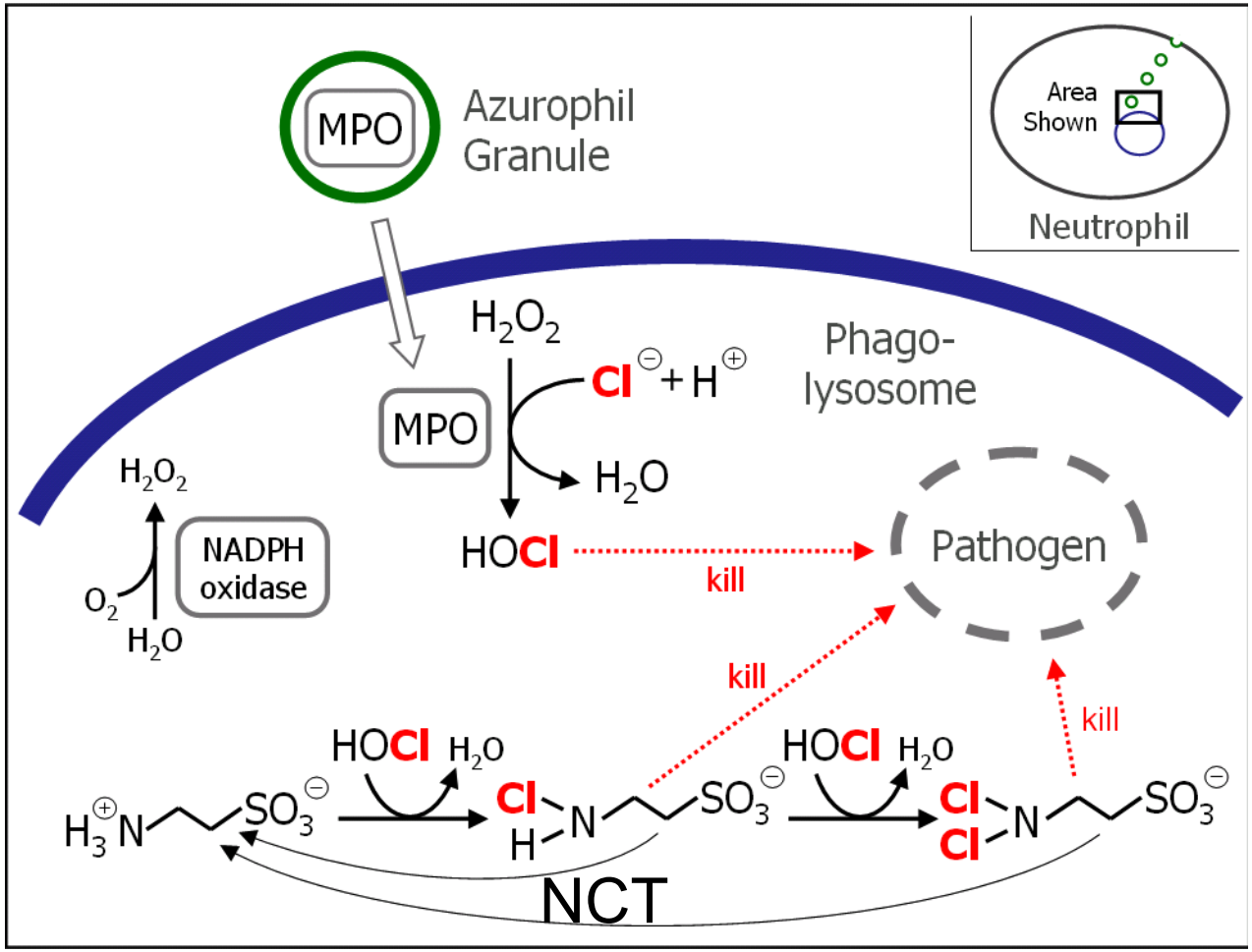
The Aganocide® Class of Antimicrobial Compounds

- Range of opportunities in topical indications
- **Taurine** present in neutrophils at high concentration
- **Chlorotaurines** formed transiently during phagocytosis
- Chlorotaurines have known antimicrobial properties
- Potential anti-inflammatory properties reported
- **Aganocides** are synthetic analogues of the chlorotaurines with broad spectrum antimicrobial activity and low potential for resistance

NVC-422 Update

- Currently in **Phase II** clinical trials (Acne, Impetigo, Viral Conjunctivitis)
- **Composition of matter IP**
- Stable analog of the natural antimicrobial dichlorotaurine
- Potent, fast acting broad spectrum non-antibiotic
- Effective against biofilm
- Our data suggest the **resistance unlikely**

Formation of N-chlorotaurines during phagocytosis



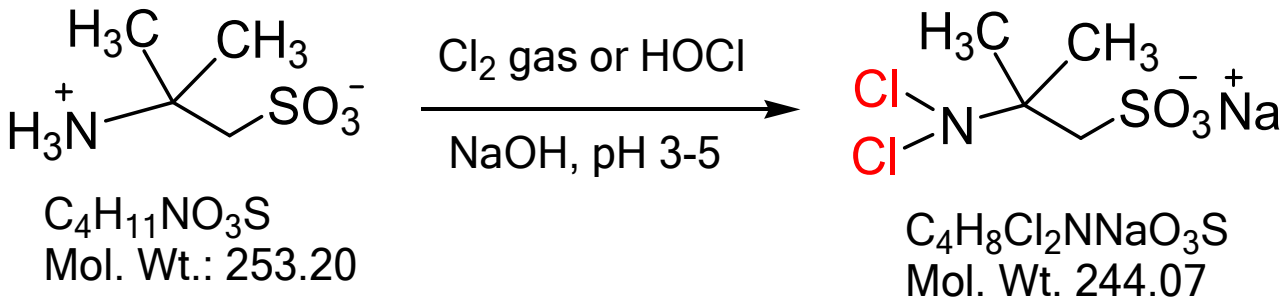
Presentation Outline

- NVC-422 synthesis and stability
- NVC-422 Broad spectrum of antimicrobial activity
- NVC-422 pH optimum
- Activity against biofilms
- NVC-422 resistance studies
- Mechanism of action

NVC-422 Synthesis

N,N-dichloro-2,2-dimethyltaurine (NVC-422)

(sodium 2-(dichloroamino)-2-methylpropane-1-sulfonate)



$C_4H_{11}NO_3S$
Mol. Wt.: 253.20

$C_4H_8Cl_2NNaO_3S$
Mol. Wt. 244.07

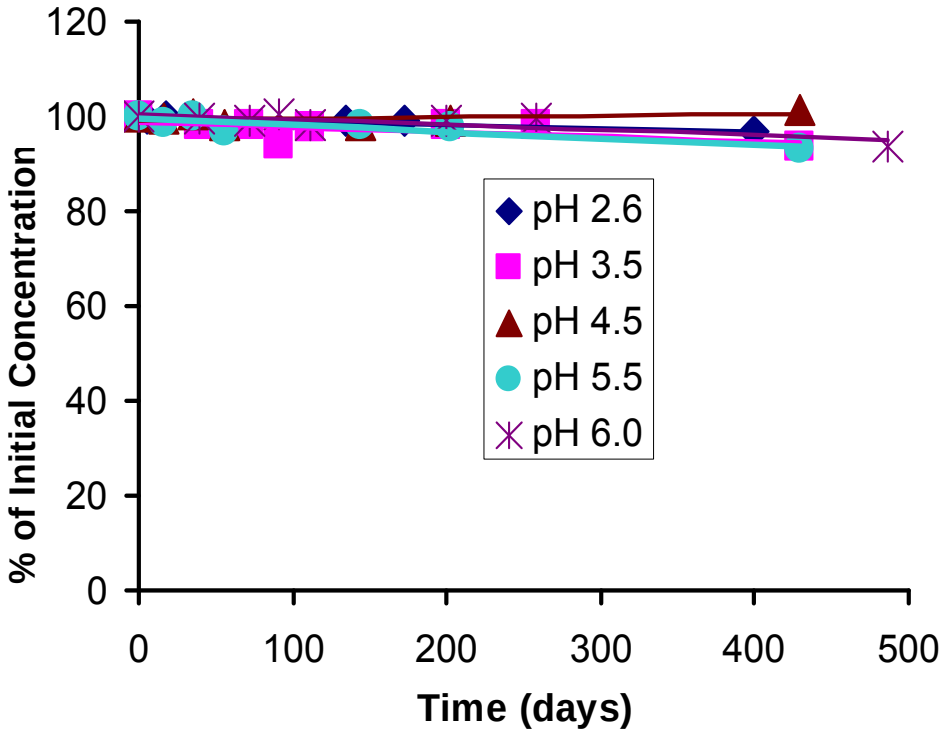
We have synthesized *N,N*-dichloro-2,2 –dimethyltaurine (NVC-422)
and show that NVC-422 has long-term stability and potent, rapid antimicrobial activity

Wang, ICAAC 2008

NVC-422 Solution Stability

$NVC\text{-}422]_{initial}$ = 2 mM, [NaCl] = 150 mM

Container: Borosilicate glass vial with Teflon/silicone liner cap



Stability of NVC-422 solutions in pH range from 2.6 to 6.0 at room temperature

Wang, ICAAC 2008

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NVC-422 has Broad Spectrum of Activity Against Bacteria and Yeast

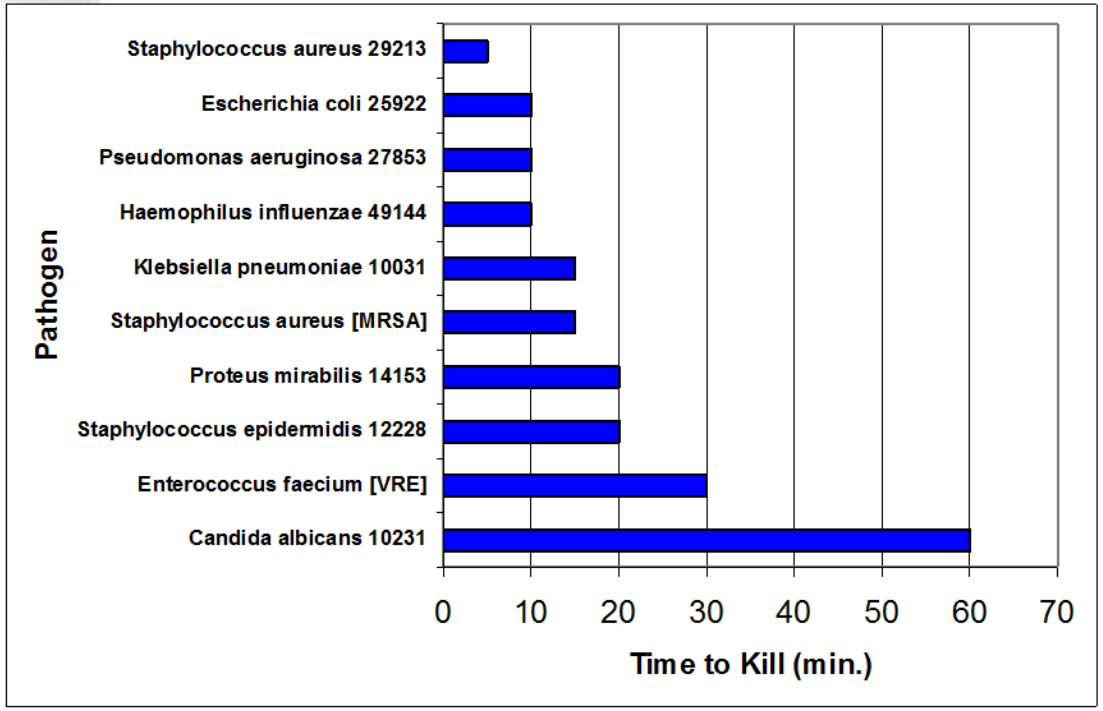
Pathogen	ATCC No.	MBC (µg/mL)
Acinetobacter baumanii	19606	4
Acinetobacter calcoaceticus	51432	2
Enterobacter aerogenes	51697	0.5
Enterococcus faecalis	29212	0.5
Enterococcus faecium [VRE]	51559	0.5
Escherichia coli	25922	2
Haemophilus influenzae	49144	0.5
Klebsiella pneumoniae	10031	0.25
Proteus mirabilis	29245	1
Pseudomonas aeruginosa	27853	1
Serratia marcescens	13880	1
Serratia marcescens	14756	2
Staphylococcus aureus	29213	2
Staphylococcus aureus	6538	2
S. aureus [MRSA]	33591	4
Staphylococcus epidermidis	12228	0.25
Staphylococcus hominis	27844	4
Staphylococcus sciuri	49575	0.12
Candida albicans	10231	32
Candida glabrata	90030	16

NVC-422 demonstrates broad spectrum antimicrobial activity. Gram positive and Gram negative bacteria including antibiotic resistance strains and yeast were shown to be susceptible to NVC-422 with a range of MBC values of 0.25 – 32 ug/mL(1 – 131 uM)

- **A fixed inoculum concentration of the microorganism of interest was tested against a variable range of antimicrobial test agent for 60 minutes at room temperature in saline, pH 4**
- **The MBC is defined as the lowest concentration of antibacterial compound resulting in >99.9% (3 logs) reduction in bacterial count**

Celeri, ICAAC 2008

NVC-422 Time-Kill at 1X MBC Against Representative Microorganisms



Kill times at concentrations of NVC-422 approximating the MBC were 5 to 30 minutes (rapid). *Candida albicans (eukaryote)*, had an MBC value of 32 µg/mL and a kill time of 60 minutes

In time kill (TK) experiments at a fixed antimicrobial test agent concentration, the rate of killing over time measured at the MBC or multiples thereof is determined over a 90 minute period at room temperature

Celeri, ICAAC 2008

NVC-422 Activity Against Antibiotics Resistant *S. aureus*

Organism	Study Isolate Id	Resistance phenotype					MBC
		OXA	MUP	LZD	VAN	DAP	
S. aureus	1674616 MRSA	R	S	-	S	S	1
S. aureus	1674625 MRSA	R	S	-	S	S	4
S. aureus	1674631 MRSA	R	S	-	S	S	2
S. aureus	1674634 MRSA	R	S	-	S	S	1
S. aureus	1674635 MRSA	R	S	-	S	S	1
S. aureus	1674604 MRSA, VISA	R	S	-	I	S	2
S. aureus	1674605 MRSA, VISA	R	S	-	I	S	2
S. aureus	1674612 MRSA, VISA	R	S	-	I	S	2
S. aureus	1674607 MRSA, Mup R	R	R	-	S	S	2
S. aureus	1674611 MRSA, Mup R	R	R	-	S	S	2
S. aureus	1744289 MRSA	R	S	-	S	NS	0.5
S. aureus	1744339 MRSA	R	S	-	S	NS	0.5
S. aureus	1744353 MRSA	R	S	-	S	NS	1
S. aureus	1744357 MRSA	R	S	-	S	NS	1
S. aureus	1674619 MSSA	S	S	-	S	S	1
S. aureus	1674624 MSSA	S	S	-	S	S	1
S. aureus	ATCC 29213 MSSA	S	-	S	S	S	2
S. aureus	1674606 MSSA, Mup R	S	R	-	S	S	1
S. aureus	1674608 MSSA, Mup R	S	R	-	S	S	2
S. aureus	1674610 MSSA, Mup R	S	R	-	S	S	1

Activity of NVC-422 against 20 antibiotic-resistant *S. aureus* strains was tested by MBC method and compared with activity against antibiotic-sensitive strains. NVC-422 was active against all tested clinical isolates.

R=Resistant; I=Intermediate; S=Sensitive
OXA = Oxacillin; MUP = Mupirocin; LZD = Linezolid; VAN = Vancomycin; DAP = Daptomycin ;

Debabov, ICAAC 2008

NVC-422 Activity Against Antibiotics Resistant Enterococci

Organism	Study Isolate Id	Resistance phenotype					MBC
		OXA	MUP	LZD	VAN	DAP	
E. faecalis	1674615 VRE	-	-	S	R	-	1
E. faecalis	1674617 VRE	-	-	S	R	-	1
E. faecalis	1674633 VRE	-	-	S	R	-	2
E. faecalis	1674646 VRE	-	-	S	R	-	1
E. faecalis	1674647 VRE	-	-	S	R	-	1
E. faecalis	1674614 VRE	-	-	S	R	-	4
E. faecalis	1674621 VSE	-	-	S	S	-	2
E. faecalis	1674632 VSE	-	-	S	S	-	4
E. faecalis	ATCC 29212 VSE	S	-	S	S	S	1
E. faecium	1674609 VRE	-	-	R	R	-	2
E. faecium	1674613 VRE	-	-	R	R	-	2
E. faecium	1674618 VRE	-	-	S	R	-	2
E. faecium	1674620 VRE	-	-	S	R	-	2
E. faecium	1744269 VRE	-	-	S	R	NS	1
E. faecium	1744278 VRE	-	-	S	R	NS	0.5
E. faecium	1744280 VRE	-	-	S	R	NS	1
E. faecium	1744284 VRE	-	-	S	R	NS	0.5
E. faecium	1744287 VRE	-	-	S	R	NS	1
E. faecium	ATCC 51559 VRE	R	-	S	R	S	1

Activity of NVC-422 against 18 antibiotic-resistant enterococci was tested by MBC method and compared with activity against antibiotic-sensitive strains. NVC-422 was active against all tested clinical isolates.

R=Resistant; I=Intermediate; S=Sensitive
OXA = Oxacillin; MUP = Mupirocin; LZD = Linezolid; VAN = Vancomycin; DAP = Daptomycin ; ;

Debabov, ICAAC 2008

NVC-422 Activity Against Gram-negative Antibiotics Resistant Strains

Organism	Study Isolate Id	Resistance phenotype					MBC
		IMI	FEP	CIP	GEN	P/T	
A. baumannii	1674622 MDR	R	R	R	R	R	0.5
A. baumannii	1674627 MDR	R	R	R	R	R	1
A. baumannii	1674628 MDR	R	R	R	R	R	1
A. baumannii	1674640 MDR	R	R	R	R	R	1
A. baumannii	1674641 MDR	R	R	R	R	R	1
A. baumannii	ATCC 19606	S	S	S	R	S	2
E. coli	1674626 MDR	S	R	R	R	R	1
E. coli	1674645 MDR	S	S	R	S	S	2
E. coli	1674630 FQ R	S	S	R	S	S	1
E. coli	1674642 FQ R	S	I	R	R	I	1
E. coli	1674643 ESBL	S	R	R	R	S	2
E. coli	1674644 ESBL	S	R	R	S	R	1
E. coli	ATCC 25922	S	S	S	S	S	2
P. aeruginosa	1674623 MDR	R	R	R	R	R	2
P. aeruginosa	1674638 MDR	R	S	R	S	S	2
P. aeruginosa	1674639 MDR	R	S	R	S	S	1
P. aeruginosa	1674629	R	R	R	R	R	2
P. aeruginosa	1674637	R	R	R	R	R	2
P. aeruginosa	ATCC 27853	S	S	S	S	S	1

Activity of NVC-422 against 19 antibiotic-resistant and MDR Gram-negative pathogens was tested by MBC method and compared with activity against antibiotic-sensitive strains. NVC-422 was active against all tested clinical isolates.

Daptomycin; IMI = Imipenem; FEP = Cefepime; CIP = Ciprofloxacin; GEN = Gentamicin; P/T = Piperacillin / Tazobactam

Debabov, ICAAC 2008

NVC-422 is Active Against Antiseptic-Resistant Strains

- 7 strains positive for qacA/B presence were tested against several antiseptics and NVC-422
- Most strains showed resistance to chlorhexidine, and all strains were resistant to pentamidine, and proflavine, confirming results reported in literature*
- All **antiseptic resistant** MRSA strains tested had the same MBC value as *S. aureus* 29213 or lower

Organism	MIC			MBC (µg/mL)
	Chlorhexidine	Pentamidine	Proflavine	NVC-422 pH 4
Reported MIC *(S. aureus)*	0.8	<50	40	ND
S. aureus ATCC 29213	0.8	12.5	25	2
MRSA 1974180	1.6	200	100	1
MRSA 1974181	1.6	200	200	1
MRSA 1974186	1.6	200	200	1
MRSA 1974189	1.6	200	200	2
MRSA 1974193	0.8	200	200	1
MRSA 1974194	1.6	200	200	1
MRSA 194214	1.6	200	100	2

*McDonnell et al 1999. Antiseptics and Disinfectants: Activity, Action and Resistance. Clinical Microbiology Reviews, Jan. 1999, p.147-199

Zuck, ICAAC 2009

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- NVC-422 mechanism of action

NVC-422 Activity: Effect of pH on MBC

Activity of NVC-422 is pH dependent for both *S. aureus* and *E. coli*: as the pH increases the MBC increases

	MBC (µg/ml)	
	S. aureus ATCC 29213	*E.coli* ATCC 25922
	NVC-422	NVC-422
pH 4.0	1	1
pH 4.7	2	2
pH 5.4	8	4
pH 5.9	16	8
pH 7.4	256	512

Diluents alone had no antibacterial activity

Wang, ICAAC 2008

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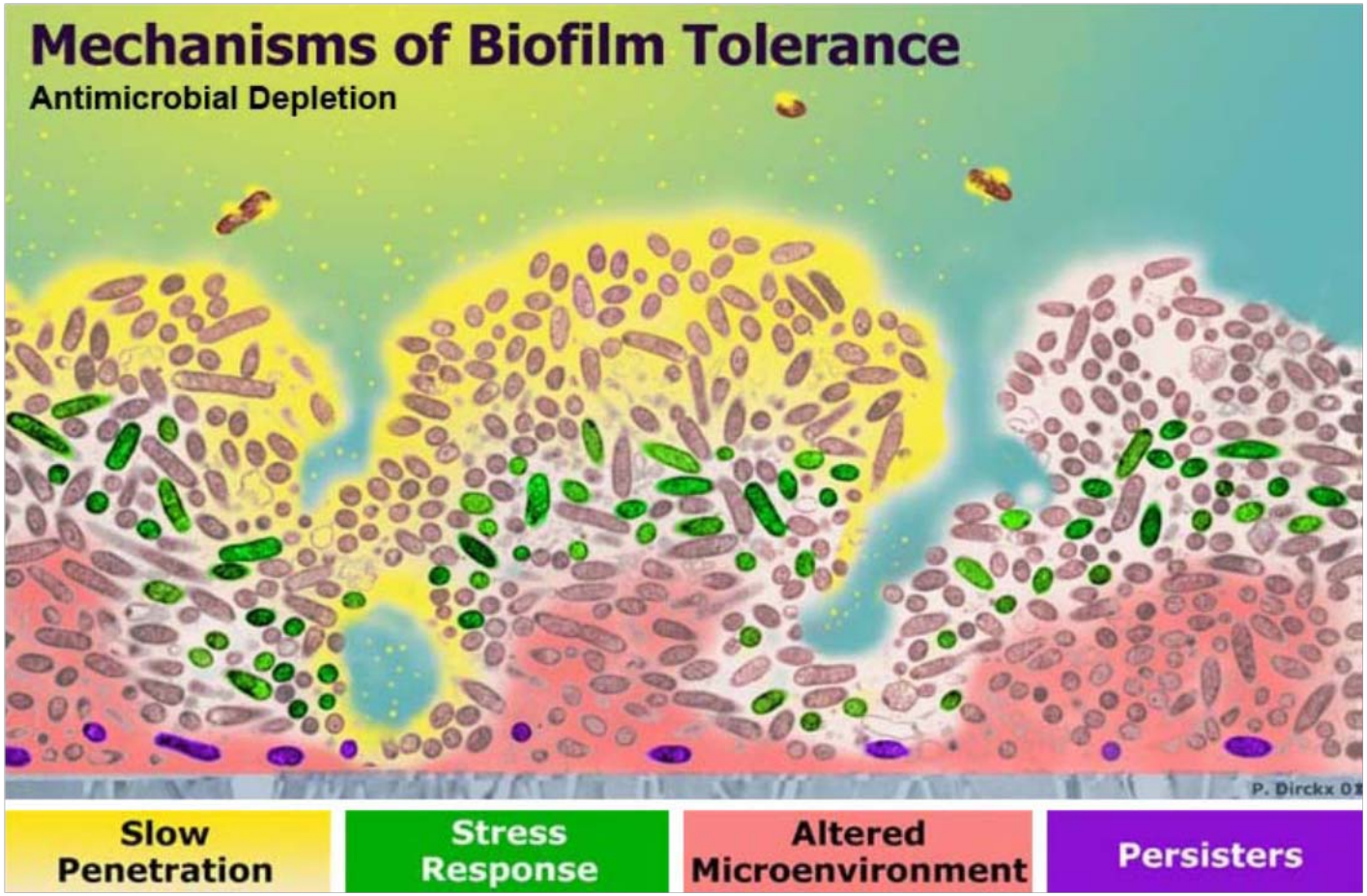
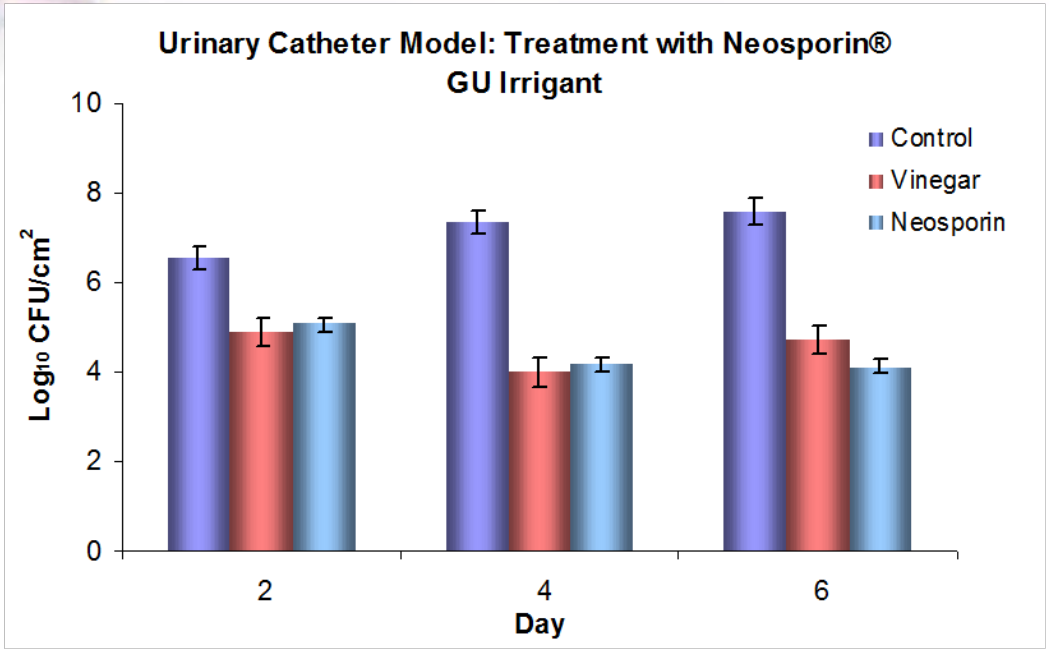
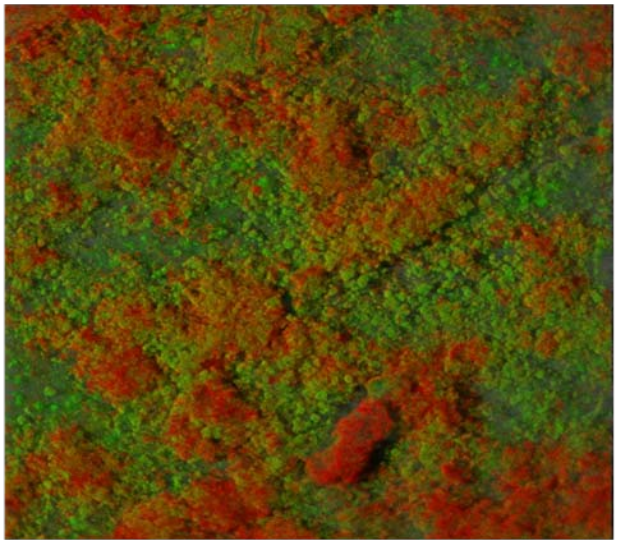
Bacteria in Biofilms are Resistant to Antibiotic Treatment

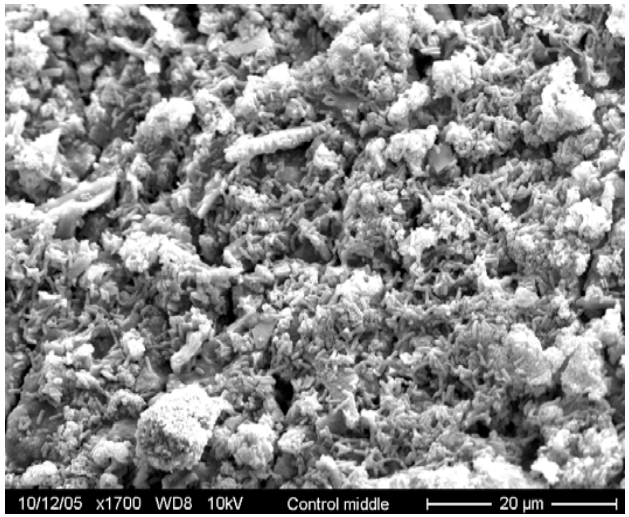


Biofilms form on **contact lens, urinary catheters, CVC catheters** and other devices

Neosporin and Vinegar have Only Minor Effect on Bacterial Biofilm in Urinary Catheter Model



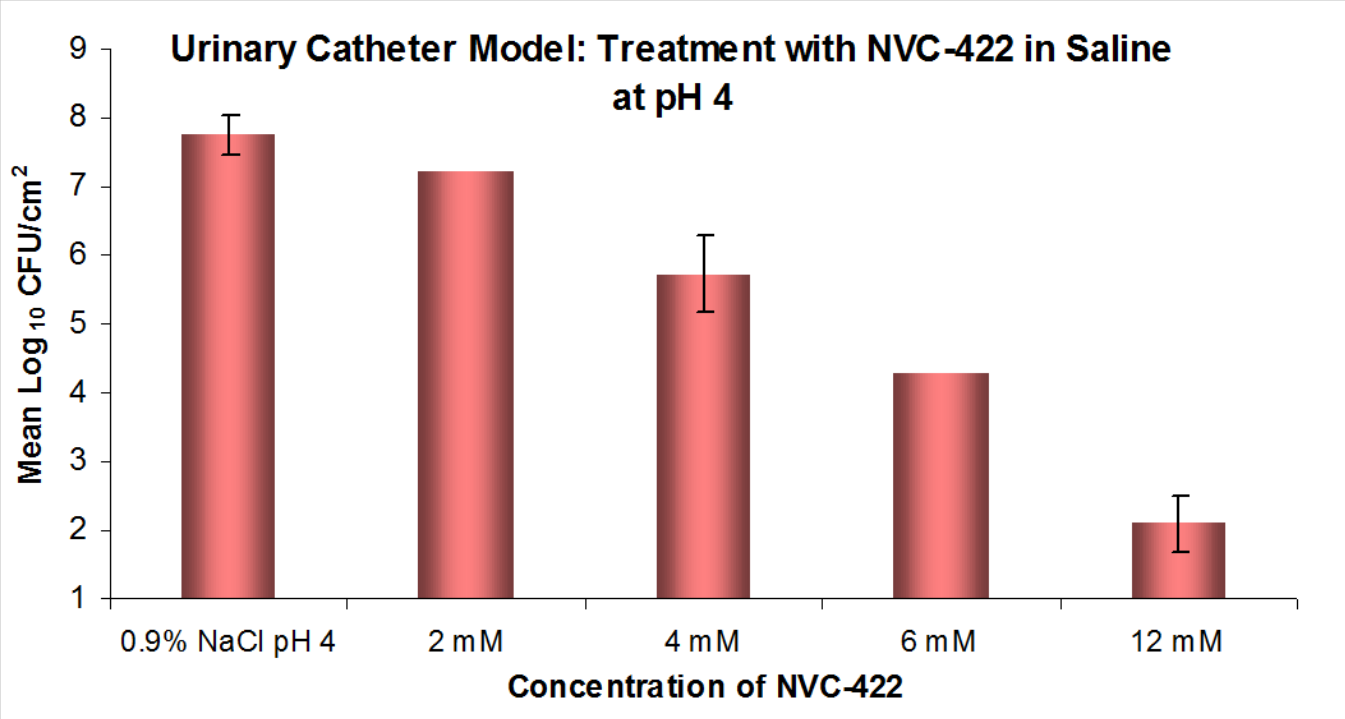




Activity of Neosporin (a mixture of Bacitracin, Neomycin and Polymixin B) against *E. coli* biofilm grown in Foley catheters. Catheters were treated for 40 mins on days 2, 4, and 6. Biofilm reduction of 1-3 logs was observed.

Rani, ASM 2008

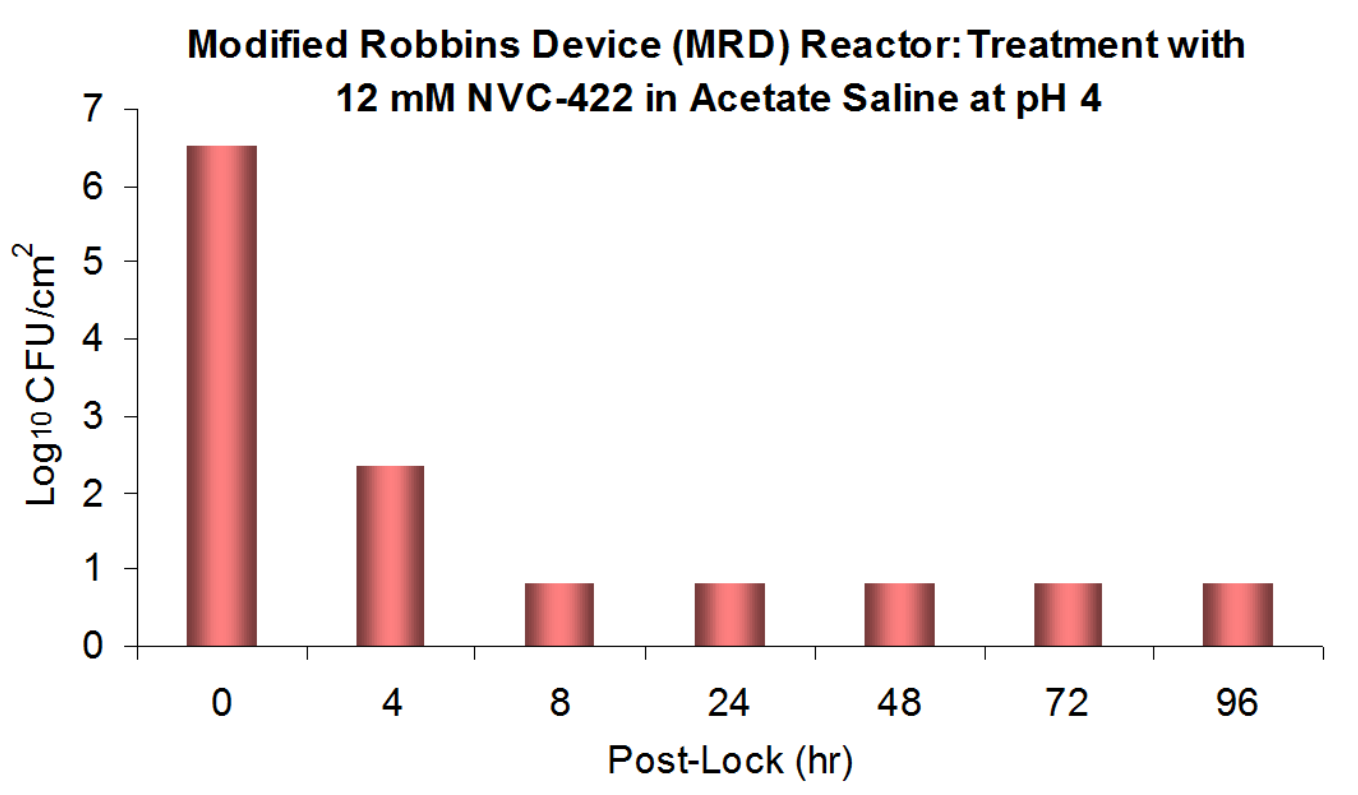
NVC-422 Action on Bacterial Biofilm in Urinary Catheter Model



Activity of NVC-422 against *E. coli* biofilm grown in Foley catheters. Catheter sections were treated for 60 mins. Biofilm reduction of up to 6 logs was observed.

Rani, ICAAC 2008

NVC-422 Action on Bacterial Biofilm in Modified Robbins Device (MRD) Reactor



Activity of NVC-422 as a catheter lock solution against *S. aureus* biofilm grown in MRD reactor. After 4 hr of NVC-422 as a lock solution, biofilm reduction of up to 4 logs was observed.

Rani, ASM Biofilm 2009

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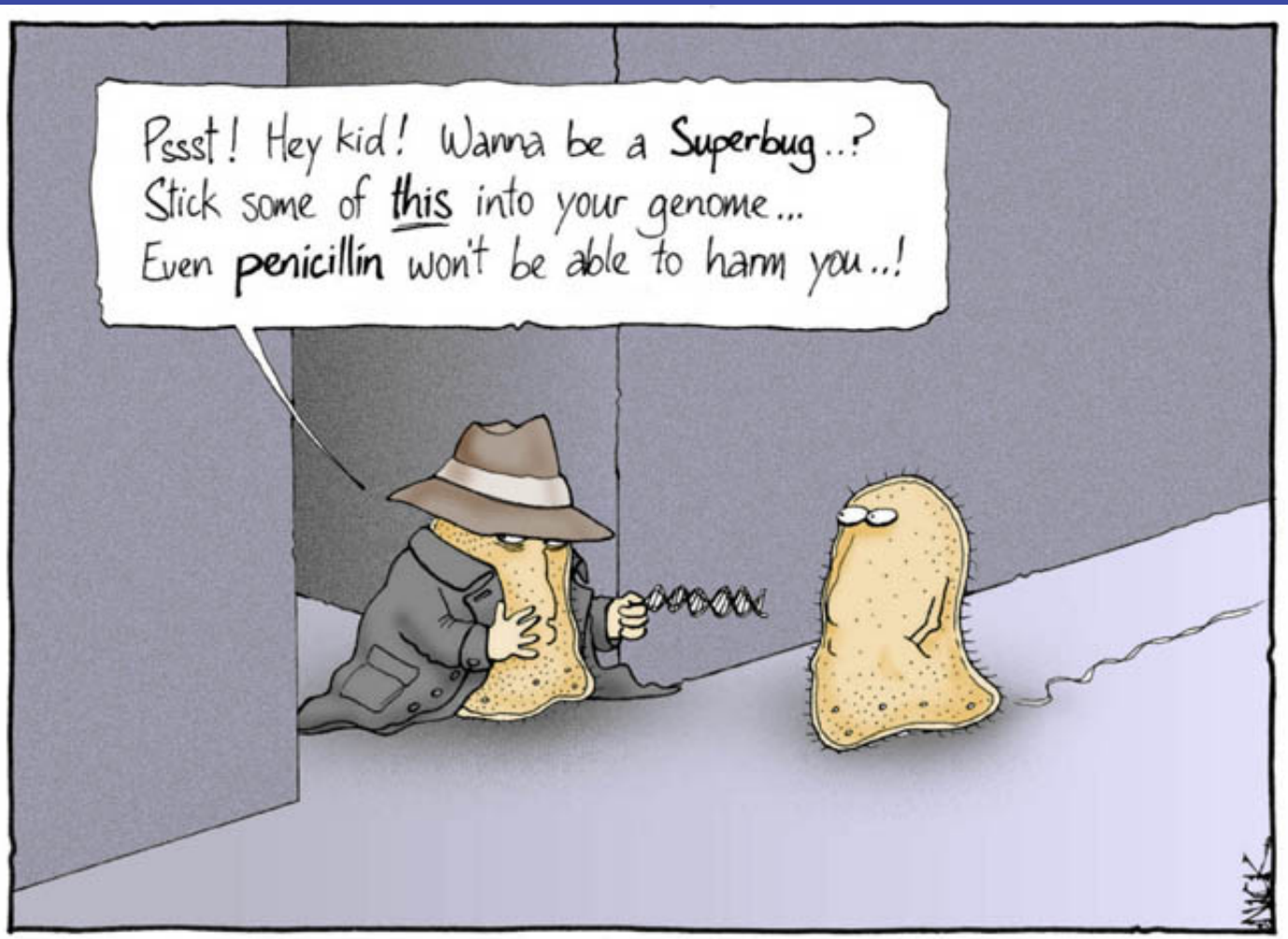
Antibiotic Resistance is a Growing Problem



It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

Why Do a Resistance Study? Why does it Matter?

- Most classes of antibiotics and some antispetics select for resistance

- To investigate the potential mechanism of resistance one would have to design an in-vitro study that will create **artificial resistance**

- In the case of antibiotics, bacteria are grown in the medium with **sublethal** concentration of antibiotic

- After several **passages** one will observe an increase in the MIC of the surviving colonies

- An antimicrobial candidate for clinical trials must undergo testing for this potential prior to it becoming widely available.

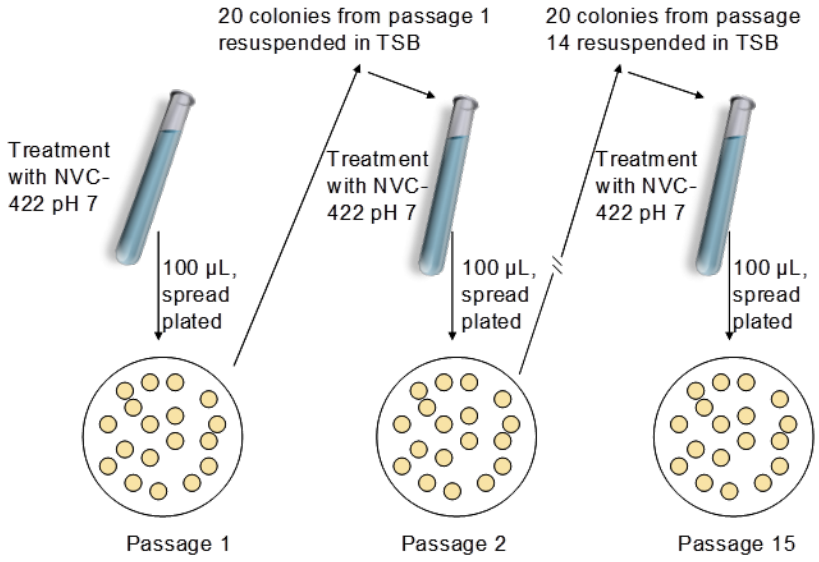
NVC-422: The Result of 15 Passages Indicate no MBC Creep

Goal: To determine if bacteria acquire resistance to NVC-422 over multiple treatments using the method of Nagl et al.*

Method:

- *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922) are treated with a sub-lethal concentration of 1mM NVC-422 (pH 7) and dilutions are plated
- 20 surviving colonies are sub-cultured and treated again. After 15 passages, surviving colonies are tested in the 96-well MBC with ATCC control to determine if resistance has occurred.

15 Passages



***M. Nagl and W. Gottardi. Enhancement of the bactericidal efficacy of N-Chlorotaurine by Inflammation Samples and Selected N-H compounds. Hyg Med 1996; 21: 597-605.**

Debabov, ICAAC 2008

NVC-422: After15 Passages no MBC Creep *was Found*

Organism	MBC (µg/mL)	
	Pre-passage	15 passages
S. aureus ATCC 29213	512	256
E. coli ATCC 25922	256	128-256

- For both *E. coli* and *S. aureus,* there was no increase in MBC observed after repeated sub-lethal dosing of NVC-422 pH 7.

Debabov, ICAAC 2008

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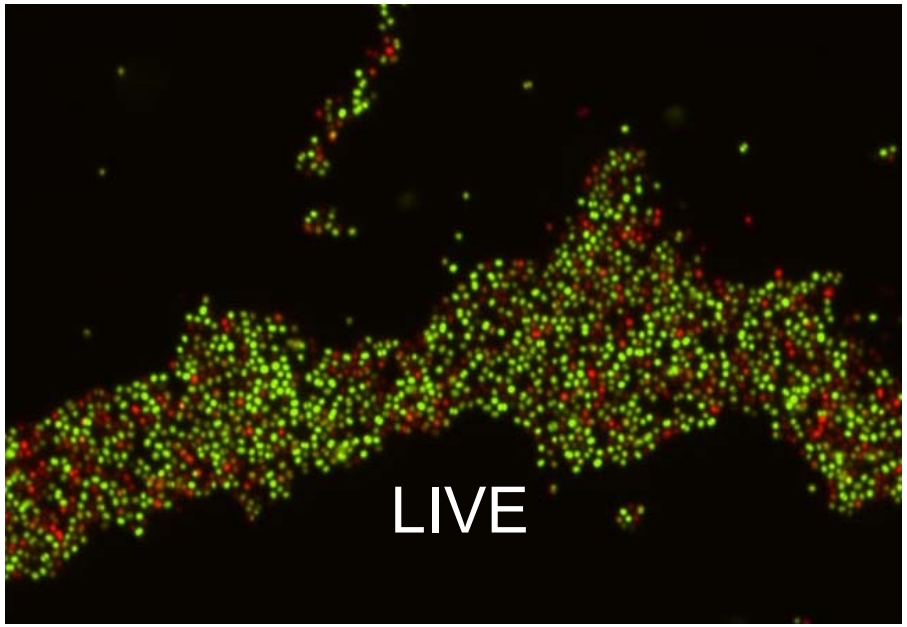
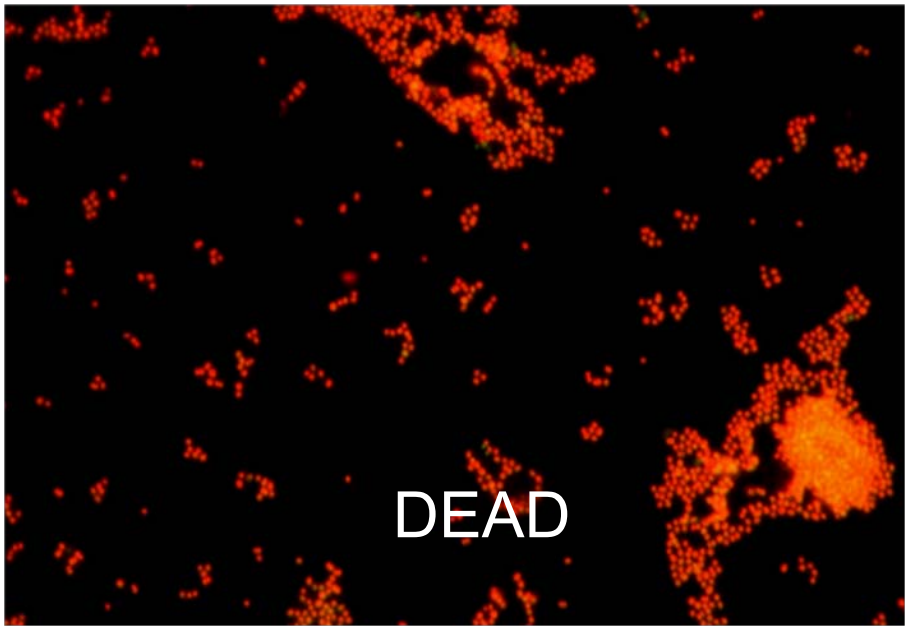
NVC-422 Mechanism of action

- Antibiotics are enzyme specific and targeted drugs
- Aganocides target and selectively oxidize key components of important enzymes; e.g. oxidize thiols and methionines affecting microbial cell-regulatory pathways
- Antibiotics in use today develop microbial resistance
- Aganocides do not show microbial resistance
- Aganocides have been shown to be well-tolerated by human tissues
- NVC-422 oxidized key compents resulting in membrane permeability in *S. aureus* (shown on the next slide)

Detection of *S. aureus* Killing by NVC-422 Using Fluorescent Microscopy with BacLight (Propidium Iodide / Syto 9) Bacterial Cell Viability Kit





Untreated (control) MRSA 33591 cells stain mostly with Syto-9 (**green**) that penetrates intact membranes and binds DNA.

After 10 minutes of treatment with 8 µg/ml of NVC-422, cells stain also with Propidium Iodide (**red**) that does not penetrate membranes unless they are damaged.

Acknowledgments

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