The Singapore Eye Research Institute

# Breaking Down barriers to promote effective antibiotic action

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**Disclaimer**

Multiple Patents in Area

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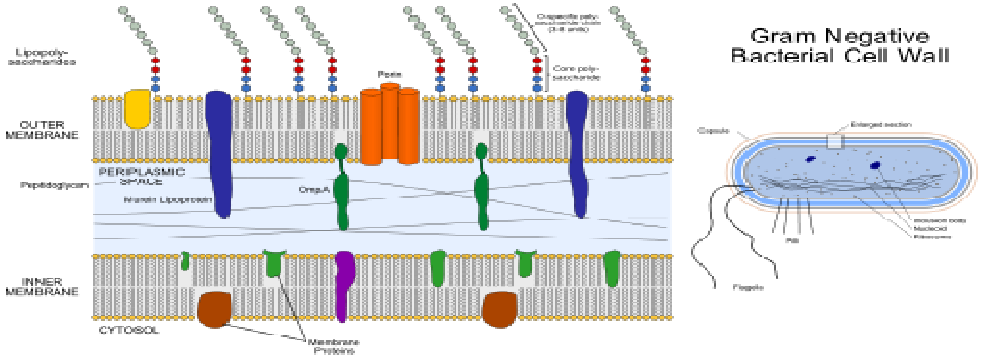
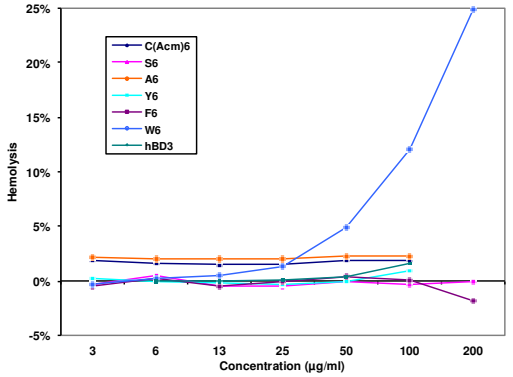
Consultant-Allergan

# All Bacteria have a Common Weakness,…their inner membrane which is markedly different than the human cell membrane

The so-called natural antibiotics-the **defensin**s have used this weakness for millions of years to kill Gram negative and Gram positive pathogens (also virus and fungus) with out developing resistance.

But they just provide some coverage and in therapeutic concentrations can be inflammatory or toxic to human cells



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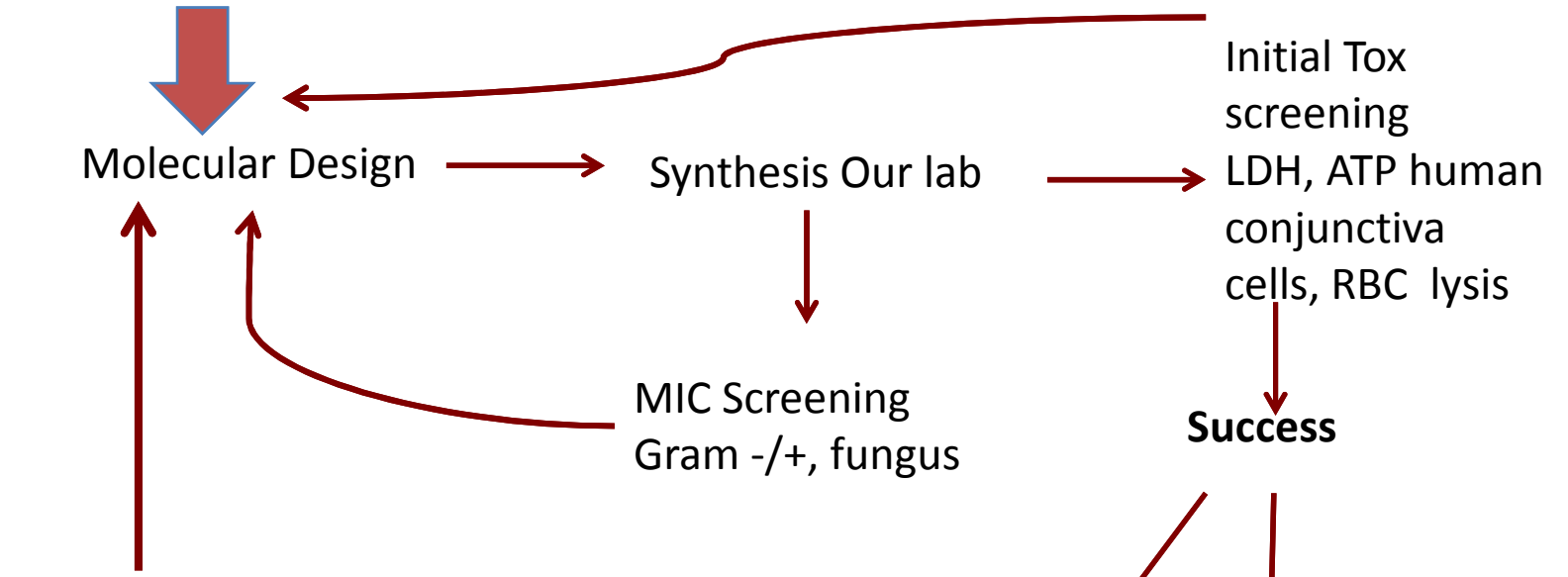



# Developmental Path- Antibiotics Targeting the Bacteria/Fungal Membrane

# MIC assay

## Extended Gram Negative Organisms

| | Test Organism | $MIC_{99}$ Value (µg/ml) |
|---|---|---|
| 1 | ***E.Coli* ATCC 25922** | **3.125** |
| 2 | ***E.Coli* ATCC 8739** | **3.125** |
| 3 | ***Klebsiella pneumoniae* ATCC 10031** | **3.125** |
| 4 | ***Serratia marcescens* ATCC 8100** | **6.25** |
| 5 | ***Citrobacter koseri* DM4432** | **6.25** |
| 6 | ***Proteus vulgaris* DM 4635** | **25** |

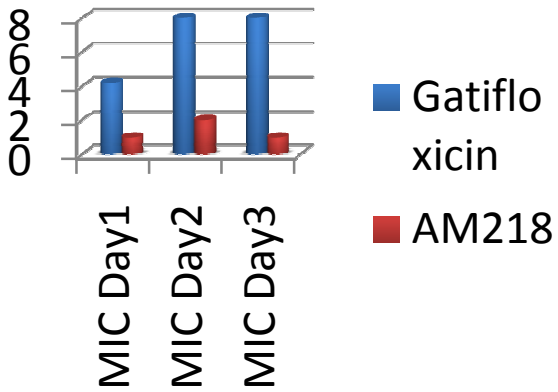
| Organism | MIC (µg/ml) SinSa | MIC (µg/ml) Gatifloxacin |
|---|---|---|
| *Pseudomonas aeruginosa* (DR 4877/07)-sputum | 12.5 | 125 |
| *Pseudomonas aeruginosa* (ATCC 9027) | 12.5 | 0.39 |
| *Pseudomonas aeruginosa* (ATCC 27853) | 6.25 | 1.56 |
| *Pseudomonas aeruginosa* (DM 23257)-eye | 12.5 | 0.78 |
| *Pseudomonas aeruginosa* (DR 5790/07 )-wound | 12.5 | 25 |
| *Pseudomonas aeruginosa* (DM 4150 R)-NA | 6.25 | 0.78 |
| *Pseudomonas aeruginosa* (DR 18531)-NA | 6.25 | 3.125 |
| *Pseudomonas aeruginosa* (DM 23104)-eye | 6.25 | 1.56 |
| *Pseudomonas aeruginosa* (DM 23155)-eye | 6.25 | 1.56 |
| *Pseudomonas aeruginosa* (DM 15013)-wound | 3.125 | > 50 |

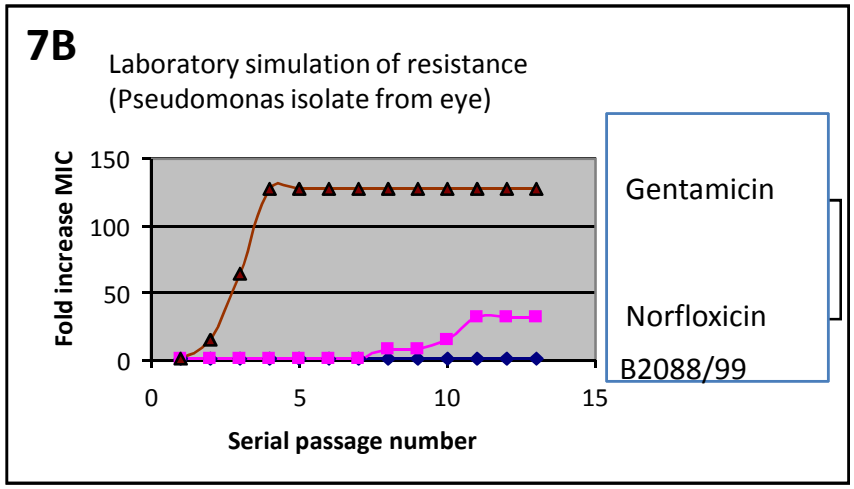
Zhou L, Liu SP, Chen LY, Li J, Ong LB, Guo L, Wohland T, Tang CC, Lakshminarayanan R, Mavinahalli J, Verma C, Beuerman RW. The structural parameters for antimicrobial activity, human epithelial cell cytotoxicity and killing mechanism of synthetic monomer and dimer analogues derived from hBD3 C-terminal region. Amino Acids. 2011 Jan;40(1):123-33.

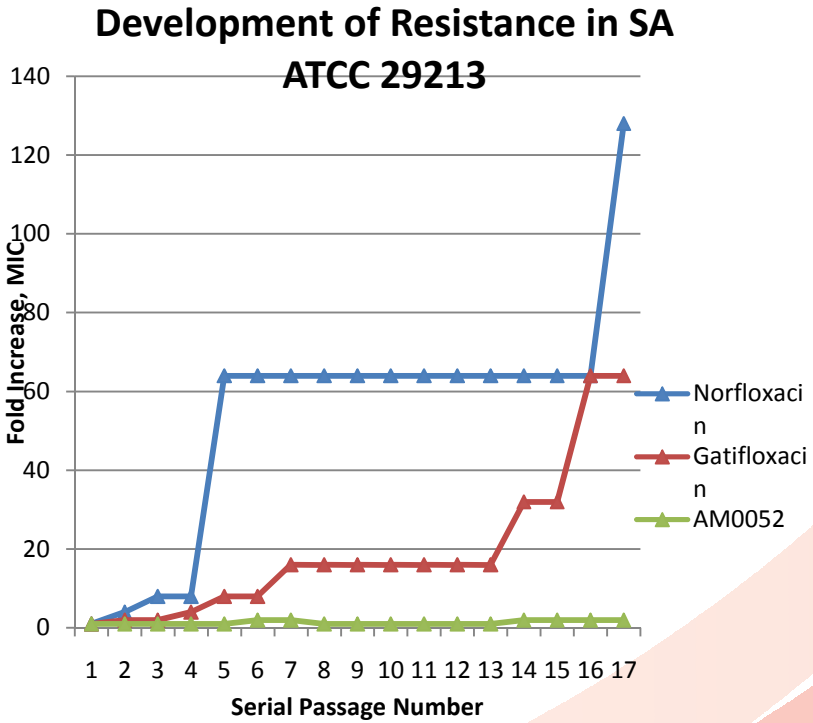
# Averting Resistance-

Simulation in the Laboratory and Induced During treatment of an experimental infection of the Mouse Cornea

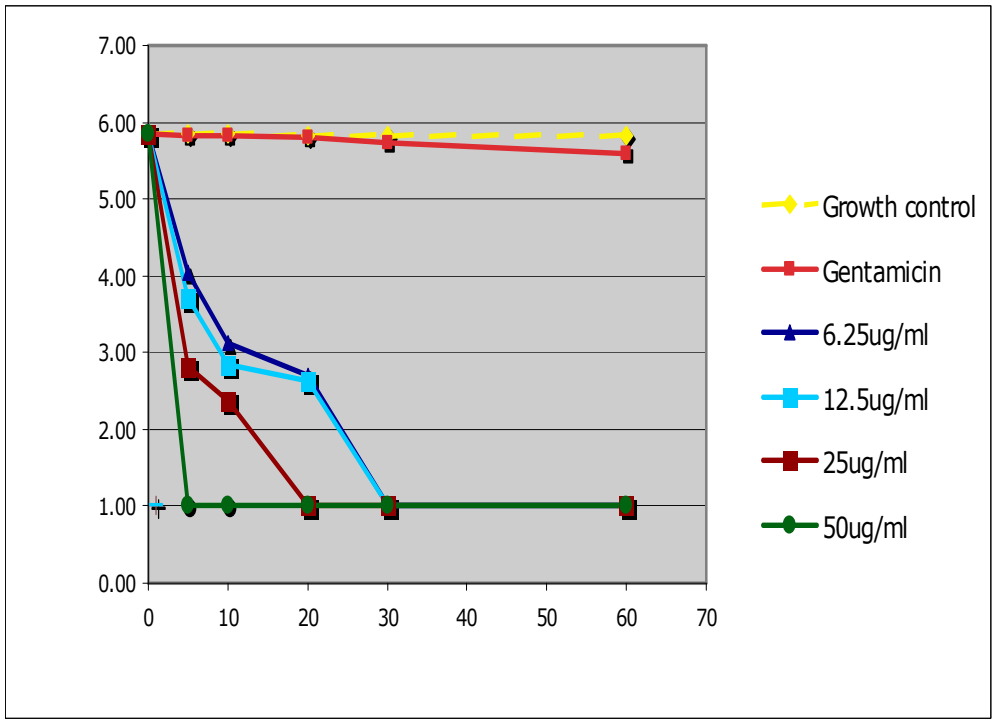
**Treatment induced resistance**
Gatifloxicin MIC increased 4-8X



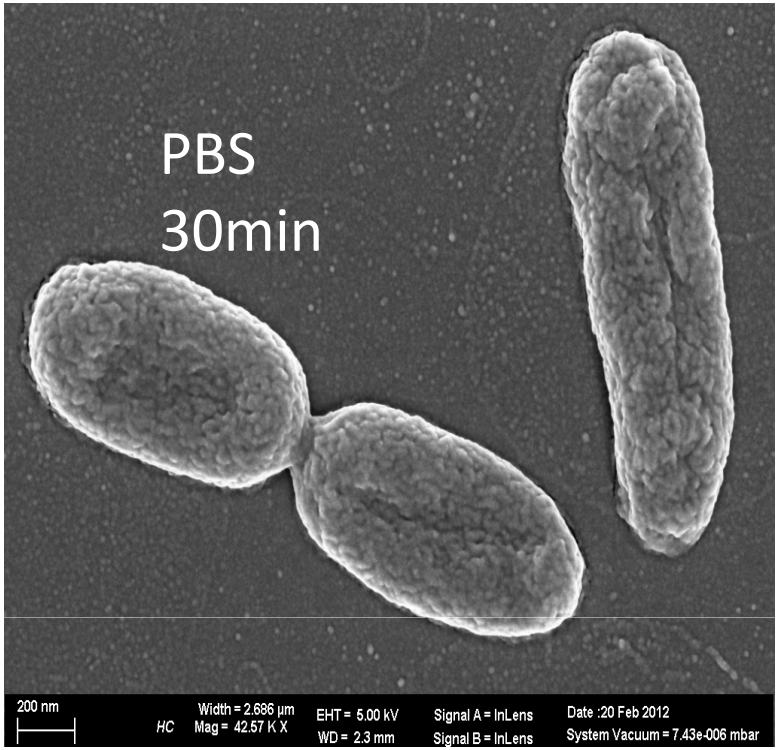



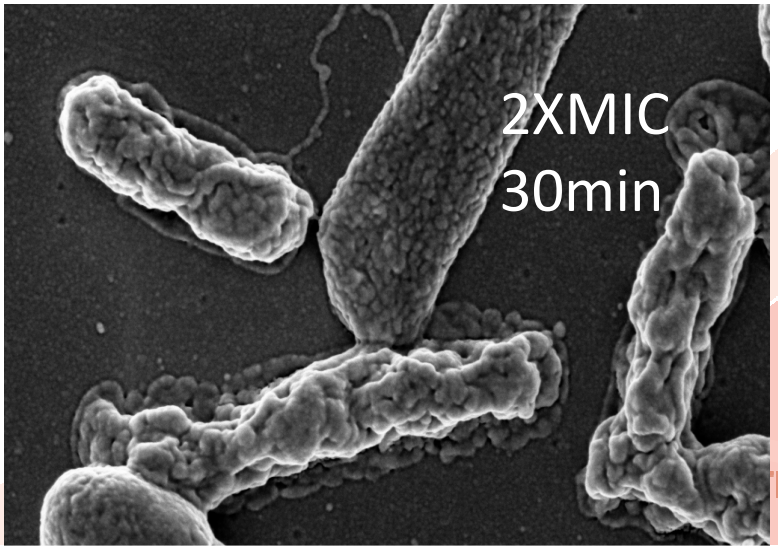



# Rapid Killing-V2D

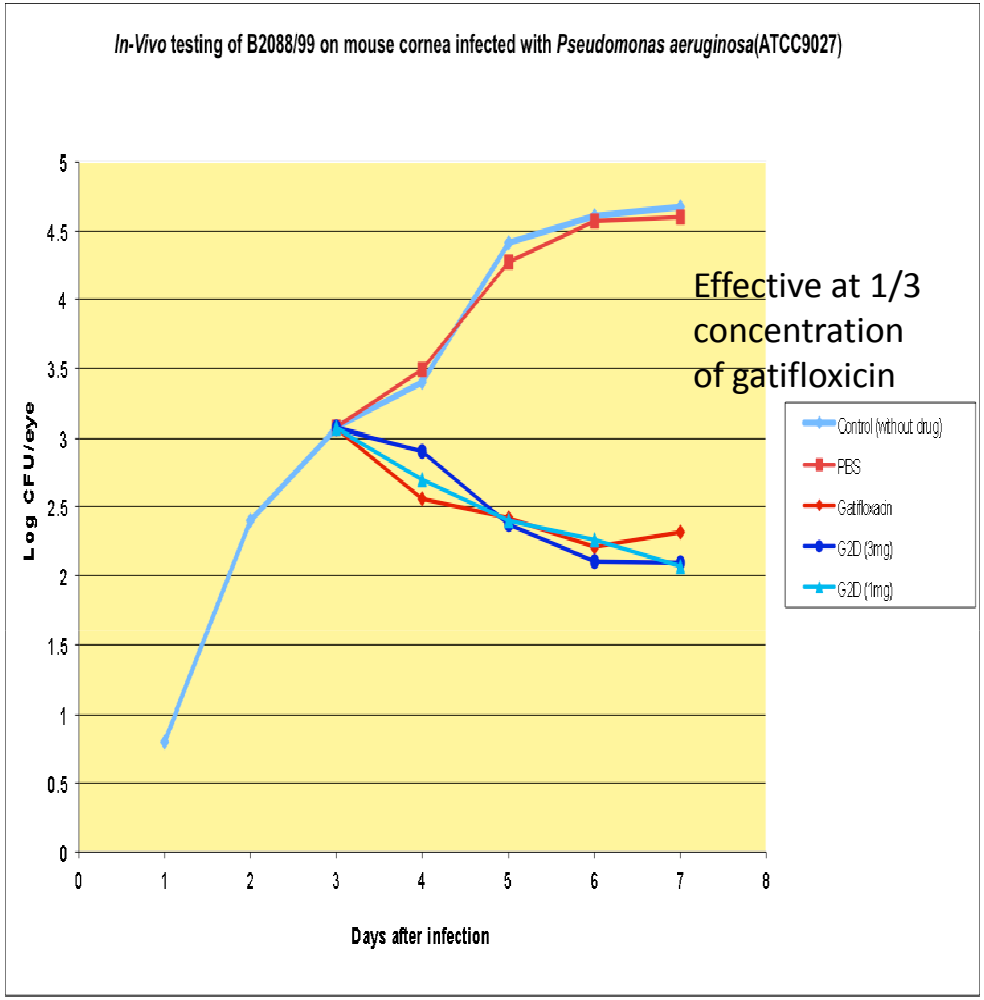


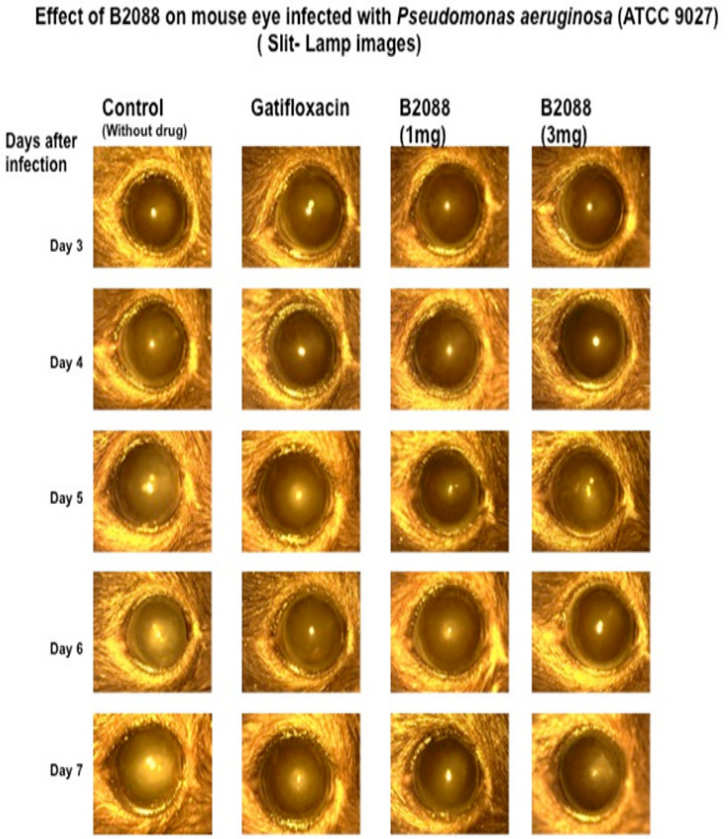

***Pseudomonas aeruginosa* ATCC 9027**




Design and synthesis of amphiphilic xanthone-based, membrane-targeting antimicrobials with improved membrane selectivity.
Zou H, Koh JJ, Li J, Qiu S, Aung TT, Lin H, Lakshminarayanan R, Dai X, Tang C, Lim FH, Zhou L, Tan AL, Verma C, Tan DT, Chan HS, Saraswathi P, Cao D, Liu S, **Beuerman** RW.
J Med Chem. 2013 Mar 28;56(6):2359-73. Compared vancomycin and daptomycin




# Mouse model of Corneal Infection

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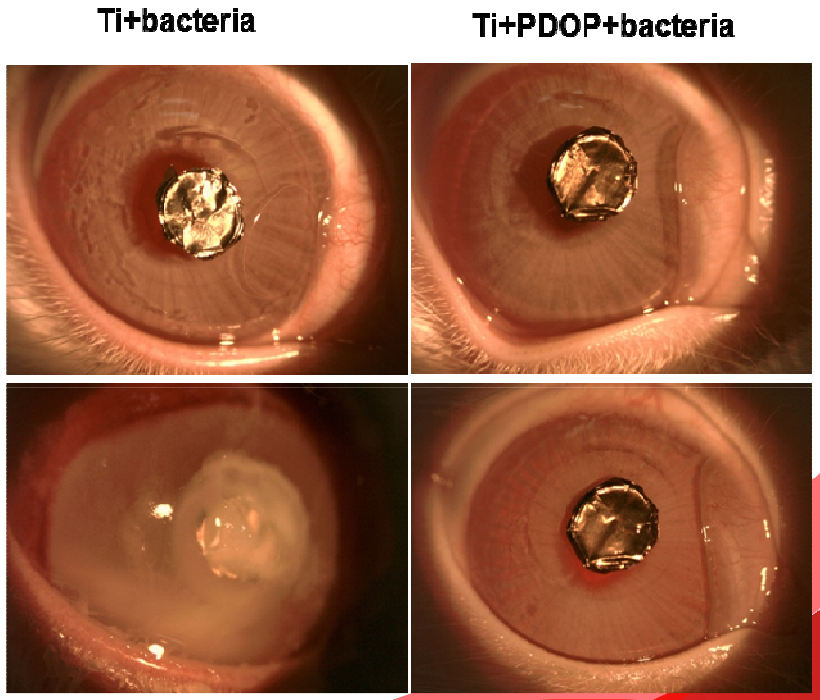
- Antimicrobial coating for medical devices.
  - E.g keratoprostheses devices for the eye and other implants
  - Effective with Pseudomonas and Staph
  - More effective than an aggressive antibiotic strategy

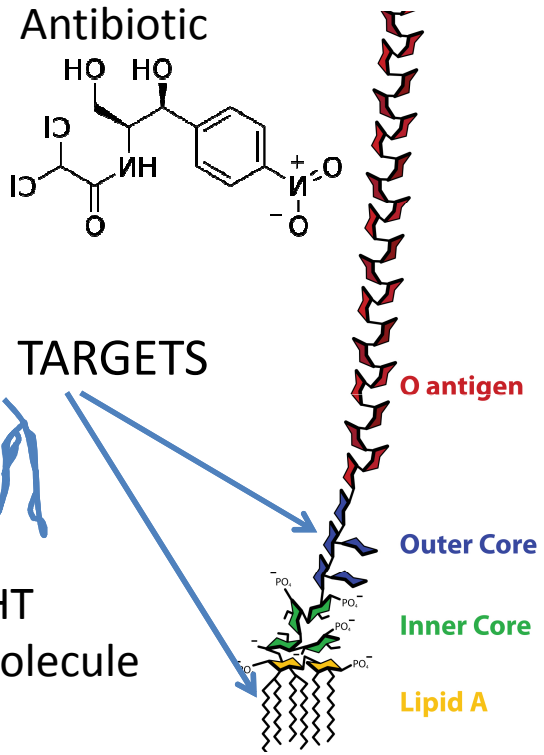


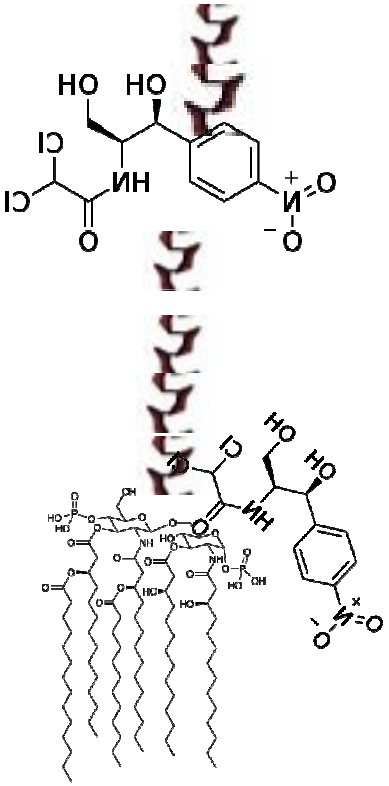
# FAS TECHNOLOGY

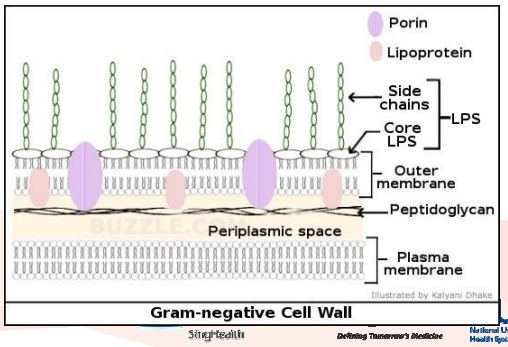
Facilitated Antibiotic Synergism, FAS, using Current Antibiotics, FAS Acts at submicrogram, sub MIC values to increase activity of existing antibiotics even on resistant forms of Pseudomonas and CREs , the carbapenem-resistant Enterobacteriaceae, such as E. coli and K. pneumoniae

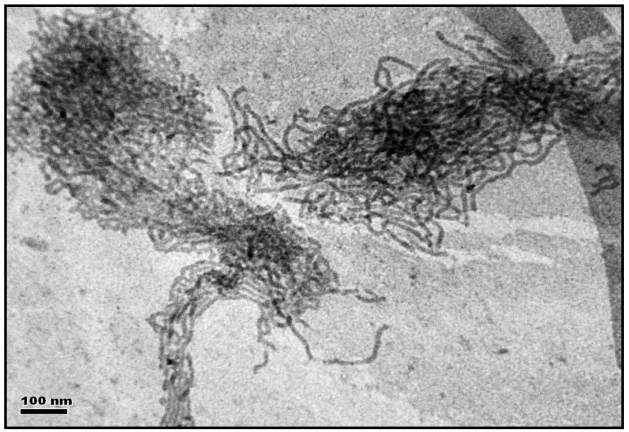
FAS Technology helps to avoid resistance: kills bugs faster and at a lower concentration of antibiotic

**SpearHead Technology**: Molecules designed by SinSa breakdown the polyanionic LPS barrier and allow entry of antibiotics. Antibiotics tend to be either hydrophobic or large hydrophilic molecules.

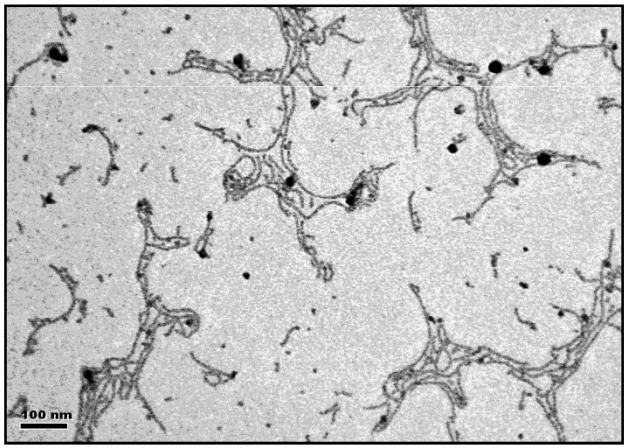





**LPS: Without SHT**




**LPS: After SHT)**

The Gram negative cell wall, at left is protected by LPS, seen above expanded. The porins allow only small hydrophilic molecules to pass into the cytoplasm.

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# FAS Technology

Effective with Different Classes of Antibiotics

Chloramphenicol
Erythromycin
Gatifloxicin
Ciprofloxicin
Gentamycin
Kanamycin
Imipenam
Tobramycin

We have developed several different classes of molecules that are effective when paired with these antibiotics

# Summary of Synergistic Action with other Antibiotics

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| Antibiotics | Class | Target | MIC of antibiotics, µg/mL | MIC (µg/mL) in the presence of B2088 | | | | FICI in the presence of B2088 | MIC in the presence of B2088_99 | | | | FICI in the presence of B2088_99 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ½ × | 1/4× | 1/8× | 1/16× | | ½ × | 1/4× | 1/8× | 1/16× | |
| **Carbenicillin** | Penicillins | Cell wall synthesis | 1600 | 200 | 800 | - | - | **0.63** | 400 | - | - | - | **0.75** |
| **Chloramphenicol** | | Protein Synthesis (23S rRNA) | 200 | 12.5 | 25 | 50 | - | **0.38** | 6.25 | 25 | 50 | - | **0.38** |
| **Erythromycin** | Macrolides | Protein Synthesis (23S rRNA | 400 | 50 | 100 | 200 | - | **0.5** | 50 | 200 | - | - | **0.63** |
| **Gatifloxacin** | Fluoroquinalones | DNA replication: topoisomerases, gyrase and topo IV | 31.25 | 7.8 | 15.6 | 15.6 | 15.6 | **0.56** | 7.8 | 15.6 | - | - | **0.75** |
| **Gentamycin[a]** | Aminoglycoside | Protein synthesis: 16S rRNA | 0.39 | 0.024 | 0.098 | 0.195 | - | **0.5** | 0.0243 | 0.098 | 0.195 | - | **0.5** |
| **Tobramycin** | Aminoglycoside | Protein synthesis: 16S rRNA | 400 | 50 | 200 | - | - | **0.63** | 200 | - | - | - | **1.0** |
| **Imipenam** | β-lactams | Cell-wall synthesis: multiple penicillin binding proteins (PBPs) | 0.78 | 0.098 | 0.195 | 0.39 | - | **0.5** | 0.098 | 0.39 | - | - | **0.63** |
| **Kanamycin** | Aminoglycoside | Protein synthesis: 16S rRNA | 3200 | 400 | 800 | 1600 | 1600 | **0.5** | 800 | 1600 | 1600 | - | **0.63** |
| **Nalidixic Acid** | Quinalones | DNA replication: topoisomerases, gyrase and topo IV | 3200 | 400 | - | - | - | **0.56** | 800 | - | - | - | **0.75** |
| **Streptomycin** | Aminoglycosides | Protein synthesis: 16S rRNA | 200 | 50 | 100 | 100 | - | **0.63** | 50 | 100 | - | - | **0.75** |

**[a]For gentamycin *P. aeruginosa ATCC 9027* was used to determine the FICI. For all other antibiotics a clinical isolate, *P. aeruginosa DR 4877* (multi drug resistant strain) was used**

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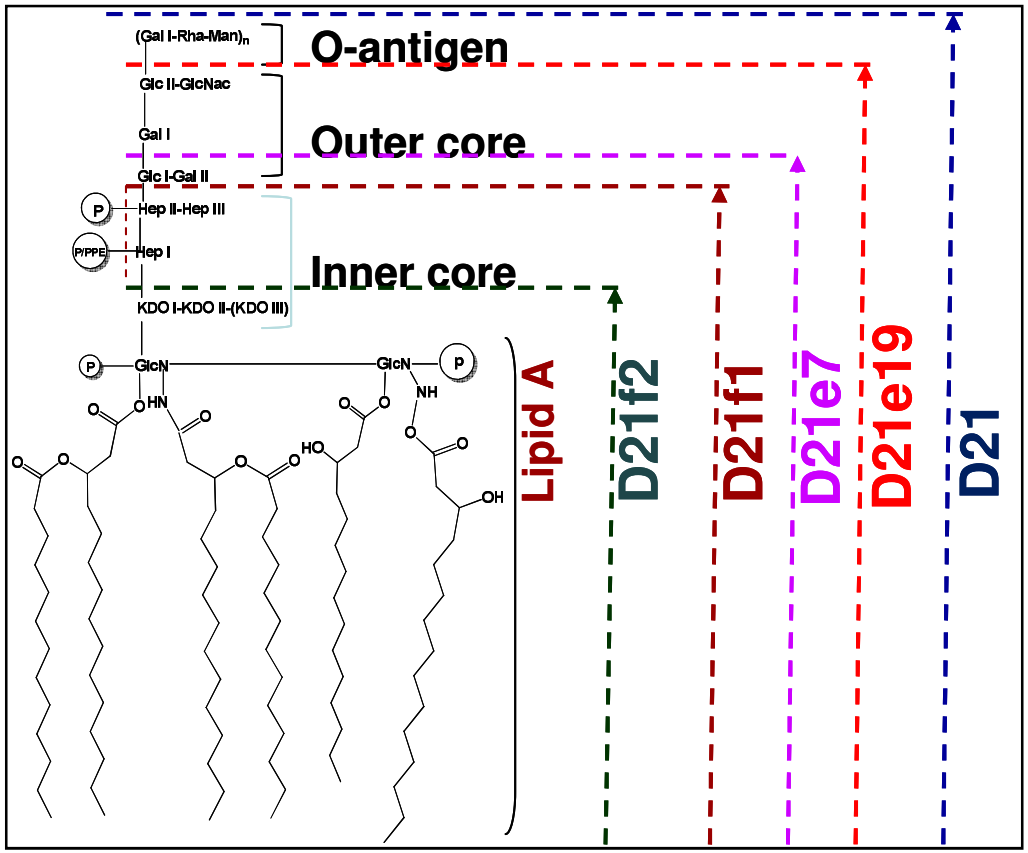
# E coli Mutants with Outer Membrane Truncation



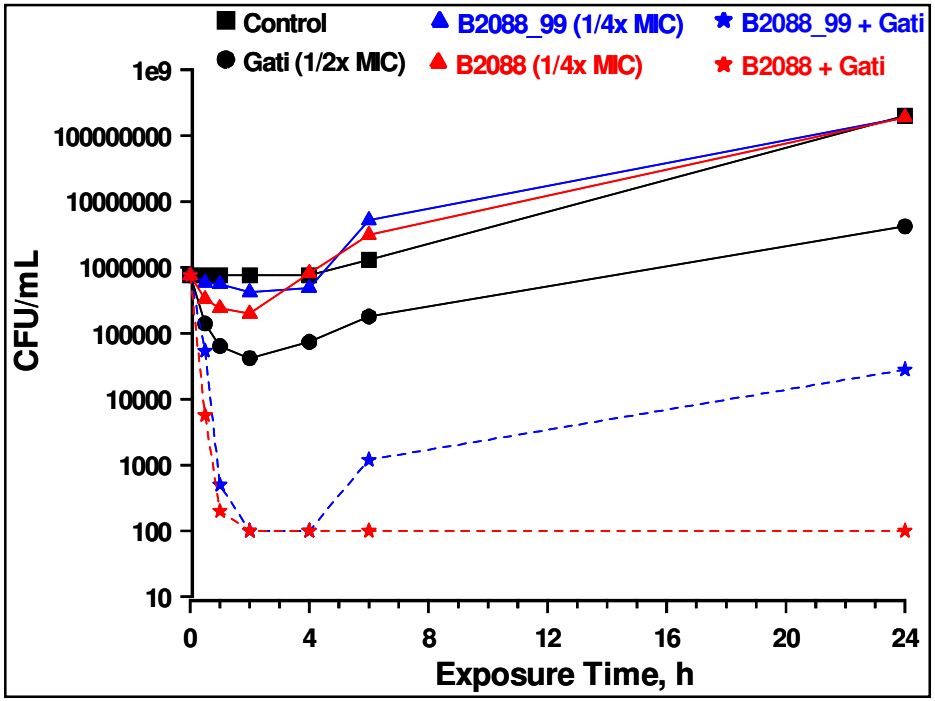

**B2088, B2088_99 and Polymyxin B binds to lipid A portion of LPS**

**B2089, B2099 bind to O-antigen domain**

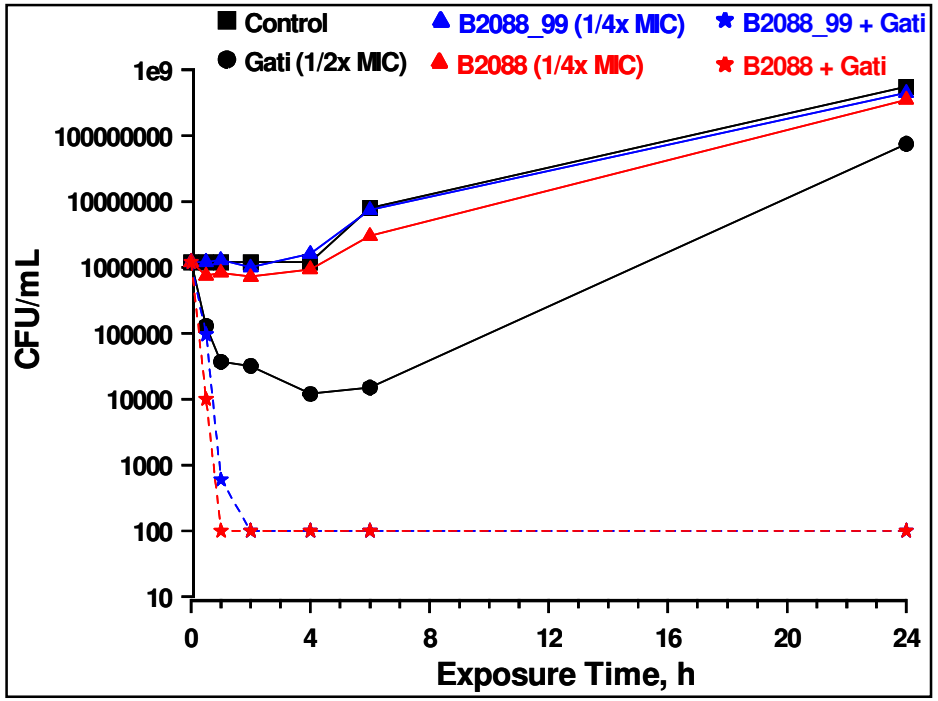
**B2499 binds to Outer core region**

# Time-Kill Kinetics of B2088/B2088_99 with Gatifloxacin

## *P. aeruginosa* 27853



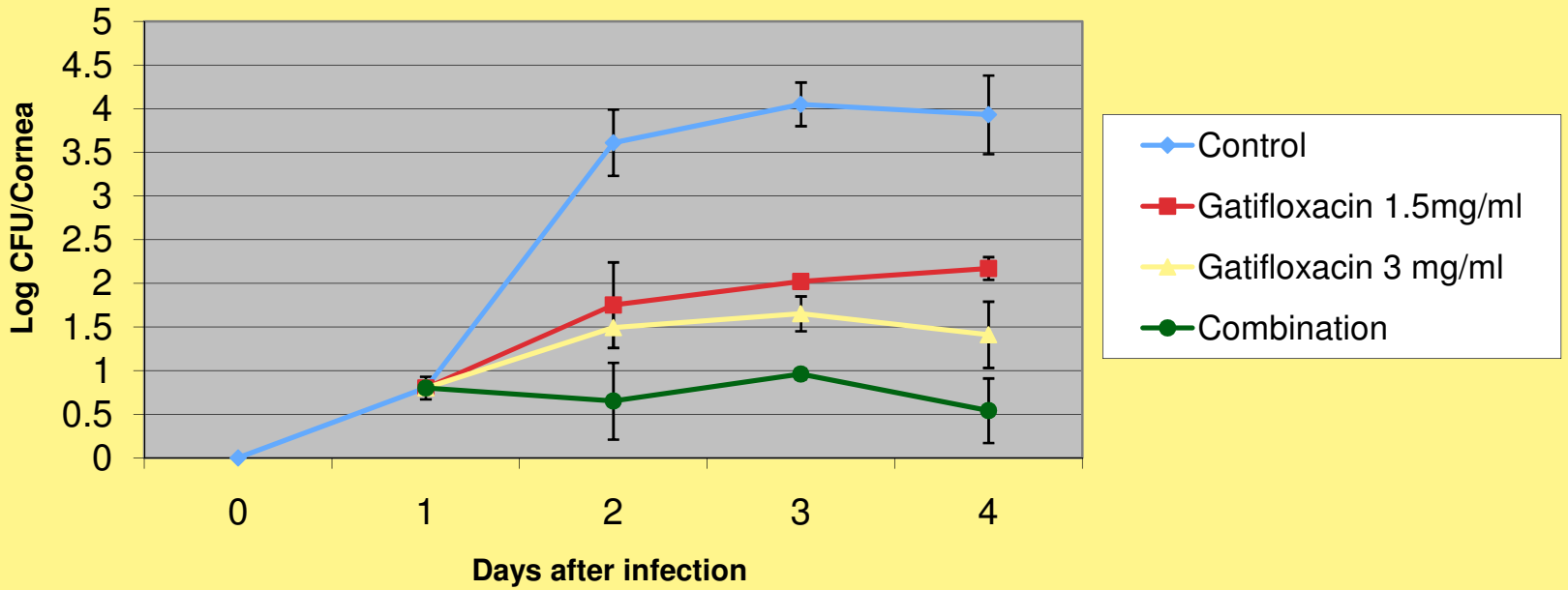

## *P. aeruginosa* 9027




**Combination of B2088/B2088_99 and Gati at Sub MIC displayed pronounced bactericidal effect**

Synergy-increase the activity of current antibiotics
ATP and LDH release-no toxicity at 1000ug/ml

**_In Vivo_ testing (linking Gatifloxacin 1.5mg/ml+B2088 0.5mg/ml) on mouse cornea infected with _Pseudomonas aeruginosa_ (ATCC 9027)**

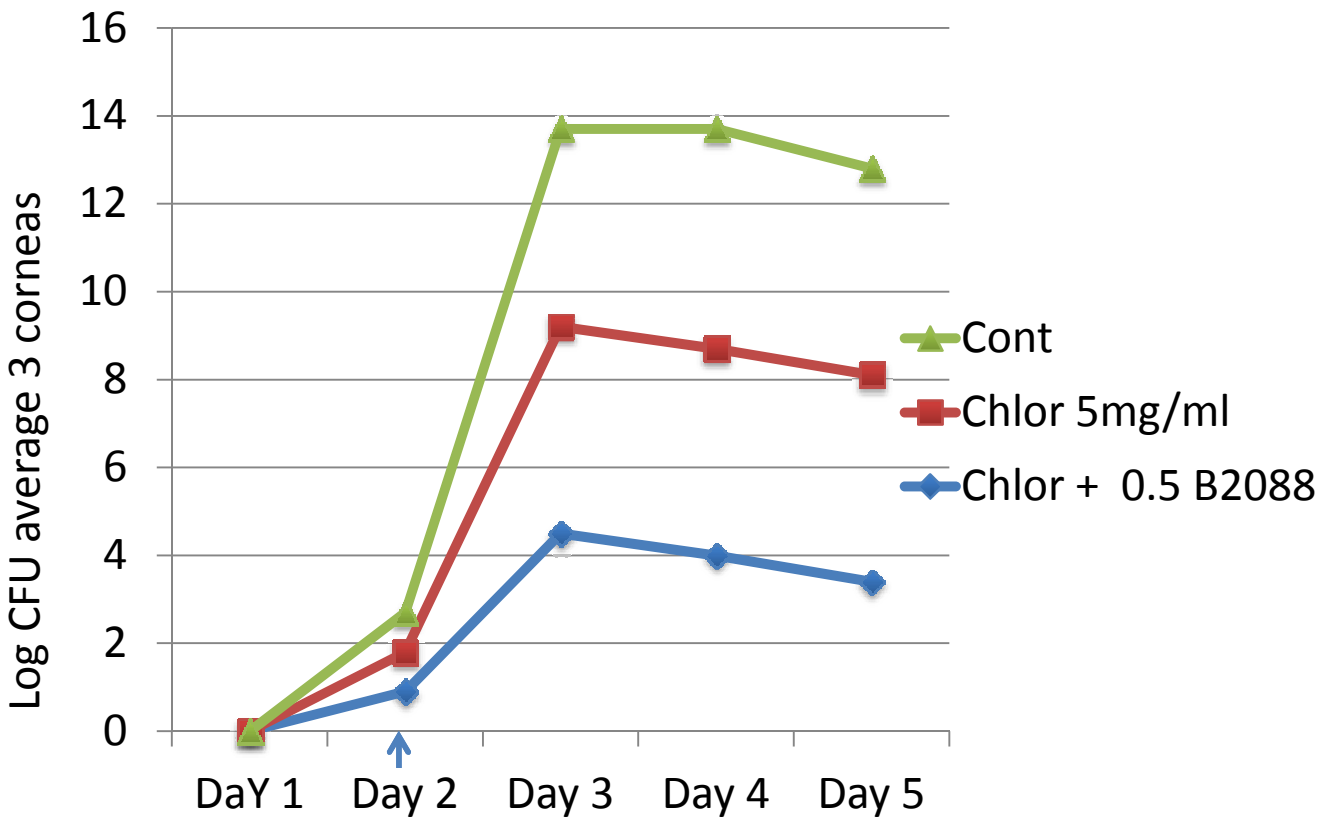


# Chloramphenicol

A good antibiotic but with limited effectiveness against

Gram Negative organisms

# Mouse Model of Cornea Infection




Infection with Pseudomonas aeruginosa ATCC 9027
Treatment 3X/day starting on Day 2

# Summary

## Facilitated Antibiotic Synergy (FAS)

**FAS Technology** deals with some of the most difficult pathogens-such as Pseudomonas and the CREs.
FAS does the following:

1-Extends the action spectrum of some antibiotics

2-In many cases it will allow the use of lower concentrations of antibiotics to achieve the same therapeutic effect

3-FAS Technology can decrease bacteria kill times from 18-22hrs to 3-4hrs

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# Summary-2

4-Importantly it has the ability to lower the cost of development, should require fewer studies for IND filing, and more rapid entry into clinical use with Phase II trials

5-Importantly for patients offers better and quicker eradication of infections

6-FAS technology works with several classes of antibiotics

7-Improved therapeutic index

8-Health care costs should be lower

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# With appreciation


## SERI

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# Thank you