NVC-422 Does Not Generate Resistance in MRSA, Staphylococcus aureus, Pseudomonas aeruginosa and **Escherichia coli after Multiple Serial Passages at Sub-Inhibitory Concentrations**

E-135

Abstract

Background: NVC-422 (*N*,*N*-dichloro-2,2-dimethyltaurine) is a potent, broad-spectrum, fast-acting antimicrobial agent with a novel mechanism of action. This study evaluated the potential of NVC-422 and comparator antibiotics ciprofloxacin, mupirocin, fusidic acid and retapamulin to generate drug resistance in Gram-negative and Gram-positive strains in a multiple serial passage study.

Methods: Serial passages using CLSI MIC methodology were used to study evolution of resistance at 0.5 MIC as determined by the previous passage. The strains used were: Escherichia coli ATCC 25922, Pseudomonas aeruginosa PAO1, Staphylococcus aureus ATCC 29213 and MRSA ATCC 33591. Stability of compounds in cation adjusted Mueller Hinton broth (CAMHB) and M9 minimal medium was studied using HPLC/UV. From our stability studies of NVC-422 in media and the ability of microbes to grow in a minimal M9 medium, we selected M9 medium for P. aeruginosa and E. coli and CAMHB for S. aureus and MRSA.

Results: MIC of ciprofloxacin increased by 256 fold in E. coli, and 32 fold in P. aeruginosa and S. aureus. Mupirocin, fusidic acid and retapamulin MICs against MRSA increased by 64, 256 and 16 fold respectively. In contrast, no increase in MIC was observed for NVC-422 in any of the 19 independent bacterial cultures tested. At 37°C the half-life of NVC-422 is 30 minutes in CAMHB, and >24 hrs in M9 media.

Conclusions: NVC-422 is a potent, broad-spectrum antimicrobial agent. This study shows that NVC-422 did not generate drug resistance in Gram-negative or Gram-positive pathogens, while comparator antibiotics ciprofloxacin, mupirocin, fusidic acid and retapamulin showed a dramatic 16-256 fold increase in MIC after repeated sub-lethal exposure over 25 to 50 passages. NVC-422 is currently in Phase II clinical trials for the treatment of impetigo and for the prevention of urinary catheter blockage and encrustation (UCBE).

Introduction

In healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming common, complicating treatment and increasing human morbidity and mortality.¹ The development of resistance to one or multiple drugs results from medical and agricultural use and overuse of antimicrobials that promote transfer of resistance by both vertical and horizontal mechanisms.^{2,3,4} As a consequence, there is an urgent, unmet medical need for safe, novel antimicrobial agents that are effective against existing resistant pathogens and that carry a low potential for the development of drug resistance.^{4,5}

NVC-422 is a rapidly bactericidal antimicrobial agent that is active against a broad range of Gram-positive and Gramnegative species, including drug resistant pathogens. NVC-422 is cidal to pathogens by the oxidative modification of sulfur-containing amino acids, such as methionine and cysteine, resulting in protein inactivation. Pathogens die within minutes after exposure to lethal concentrations of NVC-422 as measured by our time-kill assays. This novel mechanism differentiates chlorotaurines from traditional antibiotics by attacking multiple specific targets on the surface of bacteria; therefore, making it virtually impossible for pathogens to develop resistance.

We evaluated the propensity to select for resistance in vitro through multiple serial passages in the presence of subinhibitory concentrations of NVC-422, ciprofloxacin, mupirocin, fusidic acid and retapamulin. Twenty-five serial passages of two *E. coli* ATCC 25922, five *P. aeruginosa* PAO1 and five *S. aureus* ATCC 29213 independent cultures and up to 50 serial passages with seven methicillin resistant S. aureus (MRSA) ATCC 33591 independent cultures were used to study evolution of resistance to NVC-422 and other antibiotic drugs.

Materials and Methods

NVC-422 preparation: The synthesis of NVC-422 has been described previously.⁶ The purity of NVC-422 powder was determined to be 99.83% using an HPLC method.

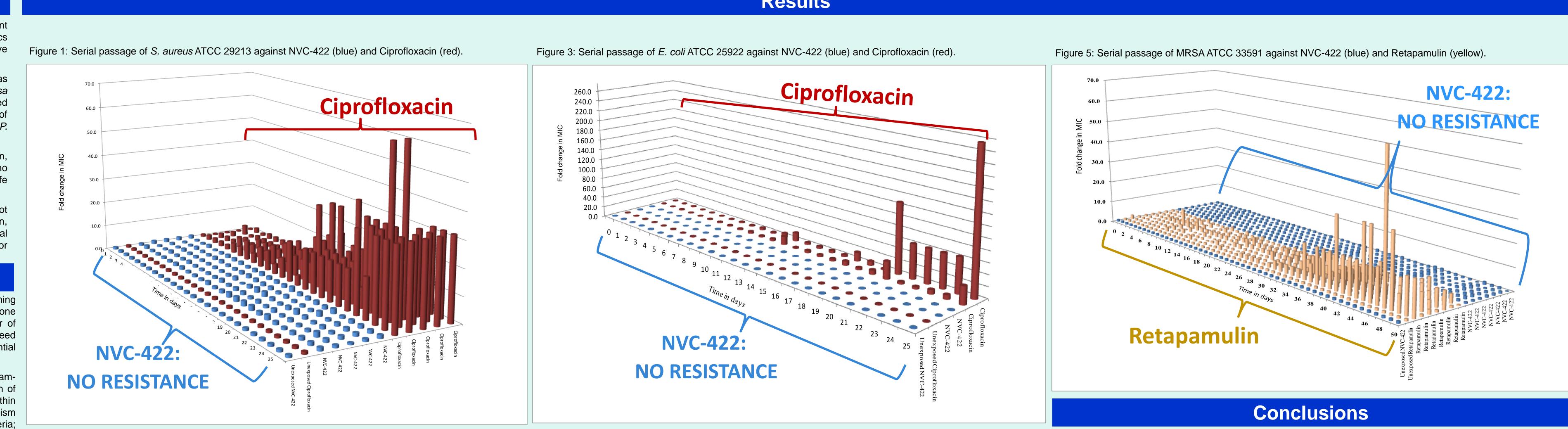
Stability testing: Stock solutions of NVC-422 were prepared by dissolving NVC-422 powder in media. Stabilities of NVC-422 (100 µg/mL) in cation adjusted Mueller Hinton broth (CAMHB) and minimal M9 medium were tested at 37°C using Agilent HPLC-1200 equipped with diode array UV detector over a period of 24 hours.

Bacterial cultures: E. coli 25922, S. aureus 29213 and MRSA 33591 were purchased from the American Type Culture Collection (ATCC) while *P. aeruginosa* PAO1 was obtained from Queens University. All strains were grown on Tryptic Soy Agar at 37 °C overnight for Day 0.

Antimicrobial agents and MIC testing: MICs were tested by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method⁷ using CAMHB or M9 minimal medium.

Multiple passage resistance selection studies: On Day 0, a single colony of the organism was inoculated in 5 ml of CAMHB or M9 medium and grown for 2-3 hours. The culture was diluted with broth to obtain a final inoculum of 1 x 10⁵ cfu/mL to 1 x 10⁶ cfu/mL. One hundred eighty µL inoculum was added to each well of the 96 well plate. Twenty µl of drug dilutions in water were added to the well. Plates were incubated at 37°C for 16-20 hours and subsequently read spectrophotometrically at O.D_{600nm}. Readings >0.05 units are considered growth. For day 1 and each subsequent passage, inoculum was prepared from the well in the 96-well MIC plate with the highest concentration of the compound that supported growth. Multiple independent cultures for each organism were prepared at day 1 and passaged separately to maximize the chance of resistance development. Serial passages were performed in CAMHB for S. aureus 29213 and MRSA 33591 and in M9 for E. coli 25922 and P. aeruginosa PAO1. Fifty passages were completed for retapamulin and NVC-422 against MRSA, while 25 passages were completed for other control antibiotics and NVC-422 against the other test organisms. A control strain previously unexposed to any antimicrobial was tested for each drug in every passage.

L. D'Lima, L. Friedman, P. Xu, L. Wang, M. Anderson, D. Debabov NovaBay Pharmaceuticals, Inc., Emeryville, CA 2011 ICAAC, Chicago; September 17-20



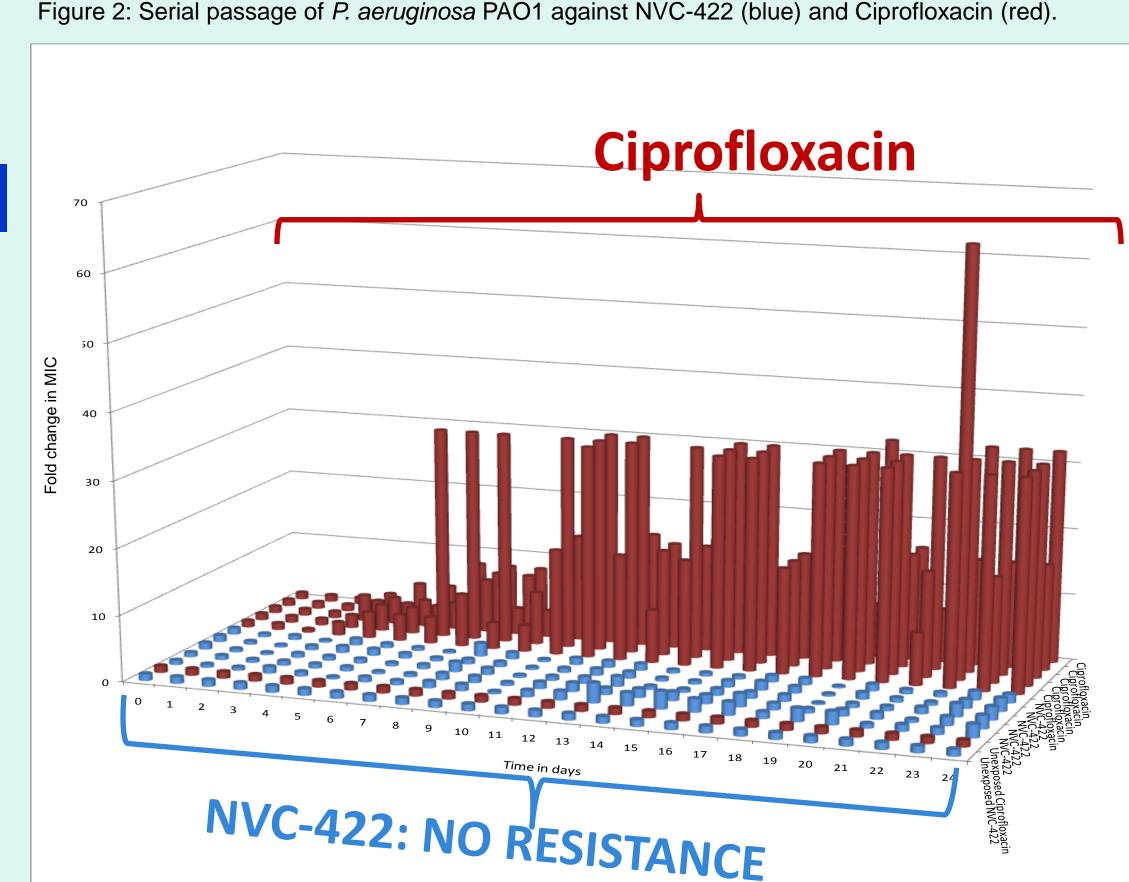
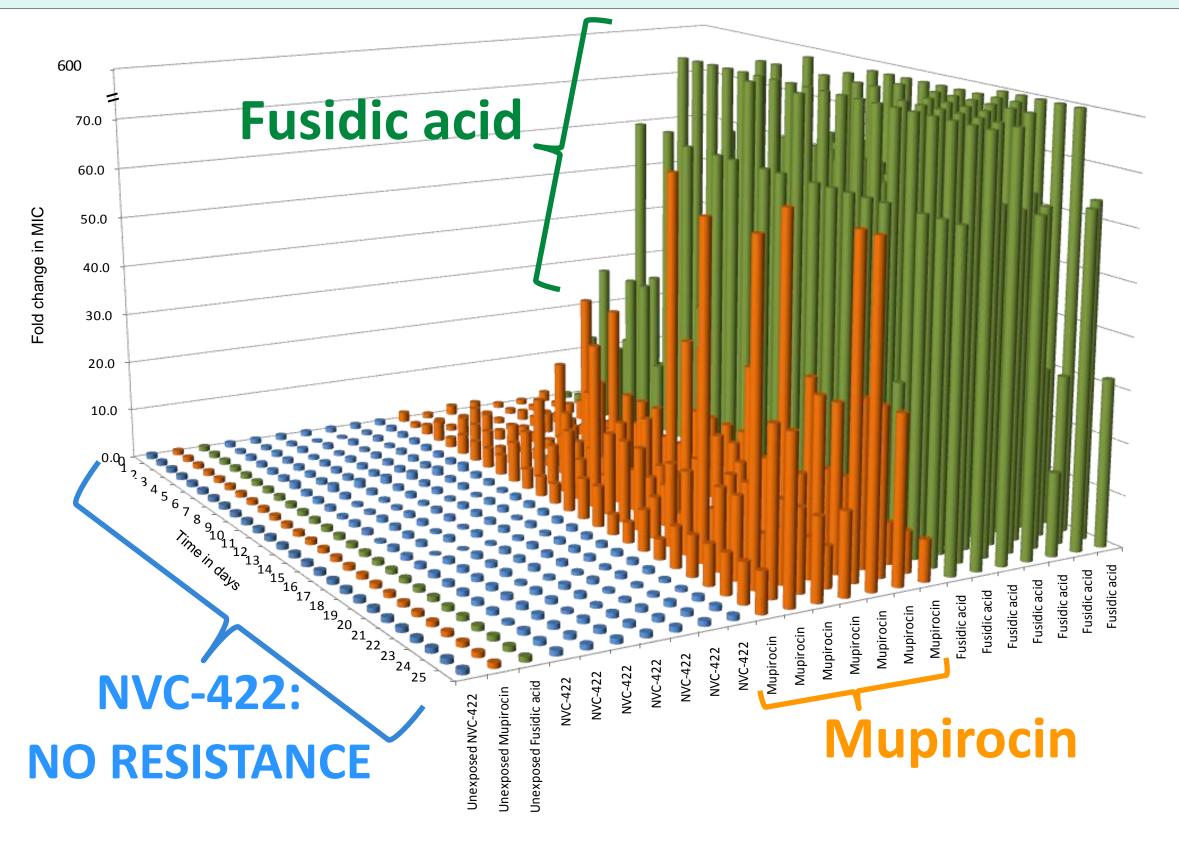


Figure 2: Serial passage of *P. aeruginosa* PAO1 against NVC-422 (blue) and Ciprofloxacin (red).

Results

Figure 4: Serial passage of MRSA ATCC 33591 against NVC-422 (blue), Mupirocin (orange) and Fusidic acid



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✤ NVC-422: No resistance was observed for any of the 19 cultures passaged independently. (Figs.1-5). Furthermore no cross-resistance was observed with mupirocin, fusidic acid or retapamulin resistant strains when tested against NVC-422 (data not shown).

* Ciprofloxacin: Resistance was generated with MIC increasing by 32-fold against S. aureus (Fig.1) and P. aeruginosa (Fig.2) and 256-fold against E. coli (Fig.3) over 25 passages.

* **Mupirocin:** Resistance was generated with a **64-fold** increase in MIC against MRSA over 25 passages (Fig.4).

Fusidic acid: Similarly, resistance was generated with a **256-fold** increase in MIC against MRSA (Fig.4).

* **Retapamulin:** Resistance was generated against MRSA with MIC increasing by **16-fold** over 50 passages (Fig.5).

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Acknowledgements

The authors would like to thank K. Poole of Queens University for providing the PAO1 strain.