

VENJAMINNE FUA





PROJECT RAISE

Sublactam-avibactam inhibitors combination strategy: understanding the mechanisms beyond their activity to combat *Acinetobacter* spp. infection

Venjaminne Fua*, Jose Cedano*, Michèle Bass*, Casin Li*, Robert A. Bonomo*, Marcelo E. Talmash*, Pastran Fernando*, Maria Soledad Ramirez*,
Center for Applied Biotechnology Studies, Department of Biological Sciences, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California, USA, *Research Service and GRECC, Louis Stokes Cleveland
Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA, †National Reference Laboratory for Antimicrobial Resistance (INRE), Servicio Antimicrobiano, Instituto Nacional de Enfermedades Infecciosas, ANLIS "Dr. Carlos G. Malbrán"

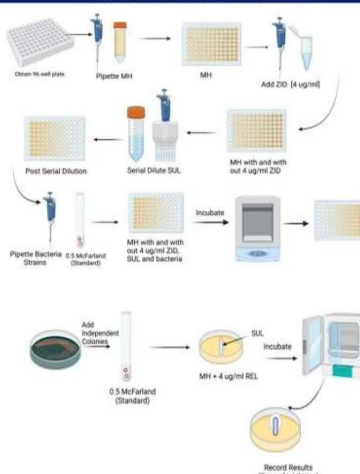



Introduction

Infections caused by antibiotic resistant bacteria are increasing in frequency, resulting in significant patient morbidity and mortality. *Acinetobacter baumannii* is a nosocomial pathogen frequently resistant to multiple drugs and causes pneumonia, bacteremia and wound infections with associated high mortality rates. In particular, carbapenem resistant *A. baumannii* strains (CRAB), mostly encode for either chromosomally or plasmid class D oxacillinases (OXA), is frequently reported among hospital settings. However, in the last years also the occurrence of NDM-1 has been increasingly observed. Few active antimicrobials remain to treat CRAB infections. Interestingly, sublactam, which is a β -lactamase inhibitor of Ambler class A enzymes, exhibited an inherent antibacterial activity against a limited number of bacterial species, including *Neisseria gonorrhoeae*, *Bacteroides fragilis* and *Acinetobacter* spp. In recent years, many studies have focused their efforts on the development of new β -lactamase inhibitors to treat MDR isolates. Avibactam, which is a class A β -lactamase inhibitor with some activity against some β beta-lactamases, showed high efficiency in combination with ceftazidime to treat infections caused by pathogens with extreme resistance being inactive against *Acinetobacter*.

Dr. Ramirez lab has observed that avibactam combine with sublactam successfully restores sublactam susceptibility in multidrug-resistant *Acinetobacter baumannii*.

Methods and Materials



Results 1

Table 1. Sublactam MICs *A. baumannii* strains on Mueller-Hinton broth with and without zidebactam supplementation

Strain	MH	MH + ZID 1 ug/ml
AMA 113 (OXA-23)	16	2
AMA 116 (OXA-23)	32	2
AMA 122 (NDM-1)	8	2
AMA 123 (OXA-23)	16	2
AMA 126 (AMP-1)	4	1/2
AMA 147 (OXA-23)	16	1/2
AMA 163 (OXA-23)	1	<0.125
AMA 165 (OXA-23)	4	2
AMA 181 (NDM-1)	4	2

Table 2. Sublactam MICs *A. baumannii* strains on Mueller-Hinton agar with and without relebactam supplementation

Strain	Sublactam MIC (ug/L) on agar	MH + SUL 4 ug/ml
AMA 113 (OXA-23)	8	16
AMA 116 (OXA-23)	8	16
AMA 122 (NDM-1)	8	16
AMA 123 (OXA-23)	8	16
AMA 126 (AMP-1)	2	12
AMA 147 (OXA-23)	2	8
AMA 163 (OXA-23)	2	12
AMA 165 (OXA-23)	2	8
AMA 181 (NDM-1)	2	8
AMA 186 (NDM-1 + OXA-23)	8	16

Results 2

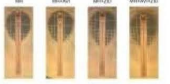


Fig. 1. Strip test of AMA 181 with: Mueller-Hinton, Mueller-Hinton/Avibactam, Mueller-Hinton/Zidebactam & Mueller-Hinton/Avibactam/Ceftazidime

Results 3

Table 3. Whole Genome Sequencing: Carbapenemase content and sequence type

Strain	Whole genome sequencing first sequence type	Resistance form
AMA 113	OXA-23	26
AMA 116	OXA-23	801
AMA 122	NDM-1	201
AMA 123	OXA-23	26
AMA 126	AMP-1	750
AMA 147	OXA-23	75
AMA 163	OXA-23	2
AMA 165	OXA-23	2
AMA 181	NDM-1	201
AMA 186	OXA-23	2

Future Work

Further WGS analysis will be performed to compare the sublactam-avibactam resistant strains to identify specific characteristic.

Single nucleotide polymorphisms (SNPs) will be used to identify for potential mutant/s genes that can explain the observed resistant.

Genomic comparison with other available genomes will be conducted to identified differences among them.

Further phenotypic studies, such as antibiotic susceptibility of ZID combined with other antibiotics will be performed

Conclusions

- Exploring novel combinations may offer new options to treat *Acinetobacter* spp. infections, especially for widespread oxacillinases and metallo- β -lactamases producers.
- From the results one can conclude that the synergy of SUL/ZID is effective in combating antibiotic resistant *Acinetobacter baumannii*.
- The combination of SUL/REL was not effective on the tested SUL/AVI resistant strains
- WGS analysis of the selected strains showed the presence of different carbapenemases and diversity of sequence types
- AMA N0+, that harbors both OXA-23 and NDM-1 is the first strains with both carbapenemases.

Acknowledgements

Project supported by Project RAISE, U.S. Department of Education HSI-STEM award number P031C160152.
Dr. Ramirez' work cited in this project was funded by Public Health Service Grants SC3GM125556 (MSR).

References

I worked on testing different strains of bacteria that can cause resistant pneumonia to antibiotics. To see which antibiotics were effective, we synergized different combinations of antibiotics and put them in their respective strains and let them grow. From there we were able to see the minimum level of a concentration that is needed to kill that strain of bacteria with the respective mix of antibiotics.

Alternate Text:

Venjaminne Fua

Quote: "I worked on testing different strains of bacteria that can cause resistant pneumonia to antibiotics. To see which antibiotics were effective, we synergized different combinations of antibiotics and put them in their respective strains and let them grow. From there we were able to see the minimum level of a concentration that is needed to kill that strain of bacteria with the respective mix of antibiotics."

Image of Venjaminne Fua

Image of text and graphic laden project presentation entitled "Sulbactam-avibactam inhibitors combination strategy: understanding the mechanisms beyond their activity to combat *Acinetobacter* spp. Infection."