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

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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, sulbactam, 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 sulbactam successfully restores sulbactam susceptibility in multidrug- resistant *Acinetobacter baumannii*.

Fig. 1. Gradient diffusion (E-test) of sulbactam/avibactam and sulbactam in selected representative strains.

To further characterized the efficacy of sulbactam/avibactam combination, we plan to study the molecular mechanisms which affect the association of sulbactam and avibactam.

In the present project we **aim** to
to test the combination of sulbactam with relebactam and zidebactam, a novel inhibitor, in strains that are resistant to sulbactam-avibactam
the identify through whole genome sequencing and phenotypic approaches the resistant mechanisms that affect the association of sulbactam-avibactam in order to prevent its emergence or dissemination

Acknowledgements

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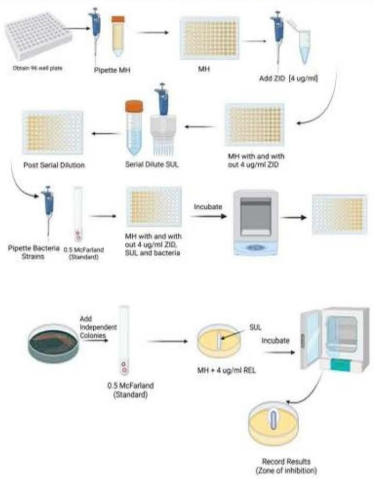
References

Methods and Materials



Results 1

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

Strain	MH	MH + ZID 4 ug/ml
AMA 113 (OXA-23)	16	1
AMA 116 (OXA-23)	32	2
AMA 122 (NDM-1)	8	2
AMA 133 (OXA-23)	16	2
AMA 136 (IMP-1)	4	0.25
AMA 147 (OXA-23)	16	2
AMA 163 (OXA-58)	1	<0.125
AMA 196 (OXA-23)	64	8
AMA 181 (NDM-1)	4	2
AMA N0+ (NDM-1+ OXA-23)	128	64

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

	Sulbactam MICs (mg/L) (E-test)	
Strain	MH	MH + REL 4 ug/ml
AMA 113 (OXA-23)	8	8
AMA 116 (OXA-23)	16	16
AMA 122 (NDM-1)	8	12
AMA 133 (OXA-23)	8	12
AMA 136 (IMP-1)	2	2
AMA 147 (OXA-23)	6	8
AMA 163 (OXA-58)	2	1.5
AMA 196 (OXA-23)	24	32
AMA 181 (NDM-1)	8	8
AMA N0+ (NDM-1+ OXA-23)	96	96

Results 2

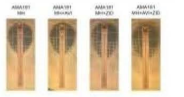


Fig. 1. E Strip test of AMA 181 with Mueller Hinton , Mueller Hinton/Avibactam, Muller Hinton /Zidebactam & Mueller Hinton/ Avibactam/Zidebactam

Results 3

Table 3. Whole Genome Sequencing- Carbepenemase content and sequence type

Whole genome sequence first analysis		
Strain	Carbapenemase	Sequence type
AMA 113	OXA-23	25
AMA 116	OXA-23	881
AMA 122	NDM-1	2vo
AMA 133	OXA-23	793
AMA 136	IMP-1	790
AMA 147	OXA-23	79
AMA 163	OXA-58	
AMA 196	OXA-23	2
AMA 181	NDM-1	2vo
AMA N0+	OXA-23/NDM-1	2

Future Work

Further WGS analysis will be performed to compare the sulbactam-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 carbepenemases.

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:

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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."