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Evaluation of the efficacy of novel drugs and combinations against extensively- and pan-drug resistant *Acinetobacter baumannii* isolates

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Introduction

Acinetobacter baumannii, a Gram-negative, nosocomial, opportunistic pathogen is commonly infectious in immunocompromised patients, more specifically in patients that are admitted to the intensive care unit (*Howard et al.*, 2012). In the hospital setting, most patients develop healthcare-associated infections (HAI) spanning beyond sepsis, pneumonia, meningitis, and urinary tract infections (*Rebic et al., 2018*). Due to its rapid ability to acquire antibiotic resistance, it has raised the necessity to discern a novel therapeutic treatment that can be effectively used against the multidrug resistant (MDR) *A. baumannii* (*Betchen et al.*, 2022).

Between 2004 and 2005, the intensive care unit of Cooper University Hospital (CUH) in Camden, NJ faced with an increased case load of patients that were infected with MDR *A. baumannii*. The patient isolates obtained from CUH were highly resistant to the 22 standard-of-care antibiotics (*Deolankar et al., 2022*). With the recent introduction of novel antibiotics released onto the market, the possibility of antimicrobial synergism between old and new drugs was tested (*Deolankar et al.,* 2022; *Halim et al., 2024*). Antimicrobial synergism, which is the combination of two drugs, has proven to be effective in preventing the emergence of further drug-resistance by improving the effectiveness of the antimicrobial agents (*Lu et al., 2022 ; Xu et al., 2018*).

Using knowledge from our previous work, this current study focuses on testing combinatorial therapies between the 22 standard-of-care antibiotics and newly marketed drugs against the patient isolates obtained from CUH. We tested to see if drug synergy existed between levofloxacin and cefepime, and levofloxacin and amikacin, based upon preliminary disc diffusion screening. In addition, we also studied the potency and potential synergism of the newer drug cefiderocol in combination with the 22 standard-of-care antibiotics. Obtaining useful dual antibiotic therapies can provide clinicians with an immediate resource to help treat patients with highly drug-resistant *A. baumannii* infections.

Methods

- ❖ 21 *A. baumannii* de-identified patient isolates (M1 to M22) were obtained during routine workup from CUH, which were comprised of extensively- (XDR) and pan drug-resistant (PDR) strains.
- ❖ Performed two trials of broth microdilution to determine minimum inhibitory concentrations (MICs) and checkerboard assays on each dual drug combination against the patient isolate (*Deolankar et al., 2022*).
- ❖ MIC determination: The antibiotic of choice was added to a 96-well plate, followed by ten 2-fold serial dilutions. Following this, cells were added at a starting optical density at 600 nm (OD_{600}) of 0.05. Plates were incubated at 37°C overnight and analyzed by measuring the OD_{600} using a microplate reader (BioTek).
- ❖ Checkerboard Assays: Two different antibiotics were serially diluted two-fold in the horizontal and vertical directions of a 96-well plate. Cells were added to each well at an OD_{600} of 0.05 and were incubated overnight at a temperature of 37°C. Plates were analyzed by measuring the OD600, as above. The fractional inhibitory concentration (FIC) index was determined to identify synergistic or additive effects of combinational therapy. Results that showed an FIC index ≤ 0.5 was defined as having synergistic effects. Results that showed an FIC index within a range of 0.5 – 1 was defined as having additive effects.

Results

Drug Combination: Levofloxacin & Amikacin

Concentration of Amikacin (ug/mL)	Concentration of Levofloxacin (ug/mL)									
	256	128	64	32	16	8	4	2	1	0
128	0.046	0.044	0.043	0.043	0.044	0.043	0.043	0.043	0.043	0.043
64	0.046	0.045	0.043	0.044	0.044	0.043	0.045	0.043	0.044	0.043
32	0.054	0.044	0.044	0.044	0.044	0.043	0.043	0.044	0.044	0.043
16	0.047	0.044	0.043	0.046	0.043	0.044	0.042	0.043	0.044	0.045
8	0.046	0.045	0.044	0.045	0.044	0.043	0.106	0.049	0.045	0.045
4	0.046	0.044	0.044	0.044	0.043	0.044	0.436	0.297	0.459	0.512
2	0.046	0.047	0.044	0.044	0.044	0.404	0.736	0.731	0.711	0.739
0	0.055	0.046	0.046	0.049	0.845	1.218	1.177	1.23	1.069	1.089

No Bacterial Growth / Additive / Synergy / Bacterial Growth

Figure 1: Checkerboard assay of the drug combination levofloxacin and amikacin tested against patient isolate strain M7. OD_{600} values are displayed. An OD_{600} < 0.1 was considered no growth. Values in red are MIC values for each drug alone. Additive combinations have an FICI of 0.5-1.

Concentration of Amikacin (ug/mL)	Concentration of Levofloxacin (ug/mL)									
	256	128	64	32	16	8	4	2	1	0
128	0.052	0.048	0.053	0.05	0.044	0.048	0.044	0.044	0.045	0.379
64	0.048	0.045	0.046	0.044	0.044	0.044	0.044	0.045	0.044	0.044
32	0.048	0.045	0.047	0.045	0.045	0.044	0.048	0.044	0.044	0.044
16	0.049	0.046	0.044	0.044	0.043	0.043	0.093	0.043	0.145	0.043
8	0.046	0.045	0.048	0.044	0.043	0.046	0.197	0.044	0.045	0.215
4	0.048	0.055	0.045	0.045	0.043	0.044	0.255	0.268	0.243	0.232
2	0.047	0.046	0.046	0.044	0.045	0.276	0.271	0.306	0.091	0.287
0	0.054	0.047	0.046	0.044	0.178	0.647	0.918	0.898	1.027	1.049

No Bacterial Growth / Additive / Synergy / Bacterial Growth

Figure 2: Checkerboard assay of the drug combination levofloxacin and amikacin tested against patient isolate strain M10. OD_{600} values are displayed. An OD_{600} < 0.1 was considered no growth. Values in red are MIC values for each drug alone. Additive combinations have an FICI of 0.5-1.

Concentration of Amikacin (ug/mL)	Concentration of Levofloxacin (ug/mL)									
	256	128	64	32	16	8	4	2	1	0
128	0.048	0.046	0.046	0.047	0.047	0.046	0.046	0.046	0.052	0.046
64	0.048	0.045	0.045	0.045	0.045	0.044	0.045	0.045	0.054	0.044
32	0.048	0.051	0.045	0.045	0.044	0.045	0.045	0.045	0.054	0.046
16	0.048	0.046	0.046	0.046	0.044	0.045	0.045	0.044	0.089	0.044
8	0.047	0.045	0.052	0.046	0.045	0.047	0.048	0.047	0.058	0.047
4	0.046	0.044	0.045	0.045	0.046	0.4	0.133	0.217	0.358	0.38
2	0.048	0.047	0.045	0.045	0.045	0.048	0.203	0.268	0.364	0.892
0	0.049	0.046	0.047	0.045	1.158	1.357	1.342	1.355	1.327	1.287

No Bacterial Growth / Additive / Synergy / Bacterial Growth

Figure 3: Checkerboard assay of drug combination levofloxacin with amikacin tested against patient isolate strain M16. OD_{600} values are displayed. An OD_{600} < 0.1 was considered no growth. Values in red are MIC values for each drug alone. Additive combinations have an FICI of 0.5-1.

Drug Combination: Levofloxacin & Cefepime

Concentration of Cefepime (ug/mL)	Concentration of Levofloxacin (ug/mL)									
	256	128	64	32	16	8	4	2	1	0
64	0.062	0.06	0.059	0.053	0.054	0.062	0.043	0.046	0.043	0.045
32	0.052	0.051	0.048	0.049	0.047	0.053	0.045	0.042	0.043	0.045
16	0.053	0.051	0.047	0.046	0.046	0.045	0.042	0.043	0.042	0.043
8	0.05	0.049	0.044	0.044	0.045	0.046	0.047	0.047	0.068	0.192
4	0.047	0.047	0.045	0.045	0.043	0.043	0.517	0.49	0.231	0.535
2	0.048	0.048	0.053	0.047	0.051	0.045	0.608	0.674	0.92	0.72
1	0.058	0.056	0.061	0.068	0.106	0.394	0.746	0.853	1.037	0.797
0	0.052	0.059	0.063	0.053	0.063	0.089	0.152	0.62	0.731	0.751

No Bacterial Growth / Additive / Synergy / Bacterial Growth

Figure 4: Checkerboard assay of drug combination levofloxacin with cefepime tested against patient isolate strain M10. OD_{600} values are displayed. An OD_{600} < 0.1 was considered no growth. Values in red are MIC values for each drug alone. Additive combinations have an FICI of 0.5-1, while synergistic combinations are < 0.5.

Concentration of Cefepime (ug/mL)	Concentration of Levofloxacin (ug/mL)									
	256	128	64	32	16	8	4	2	1	0
64	0.059	0.053	0.053	0.053	0.049	0.047	0.047	0.045	0.045	0.045
32	0.053	0.05	0.051	0.053	0.045	0.044	0.045	0.042	0.044	0.042
16	0.049	0.048	0.049	0.046	0.042	0.044	0.05	0.045	0.045	0.044
8	0.046	0.05	0.046	0.047	0.044	0.046	0.056	0.05	0.055	0.047
4	0.046	0.046	0.045	0.047	0.042	0.044	0.055	0.057	0.06	0.051
2	0.046	0.044	0.044	0.044	0.043	0.062	0.066	0.066	0.063	0.056
1	0.047	0.044	0.044	0.043	0.043	0.073	0.076	0.064	0.07	0.067
0	0.047	0.049	0.046	0.046	0.303	0.243	0.383	0.371	0.375	0.47

No Bacterial Growth / Additive / Synergy / Bacterial Growth

Figure 5: Checkerboard assay of drug combination levofloxacin with cefepime tested against patient isolate strain M11. OD_{600} values are displayed. An OD_{600} < 0.1 was considered no growth. Values in red are MIC values for each drug alone. Additive combinations have an FICI of 0.5-1.

Discussion

Previously, our lab used disc-diffusion assays to identify promising drug combinations, which required further validation by the checkerboard assays. Two drug combinations that were identified from this screen were levofloxacin and cefepime, and levofloxacin and amikacin. Testing against the 21 patient isolates showed a variable response throughout. From the collection, only patient isolates M7, M10, and M16 displayed additive effects for the drug combination levofloxacin and amikacin. For all other strains, no interaction between levofloxacin and amikacin was detected. This data suggests that this is not an effective combination for clinical use.

From the 21 patient isolates, only M10 and M11 were analyzed by checkerboard assay so far with the drug combination of levofloxacin and cefepime. Both isolates showed additive effects, having an FIC index value between 0.75-1. In addition, one synergistic combination was identified, against strain 10. As both these isolates displayed combinatorial effects, this is a promising combination for further evaluation with the rest of the collection.

Experimental studies with cefiderocol began by performing broth microdilution assays. Both trials of cefiderocol against the set of 21 patient isolates showed contamination, rendering it impractical to calculate the MIC. Initial checkerboard assays of the drug combination of cefiderocol and amikacin, also had significant contamination for patient isolates M1, M2, M3, and M4. It was discovered that the prepared media we were using was the source, and with the purchase of additional supplies, these experiment need to be repeated.

Conclusions

- ❖ Levofloxacin and amikacin had limited efficacy against the highly-drug resistant *A. baumannii* isolates. This drug combination would likely not be an effective option in a clinical setting.
- ❖ Initial studies of levofloxacin and cefepime showed promise as an effective therapeutic combination against *A. baumannii* isolates, making it worthwhile to perform further studies.

Future Directions

- ❖ We will continue to work with cefiderocol and understand its potency by performing disc diffusion assays and broth microdilution.
- ❖ We will study cefiderocol in combination with other standard-of-care antibiotics to analyze the potency of this newer drug, by performing checkerboard assays that will inform us on its synergism.
- ❖ Further evaluation with the drug combination of levofloxacin and cefepime with other patient isolates in the collection will need to be performed.
- ❖ We will continue to identify antimicrobial synergism against *A. baumannii* patient isolates by studying novel drug combinations with newly marketed drugs and standard-of-care antibiotics.

Acknowledgements

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