P2400

Assessing the Therapeutic Potential of an Apramycin-Gallium Combination for Treating Acinetobacter baumannii Lung Infections

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Background

Acinetobacter baumannii (A. baumannii) is a opportunistic pathogen commonly linked to pulmonary infections, especially in intensive care unit. *A. baumannii* has been designated by the WHO as a **critical priority** pathogen requiring the developement of new therapies due to its extensive antibiotic resistance mechanisms. **Apramycin (APR)**, an aminoglycoside with a unique chemical structure, has shown the ability to evade most resistance mechanisms encountered by other aminoglycosides (**Figure 1**). **Gallium,** in its **Ga(III) form**, exhibits bactericidal effects against various Gram-negative bacteria by mimicking ferric iron, which is essential in iron-dependent bacterial pathways. Since aminoglycosides have the potential to complex with metals, we hypothesize that APR and Ga(III) could form a stable complex with enhanced efficacy against *A. baumannii.*

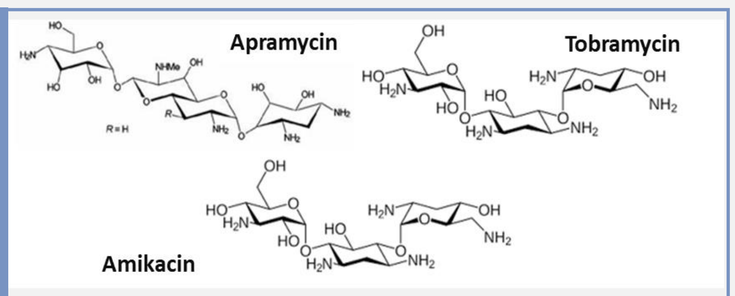
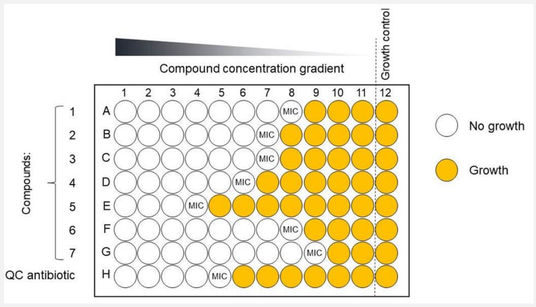


Figure 1: Apramycin, Tobramycin and Amikacin structures

Materials and Methods

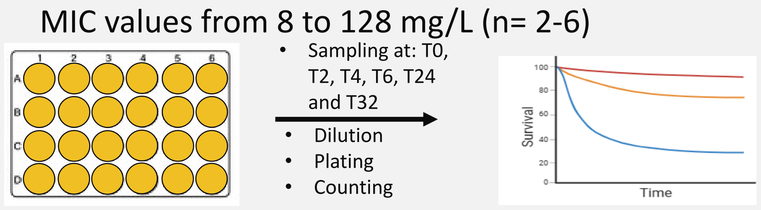
All experiments were conducted using an **APR-Ga(III) combination at a 1:1 molar ratio. The Ga(III) solution, prepared by dissolving gallium nitrate in water, was used for the combination and all experimental procedures.**

1 MICs were measured in **iron-depleted MHB** (ID-MHB) at **37°C** and read after **24 hours**.
24 *A.baumannii* clinical isolates were tested (n=2-10)



- Amikacin and Tobramycin combination were assessed on 5 clinical strains
- APR-Ga(III) were also assessed on 3 isolates of each *E. coli*, *K. pneumoniae* and *P. aeruginosa*

2 To assess the bactericidal effect, Time-Kill Curves (TKC) were performed on 4 clinical isolates with different MIC values from 8 to 128 mg/L (n= 2-6)

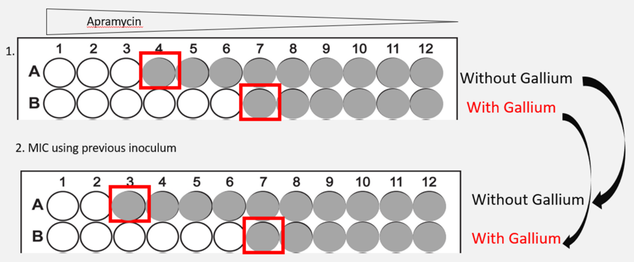


Areas under the curves of TKCs were used to compare the power of APR and APR-Ga(III) using a Emax model (γ and EC_{50})

$$E_{(c)} = E_{max} - \left(\frac{(E_{max} - E_{min}) x C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}\right)$$

$E_{(c)}$ = Effect for a concentration C
E_{max} = Maximal effect
E_{min} = Minimal effect
C^{γ} = Concentration of treatment
EC_{50}^{γ} = Concentration of treatment producing 50% of maximum response
γ = Shape parameter

3 Serial MIC assays were conducted to evaluate the impact of the combination on resistance development in 4 clinical isolates of *A.baumannii* (n=2-6)



→ Over 25 and 16 days

Results

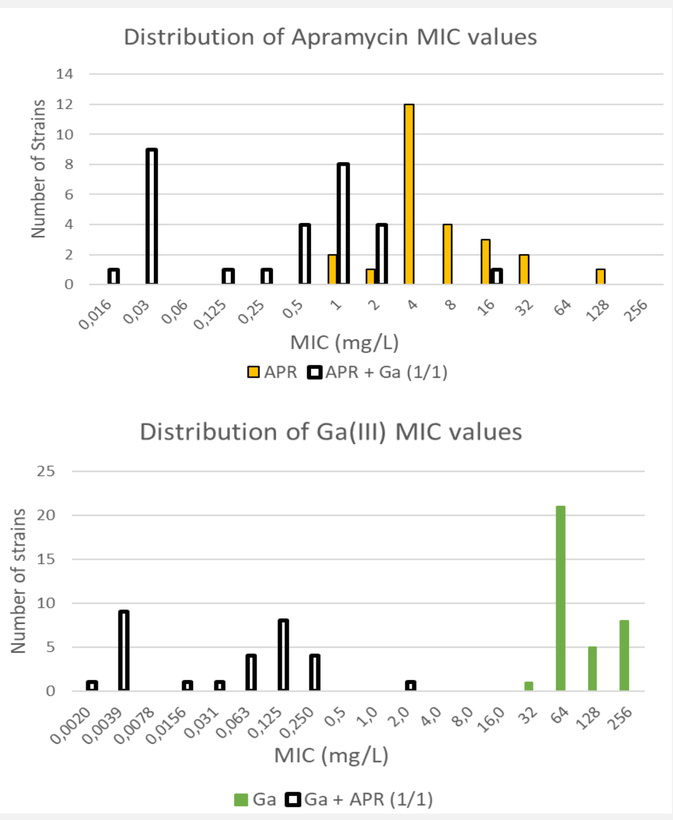


Figure 2: Distribution of MIC values for APR and Ga against *A. baumannii* isolates, either alone or in a 1:1 molar ratio combination.

As shown in Figure 2, treatment with the APR-Ga combination resulted in a **32- to 4096-fold reduction** in the APR MIC against *A. baumannii* isolates (n=24). No reduction was observed in *E. coli* or *K. pneumoniae*. A slight 4-fold decrease in the APR MIC was observed in *P. aeruginosa*. This synergy was **not observed when tobramycin or amikacin were combined with Ga(III)**.

→ **Specific action toward *A. baumannii***

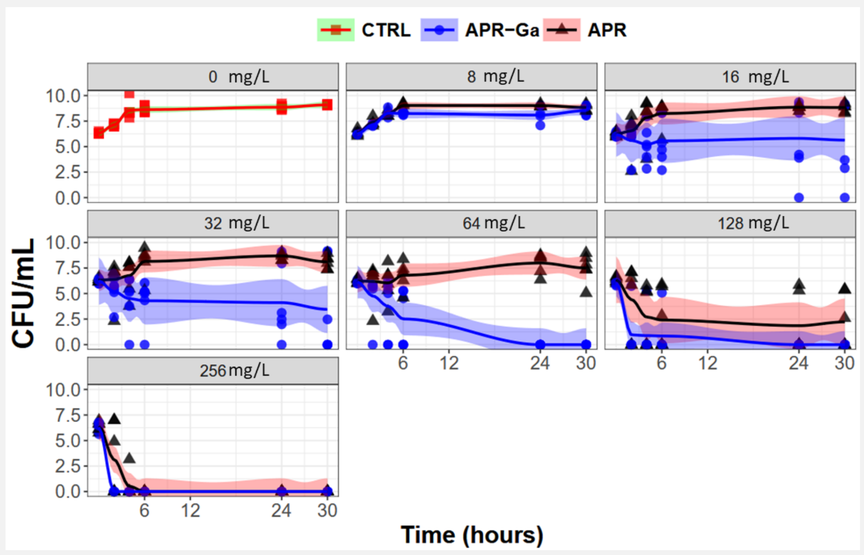
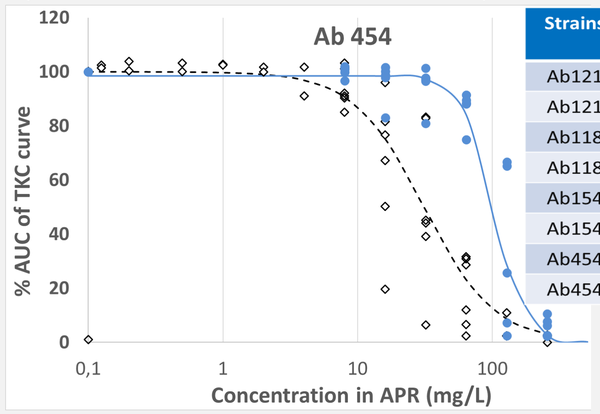


Figure 3: Time-kill curves obtained with APR alone and the APR-Ga combination against *A. baumannii* isolate 454 (n=6). Means are represented by lines and standard deviations are represented by red or blue areas.



Strains	Condition	MIC (mg/L)	EC50 (mg/L)
Ab121	APR	8	5,3
Ab121	APR/Ga	0,25	2,6
Ab118	APR	16	17,3
Ab118	APR/Ga	1	5,3
Ab154	APR	32	79,6
Ab154	APR/Ga	0,5	40,5
Ab454	APR	128	101,6
Ab454	APR/Ga	16	31,2

Figure 4: Emax model analysis of time-kill curves obtained with APR alone and the APR-Ga combination against *A. baumannii* isolates 454 (n=6). Table shows values EC50 and MIC for 4 *A. baumannii* isolates (n=2-6).

The analysis of the TKCs, shown in Figure 3, was conducted using an inhibitory Emax model, as illustrated in Figure 4. This analysis shows that the concentration of APR required to kill 50% of ***A. baumannii*** (EC_{50}) is reduced at least by half when treated with the combination.

→ **The combination treatment demonstrates a two-fold increase in bactericidal effectiveness compared to APR alone.**

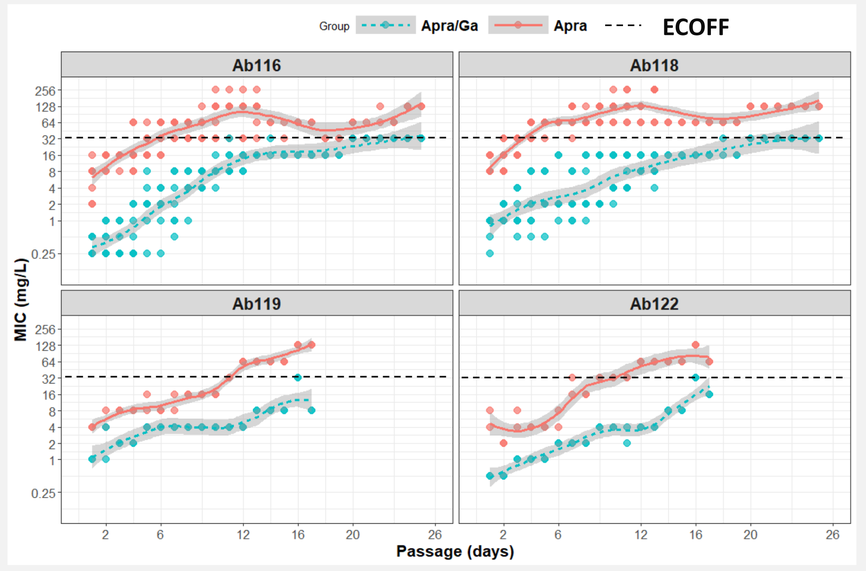


Figure 5: Serial MIC of APR or the APR-Ga combination against *A. baumannii* isolates (n=2/5). Means are represented by lines and standard deviations are represented by grey areas.

As shown in Figure 5, treatment with APR alone at sub-MIC resulted in a twofold increase in MIC by day 2 in two *A. baumannii* isolates, with a gradual rise to 64-128 mg/L at the end of the experiment.

In contrast, when treated with the APR-Ga combination at sub-MIC, the increase only reaches **16-32 mg/L by the end of the experiment, which corresponds to the potential APR ECOFF concentration** (1).

After 10 passages in pure medium, the MIC were between 64-512 mg/L for APR treatment and between 16-32 mg/L for APR/Ga

→ **This combination treatment limits the increase in APR MIC at high concentrations. Even after 16 or 25 days of treatment at sub-MIC levels, the MIC remains below the ECOFF concentration.**

Conclusion

The apramycin-gallium combination significantly enhances the *in vitro* efficacy of apramycin against *A. baumannii, with this effect appearing to be specific to A. baumannii strains*.

By markedly improving apramycin's effectiveness (up to a 4096-fold reduction in MIC), the APR-Ga combination helps delaying the development of resistance in clinical isolates. Given its promising therapeutic potential, the APR-Ga(III) combination will soon undergo *in vivo* evaluation in animal infection models.

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