Rejuvenating antibiotic activity by targeting drug efflux pumps to combat antimicrobial resistance in Indian clinical *Acinetobacter baumannii* isolates: An in-vitro and in-silico approach

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Abstract

Antimicrobial resistance (AMR) is fast becoming a medical crisis affecting the entire global population. The present study investigated over 40 synthetic compounds against the MDR Indian clinical isolates of *A. baumannii* (AB6, AB10, AB15 and AB19) for their synergistic activity with various classes of antibiotics (colistin, tigecycline, gentamycin and ciprofloxacin) using agar disc diffusion and broth dilution assay. KSA5 (30 μg) was identified as a novel compound that decreased the antibiotics MIC by 4 to 512-fold, through synergism. KSA-5 was able to inhibit the efflux of ethidium bromide (EtBr) from MDR isolate AB15, in a manner similar to the standard efflux pump inhibitor paβn. Docking studies using Schrodinger Glide indicated that KSA5 (-11.384 kcal/mol) has a higher binding affinity to the efflux pump inner membrane protein adeB than paβn (-10.534 kcal/mol). KSA5 is a new efflux pump inhibitor that has the potential to rejuvenate the antibiotics activity and overcome AMR crisis.

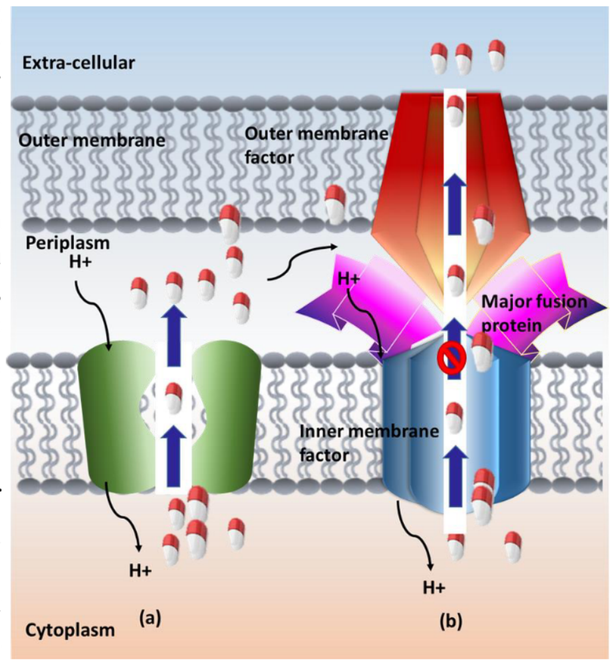
Introduction

World Health Organization (WHO) statistics show that globally 0.7 million people are dying every year due to the emergence of AMR, and by 2050, the expected lives lost will be 10 million per year. *Acinetobacter baumannii* is a dreadful nosocomial pathogen that has developed multi-drug resistance (MDR) to several currently prescribed antibiotics world over. Overexpression of drug efflux transporter is one of the mechanism of MDR development in *A. baumannii*. Therefore, blocking this drug efflux transporter can raise the efficacy of the existing antibiotics by increasing their residence time inside the bacteria.

Objectives

- Assay development and identification of potential compounds against Indian MDR isolates of *A. baumannii*.
- Computational investigation of KSA5 against RND inner membrane transporters from *A. baumanii*.

Figure 1 : Efflux pump inhibitors : How it works?



Material and methods

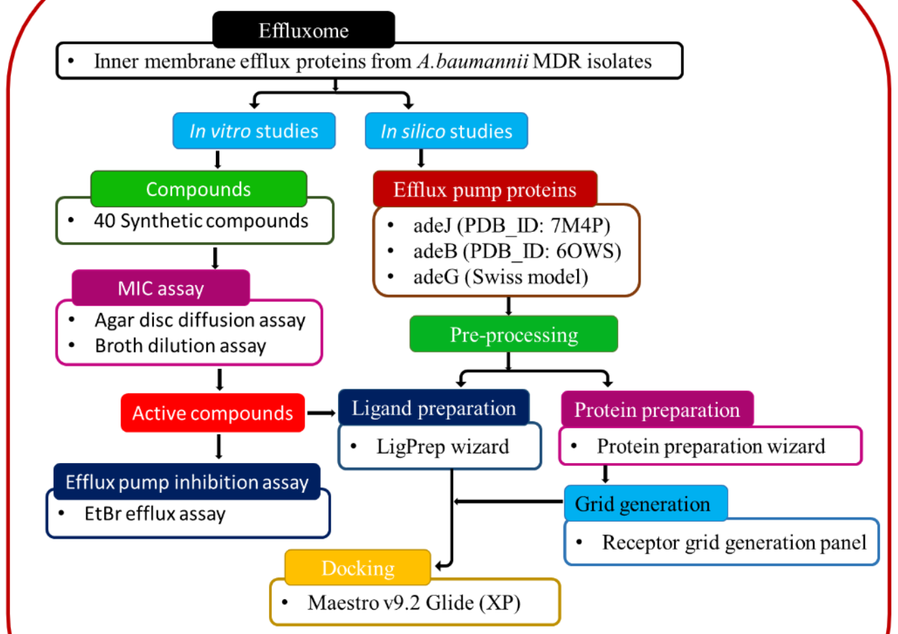


Figure 2. Workflow for in-vitro and in-silico analysis.

Results

Figure 3: Agar disc diffusion assay

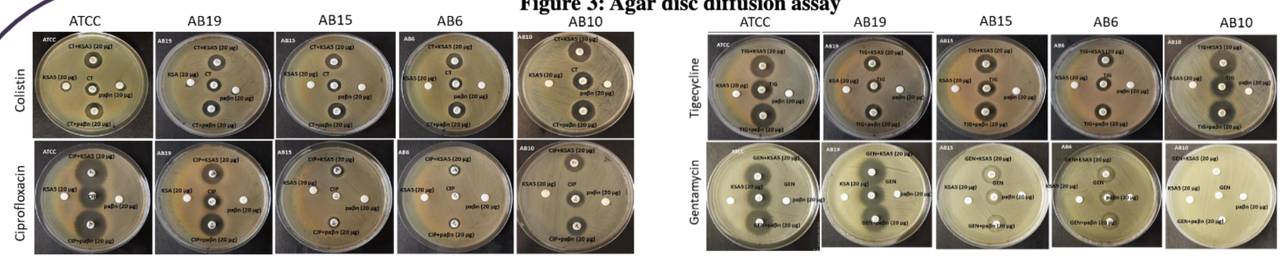


Table 1: Broth dilution assay

Bacterial strains	Antibiotics	Antibiotics individual MIC (μg)	PABN		KSA5	
			Antibiotics MIC in combination (50 μg)	Reduction in antibiotics MIC (Fold-change)	Antibiotics MIC in combination (30 μg)	Reduction in antibiotics MIC (Fold-change)*
AB ATCC (Susceptible)	Colistin	2 μg	Active	4	Active	64
	Ciprofloxacin	0.25 μg	Active	Full inhibition	Active	Full inhibition
	Gentamycin	0.25 μg	Active	Full inhibition	Active	Full inhibition
	Tigecycline	0.03 μg	Active	Full inhibition	Active	Full inhibition
AB 19 (Intermediate MDR)	Colistin	4 μg	Active	2	Active	128
	Ciprofloxacin	8 μg	Active	2	Active	8
	Gentamycin	0.25 μg	Active	Full inhibition	Active	Full inhibition
	Tigecycline	2 μg	Active	2 (partial)	Active	4
AB 15 (MDR)	Colistin	4 μg	Active	2	Active	134
	Ciprofloxacin	64 μg	Active	4	Active	64
	Gentamycin	No activity (512 μg)	No activity	-	Active	512
	Tigecycline	2 μg	Active	5	Active	67
AB 10 (MDR)	Colistin	8 μg	No activity	-	Active	80
	Ciprofloxacin	64 μg	Active	2	Active	64
	Gentamycin	No activity (512 μg)	No activity	-	Active	512
	Tigecycline	2 μg	Active	5	Active	67
AB 06 (MDR)	Colistin	4 μg	Active	2	Active	134
	Ciprofloxacin	128 μg	Active	2	Active	128
	Gentamycin	No activity (512 μg)	No activity	-	Active	512
	Tigecycline	2 μg	Active	4 (partial)	Active	16

Table 2: Glide docking of KSA5 and standard EPI against ade's efflux pump from *A. baumannii*

S.NO	Compounds	adeB		adeJ		adeG	
		Docking score	Glide ligand efficiency	Docking score	Glide ligand efficiency	Docking score	Glide ligand efficiency
1	KSA5	-11.384	-0.367	-9.423	-0.304	-9.363	-0.302
2	PAβN	-10.534	-0.319	-10.329	-0.313	-14.053	-0.426

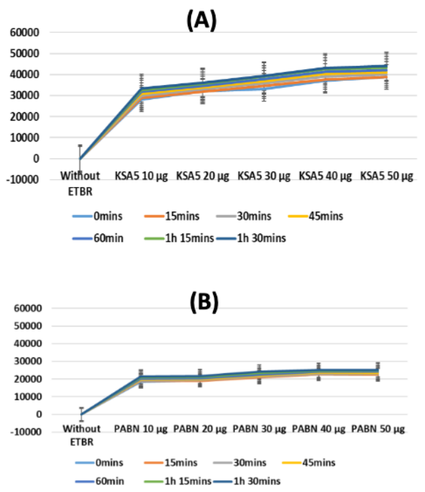


Figure 4: EtBr efflux assay on Indian clinical isolates of *A. baumannii* in combination with (A) KSA5 and (B) PAβN

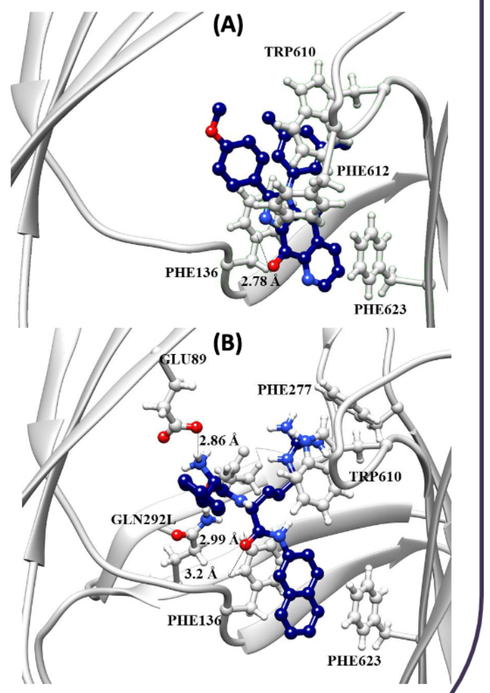


Figure 5: Docking interaction of AdeB in complex with (A) KSA5 and (B) PAβN

Conclusions

- In-vitro and in-silico study provides detailed insight into the synthetic compound KSA5 which can overcome antibiotic resistance.
- As an adjuvant KSA5 reduce the MIC of various class of antibiotics with 4 to 512-fold.
- To understand the molecular mechanism of adeB efflux pump inhibition by KSA5 and PAβN, Schrodinger glide XP docking software was used.
- Docking score and interaction of KSA5 and PAβN with the AdeB transporter in *A. baumannii* dictated that KSA5 is having better affinity than PAβN to block the transporter by interacting with the hydrophobic microenvironment.
- Therefore, KSA5 can act as an efflux pump inhibitors (EPIs), which could eventually rejuvenate the current therapeutic agents.

Reference

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Acknowledgement

I sincerely acknowledge Sponsored Research and Industrial Consultancy (SpoRIC), Vellore Institute of technology for the seed grant number SG20210103