RES-138

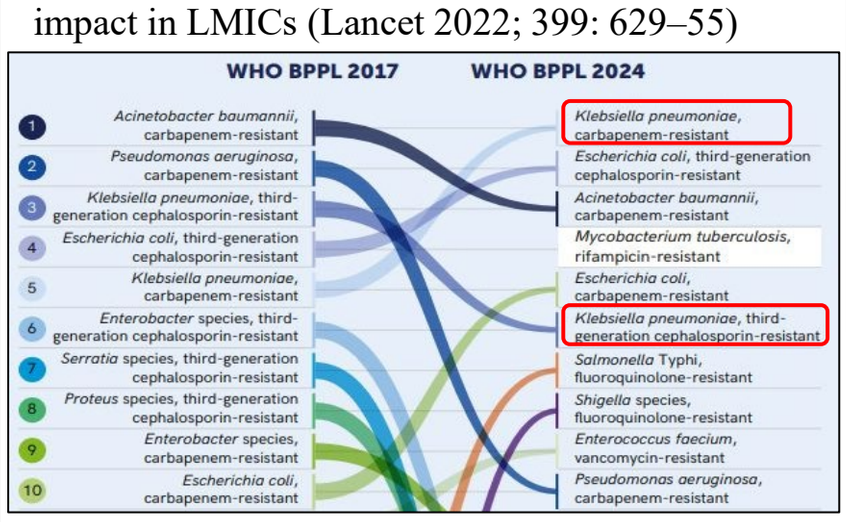
Klebicins as novel therapeutics for the treatment of infections caused by drug-resistant *Klebsiella pneumoniae*

Vivek Daniel Paul, Sukumar Hariharan and Team GangaGen

GangaGen Biotechnologies Pvt. Ltd., Bengaluru, India. www.gangagen.com

Introduction

- *Klebsiella pneumoniae* is a critical priority pathogen with high AMR burden and is a significant contributor of lung infections
- Carbapenem-resistance due to Metallo-beta lactamases (MBL) are prevalent in most LMICs with resistance rates touching 50%
- Emergence of Hypervirulent *K. pneumoniae* (hvKp) that are resistant to carbapenems have increased the risk of morbidity & mortality in hospitals (ECDC. 14 Feb 2024)
- *K. pneumoniae* is responsible for more than 700,000 deaths associated with AMR and with significant impact in LMICs (Lancet 2022; 399: 629–55)



GangaGen's Bacteriocins Platform

Identify Pathogen of interest

↓

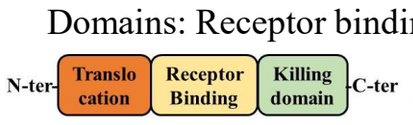
Genome mining: Identify Bacteriocins sequences

↓

Bioinformatics: Delineating conserved domains/functional boundaries

↓

Domains: Receptor binding, Translocation, Killing



↓

Identify novel sequences

↓

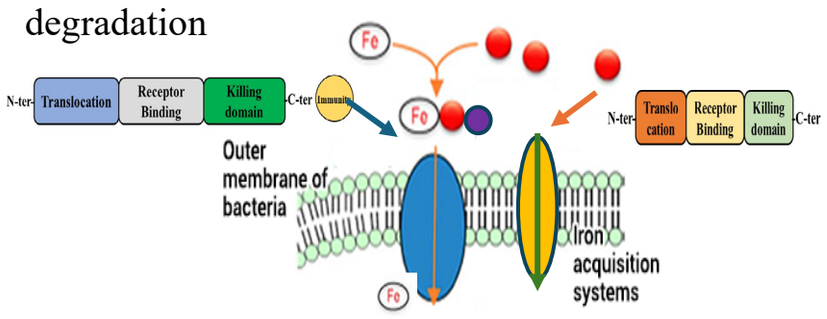
Functional characterization of bacteriocins
Or Engineered Chimeric bacteriocins with desired properties

↓

Library of novel antibacterials

Klebicins: Protein antibiotics produced by *Klebsiella* spp.

- Klebicins belong to a class of natural protein antibiotics called "Large bacteriocins" with mol.wt of 30-60 kDa
- Highly genus specific, multi-domain protein structures that specifically kill *Klebsiella* spp.
- Unique MoA: Cell surface receptor mediated cell entry, translocation to substrate compartment & killing via cell wall damage or nucleic acid degradation



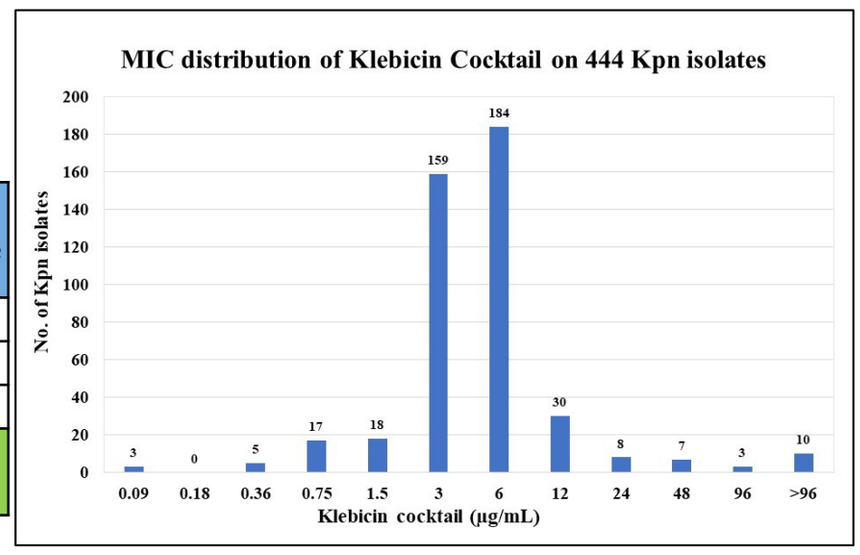
Killing mechanisms:
Pore formation
Peptidoglycan degradation
Nucleases (RNase/DNase)

➢ **We are developing a cocktail containing 3 different Klebicins**

MICs of Klebicins and Klebicin cocktail on global *K. pneumoniae* isolates

- 444 clinical *K. pneumoniae* isolates from all parts of the globe, different body locations, drug-sensitive, drug-resistant and MDR strains

Klebicins	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	% Coverage
GAN002	8	>64	60
GAN010	4	>64	65
GAN011	4	32	85
Klebicin Cocktail (GAN002+010+011)	6	**12**	**95**



- *K. pneumoniae* from all geographies and sites of infection were sensitive to Klebicin cocktail
- Klebicin cocktail covered 95% of *K. pneumoniae* isolates with MIC of ≤12µg/mL total protein (<1 µM)
- No cross-resistance observed to any antibiotic class used
- Klebicins showed potent activity even on MDR strains that are resistant to carbapenems, CAZ-AVI and colistin

FoR studies with the Klebicin cocktail

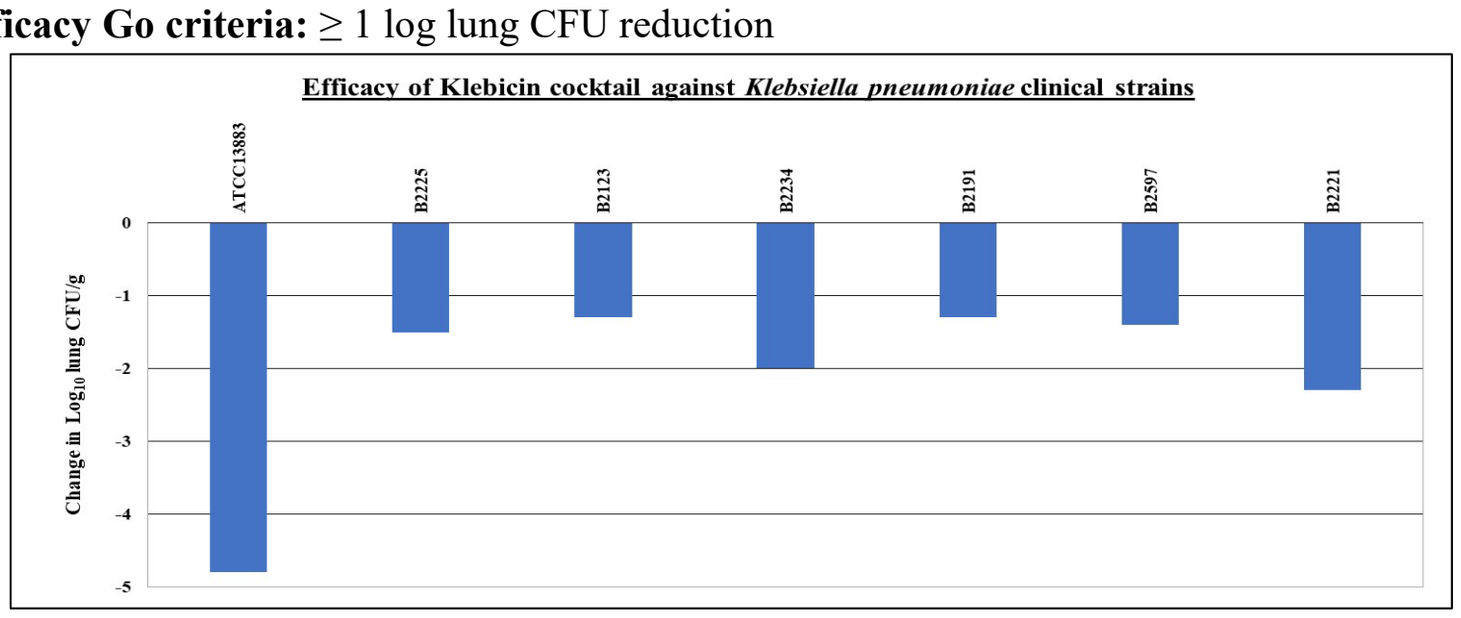
- Frequency of spontaneous resistance mutants to Klebicin cocktail was determined by FoR plating
- Klebicin cocktail was incorporated in solid agar plates at 8X MICs
- *Klebsiella pneumoniae* strains including hypervirulent and drug-resistant strains were screened at high inoculum of 10^9 - 10^{10} cells

Kpn Strains	Cocktail MIC (µg/mL)	Recovery Frequency with Klebicin cocktail at 8X MIC
ATCC13883	1	$<1.8 \times 10^{-9}$
B2191 (hvKp)	1	$<2 \times 10^{-9}$
B2192	1	$<3.2 \times 10^{-9}$
B2225	2	2.7×10^{-9}
B2230	2	$<4 \times 10^{-9}$
B2242 (Cipro^R)	4	$<2.5 \times 10^{-9}$

➢ **FoR of the Klebicin cocktail was ≤ 10^{-9} and no resistant mutants were recovered**
➢ **Low risk of resistance development**

In vivo Efficacy of the Klebicin cocktail against Kpn clinical strains

- **Non-clinical model:** 26hr Neutropenic Lung Infection Model in BALB/c mice
- **Challenge strains:** Drug-sensitive, drug-resistant, MDR, hypervirulent
- **Treatment regimen:** Single IV dosage of Klebicin cocktail administered 2h after infection
- **Efficacy Go criteria:** ≥ 1 log lung CFU reduction



➢ **Klebicin cocktail afforded significant lung bacterial load reduction with a single IV dose**

Current status- Preclinical Development

- **A cocktail of 3 Klebicins GAN002, GAN010 & GAN011 developed and optimized.**
- Klebicins are amenable to high-yield recombinant protein expression in *E. coli.*
- The Klebicin cocktail is highly potent & covers 95% of *K. pneumoniae* isolates including MDR at MIC of <1µM
- Low risk of resistance development to the Klebicin cocktail.
- Safety established for Klebicins and Klebicin cocktail by *in vitro* and *in vivo* assays.
- *In vivo* efficacy of the klebicin cocktail demonstrated in a neutropenic mouse model of lung infection using multiple strains of *K. pneumoniae* including MDR strains.

Acknowledgements: This study is supported by CARB-X grant (PCA No. 4500004357)