Deciphering the Role of Defense Systems in the Evolution of Multi-drug Resistant *Acinetobacter baumannii* ST2

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Background

•**The Threat of CRAB:** Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a WHO "critical" priority pathogen. **ST2** is the globally dominant clone (>50% globally; ~74% in China), notorious for carrying the *bla*OXA-23 gene.

•**HGT & Resistance:** The rapid spread of multidrug resistance (MDR) in CRAB is primarily driven by Horizontal Gene Transfer (HGT) via mobile genetic elements (MGEs), such as transposon Tn*2006*.

•**The Role of Defense Systems:** Bacterial innate and adaptive immune systems, particularly **Restriction-Modification (R-M)** and **CRISPR-Cas**, protect against foreign MGEs.

•**Pilot Study Insight:** Our preliminary analysis of 34 clinical isolates revealed a striking pattern: **100% of *bla*OXA-23-positive strains lacked a complete CRISPR-Cas system**, suggesting a potential evolutionary trade-off where the loss of immune defense facilitates the acquisition of MDR.

Aims & Objectives

This study aims to elucidate the relationship between bacterial defense mechanisms and HGT of antibiotic resistance genes (ARGs) in clinically important A. baumannii.

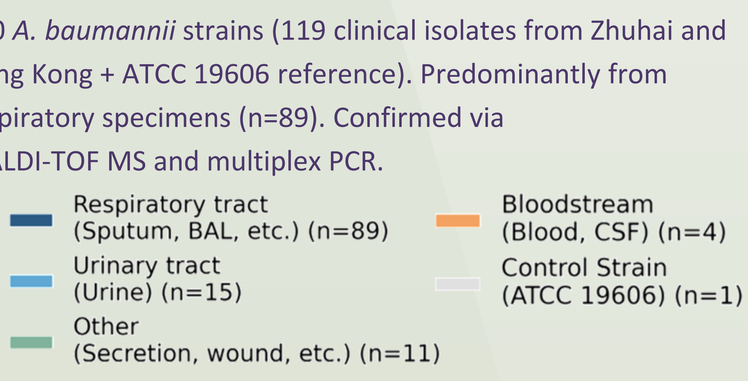
•**Obj 1**: Characterize the ARGs, defense systems, and phenotypic resistance profiles of 120 clinical strains from Hong Kong and Zhuhai.

•**Obj 2**: Investigate the specific role of CRISPR-Cas and Type I R-M system deficiencies in facilitating MGE-mediated ARG acquisition (e.g., blaOXA-23, tet(B)) in prevalent ST2 clones.

Methods

•**Isolate Collection & Identification:**

120 *A. baumannii* strains (119 clinical isolates from Zhuhai and Hong Kong + ATCC 19606 reference). Predominantly from respiratory specimens (n=89). Confirmed via MALDI-TOF MS and multiplex PCR.



•**Phenotypic AST**: Antimicrobial susceptibility testing performed via Kirby-Bauer disk diffusion across 19 antibiotics (8 classes, including carbapenems), interpreted per CLSI 2024 guidelines.

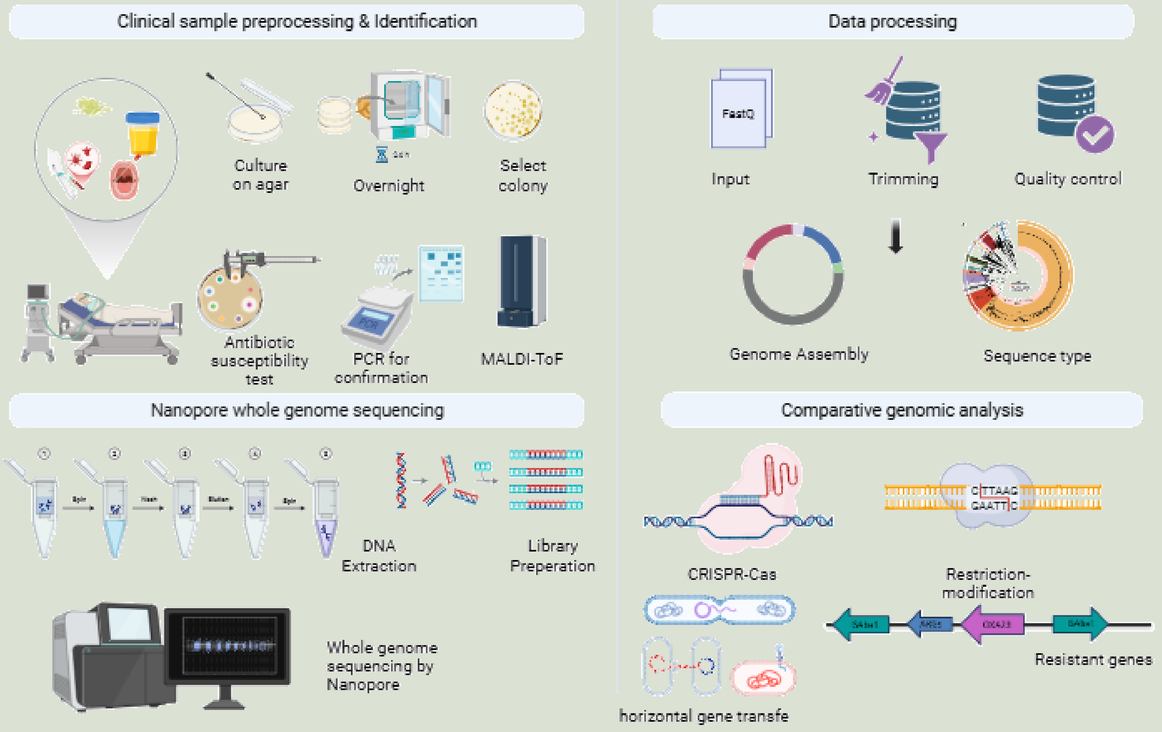
- **Carbapenems:** Doripenem | Imipenem | Meropenem
- **Aminoglycosides:** Amikacin | Gentamicin | Tobramycin
- **Fluoroquinolones:** Ciprofloxacin | Levofloxacin
- **Cephems:** Amoxicillin-Clavulanic Acid | Cefepime | Cefotaxime | Ceftazidime | Ceftriaxone
- **β-lactam Combinations:** Ampicillin-Sulbactam | Piperacillin-Tazobactam
- **Tetracyclines:** Doxycycline | Minocycline | Tetracycline
- **Folate Antagonists:** Trimethoprim-Sulfamethoxazole

•**Whole-Genome Sequencing (WGS)**: High-quality genomic DNA sequenced using Oxford Nanopore Technologies (ONT) MinION platform (Rapid Barcoding Kit V14).

•**Bioinformatics (Bactopia Pipeline)**:

- Assembly: Guppy, Flye, Medaka.
- Typing & AMR: Pasteur MLST, AMRFinderPlus, ResFinder.
- MGEs & Defense Systems: MobileElementFinder and Defense Finder (targeting CRISPR-Cas & R-M systems).

Workflow from clinical to WGS



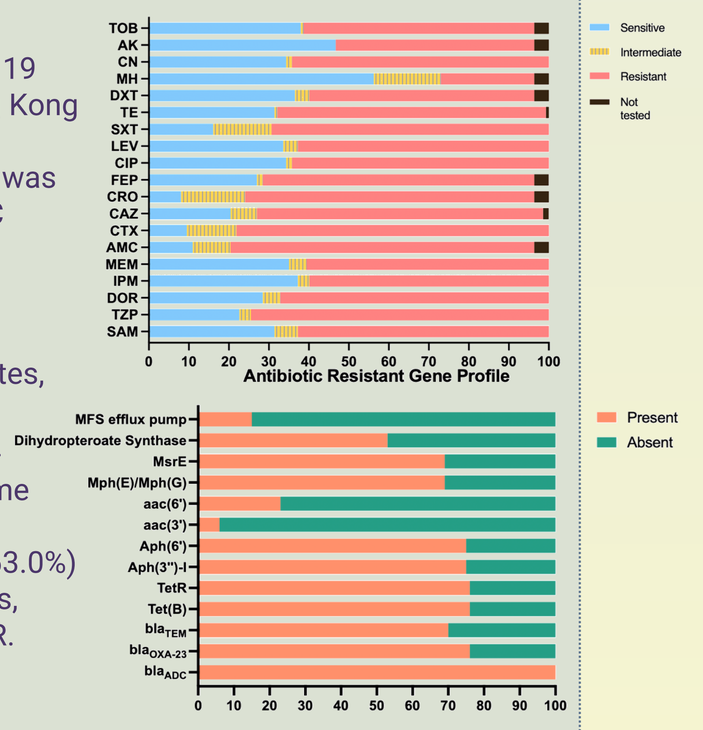
Results

Epidemiology & AMR Profiles

•**High CRAB Prevalence:** >60% of the 119 clinical isolates from Zhuhai and Hong Kong were Carbapenem-Resistant *A. baumannii* (CRAB). Highest resistance was observed against β-lactams (e.g., AMC 95.2%).

•**ST2 Global Dominant Clone:** ST2 accounted for **58.8% (70/119)** of isolates, forming a distinct MDR cluster in the phylogenetic tree (above ATCC19606).

•**Key AMR Determinants:** Whole-genome sequencing identified *bla*OXA-23 (63.8%), *tet*(B) (63.8%), and *aph(3'')-I* (63.0%) as the most prevalent resistance genes, perfectly aligning with phenotypic MDR.



Strong negative correlation between defense systems and ARGs

•CRISPR-Cas as a Susceptibility Marker: A striking inverse correlation was found. 0% (0/27) of CRISPR-Cas-positive strains harboured the *blaOXA*-23 or tet(B) genes. In contrast, CRISPR-negative strains had an >80% probability of carrying these critical ARGs ($p < 0.0001$).

•Type I R-M S/R Subunits as Genomic Gatekeepers: The absence of Type I R-M S/R subunits is a major MDR indicator. Strains lacking these subunits comprised >93% of all carbapenem-, tetracycline-, and aminoglycoside-resistant isolates.

Evolutionary Trade-off in ST2

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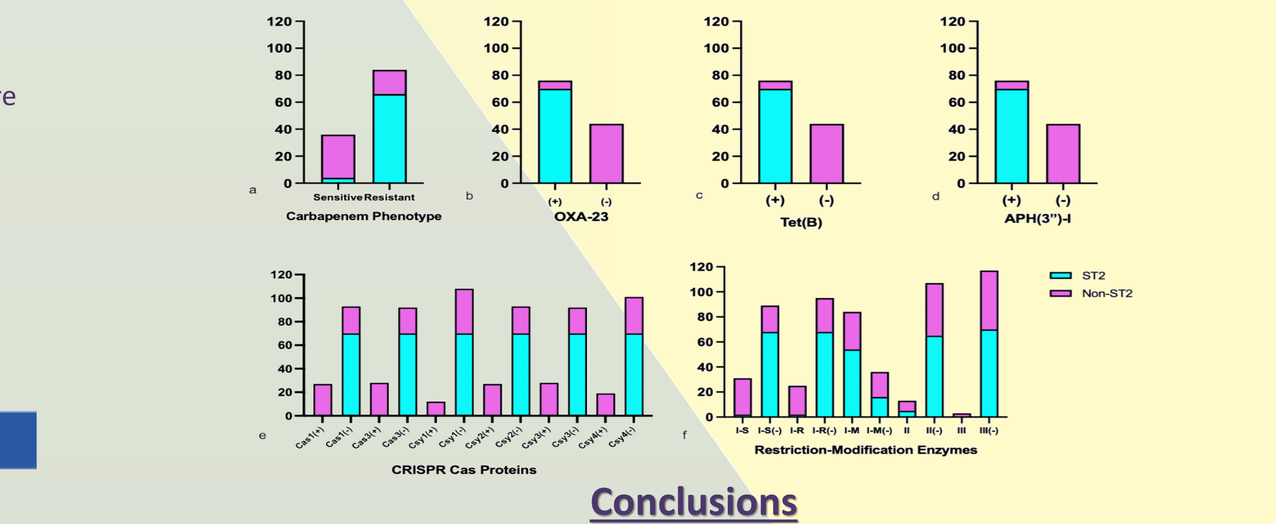

Conclusions

- **Systemic Defense Deficiency in ST2**: Functional CRISPR-Cas and Type I R-M systems are completely or nearly absent in all ST2 clones, in sharp contrast to non-ST2 strains (which maintain 46-58% carriage).
- **Genomic Plasticity Drives XDR**: The lack of this "innate immunity" clears the path for Mobile Genetic Elements (e.g., Tn2006). This allows ST2 to achieve 100% carriage of critical ARGs (like blaOXA-23), driving the XDR phenotype.
- **An Evolutionary Trade-off**: In high-antibiotic hospital environments, ST2 clones sacrifice defense for survival. They compensate for potential fitness costs (e.g., biofilm capacity) via other mechanisms to maintain high pathogenicity.
- **Clinical Implications**: The strong inverse correlation between defense systems and ARGs highlights CRISPR-Cas and R-M presence as potential biomarkers for MDR risk prediction. Furthermore, their defense deficiency exposes a potential vulnerability to targeted phage therapies.

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