Efflux Activity Drives Cefepime Heteroresistance in *Pseudomonas aeruginosa* from Hematologic Malignancy Patients

Thomas P. Murphy[1], Lynne Strasfeld MD[1], William R. Miller MD[2,3], Morgan Hakki MD[1] Rodrigo de Baptista PhD[2,3], and Stephanie L. Egge MD[1]

[1]Oregon Health & Science University, Portland, OR [2]Houston Methodist Hospital, Houston, TX [3]Weill Cornell Medical College, New York, NY

Introduction

Pseudomonas aeruginosa (*PsA*) is a high-priority pathogen, causing significant mortality, particularly in hematologic-malignancy (HM) patients with neutropenia. This population is profoundly vulnerable, with estimated 30-day *PsA* bacteremia mortality rates as high as 30-70%. Despite high *in vitro* susceptibility, clinical failures of the first-line therapy cefepime (FEP) are frequent.

Heteroresistance (HR), a phenomenon whereby a minority subpopulation has reduced susceptibility below threshold detection via standard clinical testing, is a probable source of unanticipated treatment outcomes.

Limited data suggests increased FEP-HR rates among HM patients. FEP-HR has also been linked to MexXY-OprM efflux pump upregulation, a mechanism commonly induced by fluoroquinolone (FQ) exposure and known to promote cefepime resistance.

Case Study

At our institution, FEP-HR has been linked to cases of treatment failure (example provided below in **Fig. 1**)

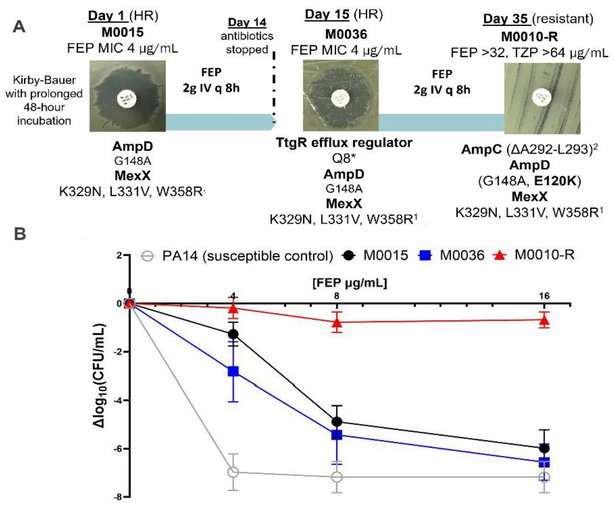

Fig. 1 Case example FEP-HR MexX efflux-associated treatment failure (A) and FEP HR to resistance convergence (A,B) in an HM patient with *PsA* bacteremia with index culture obtained in setting of fluoroquinolone prophylaxis

This case provided clinical support for further investigations of FEP-HR related treatment failure associated with fluoroquinolone exposure and efflux activity.

Hypothesis

We hypothesized that in HM patients, prior fluoroquinolone prophylaxis promotes FEP-HR by inducing MexXY-OprM efflux activity, thereby facilitating cross-resistance and development of overt cefepime resistance.

Objectives

1. Define FEP-HR incidence among HM *PsA* bloodstream infections
2. Identify associations between fluoroquinolone exposure and FEP-HR
3. Identify associations between FEP-HR and efflux upregulation in association with levofloxacin (a fluoroquinolone) exposure, both by phenotypic and genotypic analyses.

Methods

Study Population:
• 51 FEP-susceptible *P. aeruginosa* bloodstream isolates from adult hematologic malignancy patients (2016–2025).
• FEP susceptibility confirmed by broth microdilution (Clinical Laboratory and Safety Institute susceptible criteria ≤8 µg/mL).

Phenotypic & Genotypic Assessments:

- **HR detection:** Population analysis profiling (PAP)-area under the curve (AUC) was assessed across Mueller-Hinton agar gradient of 0, 4, 8, and 16 µg/mL. All isolates were tested for PAP-AUC in quadruplet to reliably confirm phenotype, **Fig 2A.**
- **FEP- HR Definition**: Detectable growth at cefepime concentrations ≥16 µg/mL, and a population analysis profile–area under the curve (PAP-AUC) whose 95% confidence interval did not overlap with the 95% confidence interval of either laboratory reference strain (PA14 or ATCC 27853) controls.
- **Efflux activity:** Subset tested with PAβN (80 µg/mL), a known efflux inhibitor; ≥2-fold decrease in MIC = significant efflux activity, **Fig 2B.**
- **Whole-genome sequencing:** Compared isolates by FEP-HR vs susceptible and by fluoroquinolone exposure status.
- **Analysis:**
  Compared HR vs susceptible for: (1) Clinical risk factors (fluoroquinolone exposure at infection, neutropenia, transplant, demographics, prior β-lactam ≤90 days).(2) First-generation β-lactam microbiologic failure (persistent/recurrent infection). Fisher's exact test was used for binary comparisons. Multivariate regression for independent associations among variables (efflux, genome features, fluoroquinolone exposure).

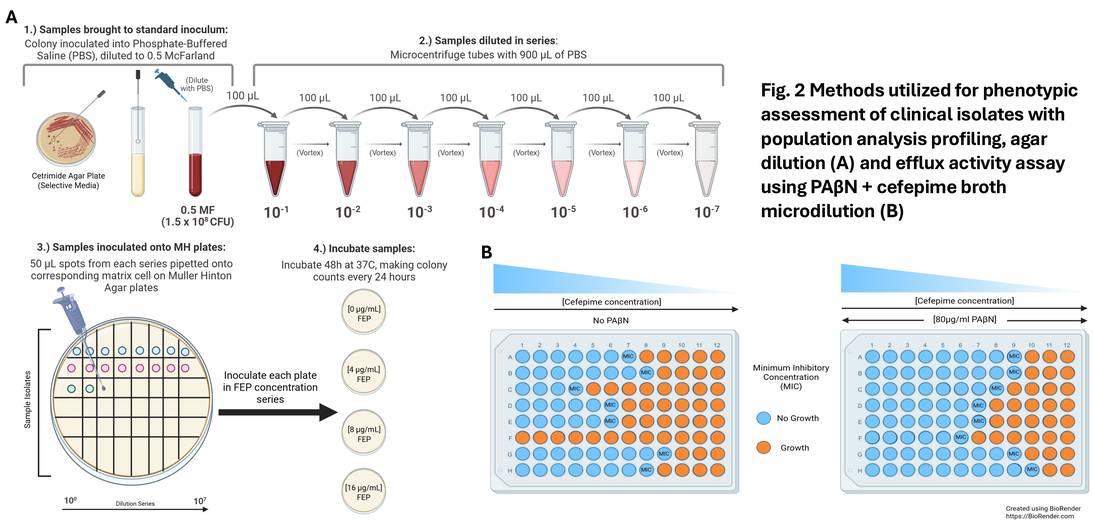

Fig. 2 Methods utilized for phenotypic assessment of clinical isolates with population analysis profiling, agar dilution (A) and efflux activity assay using PAβN + cefepime broth microdilution (B)

Results

Table 1. Clinical and microbiologic features of *Pseudomonas aeruginosa* bloodstream isolates infection patients with hematologic malignancy (2016-2025)

Microbiologic Features/Phenotype	Heteroresistant (n=27)	Susceptible (n=20)	p-value
FEP AUC (Range)	79.4-111.8	71.0-76.0	<0.0001
FEP MIC (Median, Range)	4, (2-8)	2, (0.5-2)	<0.0001
Clinical Features/Phenotype	**Heteroresistant (n=21)**	**Susceptible (n=20)**	**p-value**
Charlson Comorbidity Index (Median ± 95%CI)	4.3 ± 1.2	5.4 ± 1.4	0.24
SOFA Score (Median ± 95% CI)	5 ± 1.4	5 ± 1.6	0.88
ANC<500, N	13	9	0.13
Prior FEP or TZP exposure, 30 days	8	7	0.75
Active levofloxacin prophylaxis at time BSI	12	5	0.02
Healthcare associated bacteremia	14	9	0.08

Prevalence & Clinical Associations

- FEP-HR was prevalent **(53%)** in PsA isolates from the HM cohort.
- **FQ exposure increased odds of FEP-HR** (p=0.02, OR = 5.49 (95% CI: 1.39–21.59)
- FEP-HR infections trended toward **β-lactam failure; although not statistically significant and limited by overall study power in treatment groups** (p=0.07, n= 5/15 vs 1/17).

Efflux activity assessment

- The HR phenotype was directly linked to **increased efflux pump activity**, as confirmed by greater PAβN-mediated inhibition in HR isolates (p=0.002), **Fig. 3A.**
- A high **baseline efflux upregulation** was identified as a key characteristic of these HR isolates.
- This high efflux activity was statistically **independent of recent FQ exposure** in the multivariate model (p<0.0001), **Fig. 3B.**

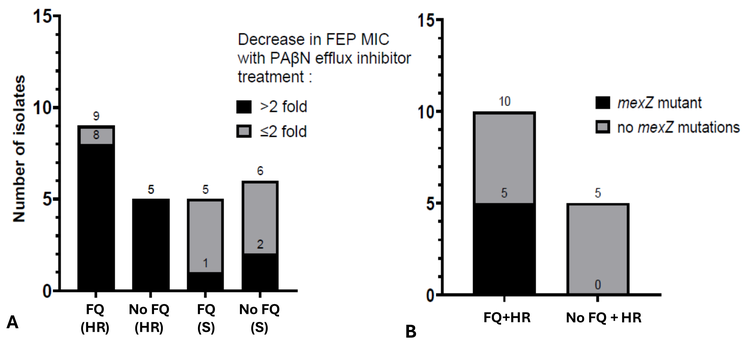

Fig. 3 FEP-HR occurs independent of FQ exposure (A) but probable loss of function mutations in *mexZ*, the master MexX transcriptional regulator, are uniquely enriched among FQ exposed FEP-HR isolates (B)

Results

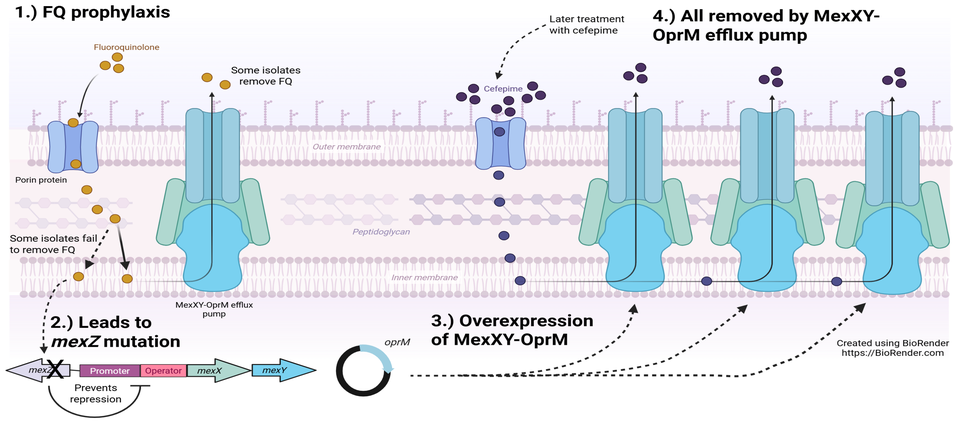

Fig. 4 Visual schematic demonstrating association of FQ, *mexZ*, and MexXY efflux interplay

- FQ exposure appears to help the **selection and stabilization** of the most robust resistance phenotypes.
  - Mutations in the ***mexZ* repressor gene** were found **exclusively in FQ-exposed isolates** (p=0.02).
    - Inactivation of MexZ (which represses MexXY-OprM) leads to constitutive overexpression; these *mexZ* mutants also corresponded to the **highest PAβN fold-change responses**.

Conclusions

FEP-HR is common among HM-associated *PsA*. Prior FQ exposure predisposes to FEP-HR and, in a subset of isolates, is associated with *mexZ* mutations that likely enhance *MexXY-OprM* expression. However, neither FQ exposure nor *mexZ* mutations fully accounts for the efflux-associated FEP-HR phenotype. FEP-HR isolates demonstrated increased FEP efflux activity, even in the absence of detectable MexXY pathway mutations or prior FQ exposure. This suggests that while FQ-mediated MexXY induction is one mechanism of FEP-HR, alternative efflux mechanisms also contribute. Given the association of FEP-HR with treatment failure, the impact of FQ prophylaxis and efflux enhancement on this phenotype warrants further investigation to guide therapeutic strategies and mitigate resistance and clinical failure.

Acknowledgement & References

This work was supported by, and funded from, the Collins Medical Trust through Oregon Health and Science University Foundations. W.R.M. funding via R21. We would like to thank the Messer lab group, and the Molecular Microbiology and Immunology department at OHSU, for their insightful discussions, technical advice, and equipment sharing. · Cebrecos C., et al. (2022) 10.3390/pathogens11101132 · Gudiol C., et al. (2024) 10.3390/microorganisms12040705 · Jia X., et al. (2020) 10.1016/j.ijantimicag.2019.10.013 · Hocquet D., et al. (2008) 10.1128/AAC.01212-07