

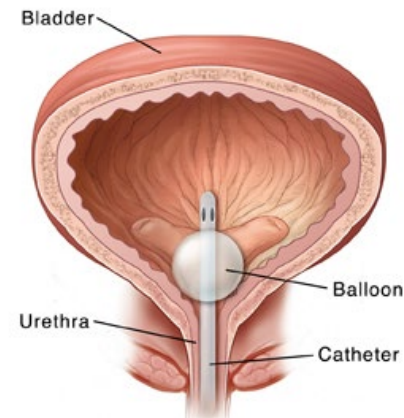
Proteus mirabilis CAUTI: Impact of Polymicrobial Interactions on Fitness Requirements

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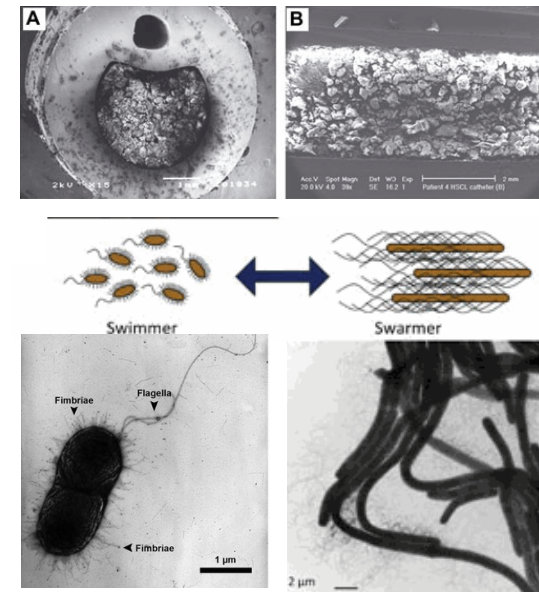
Polymicrobial bacteriuria and CAUTI

- 3-10% incidence of bacteriuria per day
 - ~100% if >14 days (common in nursing homes)
 - Polymicrobial colonization
- *Proteus mirabilis*
 - 26% of 182 recent CAUTIs in 12 nursing homes
 - 42% of these were polymicrobial
 - *Enterococcus faecalis*, *Escherichia coli*, *Providencia stuartii*, *Morganella morganii*
 - Antibiotic resistant
 - Tetracycline class antibiotics
 - Polymyxin and antimicrobial peptides
 - Emerging ESBL+ and carbapenem resistant isolates
 - Secondary bacteremia
 - 10-13% mortality
 - > if MDR and/or polymicrobial
 - No vaccine



Proteus mirabilis pathogenesis

- Targeted mutagenesis
 - ~4000 genes
 - 52 tested in murine model of UTI (1997-2017)
 - Urease enzyme
 - Flagella (swim/swarm)
 - Fimbriae (17 distinct types)
- Genome-wide assessments
 - Transcriptome analysis (UTI)
 - Pearson et al *Infect Immun* 2011
 - Signature-tagged mutagenesis (UTI)
 - Zhao et al *Microbiology* 1999
 - Burrall et al *Infect Immun* 2004
 - Himpsl et al *J Med Microbiol* 2008
- UTI vs CAUTI? Monomicrobial vs polymicrobial?

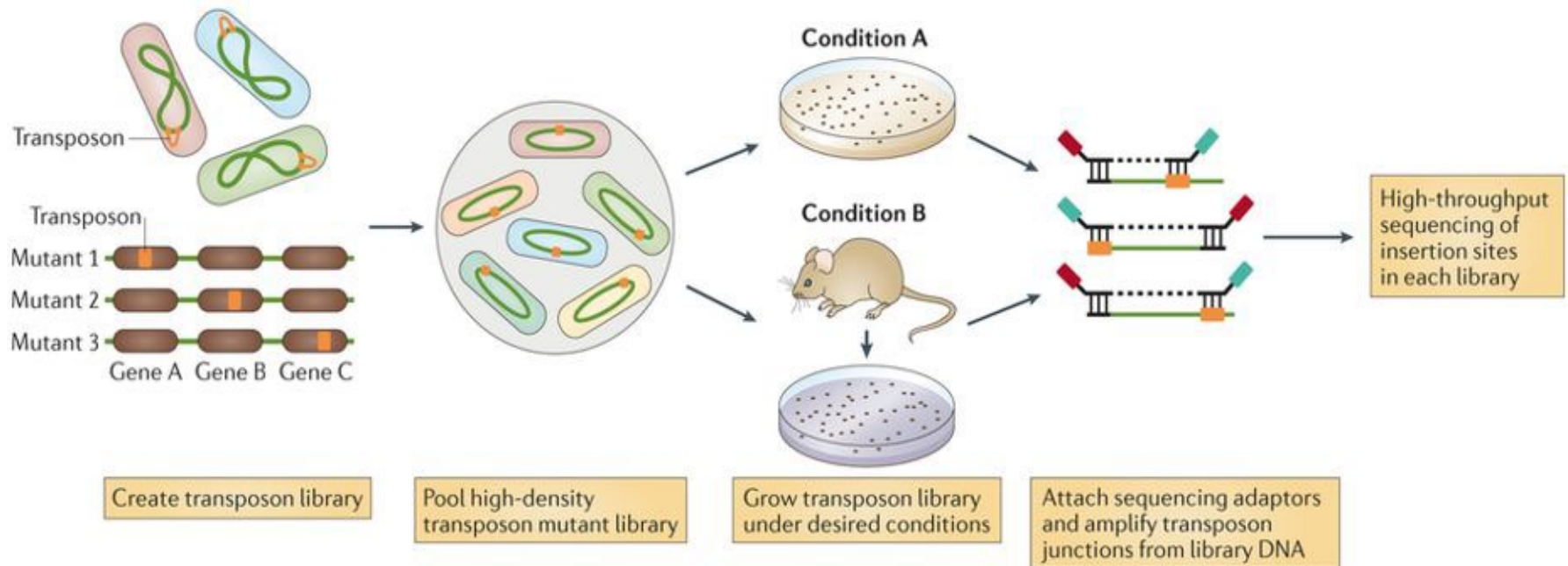


Stickler *Nat Clin Pract Urol* 2008
Armbruster, Mobley, Pearson *EcoSal Plus* 2018
Fernandez-Delgado et al *Annals of Microbiology* 2015

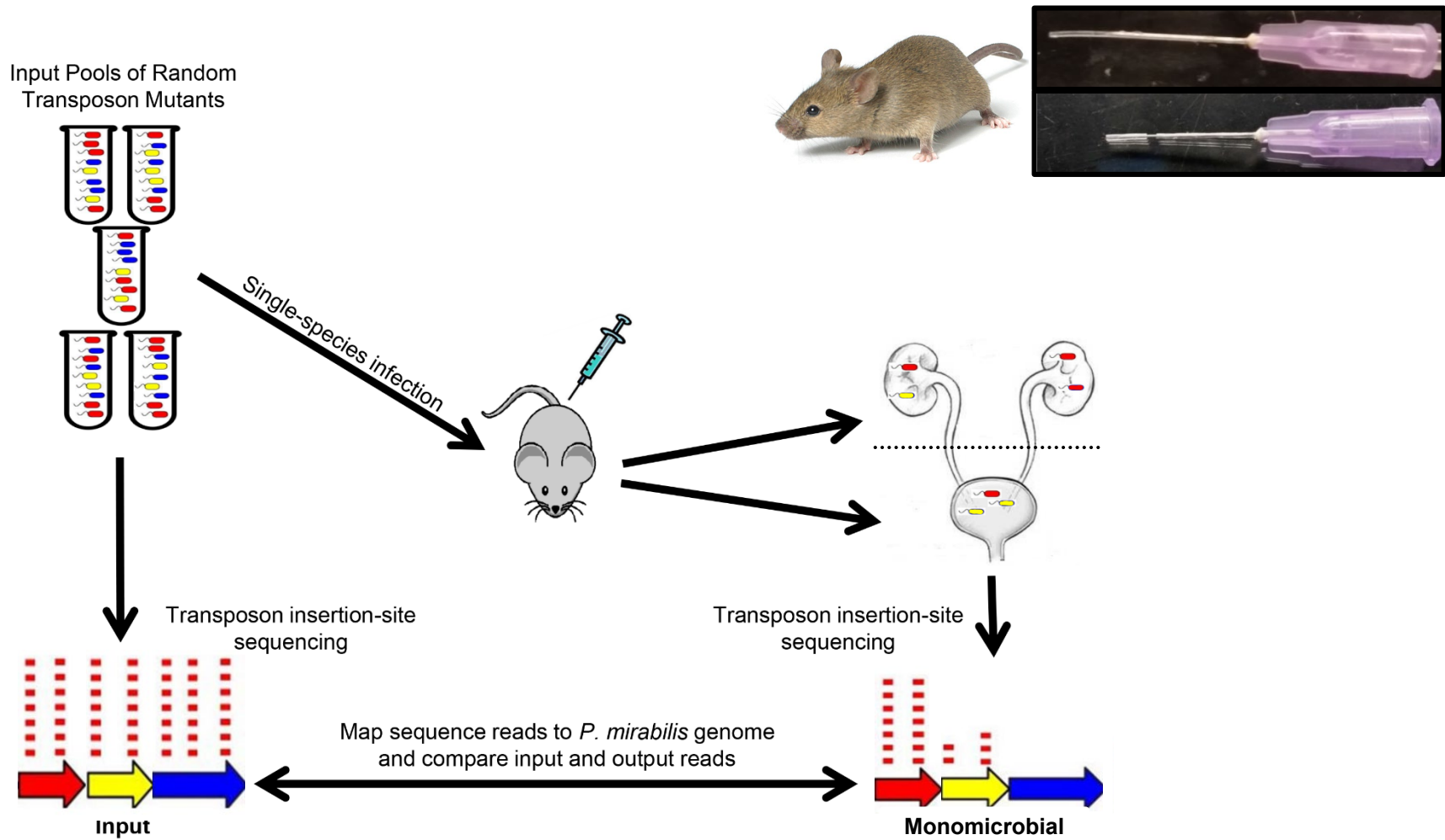
Transposon insertion site sequencing

■ Transposon insertion site sequencing (Tn-Seq)

- Genetic tool to randomly disrupt genes

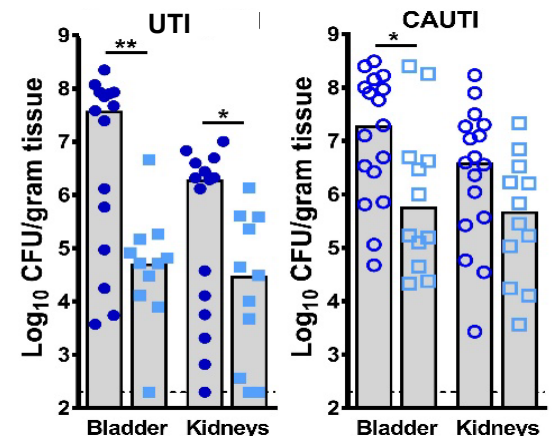
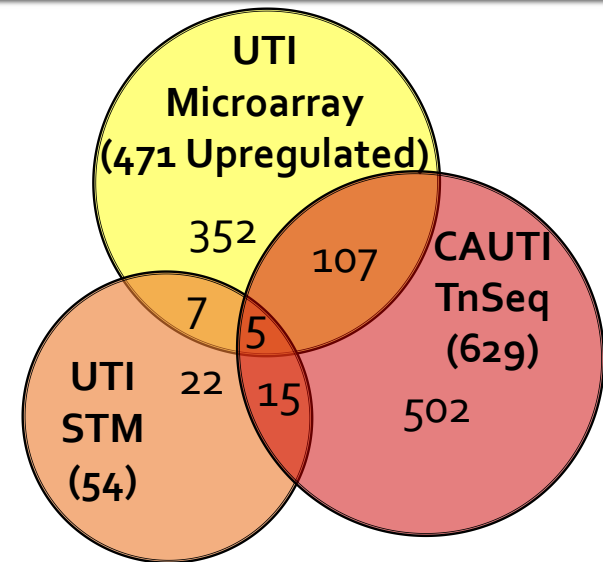


P. mirabilis CAUTI Tn-Seq

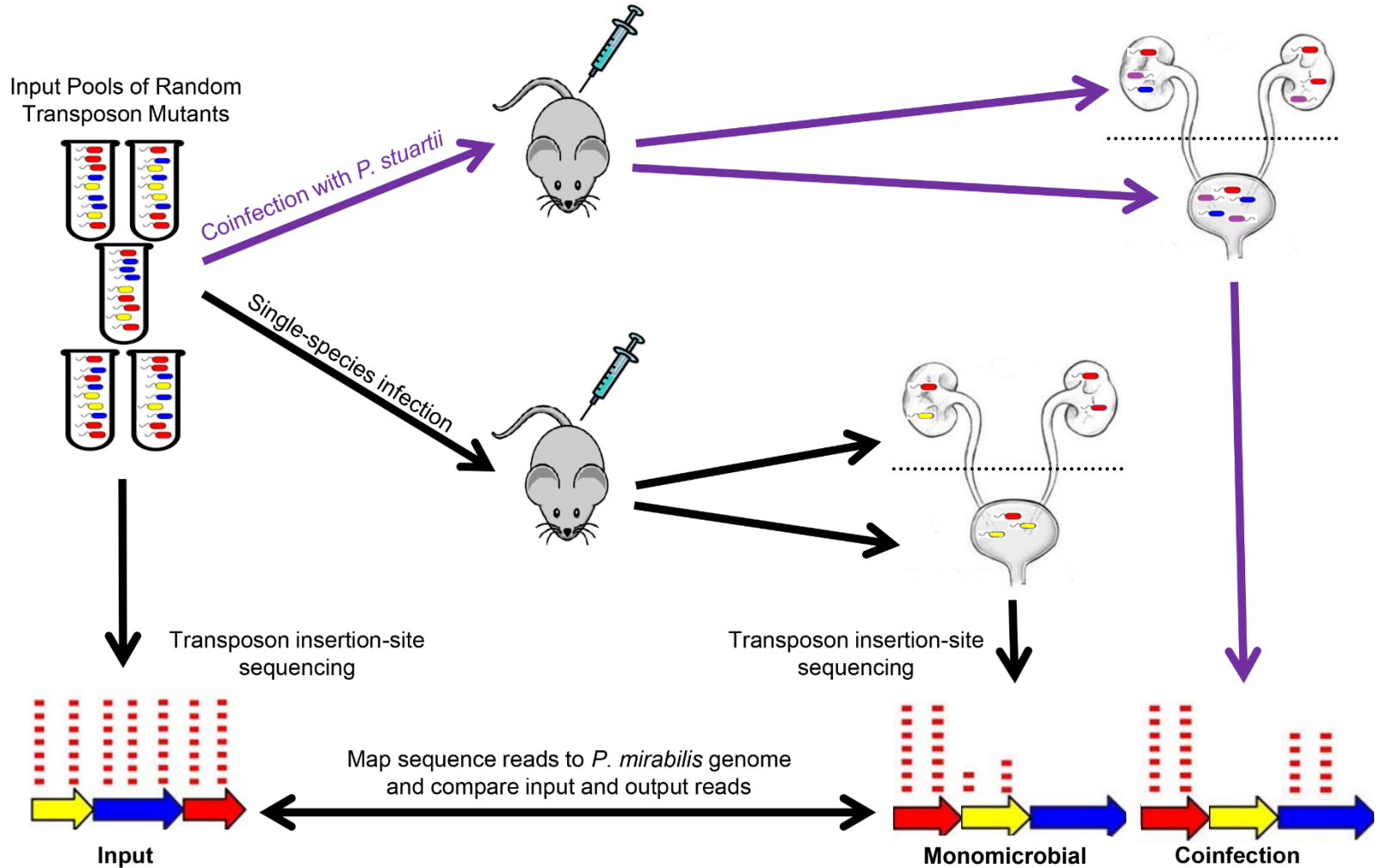


P. mirabilis requires a different set of genes for fitness during CAUTI vs UTI

- Commonalities
 - Urease
 - Flagella
 - Amino acid transport and metabolism
- Differences
 - Nitrogen assimilation pathways
 - Central metabolic pathways
 - Fimbriae



P. mirabilis CAUTI Tn-Seq: monomicrobial vs polymicrobial infection



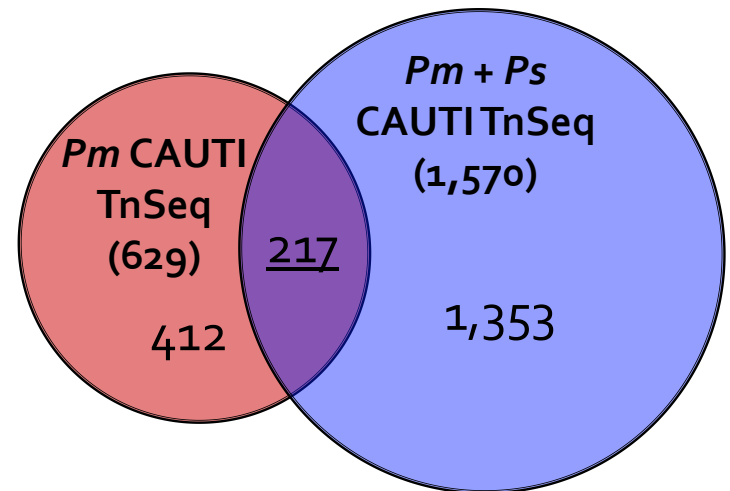
P. mirabilis requires a different set of genes for monomicrobial vs polymicrobial CAUTI

- Commonalities (217 genes)

- Urease
- Metal homeostasis
- Serine utilization

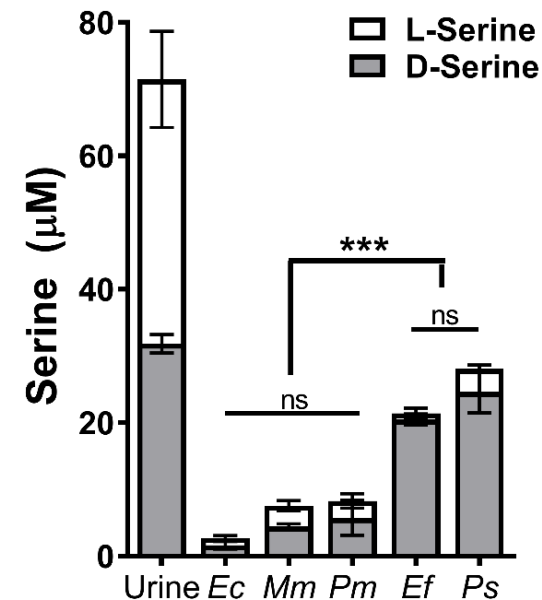
- Differences

- Fimbriae
- Iron acquisition
- Defense mechanisms
- Branched chain amino acid (BCAA) biosynthesis



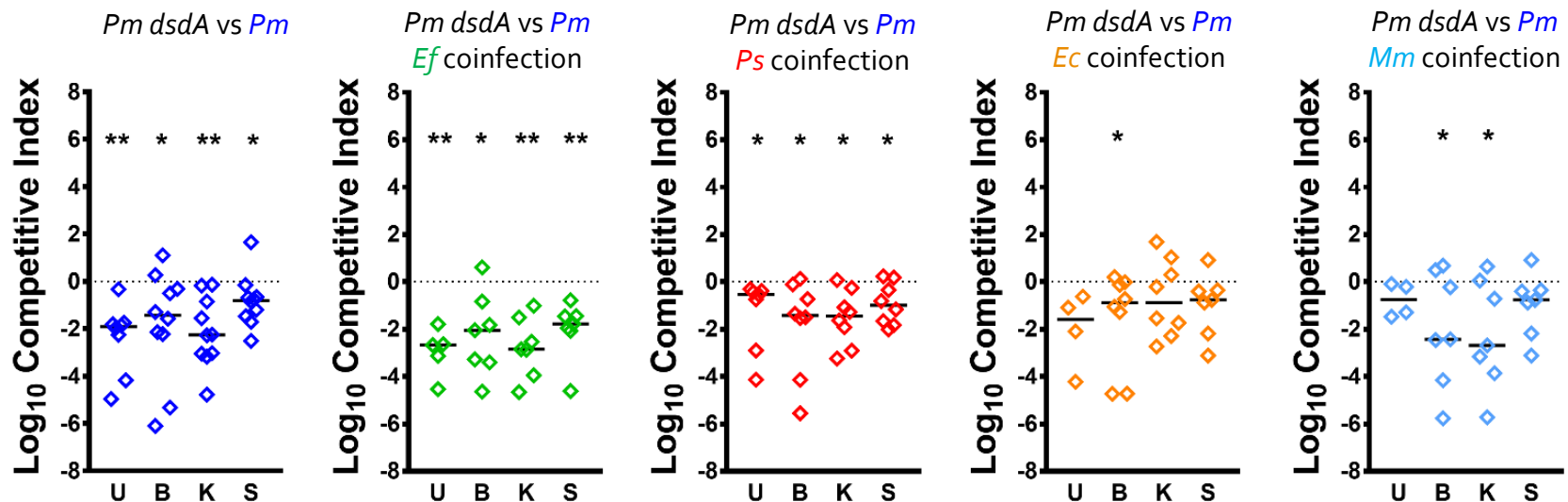
Serine utilization is a candidate *P. mirabilis* fitness factor for UTI and CAUTI

- 5 genes for DL-serine transport and metabolism were fitness factors for both monomicrobial and polymicrobial CAUTI
 - D-serine genes identified in all *P. mirabilis* genome-wide studies (UTI and CAUTI)
 - D-amino acids are rare, but D-serine is present in urine and serum at relatively high concentrations
- DL-serine depletion
 - *E. coli*, *M. morgani*, *P. mirabilis*, *E. faecalis*, *P. stuartii*



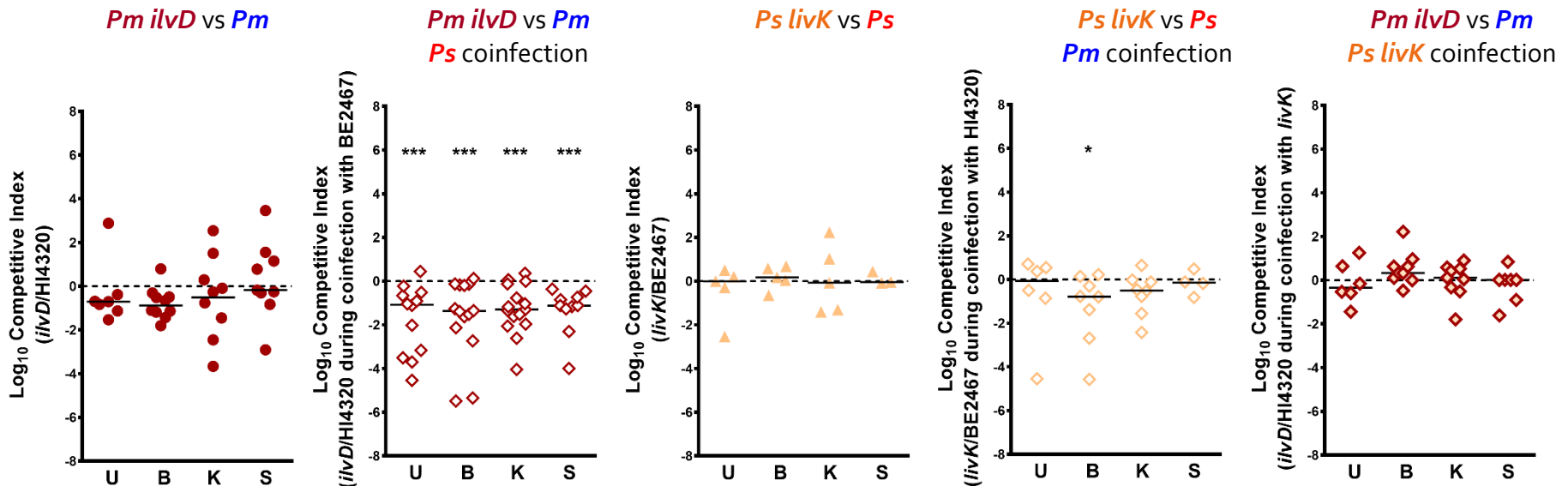
Serine utilization contributes to *P. mirabilis* fitness during monomicrobial and polymicrobial CAUTI

- D-serine ammonia lyase (*dsdA*)
 - Degrades D-serine to pyruvate and ammonia
 - Allows *P. mirabilis* to use D-serine as sole C or N
- *Pm dsdA* co-challenge experiment:
 - Competitive index = $\frac{dsdA/WT \text{ output}}{dsdA/WT \text{ input}}$



BCAA biosynthesis only contributes to *P. mirabilis* fitness during polymicrobial CAUTI

- BCAA biosynthesis genes only identified by TnSeq during coinfection
 - Upregulated during UTI
 - BCAA synthesis (*ilvD*)
- P. stuartii* BCAA import
 - High-affinity importer (*livFGHM*)
 - periplasmic binding protein (*livK*)



Summary and Conclusions

- The presence of a catheter changes the bladder environment
 - Impacts which fitness factors are required for colonization and persistence
- Polymicrobial colonization changes the bladder environment
 - Impacts which fitness factors are required for colonization and persistence
- Use the model that best mimics patient population

- Translational implications:
 - Need to understand what genes are essential for a bacterium during monomicrobial and polymicrobial CAUTI for a logical drug target strategy

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