Genetic basis of growth characteristics and trimethoprim-sulfamethoxazole susceptibility of thymine-dependent and thymine-independent serial blood culture isolates of *Escherichia coli*

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Background

In a patient with an inoperable aortic graft infection, long term antibiotic prophylaxis was initiated after an initial bacteremia episode in 2011. Multi-resistant extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* were recovered from blood cultures of this patient at recurrent bacteremia episodes from June 2014 to July 2015. The *E. coli* isolates were found to express different degree of susceptibility to trimethoprim-sulfamethoxazole (TMP-SMZ) and growth characteristics on Mueller-Hinton (MH) agar.

Objectives

The aim of this study was to investigate the molecular mechanisms of treatment-based induction of TMP-SMZ resistance and thymine auxotrophy in *E. coli* isolated from relapsing episodes of bloodstream infection.

Materials and Methods

- Cultivation and antimicrobial susceptibility testing of all *E. coli* strains (n=10) against TMP-SMZ were performed on M9 minimal agar media with thymine concentrations (20, 5, 2 and 0.5 µg/ml) and MH agar and interpreted according to EUCAST breakpoint table v.7.0.
- Multiple-locus variable number tandem repeats analysis (MLVA) was performed as previously described[1].
- All strains were whole genome sequenced using Illumina MiSeq.
- Genomic analyses were directed towards detection of mutations previously described in thymine auxotrophy, including mutations in *thyA* and deoxyribose salvage pathway genes[2,3].
- A time-comparative analysis between usage of antimicrobial therapy and isolation of *thy+* and *thy- E. coli* strains was retrospectively performed.

Results

- *E. coli* strains that were susceptible to TMP-SMZ (n=4) grew on both MH agar and M9 media. Strains that did not grow on MH agar (n=6) were found to be TMP-SMZ resistant when tested on M9 media and blood agar. All strains grew at low concentrations of thymine (0.5 µg/ml), although with different growth characteristics.
- All *E. coli* strains were identical in all ten loci by MLVA analysis, indicating that all strains belonged to the same clonal lineage.
- Genomic analysis revealed mutations in *thyA* in all resistant strains, specifically a previously described G172C substitution causing Glu-58 to Gln-58 in thymidylate synthase[4].
- Mutations affecting genes involved in the deoxyribose salvage pathway were identified in eight strains. These mutations include a C801A substitution in *deoB* and deletion in *deoC* (Figure 1).
- Time-comparative analysis indicated that TMP-SMZ prophylaxis selected for resistance at various time points, whereas sensitive strains were selected after cessation of prophylaxis (Figure 1).

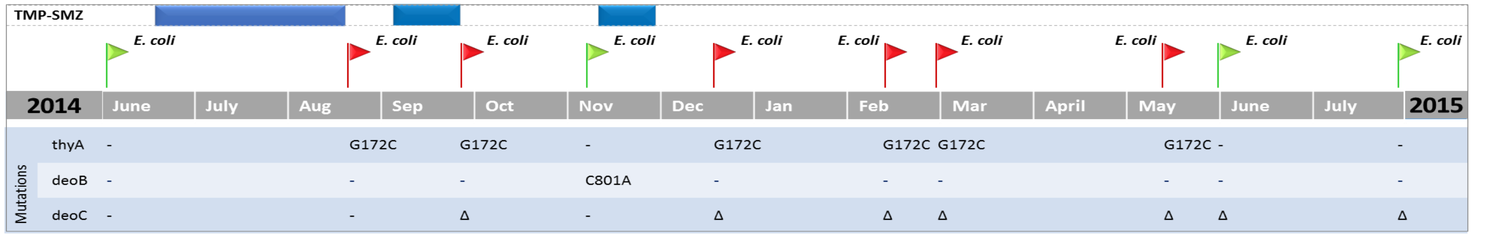

Figure 1. Timeline of TMP-SMZ prophylaxis and bacteremia episodes with susceptible (green) and resistant (red) *E. coli* strains and their corresponding mutations in *thyA* and deoxyribose salvage pathway genes. No mutations were detected in *tmk, ndk, deoA, deoD, deoR, tdk, cdd, add, dgt* and *yjjG*.

Conclusions

- A heterogeneous population originating from a single clone of *E. coli* were isolated from recurring bacteremia episodes over a 14-month period.
- A mutation in *thyA* was established as the primary cause of resistance, which was selected for upon prophylaxis with TMP-SMZ.
- The strains grew in lower thymine concentrations *in vitro* than previously reported, however the significance of this on growth *in vivo* is unknown.

References

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