

Emerging mutations causing resistance to a new antibiotic, SPR-206

The role of mutations in the basR gene in new-generation polymyxin antibiotic resistance

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A new hope for the future

Antibiotic resistance in multidrug-resistant Gram-negative bacteria is a growing issue. Polymyxins are a last-resort treatment, however resistance is rising. New polymyxin derivatives, SPR-206 and POL-7306, show promise as alternatives. **SPR-206** is a polymyxin-type antibiotic that has demonstrated significant efficacy against multidrug-resistant Gram-negative bacteria. Currently, it is in Phase II of clinical trials.

Polymyxin resistant pathogens commonly harbour basR gene mutations

Our findings, consistent with both existing literature and our previous experiments, indicate that pathogens resistant to polymyxin-type antibiotics frequently harbor mutations in the basR gene. To further investigate this phenomenon, it is crucial to elucidate the function of this gene, its role in resistance mechanisms, and its regulatory pathways.

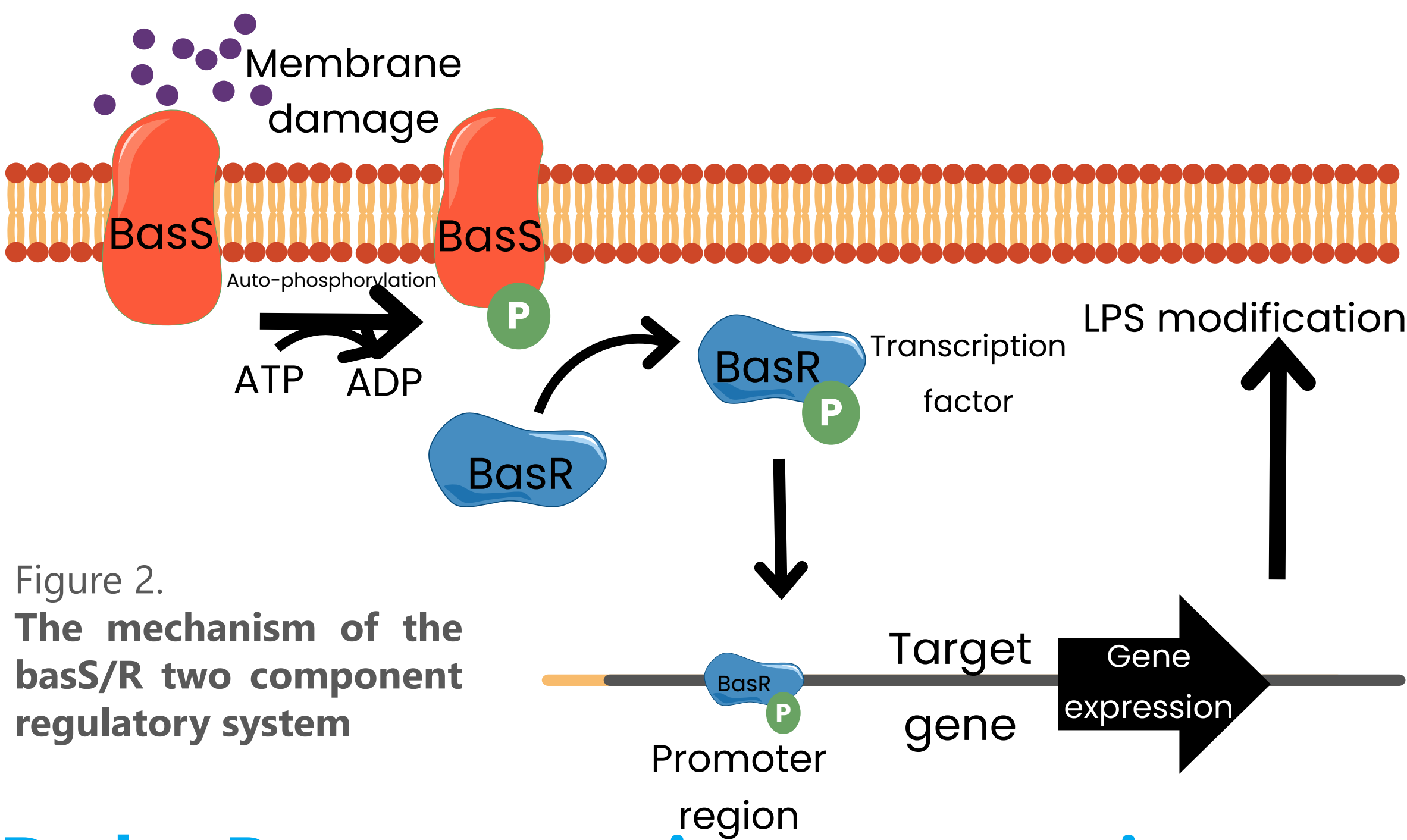


Figure 2.
The mechanism of the basS/R two component regulatory system

Do basR gene mutations cause resistance to SPR-206?

To investigate this, I generated basR mutant *E. coli* lines with a clean genetic background using DIVERGE mutagenesis¹. Following mutagenesis, I selected basR mutant strains, confirmed their genetic modifications via Sanger sequencing, and assessed their minimum inhibitory concentration (MIC) and virulence.

All basR mutant E. coli strain was resistant to SPR-206 and polymyxin-B, but not to POL-7306

The basR mutant *E. coli* strains exhibited a 32-fold increase in resistance to SPR-206 compared to the wild-type strain and an 8-fold increase in resistance to polymyxin B, a last-resort antibiotic. However, no change in resistance was observed against POL-7306.

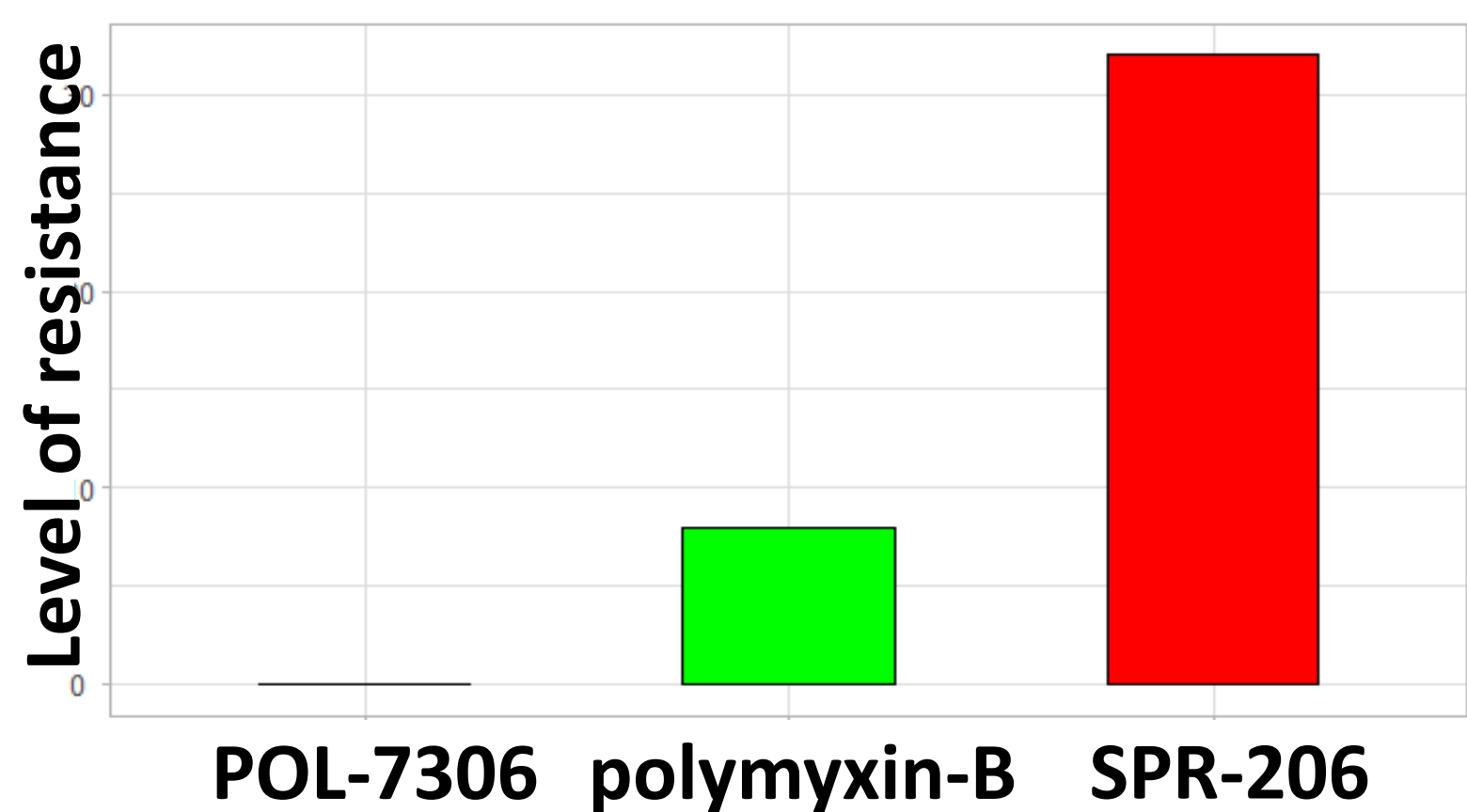


Figure 3. The MIC increases in the mutant E.coli lines

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SPR-206 resistant mutants are already present in clinical isolates

Most of the strains analyzed in this study have been previously documented in scientific literature², underscoring the significance of our research. Notably, despite the antibiotic we investigated not yet being approved for clinical use, resistance-conferring mutations are already present in these strains.

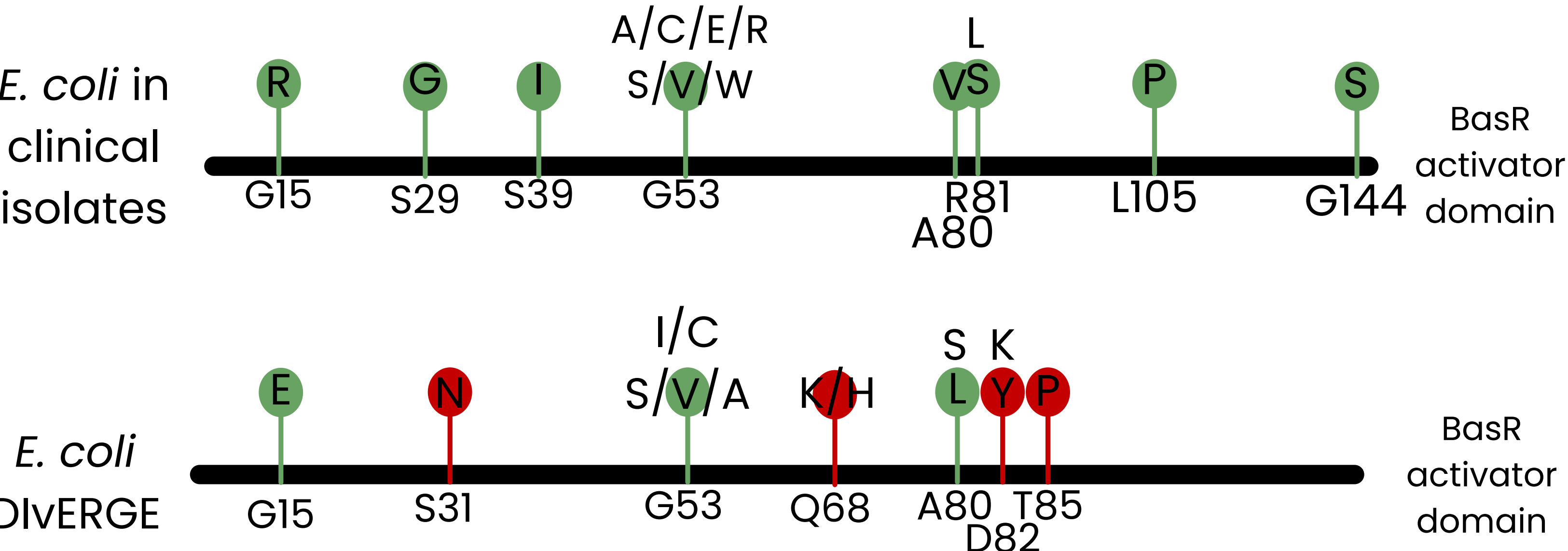


Figure 4. The mutations detected in the resistant lines Green indicates known mutations. The letter below the line is the original amino acid, the number is the mutation position, and the top letter is the new amino acid.

BasR mutations in E.coli do not lead to virulence decrease in G. mellonella

Contrary to previous literature suggesting that increased antibiotic resistance is often associated with a fitness cost, particularly a reduction in virulence, our findings did not support this trend. Despite the significant increase in resistance, the virulence of the basR mutant strains remained unchanged compared to the wild-type.

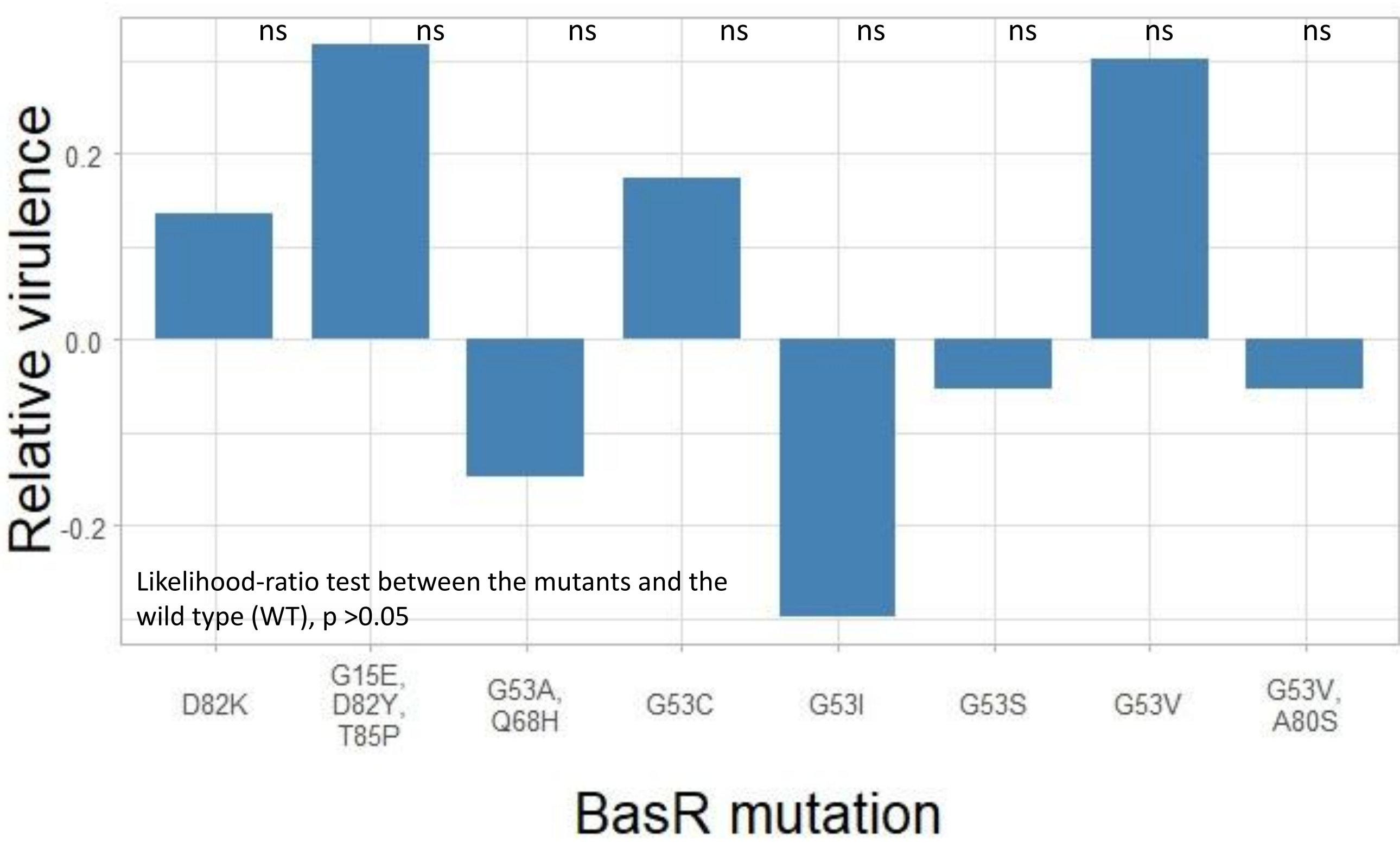


Figure 5. Relative virulence change of basR mutant *E. coli* strains. The figure shows the wild type's virulence as 0. The titles of the columns show where exactly the mutation occurred in the basR gene.

Conclusions

Most of the mutations that we found are already present in the clinical environment

Increased resistance to SPR-206 also comes with increased polymyxin-B resistance

Virulence did not decrease, even with the resistance change

However, there was no resistance increase for POL-7306

References

- (1) Nyerges, Ákos et al. "Directed evolution of multiple genomic loci allows the prediction of antibiotic resistance." Proceedings of the National Academy of Sciences of the United States of America vol. 115,25 (2018): E5726-E5735. doi:10.1073/pnas.1801646115
- (2) Janssen, Axel B et al. "Nonclonal Emergence of Colistin Resistance Associated with Mutations in the BasRS Two-Component System in Escherichia coli Bloodstream Isolates." mSphere vol. 5,2 e00143-20. 11 Mar. 2020, doi:10.1128/mSphere.00143-20