

# Possession of *exoU* by *Pseudomonas aeruginosa* is associated with increased mutations in beta-lactam resistance genes

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## INTRODUCTION

Certain pathogenic strains of *P. aeruginosa* can acquire an important virulence factor, *exoU*, that has a positive association with resistance to fluoroquinolones, and production of microbial keratitis. *ExoU* strains have more mutations in quinolone resistance-determining regions (QRDR) than non-*exoU* strains.<sup>1,2,3,4</sup> We hypothesized that possession of the *exoU* was associated with mutations not only in QRDRs but also in other drug resistance determining regions.

## METHODS

The antibiotic susceptibility and genetic characteristics of 12 strains of *P. aeruginosa* isolated from microbial keratitis (MK) and 10 from cystic fibrosis (CF) were examined. Whole genome sequencing was performed by MiSeq Illumina®. Genome assembly, annotations and variants calling were performed using SPAdes, Prokka, Bowtie2, Samtools and snpEff. Mutations were examined in QRDRs (*gyrA/gyrB/parC/parE*), the multi-drug efflux pump (*mexR*), chromosomal beta-lactamase (*ampC*) and its transcriptional regulators (*ampR*). Relative expressions of *mexR*, *ampC*, and *ampR* genes were examined by RT-qPCR using wild type non-*exoU* strains *P. aeruginosa* PAO1.

## RESULTS

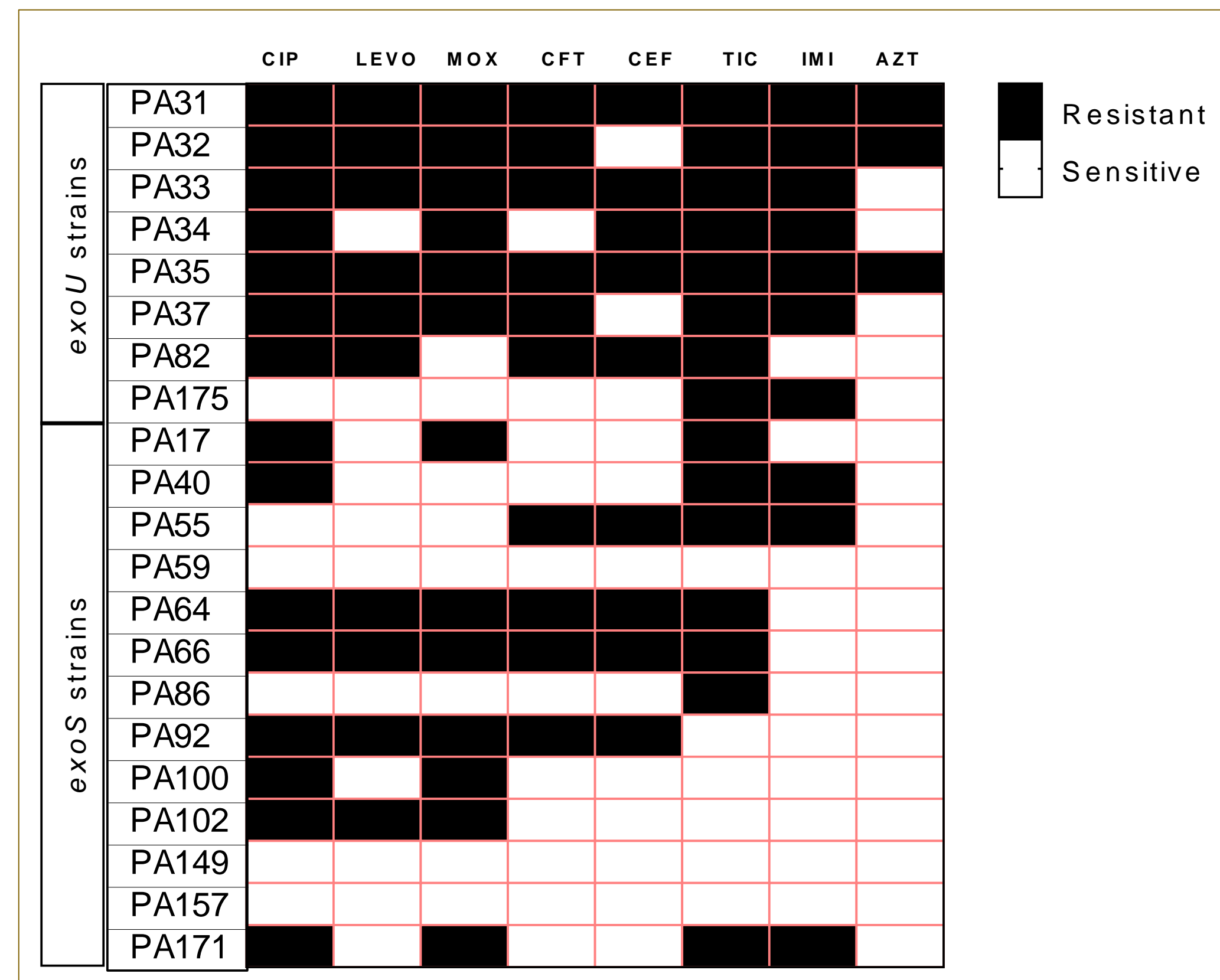


Figure 1: Antibiotic susceptibility pattern of *exoU* and *exoS* strains

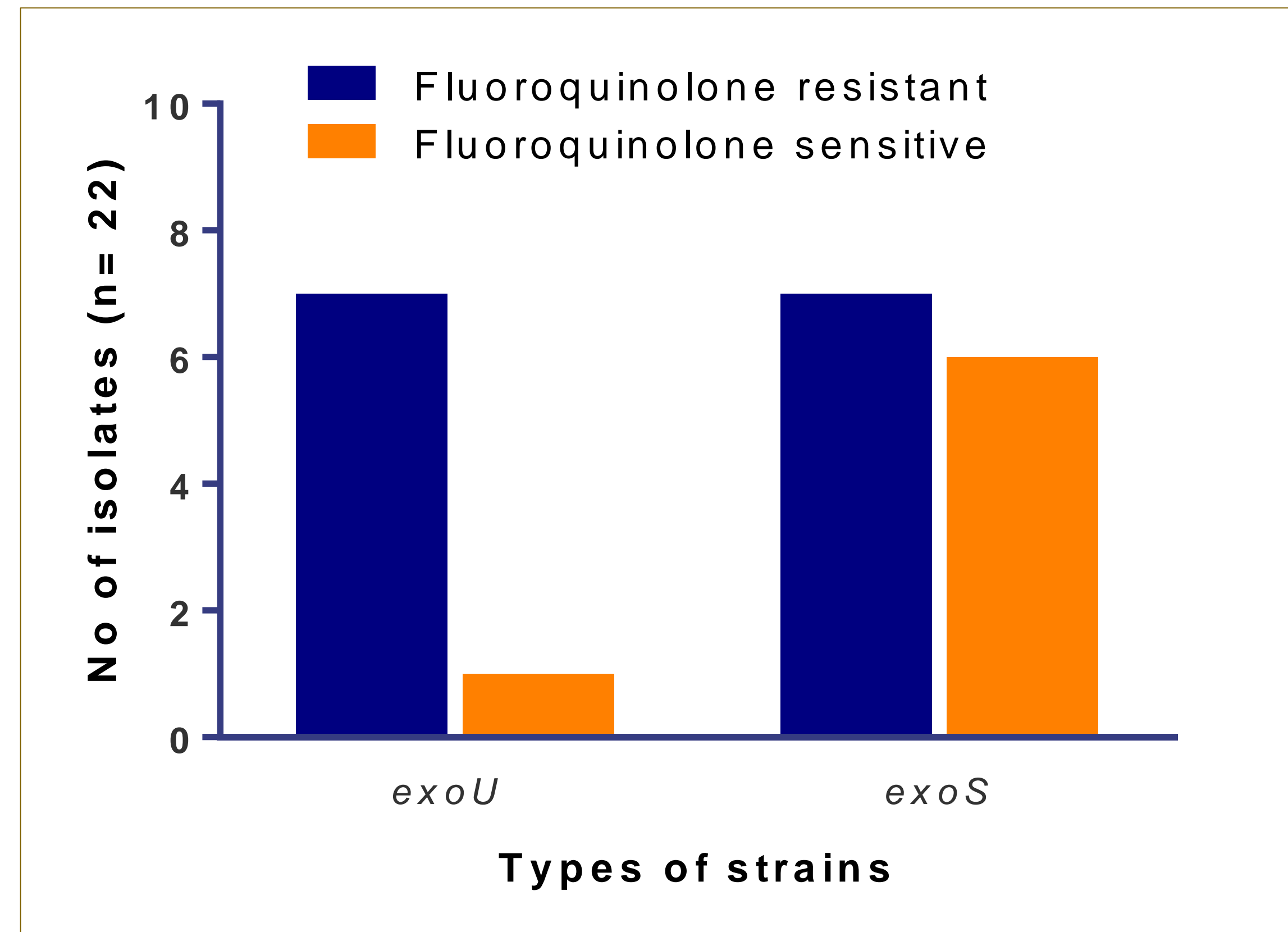


Figure 2: Susceptibility of *exoU* and *exoS* strains to fluoroquinolones

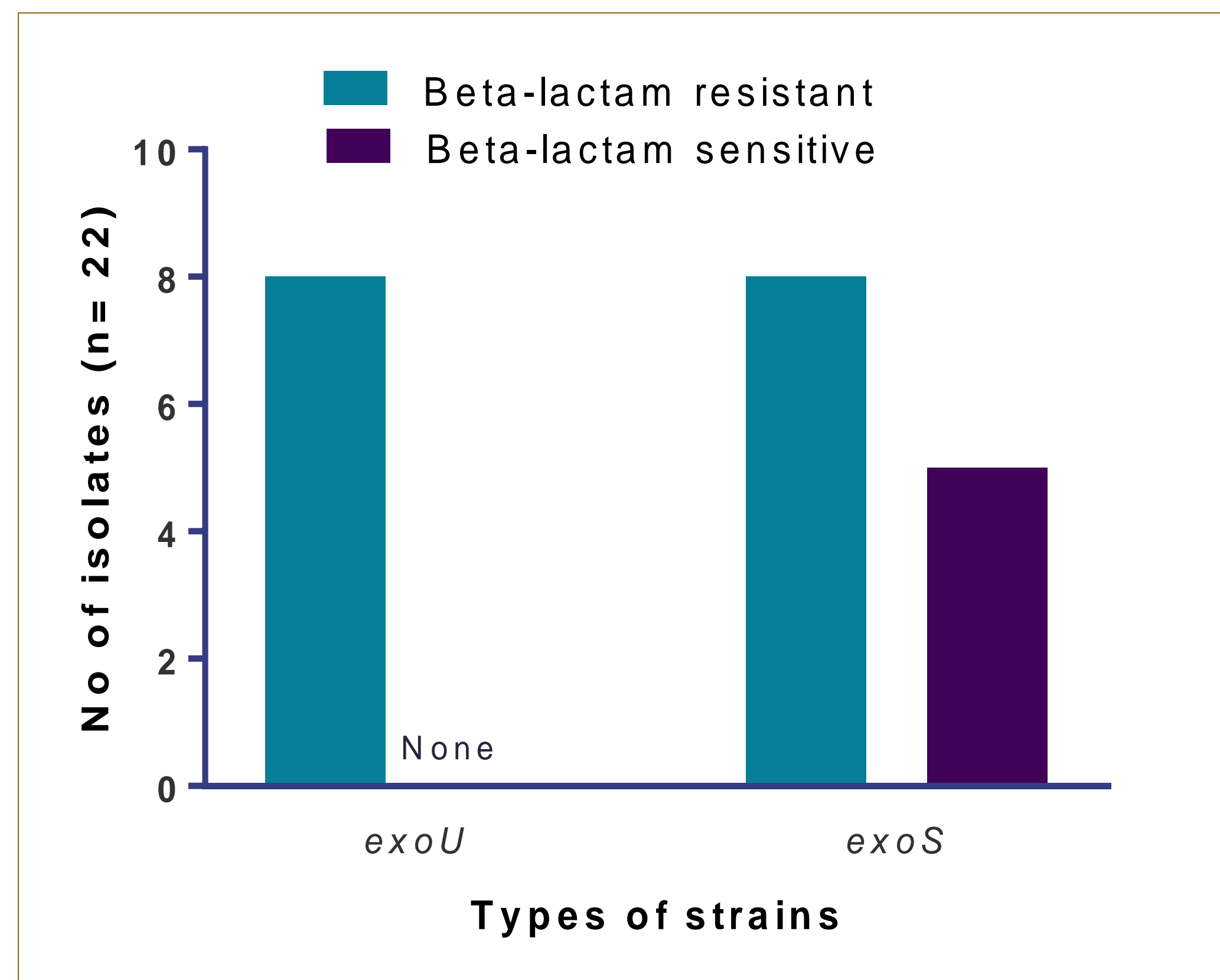


Figure 3: Susceptibility of *exoU* and *exoS* strains to beta-lactams

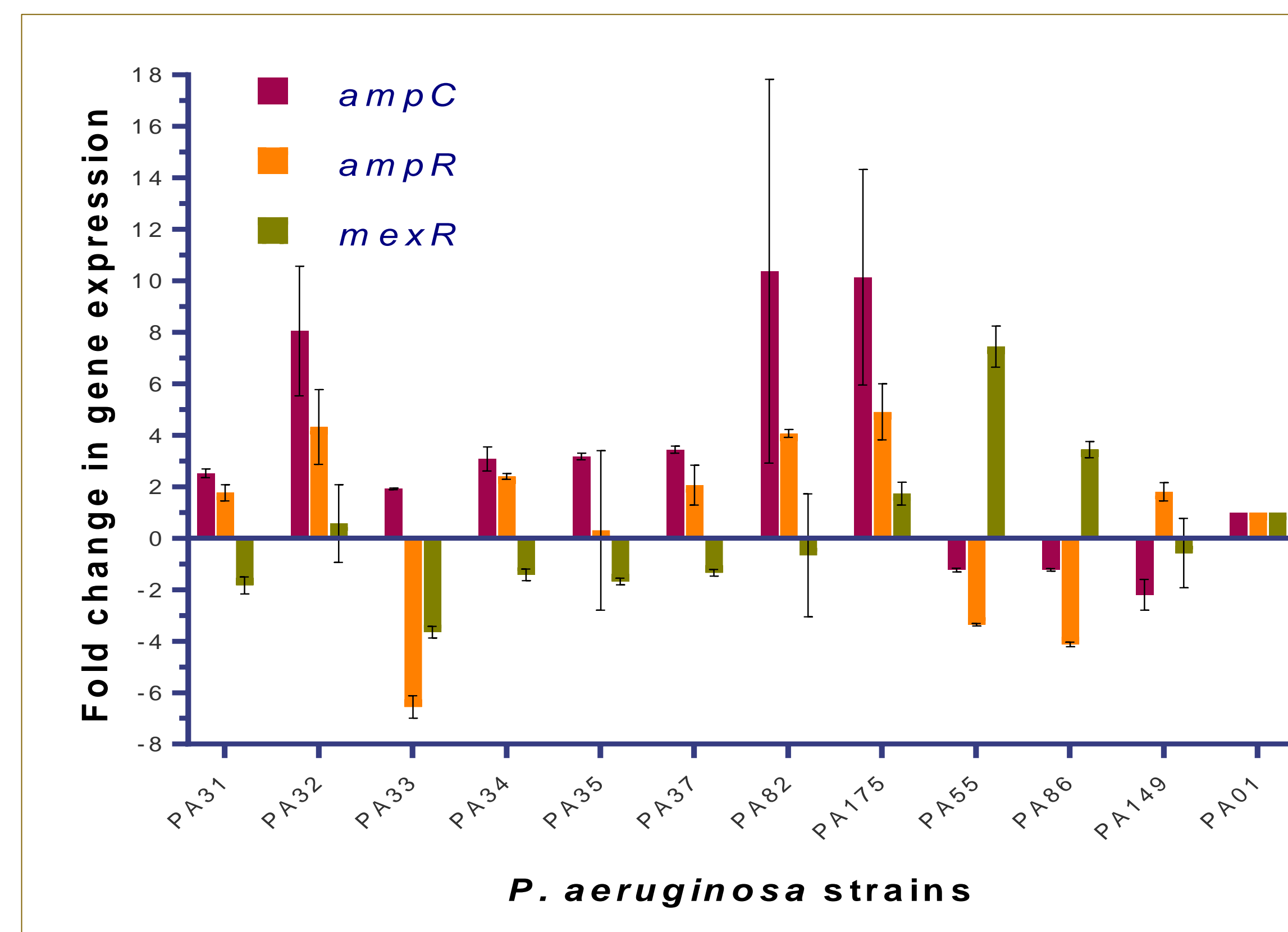


Figure 4: Expression of cephalosporinase (*ampC*) its regulator (*ampR*) and efflux pump MexAB-OprM regulator (*mexR*) in *exoU* strains

Table 1: Mutations in the drug resistance determining genes of *P. aeruginosa* strains

Resistance genes	ExoU strains							ExoS strains														
	PA31	PA32	PA33	PA34	PA35	PA37	PA82	PA175	PA17	PA40	PA55	PA59	PA64	PA66	PA86	PA92	PA100	PA102	PA149	PA157	PA171	
<i>mexR</i>	V126E	V126E	A110T, V126E	V126E	V126E	V126E	K76Q, V126E															
<i>ampC</i>	V356I	V356I		V356I	V356I	Q155I	V356I															
<i>ampR</i>	G283E, M288R	G283E, M288R	Q114A, G283E, M288R	G283E, M288R	G283E, M288R	G283E	G283E, M288R															R244W
<i>gyrA</i>	T83I	T83I	T83I		T83I	T83I		T83I														
<i>gyrB</i>														E468D	L457-458A*	S466F						
<i>parC</i>	S87L	S87L	S87L		S87L	S87L																
<i>parE</i>														A473V	A473V	A473V						

## CONCLUSION

This study has shown that the *exoU* gene carriage is associated with increased resistance to fluoroquinolones and beta-lactams. Strains having the *exoU* genotype had more mutations in genes associated with beta-lactam resistance (*mexR*, *ampC* and *ampR*) and fluoroquinolone resistance (as *gyrA* and *parC*) compared to *exoS* strains. Gene expression analysis suggests that such mutations generally lead to antibiotic resistance. These differences in mutational rate in two different genotypes indicate that the more virulent *exoU* strains may evolve in antibiotic rich environments. Understanding the relation between virulence and antibiotic resistance genes is essential for more effective management of disease caused by these strains.

## References

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