Characterization of *fosA3*, *fosA8*, and novel *fosA7.5* genes from fosfomycin-resistant *Escherichia coli* clinical isolates obtained from Canadian hospitals through the CANWARD study

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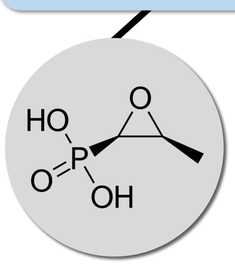
1 INTRODUCTION

Around 150 million people develop urinary tract infections (**UTIs**) annually

E. coli is the most common cause (75%-90% cases)

Fosfomycin is a first line antimicrobial for uncomplicated cystitis



Plasmid-transferrable *fosA* genes confer resistance to fosfomycin in uropathogenic *E. coli* through inactivation of the antibiotic

2 METHODS

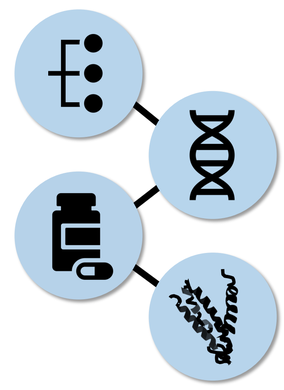
Study aim: characterization of three *fosA* genes from Canadian *E. coli* clinical isolates

1. Phylogenetic Analysis
Analysis of Fos proteins from *E. coli* clinical isolates

2. Multiple Sequence Alignment
Identification of amino acids that distinguish novel FosA variants

3. Cloning and Overexpression of *fosA* Genes
Antimicrobial susceptibility testing to confirm fosfomycin resistance

4. Homology Modelling
Comparison of FosA protein structures and active sites



3 RESULTS

Phylogenetic analysis identifies the proteins as FosA3, FosA8, and a novel FosA7.5 variant

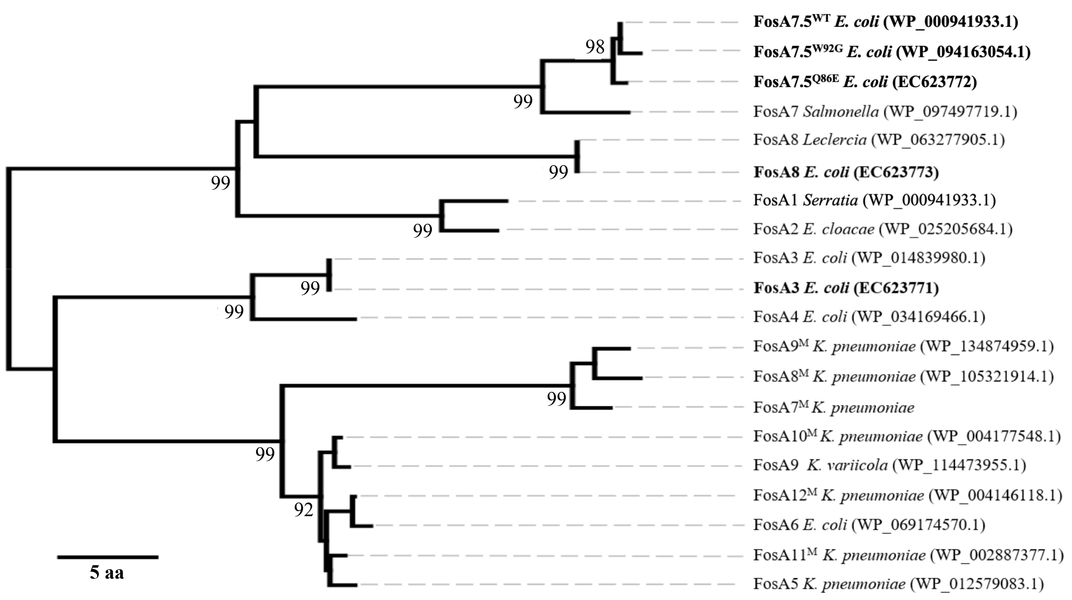


Figure 1. Phylogenetic analysis of FosA1–FosA12 protein sequences using the Neighbor-Joining distance-based method. The novel FosA7.5 (EC623772) is part of a sub-family related to FosA7, which includes at least two additional variants. Branch lengths represent amino acid differences as distance (scale bar).

FosA7.5 proteins have three distinct amino acids distinguishing them from the FosA7 family

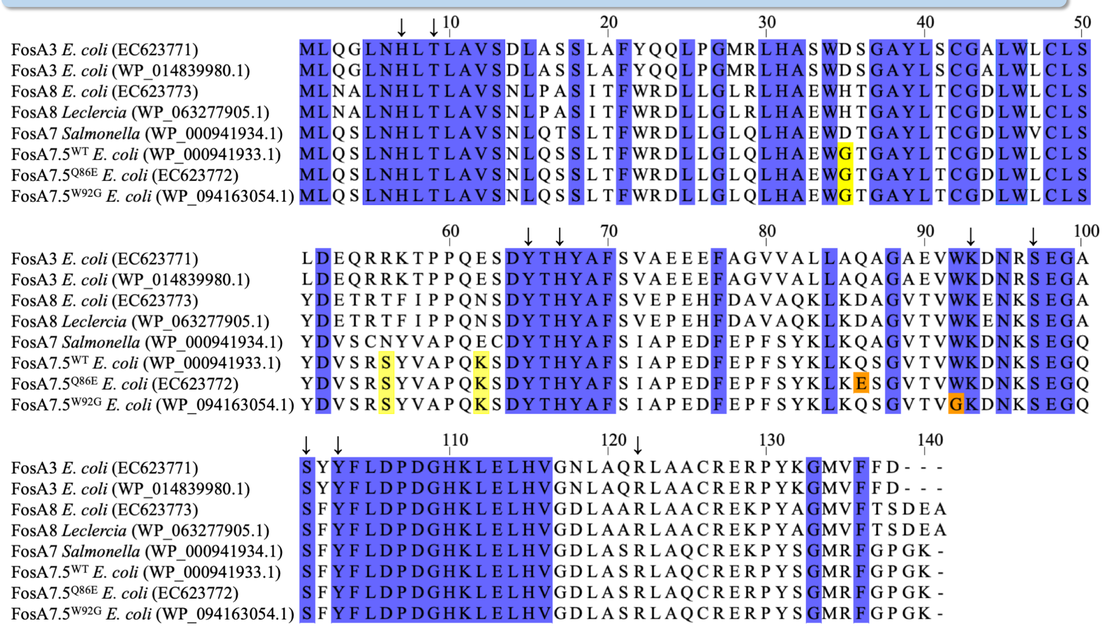


Figure 2. Multiple sequence alignment of FosA3, FosA8, and FosA7 protein sequence variants. Blue colouring indicates conserved residues. Amino acid differences that distinguish the FosA7.5 group from FosA7 are shown in yellow. Differences among FosA7.5 sequences are highlighted in orange. Arrows indicate active site residues.

E. coli K-12 BW25113 *fosA* transformants exhibit fosfomycin-resistant phenotypes

Table 1. MIC results of *E. coli fosA* transformants. All *fosA* genes confer fosfomycin resistance when transformed and individually over-expressed in *E. coli* BW25113, with the exception of *E. coli* FosA7.5^{W92G}, which has a glycine substitution at a highly conserved tryptophan residue. pMS119EH was used as the parental vector for all constructs.

	MIC (µg/mL)			
***E. coli* Transformant**	**Agar dilution**	**Disk diffusion**	**E-test**	**Result**
FosA3	>512	6mm	>1024	Resistant
FosA8	>512	6mm	>1024	Resistant
FosA7.5^{WT}	>512	6mm	>1024	Resistant
FosA7.5^{Q86E}	>512	6mm	>1024	Resistant
FosA7.5^{W92G}	32	30mm	2	Susceptible
pMS119EH (control)	2-4	30mm	0.5	Susceptible

Homology modelling demonstrates tight alignment to previously characterized FosA proteins

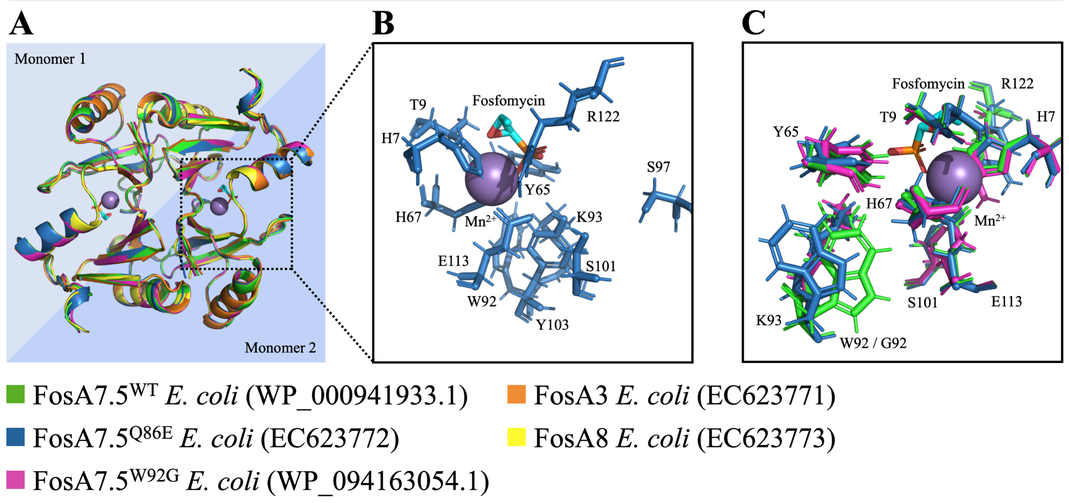


Figure 3. Homology modelling of FosA proteins. A) Overlay of FosA protein dimers. B) Active site of FosA7.5^{Q86E} from an *E. coli* clinical isolate. C) Overlay of FosA7.5^{WT}, FosA7.5^{Q86E}, and FosA7.5^{W92G} active sites rotated 120^{O} from panel B. FosA7.5^{W92G} appears to have an altered binding pocket, in agreement with MIC results from Table 1.

4 CONCLUSION

We identified and characterized the *fosA* genes from three *E. coli* clinical isolates, including the novel *fosA7.5* and its variants

Ongoing for surveillance for fosfomycin resistance is crucial to ensure that it remains effective as a first-line therapy for UTIs

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5 ACKNOWLEDGEMENTS

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