

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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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



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



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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

RESULTS

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



FosA7.5 proteins have three distinct amino acids distinguishing them from the FosA7 family								
	Ļ	$\downarrow 10$	20	30	40	5		
FosA3 E. coli (EC623771)	MLQGLNH	LTLAVSDI	LAS <mark>S</mark> LA <mark>F</mark> YQQ	L P GMR LHA S WD	SGAYLSCGALW	LCLS		
FosA3 E. coli (WP_014839980.1)	MLQGLNH	L T L A V S <mark>D</mark> I	LAS <mark>S</mark> LAFYQQ	L P GMR L H A S WD	S <mark>GAYL</mark> S <mark>C G</mark> A LW	L C L S		
FosA8 E. coli (EC623773)	MLNALNHI	L T L A V S <mark>N</mark> I	L P A <mark>S</mark> I T F WR I	LLGLRLHAEWH	I T <mark>GAYL</mark> T C G D L W	L C L S		
FosA8 Leclercia (WP_063277905.1)	ML NA LNH	L T L A V S <mark>N</mark> I	L P A <mark>S</mark> I T <mark>F</mark> WR I	D L L G L R L H A E W H	I T <mark>GAYL</mark> T <mark>C G D</mark> L W	L C L S		
FosA7 Salmonella (WP_000941934.1)	MLQS LNH	L T L A V S <mark>N</mark> I	L Q T <mark>S</mark> L T <mark>F</mark> WR I	<mark>) L L G L Q L H A</mark> E W D	T GAYL T C G D L W	V C L S		
FosA7.5 ^{WT} E. coli (WP_000941933.1)	MLQS LNH	L T L A V S <mark>N</mark> I	L Q S <mark>S</mark> L T <mark>F</mark> WR I	LLGLQLHAEWC	T G A Y L T C G D L W	L C L S		
FosA7.5 ^{Q86E} E. coli (EC623772)	MLQS LNH	L T L A V S <mark>N</mark> I	L Q S <mark>S</mark> L T <mark>F</mark> WR I	LLGLQLHAEWC	T G A Y L T C G D L W	L C L S		
FosA7.5 ^{W92G} E. coli (WP_094163054.1)	MLQS LNH	L T L A V S <mark>N</mark> I	L Q S <mark>S</mark> L T <mark>F</mark> WR I	<mark>LLGLQLHA</mark> EW <mark>C</mark>	T GAYL T CGD LW	L C L S		
		60	↓	80	90 ↓	↓ 10		
FosA3 E. coli (EC623771)	L <mark>D</mark> EQRRK	ΓΡΡQES <mark>D</mark>	YTHYAF SVAH	E E E <mark>F</mark> A G V V A L <mark>L</mark> A	Q A <mark>G</mark> A E V <mark>WK</mark> D N R	SEGA		
FosA3 E. coli (WP_014839980.1)	L <mark>D</mark> EQRRK	ΓΡΡQES <mark>D</mark> Υ	YTHYAF SVAB	E E E <mark>F</mark> AGVVAL <mark>L</mark> A	Q A <mark>G</mark> A E V <mark>WK</mark> D N R	S E G A		
FosA8 E. coli (EC623773)	Y <mark>D</mark> E T R T F	I P P Q N S <mark>D y</mark>	YTHYAF SVEF	PEH <mark>F</mark> DAVAQK <mark>L</mark> K	LDA <mark>G</mark> VTV <mark>WK</mark> E <mark>N</mark> K	S E G A		
FosA8 Leclercia (WP_063277905.1)	Y <mark>D</mark> E T R T F	I P P Q N S <mark>D y</mark>	YTHYAF SVEF	PEH <mark>F</mark> DAVAQK <mark>L</mark> K	L D A <mark>G</mark> V T V <mark>WK</mark> E <mark>N</mark> K	S E G A		
FosA7 Salmonella (WP_000941934.1)	Y D V S C N Y V	V A P Q E C <mark>D Y</mark>	<mark>YTHYAF</mark> SIAF	9 E D <mark>F</mark> E P F S Y K <mark>L</mark> K	LQA <mark>G</mark> VTV <mark>WK</mark> DNK	SEGQ		
FosA7.5 ^{WT} E. coli (WP_000941933.1)	Y <mark>D</mark> VSR <mark>S</mark> YV	V A P Q <mark>K</mark> S <mark>D </mark>	<mark>YTHYAF</mark> SIAF	9 E D <mark>F</mark> E P F S Y K <mark>L</mark> K	L Q S <mark>G</mark> V T V <mark>WK</mark> D <mark>N</mark> K	SEGQ		
FosA7.5 ^{Q86E} E. coli (EC623772)	Y <mark>D</mark> VSR <mark>S</mark> YV	V A P Q <mark>K</mark> S <mark>D </mark>	YTHYAF <mark>SIAF</mark>	P E D <mark>F</mark> E P F S Y K <mark>L</mark> K	K <mark>E</mark> S <mark>G</mark> VTV <mark>WK</mark> DNK	SEGQ		
FosA7.5 ^{W92G} E. coli (WP_094163054.1)	Y <mark>D</mark> VSR <mark>S</mark> YV	V A P Q <mark>K</mark> S <mark>D </mark>	<mark>YTHYAF</mark> SIAF	9 E D <mark>F</mark> E P F S Y K <mark>L</mark> K	LQ S <mark>G</mark> V T V <mark>G K</mark> D <mark>N</mark> K	SEGQ		
	$\downarrow \downarrow$	110	120 ↓	130	140			
FosA3 E. coli (EC623771)	SYYFLDPI	OGHKLELI	<mark>IV</mark> GNLAQRLA	A C R E R P Y K <mark>G</mark> MV	F FD			
FosA3 E. coli (WP_014839980.1)	SYYFLDPI	OGHKLELI	IV GNLAQRLA	A C R E R P Y K <mark>G</mark> MV	F FD			
FosA8 E. coli (EC623773)	SFYFLDPI	DGHKLELI	IV GDLAARLA	A C R E K P Y A <mark>G</mark> MV	F T S D E A			
FosA8 Leclercia (WP 063277905.1)	SFYFLDPI	OGHKLELI	IV GDLAARLA	A C R E K P Y A <mark>G</mark> MV	F T S D E A			

5 aa

FosA11^M *K. pneumoniae* (WP_002887377.1)

FosA5 K. pneumoniae (WP 012579083.1)

Figure 1. Phylogenetic analysis of FosA1–FosA12 protein sequences using the Neighbor-Joining distancebased 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).

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)						
<i>E. coli</i> 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			

FosA7 Salmonella (WP_000941934.1) S F Y F L D P D G H K L E L H V G D L A S R L A Q C R E K P Y S G M R F G P G K -FosA7.5^{WT} E. coli (WP_000941933.1) S F YFLDPDGHKLELHV GDLASRLAQCRERPYS GMR F GPGK -

 FosA7.5Q86E
 E. coli (EC623772)
 S
 F
 Y F L D P D G H K L E L H V
 G D L A S R L A Q C R E R P Y S
 G M R F
 G P G K

 FosA7.5W92G
 E. coli (WP_094163054.1)
 S
 F
 Y F L D P D G H K L E L H V
 G D L A S R L A Q C R E R P Y S
 G M R F
 G P G K

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.

Homology modelling demonstrates tight alignment to previously characterized FosA proteins



- FosA7.5^{WT} *E. coli* (WP_000941933.1) FosA3 *E. coli* (EC623771)
- FosA7.5^{Q86E} *E. coli* (EC623772)
- FosA8 *E. coli* (EC623773)

FosA7.5^{W92G} *E. coli* (WP 094163054.1)

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.

CONCLUSION

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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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