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Short communication

# Lack of the RNA chaperone Hfq attenuates pathogenicity of several *Escherichia coli* pathotypes towards *Caenorhabditis elegans*

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**Abstract**

*Escherichia coli* is an important agent of Gram-negative bacterial infections worldwide, being one of the leading causes of diarrhoea and urinary tract infections. Strategies to understand pathogenesis and develop therapeutic compounds include the use of the nematode *Caenorhabditis elegans* as a model for virulence characterization and screening for novel antimicrobial entities. Several *E. coli* human pathotypes are also pathogenic towards *C. elegans*, and we show here that lack of the RNA chaperone Hfq significantly reduces pathogenicity of VTEC, EAEC, and UPEC in the nematode model. Thus, Hfq is intrinsically essential to pathogenic *E. coli* for survival and virulence exerted in the *C. elegans* host.
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*Keywords:* *E. coli*; Virulence; Hfq; *C. elegans*; Antivirulence; In vivo model

## 1. Introduction

The enterobacterium *Escherichia coli* is a commensal of the human gastrointestinal tract. However, it is also a versatile pathogen causing intestinal and extraintestinal diseases in immunocompromised and healthy individuals. The wide spectrum of clinical symptoms attributable to *E. coli* is a reflection of the genetic variability within this species. Thus, disease-causing *E. coli* are generally divided into different pathotypes. The diarrhoeagenic *E. coli* constitutes a major group of several pathotypes encompassing e.g. Vero cytotoxin-producing *E. coli* (VTEC) responsible for acute and (often) bloody diarrhoea associated with the severe clinical manifestation haemolytic uremic syndrome (HUS), and enteroaggregative *E. coli* (EAEC) giving rise to acute as well as persistent diarrhoea mainly among inhabitants of developing countries but also in travellers [1]. Uropathogenic *E. coli* (UPEC), a pathotype within the extraintestinal pathogenic *E. coli* (ExPEC), is probably the most common agent of human urinary tract infections [1]. Each pathotype is described by histopathological and phenotypic appearances as well as more or less specific combinations of virulence genes. Given the high morbidity, and greatly spurred by the worldwide development of antibiotic resistance, further studies on virulence mechanisms and identification of new antimicrobial targets in *E. coli* are warranted.

A relatively novel approach in antimicrobial development is antivirulence therapy where factor(s) contributing to the pathogenesis of an infectious agent is the target of inhibition [2]. Targeting virulence would disarm a bacterial pathogen rather than kill it, which, in turn, may lower the selective pressure for evolution of resistance. The identification of an

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inhibitor of a central virulence regulator in *Vibrio cholerae* constitutes an elegant example of an antivirulence compound [3]. Identification and development of antivirulence compounds require sophisticated in vitro setups or relevant in vivo models due to the non-bacteriocidal nature of such entities. Because many biological pathways are conserved between *Caenorhabditis elegans* and humans, the nematode is being increasingly used as a simple model organism to study virulence mechanisms of both bacterial and fungal infections [4]. Due to its simplicity and ease of cultivation, it is also an organism feasible for large screenings of antimicrobials, antivirulence compounds, and other factors promoting host survival following infection [5].

The pleiotropic role played by the RNA chaperone and posttranscriptional regulator Hfq in diverse bacterial pathogens is increasingly acknowledged [6]. Though generally viable, bacterial *hfq* mutants often display a lowered stress tolerance and attenuation in animal models. Thus, the broad implication of Hfq in global gene expression and virulence has been demonstrated for bacterial pathogens as diverse as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Listeria monocytogenes* [7–9]. In the *C. elegans* infection model, Hfq was shown to be involved in virulence of *Burkholderia cepacia* and *Serratia* sp. [10,11]. A role for Hfq in multidrug resistance in *E. coli* has been described [12] adding further to the clinical significance of its regulon. Though vast amounts of biological functions of Hfq have been dissected in *E. coli*, its relevance in virulence of pathogenic *E. coli* remains less well described. One study, though, revealed a prominent role played by Hfq in fitness and virulence properties of uropathogenic *E. coli* [13]. Moreover, Hfq has also been shown to both positively and negatively affect the type III secretion systems, including effector proteins responsible for AE lesions and the hallmark pedestal formation phenotype, in different VTEC strains [14,15]. Thus, specific virulence properties may be differently integrated into the Hfq regulon even in strains of the same pathotype. Transcriptome arrays show that the Hfq regulon include *E. coli* core genome genes as well as, to a large degree, pathotype-specific genes [14]. Recently, involvement of Hfq in pathogenesis of adherent-invasive *E. coli* (AIEC) towards *C. elegans* was reported [16]. Here we tested the hypothesis that Hfq might be relevant for pathogenesis in *C. elegans* of disease-causing *E. coli* in general.

## 2. Materials and methods

### *2.1. Strains, plasmids and DNA manipulations*

The nematode strain *C. elegans* AU37 [17], a derivative of Bristol N2 carrying the mutated *sek-1* and *glp-4* alleles (MAPK kinase deficiency and temperature-sensitive sterile), were maintained on NGM media [18] supplied with the food source *E. coli* OP50 at 15 °C.

Strains of *E. coli* and their derivatives, plasmids and primers used in this study are listed in Table 1. Strains were grown overnight at 37 °C with antibiotics when appropriate at the following concentrations: ampicillin 100 μg/ml, kanamycin 50 μg/ml, chloramphenicol 12.5 μg/ml, apramycin 30 μg/ml. PCR was performed using the Expand High Fidelity PCR System (Roche). The *hfq* gene including its upstream promoter region was amplified from *E. coli* MG1655 (NCBI gene id 948689) using primer pair F_hfq/R_hfq and was cloned (Fast-Link DNA Ligation Kit, Epicentre) into pBR322 by directional cloning using BamHI and SalI restriction enzymes (Fermentas). Plasmid pBAD18-GFP was constructed by amplification of the GFPmut2 gene [19] from pGFPmut2 with primer pair F-GFPmut2/R-GFPmut2 followed by insertion into sites EcoRI and SmaI in pBAD18. The linear *hfq*

Table 1
Bacterial strains, plasmids and primers used in this study.

| Strains, plasmids, and primers | Description/sequence | Source or reference |
|---|---|---|
| *Escherichia coli strains* | | |
| OP50 | Standard food source/negative control for *C. elegans* | [18] |
| MG1655 | WT K12 isolate | Lab. stock |
| 17-2 | EAEC prototype strain | Lab. stock |
| 17-2Δ*hfq* | Strain carrying chromosomal *hfq*-deletion ($Kn^R$) | This study |
| EDL933 | VTEC prototype strain | Lab. stock |
| EDL933Δ*hfq* | Strain carrying chromosomal *hfq*-deletion ($Cam^R$) | This study |
| J96 | UPEC | Lab. stock |
| J96Δ*hfq* | Strain carrying chromosomal *hfq*-deletion ($Kn^R$) | This study |
| *Plasmids* | | |
| pKOBEGapra | Plasmid encoding arabinose-inducible λ-red functions ($Apra^R$) | J.M. Ghigo |
| pGFPmut2 | Plasmid containing gene encoding GFPmut2 | [19] |
| phfq | pBR322 encoding *hfq* under its native promoter | This study |
| pBAD18-GFP | Plasmid expressing GFPmut2 from the $P_{BAD}$ promoter | This study |
| *Primers* | | |
| F-hfq | 5′-gatacaggatcccatggctaaggggcaatc-3′ | This study |
| R-hfq | 5′-gatacagtcgacgaacgcaggatcgctggctc-3′ | This study |
| F-Kn_hfq | 5′-atggctaaggggcaatctttacaagatccgttaaagccacgttgtgtctc-3′ | This study |
| R-Kn_hfq | 5′-ttattcggtttcttcgctgtcctgttgcgcgggtcagcgtaatgctctgc-3′ | This study |
| F-GFPmut2 | 5′-gcatggaattcgaaggagatatacatatgag-3′ | This study |
| R-GFPmut2 | 5′-gctaccccgggcagttatttgtatagttcatcc-3′ | This study |

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