Serum resistance and Extra-intestinal Pathogenic *Escherichia coli* bloodstream infections

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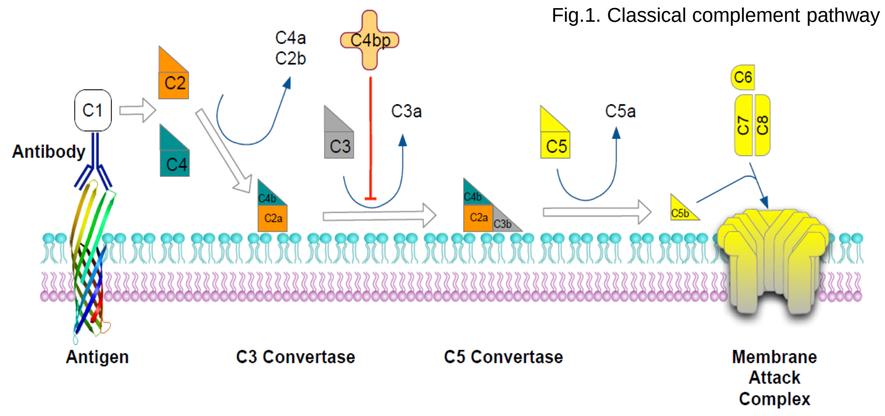
1. Introduction

Bloodstream infections are predominantly caused by extra-intestinal pathogenic *E. coli* (ExPEC). In the bloodstream, ExPEC is targeted by the innate defences present in serum including complement and anti microbial peptides. The ability to survive the bactericidal action of serum is advantageous to ExPEC that gain access to the bloodstream. Multiple virulence factors are involved in evasion of the innate immune system.

In this study the transcriptional response of ExPEC strain CFT073 was investigated in response to either human serum or serum which had been heated to inactivate complement. This revealed an important role for the Rcs regulated exopolysaccharide colanic acid.

Fig.1. Classical complement pathway



2. Genes upregulated by active serum

In response to serum in which the complement system was active, extracytoplasmic stress response pathways were induced. These included the Rcs and Cpx two component systems and the alternate sigma factor, σE. The Cpx and σE pathways regulate cell envelope structural integrity which is an important factor in serum resistance.

Gene	Function	Induction
rpoE	RNA polymerase sigma factor E	3
rseA	Anti-RNA polymerase sigma factor E	3
cpxR	Envelope stress response regulator	3
cpxP	Periplasmic stress response protein	5

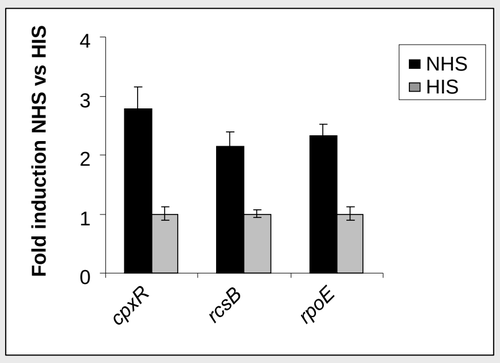


Fig.2. Gene expression measured by qRT-PCR

Strains were constructed lacking *rcsB*, *rpoE* and *cpxR* in order to determine whether the envelope stress regulators contribute to serum survival. These were compared to the WT CFT073 strain and the serum sensitive K2 capsule mutant, CFT073 Δ*kpsC*. Serum survival was significantly reduced in all three mutants indicating that these pathways are important in serum resistance.

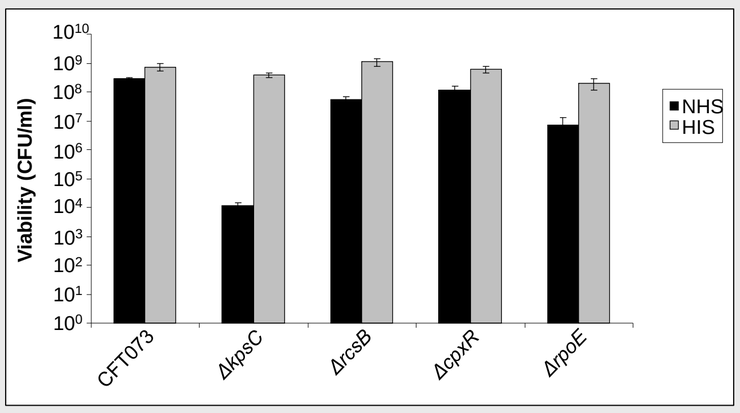


Fig.3. Response regulators contribute to serum resistance. Survival of response regulator deletion mutants was calculated following a 45 min incubation at 37 °C with 50 % human serum.

3. Role of RcsB regulated genes in serum survival

23 % of the genes induced by active serum are regulated by the Rcs two component system which controls expression of genes involved in cell envelope functions.

Strains were constructed with mutations in RcsB regulated genes *wcaDE*, *yjbE* and *mliC*. Disruption of synthesis of the exopolysaccharide colanic acid (*wcaDE)* reduced serum survival significantly (Fig.4).

Gene	Function	Induction
yjbE	Exopolysaccharide production	12
yjbF	Exopolysaccharide production	4
wcaD	Colanic acid biosynthesis	7
wcaE	Colanic acid biosynthesis	4
gmd	Colanic acid biosynthesis	3
wcaF	Colanic acid biosynthesis	3
ugd	Colanic acid biosynthesis	4
mliC	Lysozyme inhibitor	6
ivy	Lysozyme inhibitor	5

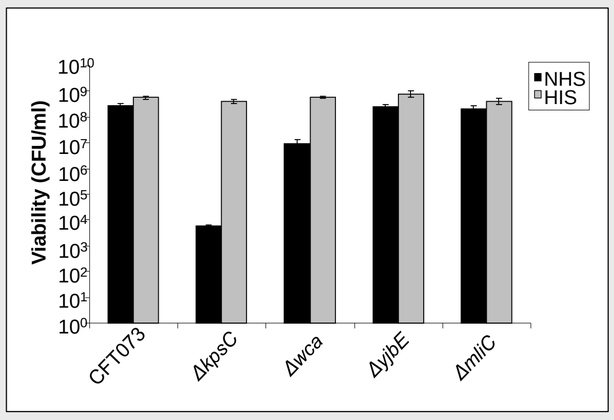


Fig.4. Role of RcsB regulated genes in serum survival. Survival of bacterial strains was calculated following a 45 min incubation at 37 °C with 50 % human serum.

Combining mutations in the K2 capsule (*kpsC*) with mutations in *rcsB* and *wcaDE* reduced serum survival below the level seen in serum sensitive strain CFT073 *kpsC* (Fig.5).

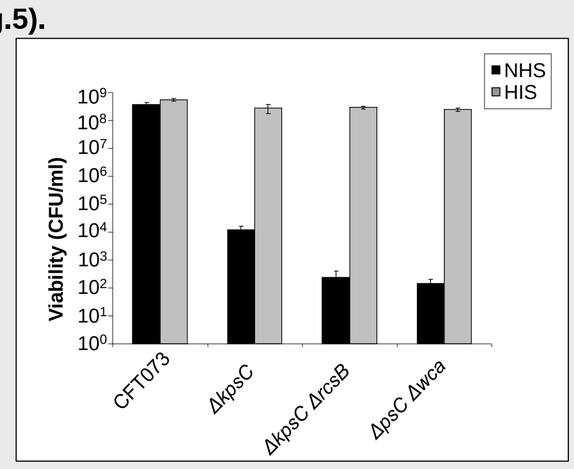
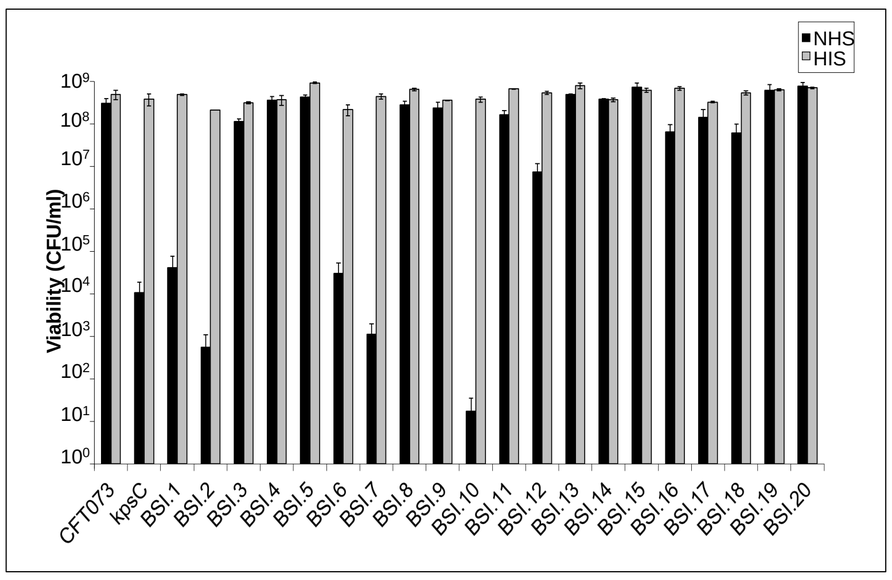


Fig.5. The K2 capsule and colanic acid contribute to serum resistance. CFT073 and mutants were incubated with 50 % NHS or HIS at 37 °C for 45 min. Bacteria were enumerated by the method of Miles and Misra after incubation with sera.

4. Characterising *E. coli* bacteraemia isolates

20 *E. coli* bloodstream isolates associated with mortality were obtained from St James's Hospital. 75 % of these isolates were serum resistant (Fig.6). Data obtained form whole genome sequencing determined that the majority of strains were of ST131. Further sequence analysis will be carried out to determine which virulence factors are present in the bloodstream isolates.



Sequence type	Number of isolates
ST62	1
ST69	3
ST73	4
ST127	1
ST130	1
ST131	5
ST393	2
ST405	2
ST453	1

Fig.6. Serum resistance of bacteraemia isolates. Survival of isolates was calculated following a 45 min incubation at 37 °C with 50 % human serum.

5. Conclusions

- **Serum resistance of CFT073 involves increased expression of envelope stress regulators (CpxR, σE and RcsB) which have a protective effect.**
- **RcsB-mediated serum resistance was conferred through induction of the exopolysaccharide colanic acid.**
- **Serum resistance is associated with *E. coli* isolates that gain access to the bloodstream**

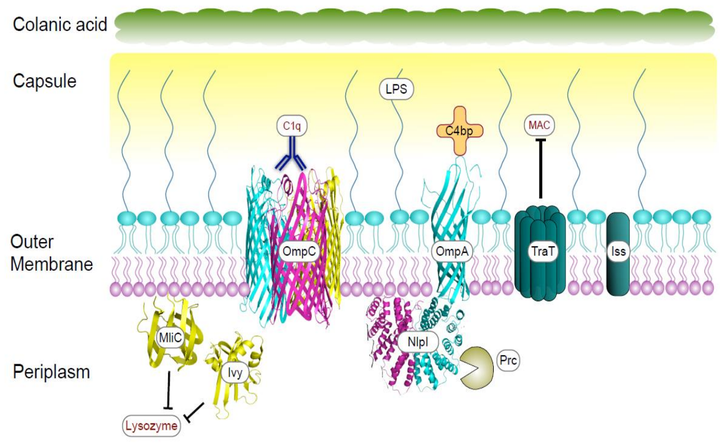


Fig.7. Serum resistance mechanisms of Escherichia coli

Miajlovic H & Smith SG (2014) FEMS Microbiol Lett DOI: 10.1111/1574-6968.12419.

Miajlovic H, Cooke NM, Moran GP, Rogers TR & Smith SG (2014) Infect Immun 82: 298-305.