Novel approaches in antimicrobial therapy

An investigation into the efficacy of waaG inhibitors against Uropathogenic *Escherichia coli* (UPEC)

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1. Introduction

- Extra-Intestinal Pathogenic *E. coli* (ExPEC) are a group of bacteria that most commonly colonise the urinary tract and are capable of gaining access to and surviving in the bloodstream (1)
- Uropathogenic *E. coli* (UPEC) is the causative agent of approximately 80% of urinary tract infections (2)
- CFT073 is a UPEC prototypic urosepsis isolate possessing virulence factors such as K2 capsule and O6 antigen that contribute to pathogenesis and bloodstream survival (1,3)
- O antigen is the major determinant of bloodstream survival of UPEC CFT073, thus playing an important role in sepsis. (3)
- The *waaG* gene encodes for a glycosyltransferase enzyme required for lipopolysaccharide (LPS) core synthesis and subsequent O antigen attachment (4)
- Mutation of *waaG* leads to significantly decreased inner core phosphorylation, resulting in outer membrane destabilisation and failure to form full-length LPS (4)
- Additionally, the Sir Patrick Dun laboratory has observed reduced capsule association with *waaG* mutation (5)

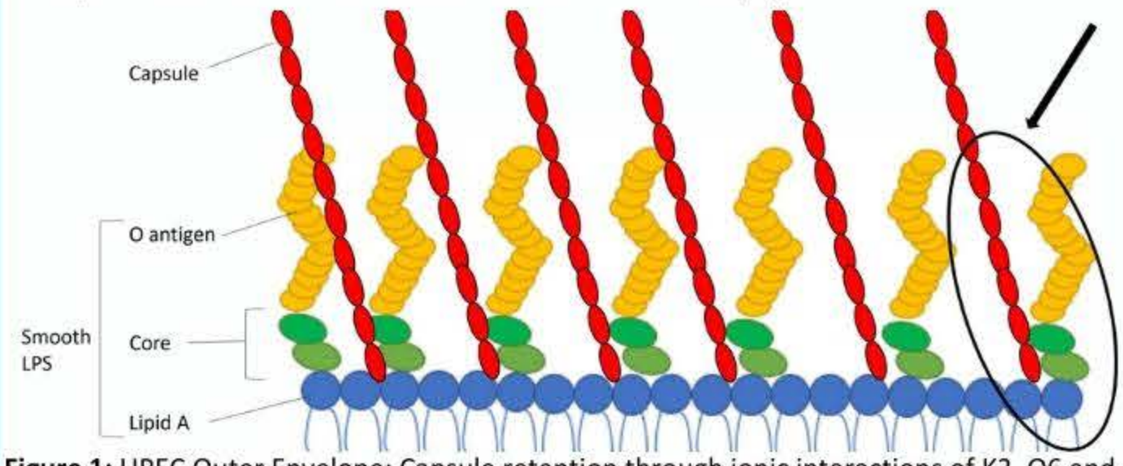

Figure 1: UPEC Outer Envelope: Capsule retention through ionic interactions of K2, O6 and core

2. Examining the effects of a WaaG inhibitor on a UPEC CFT073 urosepsis isolate

- 4-(2-amino-1,3-thiazol-4-yl)phenol, also termed L1, is a compound found previously to inhibit the activity of WaaG *in vitro* (6)
- The effects of L1 on WaaG in viable UPEC CFT073 cells were not previously examined

3. Inhibiting WaaG disturbed LPS integrity

- Change in apparent weight of Lipid A and core observed
- Long and short chain O6 antigen distorted despite similar levels of O antigen; suggests LPS disturbance

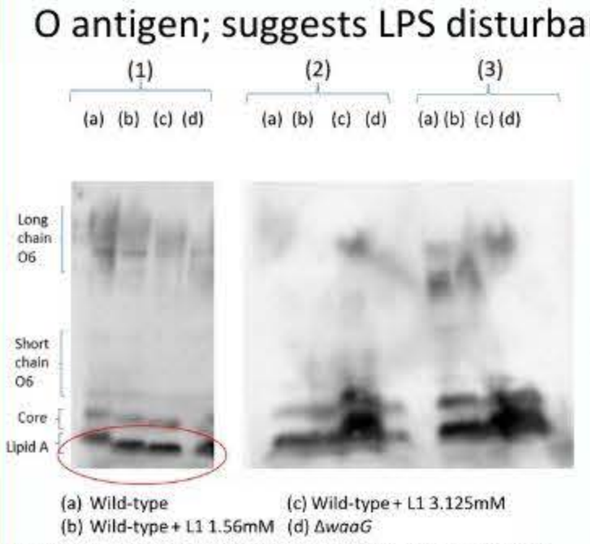

Figure 2(a): O antigen Western Blot analysis of UPEC cells in stationary phase +/- L1 inihbitor

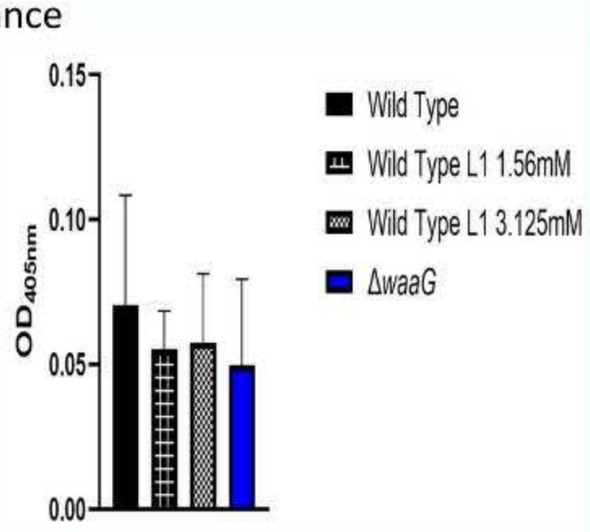

Figure 2(b): O antigen ELISA of UPEC cells in stationary phase +/- the L1 inhibitor using α-O6 antibody

4.WaaG inhibition reduces capsule association

- Capsule appears in two distinct bands at 100kDa and 150kDa (7)
- Significant decrease in capsule with increasing concentrations of L1 was observed and quantified

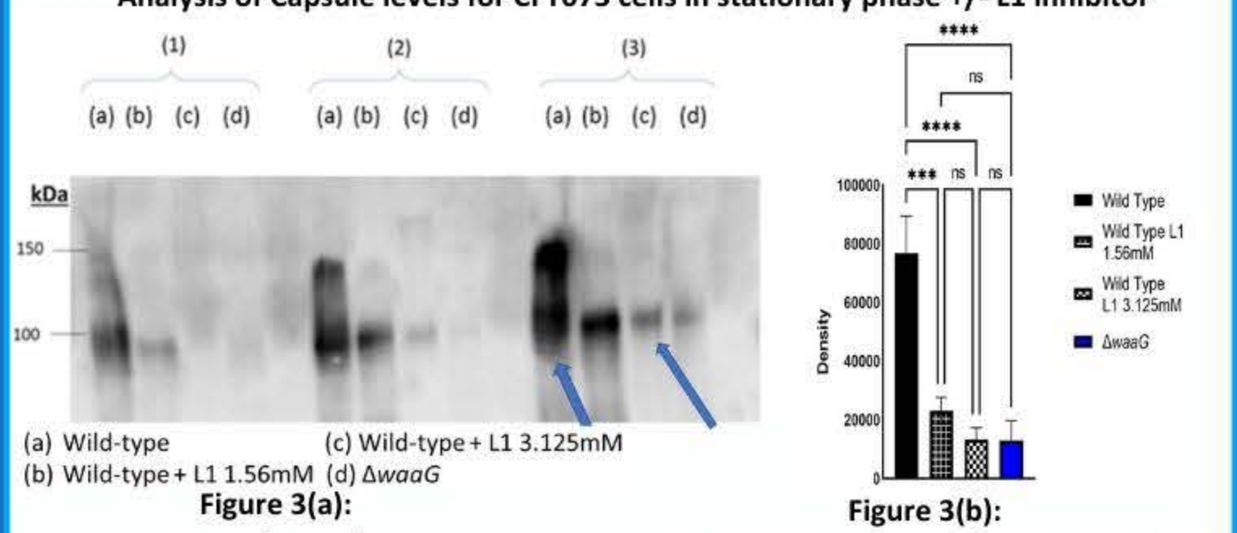

Figure 3(a): Western Blot Analysis

Figure 3(b): Densitometry for Western Blot quantification

5. L1 decreases resistance to serum

- Statistically significant reduction in serum survival seen when comparing CFT073 survival in the absence of inhibitor vs in the presence L1 inhibitor at 3.125mM concentration

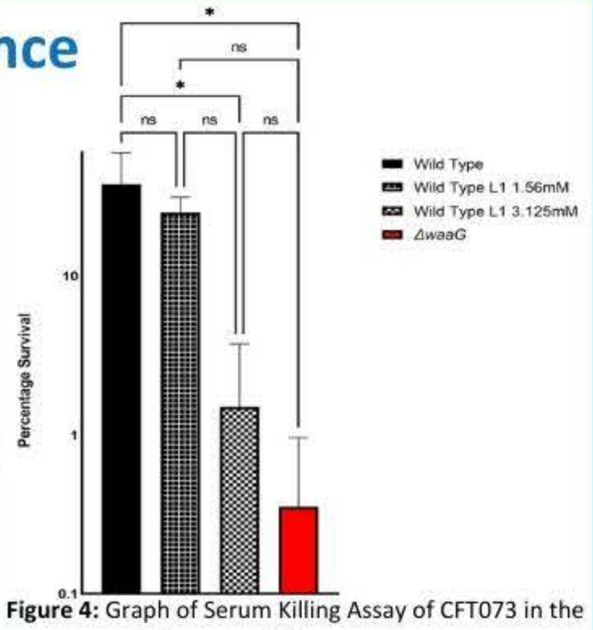

Figure 4: Graph of Serum Killing Assay of CFT073 in the presence and absence of L1

6. WaaG inhibition results in punctate capsule pattern

- Normal uniform capsule seen in the wild-type
- Punctate pattern of capsule expression observed in cultures treated with L1 and in *waaG* mutant strains

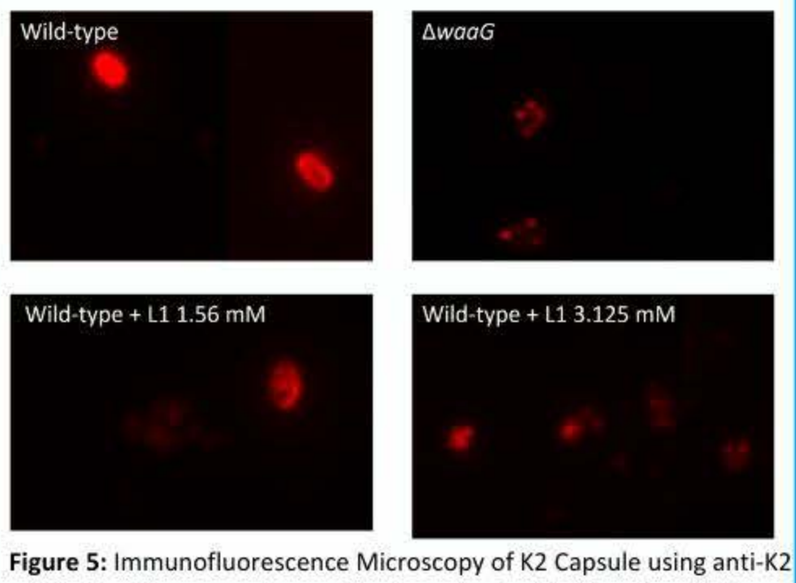

Figure 5: Immunofluorescence Microscopy of K2 Capsule using anti-K2 antibody with a secondary antibody conjugated to Alexafluor-594

7. Conclusions

- LPS integrity and capsule association are interfered with when waaG is inhibited
- This causes a reduction in the virulence and serum resistance of UPEC
- L1 is potentially a promising candidate for treating UPEC infections, especially given the importance of LPS and capsule in serum survival
- Although L1 is non-bactericidal, the compound appears to delay growth; unexpected potential for L1 as a bacteriostatic agent

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