Common anti-haemostatic medications increase the severity of systemic infection by uropathogenic *Escherichia coli*

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Abstract

Uropathogenic *Escherichia coli* (UPEC) causes urinary tract infections that can result in sepsis. The haemostatic system is protective in the pyelonephritis stage of ascending UPEC infection, but the role of the haemostatic system has not been investigated during sepsis. Here we utilize a zebrafish-UPEC systemic infection model to visualize infection-induced coagulation and examine the effects of commonly prescribed anti-haemostatic medications on the infection severity. Treatment of systemically infected zebrafish with warfarin, aspirin, or ticagrelor reduced host survival, while stabilization of clots with aminocaproic acid increased host survival. Anti-haemostatic drug treatment increased UPEC burden. Our findings provide evidence that commonly prescribed anti-haemostatic medications may worsen the outcome of severe UPEC infection.

Keywords

Infection, coagulation, thrombocyte, uropathogenic *Escherichia coli*, zebrafish

Introduction

Uropathogenic *Escherichia coli* (UPEC) is the primary pathogen responsible for urinary tract infection (UTI), which is among the most common bacterial infections worldwide. UPEC most commonly initiates self-limiting cystitis inside the urinary tract, but can ascend the ureters to the kidneys as acute pyelonephritis and potentially progress into sepsis (Wiles, Kulesus et al. 2008, Ulett, Totsika et al. 2013).

The haemostatic system plays an evolutionarily conserved role in both immune control of infections and the pathogenesis of infectious complications from a wide range of pathogens (Frick, Bjorck et al. 2007, Stroo, Zeerleder et al. 2017, Hortle, Johnson et al. 2019). In the case of UPEC, treatment of rodent UPEC pyelonephritis models with the injectable anticoagulant heparin demonstrates an important role of coagulation in preventing the progression of pyelonephritis to sepsis via an α-hemolysin/renal epithelial cell CD147/Tissue Factor axis (Melican, Boekel et al. 2008, Schulz, Chuquimia et al. 2018).

55 The prevalence of urinary tract infections rises from ~10% in the general population of women to ~20% in
56 women over 65 (Medina and Castillo-Pino 2019). Catheterization further increases the risk of urinary tract
57 infections, with a 10-25% risk of a catheter-associated urinary tract infection (Medina and Castillo-Pino
58 2019). The rise in prevalence of chronic cardiovascular conditions, such as atrial fibrillation (Alcusky,
59 McManus et al. 2019), causes a strong correlation between age and the use of anti-haemostatic
60 medications which might compound the risk and severity of UPEC urosepsis in the elderly. Here, we have
61 used a larval zebrafish model of systemic UPEC infection to study the interaction between commonly
62 prescribed anti-haemostatic medications and the severity of UPEC sepsis (Wiles, Bower et al. 2009).
63
64 **Materials and methods**
65 *Zebrafish husbandry*
66 Adult zebrafish were housed at the Centenary Institute (Sydney Local Health District AWC Approval 2017-
67 036). Zebrafish embryos were produced by natural spawning and raised at 28°C in E3 media.
68
69 *Zebrafish lines*
70 Wild type zebrafish are the AB background. Transgenic lines are: *Tg(fabp10a:fgb-EGFP)*^{mi4001} which was
71 used to visualize clot formation (Vo, Swaroop et al. 2013), and *Tg(-6.0itga2b:eGFP)*^{la2} which was used to
72 visualize thrombocytes (Lin, Traver et al. 2005).
73
74 *Infection of zebrafish larvae*
75 Stationary phase *Escherichia coli* MG-1655 or UTI89 carrying the pGI6 plasmid were outgrown in LB broth
76 supplemented with 50 µg/ml spectinomycin for three hours at 37°C. Bacteria were pelleted, washed in PBS,
77 and either resuspended in phenol red dye (0.5% w/v in PBS) for immediate microinjection or supplemented
78 with 10% v/v glycerol and frozen at -80°C (Wright, de Silva et al. 2021). 10-15 nL was injected into the
79 caudal vein or trunk of M-222 (tricaine)-anaesthetized 5 dpf larvae resulting in infectious doses as reported.
80 Larvae were recovered into E3 and housed at 32°C.
81

82 *CFU recovery assay*

83 Groups of 5 zebrafish larvae were pooled and homogenized by pipetting through a P200 tip followed by
84 shearing through 23 G and 28 G needles. Homogenate was serially diluted and plated on LB agar
85 supplemented with 50 µg/ml spectinomycin to select *E. coli* carrying the pGI6 plasmid.

86

87 *Drug treatments*

88 Larvae were treated by immersion exposure to vehicle control (DMSO or water as appropriate), 10 µg/ml
89 aspirin in 0.1% final DMSO, 20 µg/ml ticagrelor in 0.1% final DMSO, 20 µM warfarin in water, or 100 mM
90 amino caproic acid (ACA) in water immediately after infection. Drugs were refreshed after two days only for
91 the survival experiments lasting longer than two days, all other experiments were performed with a single
92 dose.

93

94 *Imaging*

95 Imaging was carried out on larvae anaesthetized in M-222 mounted in 0.75% low melting point agarose on
96 a Deltavision Elite fluorescence microscope for 24 hours. Editing and bacterial fluorescent pixel count was
97 carried out with Image J Software Version 1.51j (Matty, Oehlers et al. 2016).

98

99 *Wound haemostasis assay*

100 We transected the tails of M-222 anesthetized larvae with a scalpel at the ventral pigment gap. This severs
101 the dorsal aorta and posterior cardinal vein resulting in rapid haemostasis in control larvae. Larvae were
102 recovered to E3 prior to imaging at 2 hours post wounding.

103

104 *Statistics*

105 Survival analyses were performed by Log-rank tests in GraphPad Prism. Fluorescent pixel count analyses
106 were performed by Student's *t*-test or ANOVA in GraphPad Prism as appropriate. Error bars represent
107 standard error of the mean.

108

109 **Results**

110 *Characterization of infection kinetics in zebrafish larvae*

111 Prior reports extra intestinal *E. coli* infection have largely used embryo stage zebrafish prior to 3 dpf,
112 however these early stages are not amenable to live imaging of haemostasis as liver development is
113 insufficient to produce sufficient Fgb-GFP fusion protein in the *Tg(fabp10a:fgb-EGFP)*^{mi4001} transgenic line
114 and there are few circulating thrombocytes visible yet in the *Tg(-6.0itga2b:eGFP)*^{la2} transgenic line (Lin,
115 Traver et al. 2005, Vo, Swaroop et al. 2013). To facilitate live imaging with these lines we first characterised
116 systemic infection of 5 dpf zebrafish larvae with *E. coli* strains MG1655 and UTI89. Systemic infection with
117 2000 CFU MG1655 did not cause appreciable mortality while a bolus of 2000 CFU UTI89 resulted in
118 mortality starting around 1 day post infection (dpi) and continuing until 3 dpi (Figure 1A). These survival
119 phenotypes correlated with recovery of each strain, with recoverable MG1655 burden rapidly depleting
120 within 1 dpi (Figure 1B), while UTI89 burden peaked at 1 dpi before declining but remaining detectable until
121 at least 3 dpi (Figure 1C).

122

123 *UPEC induces coagulation in zebrafish larvae*

124 UPEC infection-induced clotting has been observed in live rats (Melican, Boekel et al. 2008, Schulz,
125 Chuquimia et al. 2018). To investigate if host haemostasis played a role in the superior survival of UPEC, we
126 infected *Tg(fabp10a:fgb-EGFP)*^{mi4001} zebrafish larvae, where clots can be visualized by GFP fluorescence,
127 with UTI89 UPEC carrying the pGI6 plasmid, allowing visualization of red fluorescent UPEC, and performed
128 timelapse microscopy across the first day post infection.

129

130 We observed the progressive formation of both arterial and venous clots in infected larvae in close
131 proximity to fluorescent UPEC (Figure 2A). We did not observe widespread clotting throughout the
132 vasculature or clotting not associated with fluorescent UPEC. We were unable to visualise fluorescent
133 MG1655 in the same assay, most likely due to the rapid killing of MG1655 by the host.

134

135 *Warfarin increases the severity of systemic UPEC infection in zebrafish larvae*

136 Inhibition of clotting with heparin has been reported to worsen UPEC infection in mice (Melican, Boekel et
137 al. 2008, Schulz, Chuquimia et al. 2018). We sought to determine if commonly prescribed anticoagulants
138 had a similar effect in our larval zebrafish model using warfarin, a vitamin K antagonist and a commonly
139 prescribed anticoagulant.
140
141 We have previously demonstrated that warfarin reduced mycobacterial infection-induced coagulation in
142 zebrafish larvae (Hortle, Johnson et al. 2019), here we demonstrate that warfarin reduced Fgb-GFP
143 fluorescence around fluorescent UPEC while aminocaproic acid (ACA) treatment stabilized clots (Figure 2B).
144 Treatment with warfarin decreased larval survival following systemic UPEC infection while treatment with
145 ACA conversely increased larval survival following systemic UPEC infection (Figure 2C). The decreased
146 survival of warfarin-treated larvae correlated with increased UPEC burden at 18 hours post infection (hpi),
147 while ACA-treated larvae had comparable levels of UPEC to control larvae (Figure 2D). Addition of warfarin
148 and ACA to LB broth cultures of UPEC had no effect on the *in vitro* growth (Figure 2E).
149
150 *Zebrafish thrombocytes interact with UPEC*
151 Clotting and thrombosis are coordinated during infection-induced haemostasis (Frick, Bjorck et al. 2007,
152 Hortle, Johnson et al. 2019). To determine if zebrafish thrombocytes could play a role in controlling
153 systemic UPEC infection, we infected *Tg(-6.0itga2b:eGFP)*^{la2} larvae, where thrombocytes can be visualized
154 by GFP expression, with UPEC-pGI6. We observed transient interactions between zebrafish thrombocytes
155 and clumps of UPEC, however these events were comparatively rare occurring in only 3/10 larvae imaged
156 (Figure 4A).
157
158 *Aspirin and ticagrelor increase the severity of systemic UPEC infection in zebrafish larvae*
159 Having shown a negative effect of inhibiting clotting, we next investigated the effect of commonly
160 prescribed anti-platelet medications on systemic UPEC infection.
161

162 Inhibition of thrombocytes with either aspirin or ticagrelor reduced survival of infected larvae compared to
163 DMSO-treated control larvae (Figure 4B and 4C). Treatment with either aspirin or ticagrelor increased UPEC
164 burden at 6 hpi compared to DMSO-treated control larvae (Figure 4D). Addition of aspirin or ticagrelor to
165 LB broth cultures of UPEC had no effect on *in vitro* growth (Figure 3E).
166
167 **Discussion**
168 We have found that common anti-haemostatic medications worsen the survival of UPEC-infected zebrafish.
169 Anti-haemostatic drug use is highly prevalent in the elderly as a preventative measure against heart attack
170 and stroke. Alongside this, the risk of urinary tract infections also increases from middle to old age and
171 there is elevated risk of recurrent urinary tract infections in aged care settings (Bennett, Johnson et al.
172 2016). Our data illustrates an association between preventative anti-haemostatic medication usage and the
173 severity of UPEC sepsis.
174
175 Our results add to a growing body of literature that the haemostatic system, and specifically clotting, is
176 crucial to the early containment of blood born UPEC (Melican, Boekel et al. 2008, Schulz, Chuquimia et al.
177 2018). Clinical trials of anticoagulants to treat sepsis-induced coagulopathy have delivered inconsistent
178 results, however an emerging theme is that early coagulation is host protective while late coagulation
179 drives pathology (Umemura, Yamakawa et al. 2016). Subsequently, administration of anticoagulants at
180 early stage can be detrimental to the clearance of pathogen by the native immune system (Scarlatescu,
181 Tomescu et al. 2017). There is little evidence of a beneficial effect of anticoagulants across the total range
182 of patients with sepsis, however anticoagulants are beneficial for the treatment of critically ill subgroups
183 with sepsis-induced disseminated intravascular coagulation (Iba, Saitoh et al. 2014, Umemura, Yamakawa
184 et al. 2016).
185
186 Pathogen-specific effects may drive the heterogeneous host response to anticoagulant therapy. For
187 example, Factor XI aided the containment of *Klebsiella pneumoniae* and *Streptococcus pneumoniae* in mice,
188 while knockout of Factor XII protected mice against *K. pneumoniae* but not *S. pneumoniae* (Stroo, Zeerleder

189 et al. 2017). The genetic conservation of vertebrate coagulation factor function and the amenability of
190 zebrafish to rapid CRISPR-Cas9 mutagenesis suggest zebrafish could be a cost-effective platform to further
191 delineate exact components of the coagulation cascade which we have bluntly targeted with warfarin in
192 this study.
193
194 The negative effects of the antiplatelet drugs aspirin and ticagrelor demonstrate a host-protective role of
195 zebrafish thrombocytes during systemic UPEC infection. Degranulation of activated mammalian platelets
196 releases important inflammatory mediators such as antimicrobial proteins, cytokines, and ADP/ATP which
197 can directly kill pathogens and activate cellular immunity (Deppermann and Kubes 2016). Clinical trials with
198 antiplatelets, especially aspirin, have demonstrated an improvement in mortality of severe septic patients
199 which, similar to anti-coagulant therapy, may reflect an effect of timing (Davis, Miller-Dorey et al. 2016). A
200 limitation of our study is that we were unable to directly quantify thrombocyte-UPEC interactions and
201 determine if the increased susceptibility of aspirin and ticagrelor-treated larvae was due to localized or
202 systemic changes to thrombocyte biology.
203
204 Our CFU recovery experiments confirm warfarin treatment results in increased UPEC growth in systemic
205 infection of zebrafish larvae. This was expected from the literature where heparin treatment facilitates
206 increased UPEC growth in mammals (Melican, Boekel et al. 2008, Schulz, Chuquimia et al. 2018). In parallel,
207 we observed increased UPEC burden in larvae treated with anti-platelet medications. This suggests the
208 haemostatic system either directly controls the growth of UPEC in zebrafish or assists the zebrafish innate
209 immune system in efforts to control systemic UPEC infection.
210
211 An important limitation of our study is that we have not established equivalency of systemic UPEC infection
212 in zebrafish larvae with sepsis seen in mammals. Additionally, our method of infusing UPEC directly into the
213 bloodstream of zebrafish larvae removes key steps of the natural ascending infection route via bladder
214 colonization and pyelonephritis which account for the majority of UPEC morbidity. Zebrafish larvae are also
215 highly tolerant of coagulopathy, as seen in an antithrombin III-deficient mutant (Liu, Kretz et al. 2014), and

216 this may cause unexplored differences in their use of the haemostatic system to control systemic
217 infections.
218
219 The internal concentrations of drugs achieved by immersion exposure of zebrafish larvae to drugs needs to
220 be determined for individual substances, however previous reports have reported a range of 1-20% peak
221 absorption of small molecules (Zhang, Qin et al. 2015, Kirla, Groh et al. 2018). Our 10 µg/ml dose of aspirin
222 delivered by immersion exposure is likely to be at the low end of the range of peak human therapeutic
223 plasma concentrations 2-20 µg/ml (Rosenkranz and Frolich 1985, Benedek, Joshi et al. 1995), while our 20
224 µg/ml dose of ticagrelor is likely to be on the high end of the peak human ticagrelor plasma concentration
225 of 3.6 µg/ml for a 400 mg/day dose (Dobesh and Oestreich 2014), and our 33 µM, roughly equivalent to 10
226 µg/ml, dose of warfarin is likely to be close to therapeutic human plasma levels <1 µg/ml (Routledge,
227 Chapman et al. 1979, Sun, Wang et al. 2006).
228
229 Future studies should investigate the interaction between the haemostatic system and the natural course
230 of UPEC infection in both animal models and by retrospective clinical chart review. The potential for an
231 association between aspirin and warfarin, two of the most commonly prescribed anti-haemostatic
232 medications, with catherization-associated UPEC infections involves a significant proportion of aged care
233 patients and may represent an important cause of excess morbidity.
234
235 **Conclusions**
236 We find an association between the use of anti-haemostatic medication and the severity of UPEC sepsis in a
237 zebrafish infection model.
238
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Author contributions

E.H., and S.H.O designed the experiments. V.T., E.H., and S.H.O performed the experiments. V.T., E.H., and S.H.O wrote the paper. E.H., W.J.B., and S.H.O. supervised the project.

Declaration of Interests

The authors declare no competing interests.

References

Alcusky, M., D. D. McManus, A. L. Hume, M. Fisher, J. Tjia and K. L. Lapane (2019). "Changes in Anticoagulant Utilization Among United States Nursing Home Residents With Atrial Fibrillation From 2011 to 2016." J Am Heart Assoc **8**(9): e012023.

Benedek, I. H., A. S. Joshi, H. J. Pieniaszek, S. Y. King and D. M. Kornhauser (1995). "Variability in the pharmacokinetics and pharmacodynamics of low dose aspirin in healthy male volunteers." J Clin Pharmacol **35**(12): 1181-1186.

Bennett, N. J., S. A. Johnson, M. J. Richards, M. A. Smith and L. J. Worth (2016). "Infections in Australian Aged-Care Facilities: Evaluating the Impact of Revised McGeer Criteria for Surveillance of Urinary Tract Infections." Infect Control Hosp Epidemiol **37**(5): 610-612.

Davis, R. P., S. Miller-Dorey and C. N. Jenne (2016). "Platelets and coagulation in infection." Clin Transl Immunology **5**(7): e89.

Deppermann, C. and P. Kubes (2016). "Platelets and infection." Semin Immunol **28**(6): 536-545.

Dobesh, P. P. and J. H. Oestreich (2014). "Ticagrelor: pharmacokinetics, pharmacodynamics, clinical efficacy, and safety." Pharmacotherapy **34**(10): 1077-1090.

Frick, I. M., L. Bjorck and H. Herwald (2007). "The dual role of the contact system in bacterial infectious disease." Thromb Haemost **98**(3): 497-502.

Hortle, E., K. E. Johnson, M. D. Johansen, T. Nguyen, J. A. Shavit, W. J. Britton, D. M. Tobin and S. H. Oehlers (2019). "Thrombocyte Inhibition Restores Protective Immunity to Mycobacterial Infection in Zebrafish." J Infect Dis **220**(3): 524-534.

Iba, T., D. Saitoh, H. Wada and H. Asakura (2014). "Efficacy and bleeding risk of antithrombin supplementation in septic disseminated intravascular coagulation: a secondary survey." Crit Care **18**(5): 497.

Kirla, K. T., K. J. Groh, M. Poetzsch, R. K. Banote, J. Stadnicka-Michalak, R. I. L. Eggen, K. Schirmer and T. Kraemer (2018). "Importance of Toxicokinetics to Assess the Utility of Zebrafish Larvae as Model for Psychoactive Drug Screening Using Meta-Chlorophenylpiperazine (mCPP) as Example." Front Pharmacol **9**: 414.

Lin, H. F., D. Traver, H. Zhu, K. Dooley, B. H. Paw, L. I. Zon and R. I. Handin (2005). "Analysis of thrombocyte development in CD41-GFP transgenic zebrafish." Blood **106**(12): 3803-3810.

Liu, Y., C. A. Kretz, M. L. Maeder, C. E. Richter, P. Tsao, A. H. Vo, M. C. Huarng, T. Rode, Z. Hu, R. Mehra, S. T. Olson, J. K. Joung and J. A. Shavit (2014). "Targeted mutagenesis of zebrafish antithrombin III triggers disseminated intravascular coagulation and thrombosis, revealing insight into function." Blood **124**(1): 142-150.

Matty, M. A., S. H. Oehlers and D. M. Tobin (2016). "Live Imaging of Host-Pathogen Interactions in Zebrafish Larvae." Methods Mol Biol **1451**: 207-223.

Medina, M. and E. Castillo-Pino (2019). "An introduction to the epidemiology and burden of urinary tract infections." Ther Adv Urol **11**: 1756287219832172.

Melican, K., J. Boekel, L. E. Mansson, R. M. Sandoval, G. A. Tanner, O. Kallskog, F. Palm, B. A. Molitoris and A. Richter-Dahlfors (2008). "Bacterial infection-mediated mucosal signalling induces local renal ischaemia as a defence against sepsis." Cell Microbiol **10**(10): 1987-1998.

Rosenkranz, B. and J. C. Frolich (1985). "Plasma concentrations and anti-platelet effects after low dose acetylsalicylic acid." Prostaglandins Leukot Med **19**(3): 289-300.

Routledge, P. A., P. H. Chapman, D. M. Davies and M. D. Rawlins (1979). "Pharmacokinetics and pharmacodynamics of warfarin at steady state." Br J Clin Pharmacol **8**(3): 243-247.

Scarlatescu, E., D. Tomescu and S. S. Arama (2017). "Anticoagulant Therapy in Sepsis. The Importance of Timing." J Crit Care Med (Targu Mures) **3**(2): 63-69.

Schulz, A., O. D. Chuquimia, H. Antypas, S. E. Steiner, R. M. Sandoval, G. A. Tanner, B. A. Molitoris, A. Richter-Dahlfors and K. Melican (2018). "Protective vascular coagulation in response to bacterial infection of the kidney is regulated by bacterial lipid A and host CD147." Pathog Dis **76**(8).

Stroo, I., S. Zeerleder, C. Ding, B. M. Luken, J. Roelofs, O. J. de Boer, J. C. M. Meijers, F. J. Castellino, C. van 't Veer and T. van der Poll (2017). "Coagulation factor XI improves host defence during murine pneumonia-derived sepsis independent of factor XII activation." Thromb Haemost **117**(8): 1601-1614.

Sun, S., M. Wang, L. Su, J. Li, H. Li and D. Gu (2006). "Study on warfarin plasma concentration and its correlation with international normalized ratio." J Pharm Biomed Anal **42**(2): 218-222.

Ulett, G. C., M. Totsika, K. Schaale, A. J. Carey, M. J. Sweet and M. A. Schembri (2013). "Uropathogenic Escherichia coli virulence and innate immune responses during urinary tract infection." Curr Opin Microbiol **16**(1): 100-107.

Umemura, Y., K. Yamakawa, H. Ogura, H. Yuhara and S. Fujimi (2016). "Efficacy and safety of anticoagulant therapy in three specific populations with sepsis: a meta-analysis of randomized controlled trials." J Thromb Haemost **14**(3): 518-530.

Vo, A. H., A. Swaroop, Y. Liu, Z. G. Norris and J. A. Shavit (2013). "Loss of fibrinogen in zebrafish results in symptoms consistent with human hypofibrinogenemia." PLoS One **8**(9): e74682.

Wiles, T. J., J. M. Bower, M. J. Redd and M. A. Mulvey (2009). "Use of zebrafish to probe the divergent virulence potentials and toxin requirements of extraintestinal pathogenic Escherichia coli." PLoS Pathog **5**(12): e1000697.

Wiles, T. J., R. R. Kulesus and M. A. Mulvey (2008). "Origins and virulence mechanisms of uropathogenic Escherichia coli." Exp Mol Pathol **85**(1): 11-19.

Wright, K., K. de Silva, K. M. Plain, A. C. Purdie, T. A. Blair, I. G. Duggin, W. J. Britton and S. H. Oehlers (2021). "Mycobacterial infection-induced miR-206 inhibits protective neutrophil recruitment via the CXCL12/CXCR4 signalling axis." PLoS Pathog: 2020.2012.2014.422665.

Zhang, F., W. Qin, J. P. Zhang and C. Q. Hu (2015). "Antibiotic toxicity and absorption in zebrafish using liquid chromatography-tandem mass spectrometry." PLoS One **10**(5): e0124805.

332 **Figure Legends**

333 Figure 1: Characterisation of larval zebrafish *E. coli* systemic infection model

334 (A) Survival analysis of 5 dpf zebrafish larvae infected with 2000 CFU MG1655 or UTI89. (B) CFU recovery
335 from 2000 CFU MG1655-infected larvae, t0 is approximately 2 hours after infection. (C) CFU recovery from
336 2000 CFU UPEC UTI89-infected larvae, t0 is approximately 2 hours after infection. Data is representative of
337 three independent experiments.

338

339 Figure 2: Clotting is protective against systemic UPEC infection.

340 (A) Intravital microscopy of Fgb-GFP clot formation around UPEC UTI89 pGI6 (red) in a *Tg(fabp10a:fgb-*
341 *EGFP)*^{mi4001} larva. Filled arrowhead indicates site of arterial clot formation, hollow arrowhead indicates site
342 of venous clot formation. Scale bar indicates 10 µm. (B) Maximal Fgb-GFP fluorescent intensity around
343 fluorescent UPEC from 4 hpi larvae. Each data point represents one larva. (C) Survival of 2000 CFU UPEC
344 UTI89-infected larvae treated with warfarin or ACA. n>=20 per group, data shown is from one experiment
345 that is representative of two biological replicates. (D) UPEC CFU recovery from 18 hpi 2000 CFU UPEC
346 UTI89-infected larvae, data shown is pooled from two independent experiments. (E) Optical density at 600
347 nm readings from *in vitro* LB broth growth of UPEC UTI89 strain treated with drugs at concentrations
348 indicated.

349

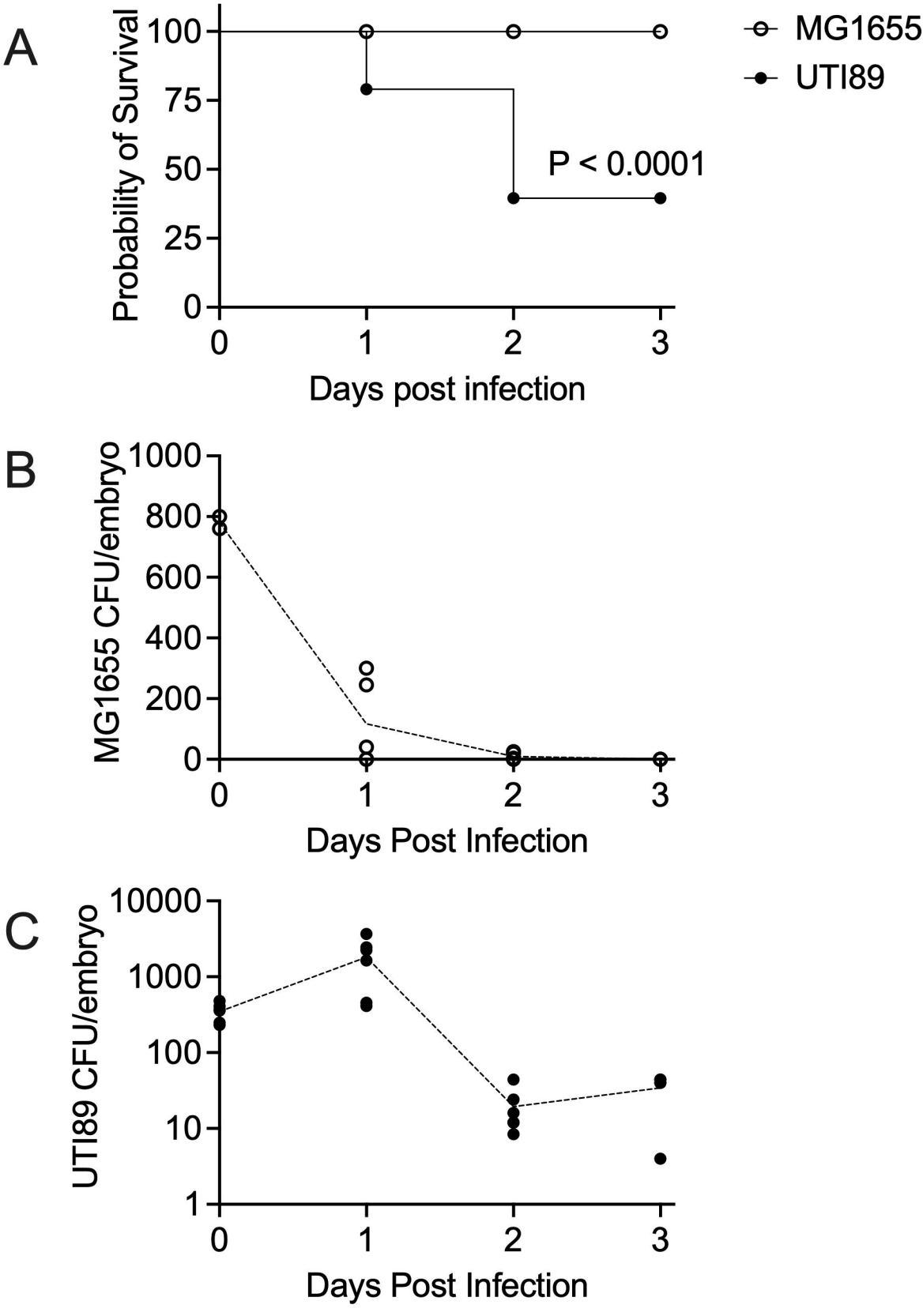
350 Figure 3: Thrombocyte activation is protective against systemic UPEC infection.

351 (A) Intravital microscopy of green thrombocytes interacting with UPEC pGI6 (red) in a *Tg(-6.0itga2b:eGFP)*^{la2}
352 larva. Filled arrowhead indicates first thrombocyte that interacts with clump of UPEC for 30 minutes,
353 hollow arrowhead indicates a second thrombocyte that interacts with the same clump of UPEC for 21
354 minutes. Unindicated green cells are thrombocytes in circulation. t0 is approximately 1 hour post infection.
355 Scale bar indicates 10 µm. (B) Survival of 1000 CFU UPEC UTI89-infected larvae treated with aspirin. n>=25
356 per group, data shown is from one experiment that is representative of two biological replicates. (C)
357 Survival of 1000 CFU UPEC UTI89-infected larvae treated with ticagrelor. n=25 per group, data shown is

358 from one experiment that is representative of two biological replicates. (D) CFU recovery from 6 hpi 1000

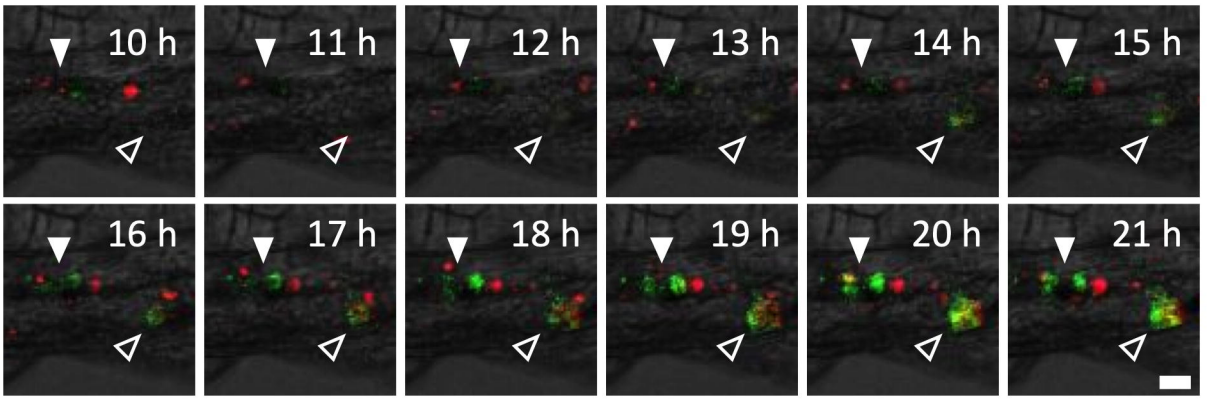
359 CFU UPEC UTI89-infected larvae, data shown is pooled from two independent experiments.

360


A
Probability of Survival
100
75
50
25
0
0
1
2
3
Days post infection
MG1655
UTI89
P < 0.0001
B
MG1655 CFU/embryo
1000
800
600
400
200
0
0
1
2
3
Days Post Infection
C
UTI89 CFU/embryo
10000
1000
100
10
1
0
1
2
3
Days Post Infection

A

Tg(fabp10a:fgb-EGFP)mi4001 UPEC-pGI6



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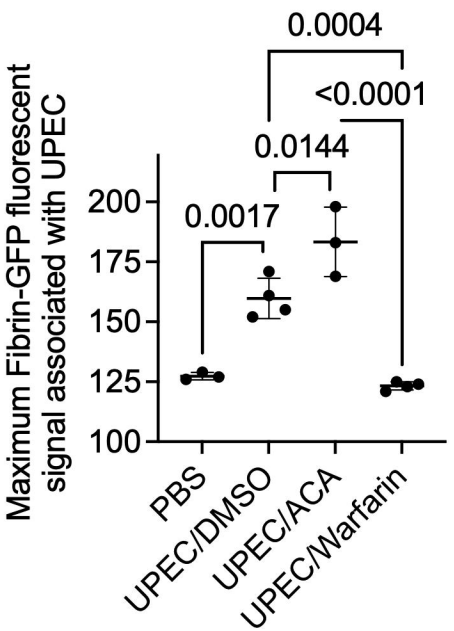

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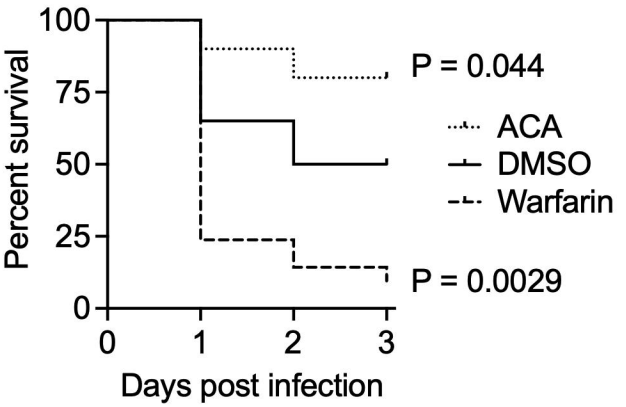
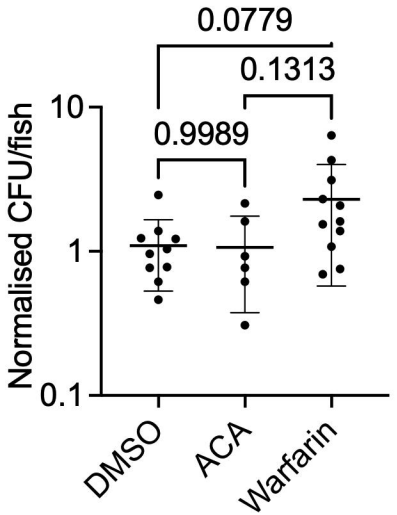
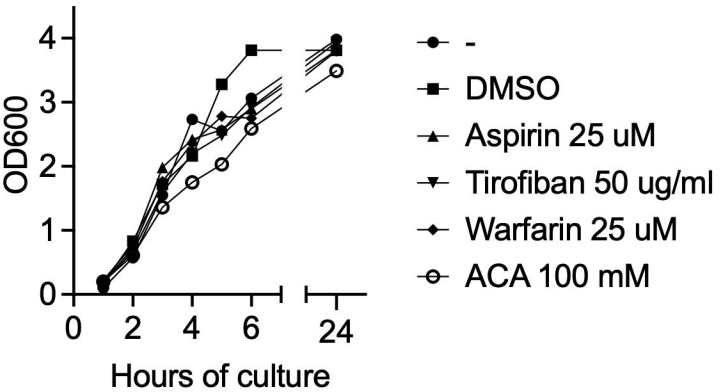
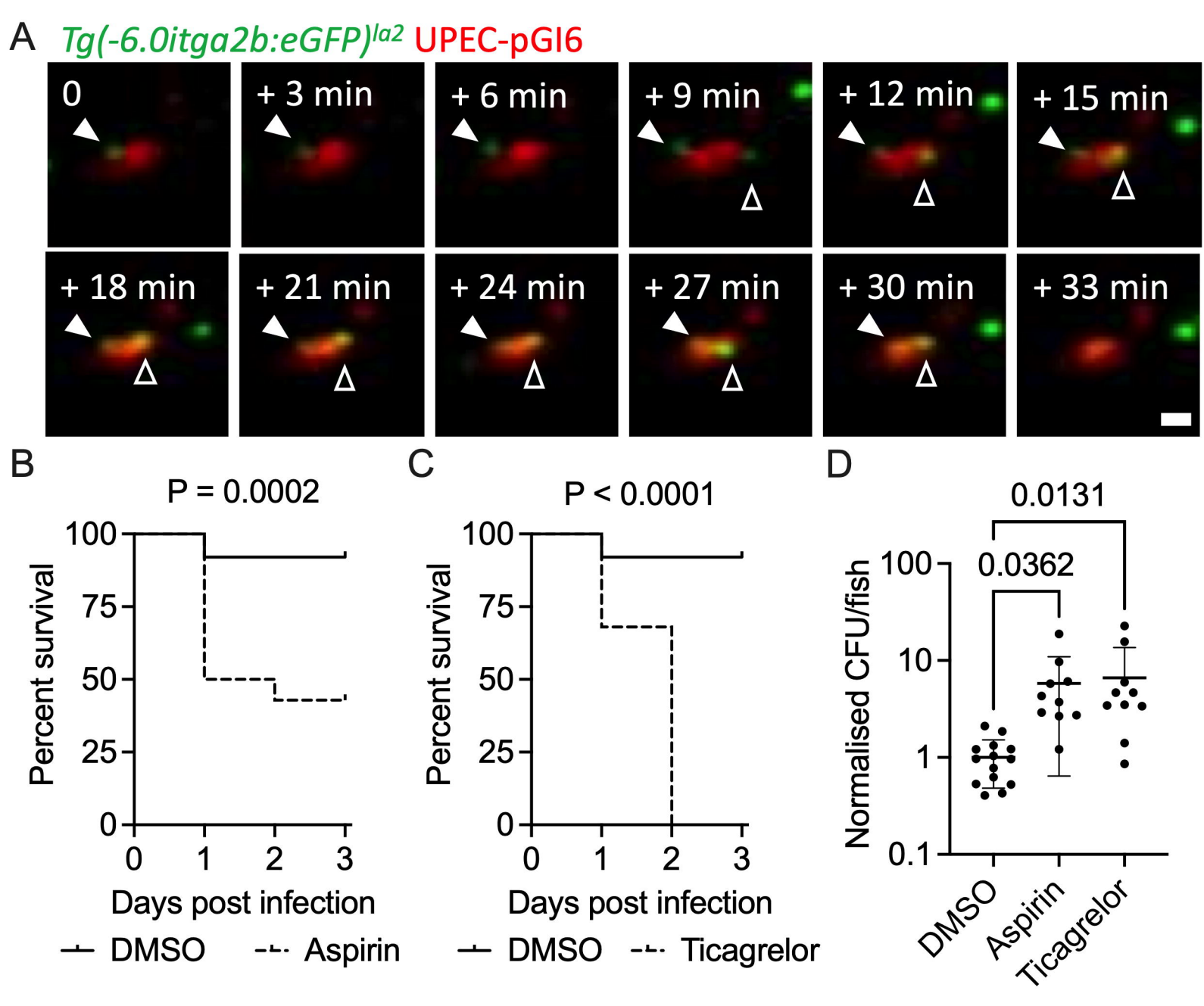


D



E




A
Tg(-6.0itga2b:eGFP)la2
UPEC-pGI6
0
+ 3 min
+ 6 min
+ 9 min
+ 12 min
+ 15 min
+ 18 min
+ 21 min
+ 24 min
+ 27 min
+ 30 min
+ 33 min
B
P = 0.0002
Percent survival
100
75
50
25
0
0
1
2
3
Days post infection
DMSO
Aspirin
C
P < 0.0001
Percent survival
100
75
50
25
0
0
1
2
3
Days post infection
DMSO
Ticagrelor
D
0.0131
0.0362
Normalised CFU/fish
100
10
1
0.1
DMSO
Aspirin
Ticagrelor