Pathogenesis of *Escherichia coli* from polymicrobial Urinary tract infections

Response to Piatti:

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On behalf of the authors of our manuscript on pathogenesis of *E. coli* from polymicrobial urinary tract infections (Croxall *et al.*, 2011), we present a response to the correspondence submitted by Gabriella Piatti. We thank the author for their interest in our work, and especially welcome their comment on the importance of studying such infections. We also hereby attempt to clarify some points of confusion in the authors interpretation of the data displayed in the initial publication.

Piatti begins by stating that our analysis of antimicrobial resistance data was (probably) performed regardless of patient group. We clearly state in our manuscript that there was no difference in levels of antimicrobial resistance between polymicrobial and monomicrobial samples. As there was no statistically significant difference we chose not to present that data in detail given the amount of data we had to present on what we considered our significant findings, namely that poylmicrobial UTI contain high numbers of organisms with significant levels of antimicrobial resistance and high levels of invasiveness and are going untreated in clinical settings. The author is correct that this is not in agreement with previous literature, and that is a key reason why we believe our study and others like it need to be conducted and published. Only by performing controlled, co-ordinated equivalent studies across multiple sites can a true evaluation of organisms circulating in polymicrobial UTI be determined and compared. We believe this to be of immediate requirement in the field, particularly when one considers comparisons of primary secondary and tertiary healthcare facilities, as well as geographical variations in circulating bacterial populations.

Piatti goes on to explain that the simultaneous discovery of enhanced ciprofoloxacin resistance and increased pathogenic potential is in their opinion contradictory, and that we have not compared "virulence genes" across our isolates. Additionally there is a suggestion that without full analysis there is "doubt on the real significance of our findings". Firstly, we do not consider enhanced resistance and pathogenesis within a population of *E. coli* as contradictory at all. Particular attention has recently been paid to the emergence of strains such as *E. coli* ST131 which is both highly resistant and pathogenic. Additionally when viewing our results as a population it is clear there will be some strains which have increased resistance and some which have increased pathogenesis, and that the results cannot be generalised as all *E. coli* displaying both phenotypes. The study of the population was outwith the scope of our manuscript, which we state clearly in abstract, introduction and discussion is about highlighting the presence of potentially dangerous pathogens in UTI samples that go untreated. Similarly the presence of "virulence genes" would need to have been conducted at the level of our *E. coli* population, outwith the scope of our manuscript. The author will be pleased to know that a comprehensive analysis of the *E. coli* population was conducted, and is currently under review for publication in another journal. In that

work the entire population is genotyped by MLST and the VAG multiplex PCR they refer to (Johnson & Stell, 2000) and correlated to antimicrobial resistance on a strain by strain basis. The debate of "virulence genes" is a contentious one also, particularly in ExPEC where it would appear there is no real virulence gene signature to speak of, rather that *E. coli* has a vast array of tools to choose from the accessory gene pool within which there is enormous overlap and redundancy (Barl *et al.*, 2008, Bielaszewska *et al.*, 2007, Brzuszkiewicz *et al.*, 2006, Dobrindt *et al.*, 2004).

One final point from Piatti is that when studying ciprofloxacin resistance we did so only in elderly patients and did not analyse catheterised –v- non-catheterised patients. We state in the manuscript a comparison between those 2 groups showing no significant difference. Indeed most of our comparative analyses across those 2 groups were not significant, primarily due to the sampling bias given to allow collection of primarily polymicrobial samples, and therefore far fewer catheterised patients. The reason this was done is the same as the reason we studied elderly patients, namely that the funding generously received from the Dowager Countess Eleanor Peel trust was to study the potential pathogens present in, and going untreated in, polymicrobial UTI in elderly patients, as made clear throughout the presentation of our work. Undoubtedly our findings have opened up the possibility of similar studies across wider cohorts, and we encourage Piatti and others to join us in studying this topic to a much greater level.

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