An Ecological Perspective on Delirium in Urinary Tract Infections and Asymptomatic Bacteriuria: The Role of *Escherichia coli* and Host-Microbe Inflammatory Responses

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Abstract

Delirium is a prevalent condition among elderly individuals. Urinary tract infections (UTIs) are often assumed to contribute to delirium onset. Given the recent discovery of the urinary microbiome, making a distinction between symptomatic UTIs and asymptomatic bacteriuria (AB) is crucial to investigate their roles in delirium. This study investigates the role of *E. coli* which is commonly found in UTIs and AB as a member of the urinary microbiome, and their interaction with the host, particularly through the interleukin-6 (IL-6) pathway, to understand their potential roles in delirium onset.

Analysis of microbiota in women with UTIs and AB reveals diverse urobiome compositions. Sole reliance on microbiota profiles or E. coli strain diversity may however not sufficiently differentiate between UTI and AB. Uropathogenic strains of *E. coli* are likely to trigger host responses through urovirulence. Biomarkers such as IL-6 show promise in improving UTI diagnostics, and might even be used as biomarkers for delirium. However, applying findings from IL-6-related delirium studies to UTI-related delirium warrants cautious consideration due to varying study contexts.

While this study hints that the host-microbe interactions in UTI onset may also play a role in the development of delirium, further research is crucial to elucidate the dynamics of UTI-induced IL-6 production and its specific implications for the onset of delirium to uncover potential mechanisms linking bacterial virulence factors with neuroinflammatory responses in elderly patients.

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Introduction

Delirium is a common condition in the elderly population, with prevalences varying from 18% – 35% in general medical wards, 20% – 22% in nursing homes, 25% in geriatric units, and 7% – 50% in intensive care units (Krinitski et al., 2021). This acute confusional state, characterised by sudden onset confusion, fluctuating severity, inattention, and abnormal consciousness levels, significantly impacts patient outcomes and healthcare resources (Mattison, 2020; Dutta et al., 2022). Delirium is often associated with prolonged hospital stays, complicated recovery, considerable difficulties for caregivers, institutionalisation, increased healthcare costs, and high mortality rates.

While the aetiology of delirium remains largely unclear, one widely explored hypothesis suggests that approximately 49.5% of delirium cases result from infections, particularly urinary tract infections (UTIs) (Dutta et al., 2022). UTIs, affecting 150 million individuals worldwide each year, are especially common in the elderly population, aged 65 and older (Neugent et al., 2020; Dutta et al., 2022). In community-dwelling populations UTI occurrence could range from 12% – 29%, while in long-term care facilities this increases up to 44% – 58%. UTIs, significantly affecting women more than men probably due to anatomical differences between the sexes. UTIs are associated with localised symptoms, like dysuria, worsening urinary urgency or frequency, and suprapubic pain, but also more serious health issues, including bacteremia, hospitalisation, decreased functional status, urosepsis, and mortality (Dutta et al., 2022; Zeng et al., 2020).

Diagnosis of UTIs – Mind the Urobiome and Host Responses

Diagnosing UTIs traditionally relied on detecting urinary pathogens at a threshold of $\geq 10^5$ colony-forming units (CFU) per mL in standard urine cultures, alongside genitourinary symptoms (Ipe et al., 2013). However, about 67% of guidelines suggest lower thresholds, and varying recommendations for different patient groups (Hilt et al., 2023). For example, men and high-risk patients may require lower thresholds ($10^2 – 10^5$ CFU/mL and $10^2 – 10^4$ CFU/mL, respectively), compared to women (10^5 CFU/mL). In addition, the culture conditions and media used in standard urine cultures (SUC) are primarily designed to detect growth of common uropathogenic Gram-negative bacteria, such as *Eschericha coli, Klebsiella* species, and *Enterobacter* species (Chai et al., 2024). In contrast, newer methods like the 'expanded quantitative urine culture' (EQUC), which uses greater urine volumes, diverse culture media, varied atmospheric conditions, and prolonged incubation, seem to be more sensitive in detecting a wider range of both uropathogenic and non-uropathogenic bacteria, also in the bladder of healthy asymptomatic women. The increased sensitivity of EQUC does not necessarily indicate that the identified species are causative of UTIs.

Recent research highlights diverse microbial communities in the urinary tract (Neugent et al., 2020; Zandbergen et al., 2021). Although the human urinary tract (UT) contains less biomass than other microbial niches, such as the gut, it hosts numerous genera and species as core components of the uro-microbiome. The species seem to form microbial ecosystems, living as communities in the UT (Zandbergen et al., 2021). Interestingly, *E. coli* – which is usually known as uropathogen – has previously been observed within healthy female bladders, and is likely a common inhabitant of the female urinary tract (Price et al., 2019; Zandbergen et al., 2021; Jones-Freeman et al., 2021; Adu-Oppong et al. 2022; Shanbhag et al., 2023 [preprint]; Chai et al., 2024). This raises questions about the role of *E. coli* in urinary health and disease,

especially given that the species was both reported in asymptomatic and inflammation-related microbiota, implying the presence of particular microbial species alone may not be sufficient to explain the onset of urinary symptoms. Given these complexities, *E. coli* will be the main focus of my investigation into the microbial ecology of UTI-induced delirium.

Furthermore, it is often proposed that rather than the presence of bacteria alone, the host's inflammatory response to bacterial colonisation of the urinary tract may determine symptom manifestation, although the specific mechanisms behind this are not well understood (Adu-Oppong et al., 2022; Chai et al., 2024). In their interactions, hosts and their resident microbes appear to be a composite organism, the holobiont (Neugent et al., 2020). Given this mutual relationship, changes in microbial ecology likely impact the entire system. Understanding these microbial ecosystems and the role of host-microbe interactions in UTI pathogenesis is crucial, as it possibly provides valuable insights into UTI development and management. For example, combining EQUC with host response tests measuring urinary cytokines, such as interleukin-6 (IL-6) (Ching et al., 2018), could possibly enhance diagnostic specificity and sensitivity for UTIs (Chai et al., 2024).

Is There Really a Correlation Between UTI and Delirium?

The correlation between UTI and delirium has been widely studied for many years. For example, in the late 1980's Levkoff et al. (1988) found that a UTI at any time during hospitalisation was the single most important factor associated with delirium. More recently, although they did not provide a clear definition of a UTI, Toure et al. (2024) found a significant association between urinary tract infections and increased risk of delirium in geriatric trauma patients.

The association between UTIs and delirium may however be overestimated, as some of these studies are reported to contain methodological flaws and biases. Balogun and Philbrick (2013) found that most studies *lacked adequate statistical adjustment* for risk factors. Secondly, there was often a *lack of objective diagnostic criteria*, increasing the risk of bias. Mayne et al. (2019) revealed that the majority lacked proper definitions of delirium, nor had valid criteria for UTI, leading to unreliable associations. Finally, there was often *diagnostic suspicion bias* (knowledge of abnormal urine leads to delirium documentation) and *exposure suspicion bias* (knowledge of delirium leads to urine abnormality documentation) (Balogun & Philbrick, 2013). Both could lead to the overestimation of the association.

Furthermore, the association between UTI and delirium may be overestimated due to the high prevalence of asymptomatic bacteriuria (AB) in the elderly (Balogun & Philbrick, 2013). AB, occurring in up to 50% of long-term care residents and 100% of individuals with indwelling catheters, is characterised by isolated urinary microbial cultures ($\geq 10^5$ CFU/mL) without UTI symptoms (Phillips et al., 2012; Wullt & Svanborg, 2016; Schonnop et al., 2022; Ipe et al., 2013). AB resembles commensalism at other mucosal sites and may even protect against symptomatic UTIs (Wullt & Svanborg, 2016), yet it is often confused with UTIs due to lack of stratifying diagnostics.

Diagnosing UTI and AB in elderly patients with delirium or dementia is challenging due to their difficulties expressing the absence or presence of symptoms (Krinitski et al., 2021). Delirium not only complicates determining physical symptoms, the condition itself and the underlying cause require much time and effort to diagnose correctly. Because delirium is often seen as a symptom of a UTI, a – relatively time-efficient – urine investigation is often used for delirium diagnosis. An abnormal urinalysis may be misattributed as UTI,

overlooking the possibility of a comorbidity between AB and delirium without a clear case. This misattribution could falsely link delirium to UTI, despite the two clinical manifestations potentially being unrelated.

Due to the conflicting evidence for the link between UTI and delirium, Krinitski et al. (2021) conducted a meta-analysis, and found significant heterogeneity and publication bias among different studies. After compensation for these biases, they still found a significant association between UTI and delirium, irrespective of potential confounders. This association remained significant when they restricted their analysis to (1) only studies where delirium diagnosis followed formal diagnostic criteria and (2) UTI diagnosis followed formal diagnostic criteria, e.g. by relying on positive results from microbiological urine tests alongside the presence of UTI symptoms. This approach minimises the potential bias from suboptimal detection methods for delirium and UTI.

Consequences of Misattributing Delirium to Asymptomatic Bacteriuria

Krinitski et al. (2021) also explored the association between AB and delirium, and found only one relevant study that showed no significant link. This lack of evidence suggests there is no substantial connection between AB and delirium. Similarly, Dutta et al. (2022) found no significant connection between AB and delirium in a systematic review, contrasting with their established significant association between UTI and delirium. They argue that it is reasonable to assume that bacteriuria without symptoms does not contribute to delirium development. Furthermore, given the high prevalence of AB in the elderly, it is highly likely that individuals who develop delirium due to unrelated causes may also develop AB by chance alone (McKenzie et al., 2014).

Moreover, there is no evidence that delirium symptoms improve when patients with AB are treated with antibiotics (Schonnop et al., 2022; McKenzie et al., 2014). Misdiagnosing AB as a UTI could lead to over-investigation and over-treatment, increasing the risk of recurrent UTI, acute pyelonephritis, drug interactions due to polypharmacy, gut dysbiosis leading to *Clostridioides difficile* infections, higher morbidity and healthcare costs (Wullt & Svanborg, 2016; Bilsen et al., 2024; Schonnop et al., 2022). Especially with the increasing appreciation of the role of commensal members of the host microbiota, over-treatment with antimicrobials should be limited to maintain ecological balance within the urinary tract (Klein & Hultgren, 2020). Additionally, unnecessary antibiotics treatment is associated with an increased risk of delirium and could contribute to the development and spread of resistant microbial strains (Ipe et al., 2013), posing threats to both human and environmental health by risking the inability to control infectious diseases and by disrupting ecological balances (Wang et al., 2023).

Present Study

To cease unnecessary antimicrobial treatment for asymptomatic bacteriuria, understanding its underlying ecology and microbial distinctions from UTIs is highly relevant, along with exploring their roles in delirium development. To my knowledge, so far there has not been much research about the connection between delirium and UTI/AB from an ecological point of view. With advancing knowledge of the urinary microbiome, questions arise: Are AB and UTI synonymous in terms of microbial communities? If the microbial communities are similar, why does the host experience symptoms in one case but not the other? And how does this contribute to the development of delirium? This study aims to address the following research question: How do the urinary microbiome, with a focus on *Escherichia coli*, and host-microbe interactions, particularly the interleukin-6 pathway, differ between patients with

asymptomatic bacteriuria and symptomatic urinary tract infection, and how do these differences affect the occurrence of delirium? Since asymptomatic bacteriuria has been shown to protect the host against symptomatic UTI (Wullt & Svanborg, 2016), I expect to find differences in microbial communities between the two conditions. With that, it might be possible that there are different host-microbe interactions, which in turn may result in different host symptoms.

Method – Search Strategy

I performed a non-systematic literature search Pubmed, Google Scholar and SmartCat in May and June 2024. Specifically, I performed a free text search for terms such as “urinary tract infection”, “UTI”, “asymptomatic bacteriuria”, “delirium”, “acute confusion”, “urinary tract microbiome”, “microbial ecology”, “microbiota”, “urobiome”, “microbial community”, “inflammation”, “interleukin-6”, “IL-6”, “biomarker”, as well as combinations of these terms. Finally, I restricted my results by selecting only literature published after 2010. I screened all the abstracts to identify the relevant studies.

Exploring Microbial Composition, Urotypes and *E. coli* Strain Variation in UTI and AB

Microbiota Composition of Symptomatic and Asymptomatic Women

Diagnosing UTIs and AB is challenging, partly due to the lack of a clear definition of the healthy urinary microbiome (Price et al., 2019; Adu-Oppong et al., 2022; Ljubetic et al., 2023). Traditional diagnostic methods have focused on common uropathogens like uropathogenic *Escherichia coli* (UPEC), which cause 80% of outpatient UTIs, with other bacteria such as i.e. *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Acinetobacter spp.* causing the remaining 20%. To place UTIs in the context of the regular asymptomatic urobiome, advanced techniques like EQUC, 16S rRNA sequencing or metagenomic shotgun

sequencing are increasingly used to establish baseline asymptomatic urobiome compositions in healthy individuals.

When applying or combining these methods, a lot of individual variation was found in urobiomes, but the most common genera in asymptomatic women, reported in different studies, were Gram-positive *Lactobacillus*, *Corynebacterium*, *Streptococcus*, *Actinomyces*, *Gardnerella* and *Staphylococcus*, as well as Gram-negative *Escherichia* (Price et al., 2019; Zandbergen et al., 2021; Jones-Freeman et al., 2021; Ljubetic et al., 2023; Chai et al., 2024). While *Lactobacillus* is sometimes thought to be less common in postmenopausal women, Price et al. (2019) found no evidence for this. In more than half of their study cohort *Lactobacillus* predominated the urobiome, which is not very surprising considering its reported beneficial role in many other ecological niches and its association with lower prevalence of urinary symptoms. On the other hand, *Escherichia coli* seemed to be associated with older, postmenopausal women, while *Gardnerella vaginalis* was found more often in younger women. It stands out that *E. coli* – which is usually known as uropathogen – is quite prevalent in asymptomatic women. This raises the question which symptoms are related, or caused, by which microbes or microbial communities.

It has been suggested that urinary disorders, like UTIs, could be a consequence of ecosystem disruptions or dysbiosis of the urinary tract (Ljubetic et al., 2023). Some argue that UTIs should be understood through the lens of 'urotypes' – specific microbial compositions in individual urinary tracts – on a spectrum of urinary microbiome health. Using metagenomic sequencing of clinical samples, Adu-Oppong et al. (2022) identified 16 different urotypes, often dominated by a single bacterial taxon. Most 'culture-positive' samples ($\geq 10^5$ CFU) from patients with suspected UTIs were dominated by uropathogenic *E. coli* (UPEC),

compared to less common uropathogens like *Klebsiella spp.*, *Gardnerella spp.*, *Proteus spp.*, and *Citrobacter spp*. In contrast, symptomatic 'no-growth' samples were dominated by common urinary tract inhabitants such as *Lactobacillus spp.*, *Gardnerella spp.*, and *Staphylococcus spp*. This means that different urotypes resulted in similar manifestations of symptoms, to varying degrees. In asymptomatic individuals, significant shifts in dominant taxa were common, often from nonpathogenic species to – previously undetected – potential uropathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, and *Enterobacter cloacae*, implying that uropathogens do not always result in symptoms.

Subsequently, Adu-Oppong et al. (2022) compared urotypes of symptomatic women to those of asymptomatic women. While 'insignificant' ($< 10^5$ CFU) and 'no-growth' suspected UTI specimens generally fell within the range of asymptomatic microbiota compositions, a distinct cluster of predominantly 'culture-positive' specimens was found outside this range, showing higher *Escherichia spp.* abundance than asymptomatic states. Other potential uropathogens like *Klebsiella spp.* and *Proteus spp.* were more common among asymptomatic microbiota. Samples indicative of urinary tract inflammation were evenly distributed among microbiota that were either concordant or conflicting with the compositions of asymptomatic urotypes. That there were no significant community differences between asymptomatic and inflammation-related microbiota implies that urinary symptoms cannot be attributed to genitourinary microbiome community dysbiosis alone.

This study highlights that patients with a suspected UTI often have diverse genitourinary microbiota compositions, including common taxa like *Enterobacteriales*, *Klebsiella*, *Lactobacillus*, *Gardnerella*, and *Staphylococcus* (Adu-Oppong et al., 2022). Analysis of asymptomatic specimens showed high inter- and intrapersonal variability in urobiome

composition, challenging the notion of a single ‘asymptomatic’ urotype. Despite the presence of potentially uropathogenic bacteria like *E. coli* in asymptomatic individuals, their overrepresentation in symptomatic cases supports their role as primary pathogens. However, the overlap in microbiota compositions between asymptomatic and symptomatic states, along with varying genitourinary inflammation in suspected UTIs, suggests that microbiota composition alone may not be sufficient to explain the onset of urinary symptoms.

Strain Diversity in the Microbiota Composition – Differences in E. coli Strains?

Perhaps just assessing the microbiota composition on a species level may not provide a complete picture; instead, a more detailed examination of the strain-specific composition (genetic variants within a species) of these urotypes may be necessary to better understand the manifestation of urinary symptoms. In individuals with asymptomatic bacteriuria, the same strain of bacteria often persists for months or years without causing symptoms (Wullt & Svanborg, 2016). Shanbhag et al. (2023 [preprint]) investigated strain diversity of species within the urinary microbiota, building upon previous confirmation of strain persistence via culture-based methods. In UTI-negative urinary samples they most frequently identified common urobiome species, such as *E. coli* and *G. vaginalis,* that were prevalent despite the absence of UTI symptoms. Multiple urine samples were found to harbour multiple strains of the same species, notably of *E. coli* and *Citrobacter spp.*, both members of *Enterobacteriaceae.* Thus, although the urobiome of healthy individuals is a low-biomass niche, strain diversity seems to persist.

In particular, the presence of *E. coli* phylogroups in urinary samples of asymptomatic women highlights the potential impact of strain variation on urinary tract health (Shanbhag et al., 2023 [preprint]), raising questions about differences in strain composition between symptomatic and asymptomatic states. Uropathogenic *E. coli* (UPEC) strains can be

categorised into several phylogenetic groups (A, B1, B2, C, D, E, and F), based on four genetic markers (Ljubetic et al., 2023). In particular, B2 strains, especially when co-occurring with D strains, are significantly more frequent in UTIs than in AB. However, the presence of these phylogroups in AB – although in low frequencies – suggests that phylogroups alone may be insufficient for differentiation between symptomatic and asymptomatic states. Moreover, UPEC strains show significant geographical variation in phylogroup distribution and virulence factor carriage, complicating the identification of consistent genomic markers for UTI onset (Klein & Hultgren, 2020).

Summary of Findings – Microbial Communities and Strains May Not Be The Absolute Answer

The study of microbiota composition in symptomatic and asymptomatic women reveals highly diverse urobiome compositions, highlighting predominant genera such as *Lactobacillus*, *Gardnerella*, and *Escherichia* in asymptomatic women (Price et al., 2019; Zandbergen et al., 2021; Jones-Freeman et al., 2021; Ljubetic et al., 2023; Chai et al., 2024). Variability in these microbial communities challenges the concept of a 'singular' asymptomatic urotype. Moreover, the overlap in microbiota compositions between asymptomatic and symptomatic states suggests that microbiota composition alone may not be sufficient to explain the onset of urinary symptoms (Adu-Oppong et al., 2022).

Strain-specific analyses underscore the diversity of *E. coli* strains, as well as strains from other species, within asymptomatic individuals, suggesting that strain variation may play a role in UTI pathogenesis. However, similar to microbiota composition, phylogroups alone may also be insufficient for differentiation between symptomatic and asymptomatic states (Ljubetic et al., 2023), which raises the question whether bacterial properties or host-microbe responses might tell us more about this difference.

E. coli Virulence and Host Responses in UTI Pathogenesis and Diagnostics

Virulence Factors in Uropathogenic and Nonpathogenic Eschericia coli

A wide range of virulence factors are used by UPEC to overcome the host's innate and adaptive immune responses and facilitating bladder colonisation during UTIs (Klein & Hultgren, 2020), raising questions about differences in virulence factors between symptomatic and asymptomatic strains. Although B2 *E. coli* strains generally carry more putative urovirulence factors than non-B2 strains, both B2 and non-B2 strains can efficiently colonise the bladder (Ljubetic et al., 2023). Furthermore, the urovirulent *fim* gene was reported to be present in both ASB and UTI strains, while the *PapG allele II*, both involved in adherence, seemed more prevalent in UTI strains. This seems to align with the finding that 60-75% of the genome of UTI and reference strains consist of shared genes, with 25-40% variation among strains (Klein & Hultgren, 2020). Additionally, the expression of these pili coding genes, rather than their mere presence, may be crucial for pathogenicity (Ljubetic et al., 2023). Bacterial gene expression may even vary under different culture conditions and in different hosts based on their genetic background (Hernández-Hernández et al., 2021). Thus, the ability of individual *E. coli* strains to infect and colonise the urinary tract likely depends on the interaction between urovirulence factors and individual host susceptibility.

While Ljubetic et al. (2023) mainly focused on UPEC strains, Wullt and Svanborg (2016) found that *E. coli* strains isolated from patients with AB sometimes seem to differ from the strains commonly found in UTI, mainly in terms of virulence. It seems that chromosomal copies of the virulence genes of UPEC are present in about 60% of AB-related *E. coli* (ABEC) strains. About 50% – 60% of ABEC strains seem to be UPEC strains that have reductively evolved. The ABEC genome was likely altered due to multiple deletions and mutations into smaller genomes with non-functional virulence genes. The researchers

hypothesise this reductive evolution was possibly an adaptation of the AB-strain to the human urinary tract. A well-known ABEC strain is *E. coli 83972*, which was first isolated from the urine of a young girl, and is well adapted to growth in human urine. The strain was previously shown to outcompete UPEC strains during *in vitro* growth. Moreover, several studies have reported a protective effect of *E. coli 83972* against urinary symptoms. In contrast to UPEC strains, *E. coli 83972* has a large *fim* deletion and several *PapG* point mutations (Wullt and Svanborg, 2016). The absence of these genes in *E. coli 83972*, along with the finding that the expression of these genes may be crucial for pathogenicity (Ljubetic et al., 2023), raises questions about their role and the mechanisms behind UTI pathogenesis.

The Role of E. coli and Host-Microbe Interactions in UTI Pathogenesis

Insights from mouse models, successfully applied to humans, show that uropathogenic *E. coli* initially adheres to urothelial umbrella cells using FimH adhesin at the tip of Type 1 pili, encoded by the *fim* gene (Klein & Hultgren, 2020; Hernández-Hernández et al., 2021). Similar to the PapG adhesin on P pili, Type 1 pili belong to the chaperone-usher pathway (CUP) found on the outer membrane of Gram-negative bacteria. To study the role of *E. coli* adhesion in human UTI pathogenesis, researchers created variants of *E. coli 83972*, which naturally has a large *fim* deletion and multiple *PapG* point mutations, by restoring *PapG* or *fim* expression (Wullt and Svanborg, 2016). These studies revealed that, while all patients remained asymptomatic, P-fimbriated strains established bacterial persistence more rapidly and triggered higher immune responses (mainly IL-6 and IL-8) compared to wild-type and Type 1-fimbriated strains, suggesting that P and Type 1 pili might activate different immune response pathways.

In particular, the immune response that was found to be activated by P-fibrilated strains, but Type 1-fibrilated strains, was Toll-like receptor 4 (Tlr4) pathway (Wullt & Svanborg, 2016). In this innate immune reaction, the bacteria's endotoxins signal through Tlr4 to induce interleukin-6 (IL-6) production, which stimulates the activation of Signal Transducer and Activator of Transcription 3 (Stat3) (Ching et al., 2018). This results in the production of antimicrobial peptides (AMPs) that perform bactericidal, bacteriostatic, and immunomodulatory activities. Notably, the AMPs hepcidin, RegIIIβ, and RegIIIγ play roles during UTIs. Hepcidin exhibits bacteriostatic activity against E. coli, while RegIIIβ and RegIIIγ have bactericidal effects against Gram-negative and Gram-positive bacteria, respectively. These innate host immune responses may vary individually due to Tlr4 promoter polymorphisms in different UTI-prone patients; promoter variants reducing Tlr4 expression are associated with AB (Wullt & Svanborg, 2016).

Additionally, bacterial properties may also contribute to immune response variability. UPEC strains in vitro were found to suppress IL-6 secretion by urothelial cells to evade the host immune response, and UPEC virulence inversely correlated with IL-6 production (Ching et al., 2018). After invading urothelial cells, UPEC multiplies intracellularly, forming biofilm-like intracellular bacterial communities (IBCs), which are associated with increased fitness and antimicrobial resistance (Klein & Hultgren, 2020). This IBC formation likely helps UPEC evade Tlr4-mediated immune responses. However, Ching et al. (2018) found that *in vivo* UPEC likely does induce IL-6 excretion, triggering the Tlr4 immune pathway.

Host Response Beyond Defense – Unveiling The Diagnostic Potential of Biomarkers

Because in clinical practice UTI symptoms can be difficult to interpret in older patients, due to cognitive impairment, the biomarkers that are excreted in host-microbe immune responses

are increasingly used for the diagnosis of UTI (Kjölvmark et al. 2016; Martino & Novara, 2022). For example, IL-6, which is excreted after activation of the Tlr4 immune pathway by UPEC, is commonly used as a biomarker in UTI diagnosis. Studies have consistently shown significantly elevated urinary levels of IL-6 in UTIs, compared to culture-negative controls and asymptomatic bacteriuria (Kjölvmark et al. 2016). These relatively low urinary IL-6 levels in AB may partly explain the absence of inflammatory symptoms in this condition. However, Martino and Novara (2022) found considerable heterogeneity among studies investigating urine IL-6 levels as valid diagnostic tools. While some studies showed significant associations between elevated IL-6 levels and lower UTIs, pyelonephritis and even bacteremia, others found no significant urinary IL-6 concentration differences between UTI and AB. In contrast, serum IL-6 seemed to consistently rise during UTI. Martino and Novara (2022) hypothesise that urine IL-6 increases proportionally to the inflammatory status of the UTI, explaining the variation in different clinical settings.

Given this reported heterogeneity, Martino and Novara (2022) argue that combining multiple biomarkers might enhance diagnostic accuracy. Similar to IL-6, increased levels of azurocidin/heparin binding protein (HBP) have been observed in urine (U-HBP) during UTI (Kjölvmark et al., 2016). HBP is released after neutrophil activation and likely exhibits antibacterial effects through monocyte chemotaxis (Martino & Novara, 2022). Kjölvmark et al. (2016) found significantly higher U-HBP levels in patients with UTI than with AB or culture-negative controls. However, when looking at the concentration threshold for diagnosis (30 ng/mL), a large proportion of AB patients exceeded it, with values in the same range as UTI patients. This indicates that HBP is released both during symptomatic and asymptomatic states, suggesting it may be an unreliable biomarker for distinguishing between AB and UTI.

Bilsen et al. (2024) investigated the diagnostic accuracy of twelve common urine biomarkers that are associated with inflammation. Two of the twelve biomarkers were absent in the tested urine samples of elderly women. Among the remaining ten, eight biomarkers showed significant differences in concentrations between UTI cases and asymptomatic controls. In particular, IL-6, azurocidin (U-HBP), NGAL, TIMP-2, and CXCL-9 showed outstanding discriminative ability. A model combining different biomarkers (IL-6, xanthine oxidase, U-HBP, NGAL, TIMP-2, CXCL-9, and uromodulin) showed the highest diagnostic accuracy. However, this accuracy did not significantly differ from the most accurate univariate model, containing only U-HBP, a finding that somewhat contrasts with Kjölvmark et al. (2016) who found U-HBP to be an unreliable discriminator. As far as I know, Bilsen et al. (2024) did not look at the diagnostic threshold, which may explain their result. In addition, both IL-6 and U-HBP demonstrated high specificity (90% and 89%, respectively) and sensitivity (76% and 86%, respectively). These findings show that five urine biomarkers – in particular IL-6 and potentially U-HBP – are likely effective tools for distinguishing women with UTIs from asymptomatic women.

Summary of Findings – Host-Microbe Mechanisms May Explain Symptom Onset in UTI

The comparison of uropathogenic and nonpathogenic *Escherichia coli* strains reveals differences in virulence factors, particularly in their ability to express urovirulence genes that seem to impact UTI pathogenesis (Wullt and Svanborg, 2016). Despite sharing many core genes, AB strains often undergo reductive evolution, leading to non-functional virulence factors. Since virulence factors, such as *PapG*, seem to impact the Trl4 immune pathway, this might – at least partly – explain the absence of symptoms in this AB. Accordingly, diagnostic biomarkers like IL-6 and U-HBP have shown promise in distinguishing symptomatic UTIs

from AB, although their effectiveness can vary. The integration of these multi-biomarker approaches into the clinic might enhance diagnostic accuracy, especially in older patients with cognitive impairments where UTI symptoms can be harder to detect.

Exploring Biomarkers for Delirium and the Neuroinflammation Hypothesis

Delirium is influenced by multiple factors, and its underlying neurobiological mechanisms are complex and incompletely understood (Lozano-Vicario et al., 2023). Given the complexity of these processes, biomarkers are increasingly used for delirium diagnosis, in a similar way to UTI diagnosis. For example, there seems to be an association between biomarkers of neuroinflammation and delirium. Van Munster et al. (2011) conducted a post-mortem case-control study in which they compared brains of deceased patients with and without delirium. They found significantly higher markers of microglial and astrocyte activity in delirious patients, especially in the hippocampus and frontal cortex. Moreover, IL-6 immunoreactivity was significantly increased across all brain areas in patients with delirium, compared to non-delirious patients. This indicates a potential association between IL-6 and delirium in elderly individuals, and suggests that inflammatory mechanisms may be involved in the pathogenesis of delirium.

The “neuroinflammatory hypothesis” (NIH) of delirium suggests a connection between delirium and various infectious and inflammatory abnormalities, proposing that the central nervous system (CNS) and the peripheral immune system interact to coordinate the innate immune response (Maldonado, 2020). The degree of this cerebral inflammatory response to stress seems to vary with age, with evidence suggesting increased neuroinflammation in the elderly, potentially contributing to their higher susceptibility to delirium (Liu et al., 2013). The NIH posits that delirium represents the CNS manifestation of systemic disease, with

potential triggers including bodily trauma, peripheral infections, and surgical procedures (Maldonado, 2020). Inflammation induced by these triggers might initiate a proinflammatory pathway, leading to the suppression of anti-inflammatory markers and activation of tissue macrophages and blood monocytes (Lozano-Vicario et al., 2023). Consequently, inflammatory mediators like IL-6 – involved in both host immune response as well as adult neurogenesis – could permeate the blood-brain barrier (BBB), causing cerebral injury through microglial activation, ultimately resulting in brain dysfunction and delirium. However, this mechanism warrants further investigation.

To test the association between inflammation and delirium, in a mouse model Rashid et al. (2021) experimentally examined whether systemic IL-6 inhibition would mitigate delirium-like phenotypes in mice with and without UTI. Similar to human studies, plasma IL-6 levels were significantly elevated in UTI mice compared to non-UTI controls. Moreover, compared to non-UTI mice, mice with UTI showed significantly greater impairments in frontal and hippocampus-mediated behaviours (delirious-like symptoms). Treatment of UTI mice with systemic anti-IL-6 fully reversed these functional impairments, suggesting a possible role of IL-6 in the onset of delirium-like phenotypes. Although this study was an animal model, there are implications that IL-6 might play a role in the mitigation of delirium from UTI.

While the impact of UTI-induced IL-6 on delirium onset in humans remains understudied, extensive research has explored the association between surgery-induced IL-6 levels and the development of delirium. For example, Liu et al. (2013) explored the link between serum IL-6 levels and delirium occurrence following major noncardiac surgery. First, they found significantly elevated post-surgery serum IL-6 levels compared to their pre-surgery baseline

measurement, possibly due to the stress of the procedure. In addition, these high postoperative IL-6 levels were consistently correlated with increased delirium development, independent of confounding factors.

Furthermore, in a meta-analysis, Lozano-Vicario et al. (2023) showed a significant increase in serum biomarkers of CRP, TNF-α, and IL-6 in delirious postoperative patients. When only looking at IL-6 in patients who developed delirium, this increase remained significant. According to Kuswardhani and Sugi (2017), IL-6 is not only associated with the risk for developing delirium, but also seems to serve as a predictor of the severity of the condition, as IL-6 concentrations (pg/mL) showed a significant correlation with Memorial Delirium Assessment Scale (MDAS) scores. Higher levels of IL-6 were associated with higher MDAS scores. However, it is important to keep in mind that all this research involved different conditions from the scope of this study. Therefore, these results should be interpreted with great care. I can only wonder if the link between elevated IL-6 levels and postoperative delirium can be extended to UTI-induced IL-6.

Summary of Findings – Putative Association Between IL-6 and Delirium

Diverse studies highlight the association between IL-6 and delirium, from experimental animal models to human studies exploring surgery-induced inflammation. IL-6, a marker of neuroinflammation, seems to be consistently elevated delirium states, suggesting its potential role in the pathogenesis of delirium. The neuroinflammatory hypothesis states that systemic triggers like infection and surgery may induce cerebral injury, ultimately resulting in brain dysfunction and delirium, particularly the elderly. However, while IL-6 shows potential as a biomarker for delirium, given the research conditions – which all differ from UTI-induced IL-6 – great care should be taken when interpreting these results. Further research is crucial

to uncover the exact role and therapeutic implications of IL-6 in the development of delirium, especially when generalising these results to different clinical contexts.

Conclusion and Discussion

This study aimed to answer the question of how the urinary microbiome, mainly *E. coli*, and host-microbe interactions, particularly the interleukin-6 pathway, differed between patients with AB and UTI, and how these differences impact the occurrence of delirium. Research on microbiota in women with UTIs and AB revealed diverse urobiome compositions, challenging the idea of a singular asymptomatic urotype. Only looking at microbiota compositions or *E. coli* strain diversity seemed insufficient to differentiate between symptomatic and asymptomatic states. The uropathogenic virulence of *E. coli* strains might play a role through their interaction with the host innate immune response, particularly the Trl4 pathway that induces interleukin-6 expression. Biomarkers like IL-6 and U-HBP show potential for enhancing UTI diagnostic accuracy. Additionally, studies on IL-6 as a marker for delirium highlight its consistent elevation in neuroinflammatory conditions, suggesting a role in delirium pathogenesis across diverse clinical settings. However, applying findings from IL-6 research to UTI-induced scenarios requires cautious consideration due to varying research conditions and contexts. In conclusion, I speculate whether the onset of UTI-related delirium might potentially stem from neuroinflammation induced by IL-6 excreted from the urothelium through Trl4 pathways, triggered by virulence factors of uropathogens such as *E. coli*, and whether this might explain the difference in delirium-like phenotypes between UTI and AB.

Further research is needed to investigate the role of UTI-induced IL-6 in the onset of delirium; it would be interesting to research the link between IL-6 and delirium, while taking

the presence or absence of UTI into account. Given the high prevalence and adverse effects of both UTI and delirium, especially in the elderly population, it is highly relevant to uncover the mechanisms underlying these conditions, to improve diagnosis, prevalence and treatment. These insights are especially relevant as wrongfully diagnosed UTIs could cause antimicrobial over-treatment, which could have negative consequences, such as dysbiosis of the urinary microbiome resulting in recurrent UTI, as well as the development and spread of resistant microbial strains (Ipe et al., 2013), posing threats to both human and environmental health (Wang et al., 2023).

Limitations

There were a number of limitations to this study. First, given the close proximity of the urinary, reproductive, and gut tracts in the female anatomy, it is plausible that these microbiomes interact (Chai et al., 2024). The urinary and vaginal microbiota were reported to greatly overlap, while both seem to differ from the gastrointestinal tract microbiota. Uropathogenic *E. coli* likely stem from the gastrointestinal tract, while protective *Lactobacillus* may stem from the vaginal microbiome. However, the compositions and interactions between the microbes in these different ecological niches were not investigated in this study, which might mean that the picture of microbiota compositions presented here is incomplete. Although the gastrointestinal microbiome is dissimilar to the urinary microbiome, studies have suggested that altering the gut microbiome affects UTI risk. The vaginal microbiome, similar to the urinary microbiome, likely contributes to UTI pathogenesis. For example, studies have shown that low-dose intravaginal oestrogen could reduce UTI recurrence in post-menopausal women, with observed changes in both vaginal and urinary microbiomes. These interactions should be taken into account in further research.

Secondly, the majority of studies investigating biomarkers for delirium, focused on a limited range of inflammatory markers presumed to be involved in delirium pathophysiology, leaving potential for bias. Kalantar et al. (2018) adopted a more unbiased approach, and used whole RNA sequencing to identify gene expression patterns underlying delirium in the context of UTIs. They revealed that delirious patients with an UTI had significant activation of the interferon signalling pathway, upstream cytokines, and transcriptional regulators, as well as repression of integrin, actin cytoskeleton, paxillin, and glioma invasiveness signalling, compared to those without UTIs. When comparing all patients with an UTI to all patients without an UTI, interferon signalling was also significant, as expected with infection. In addition, the activation of the complement system was consistent across all delirious patients, irrespective of UTI. Moreover, while previous studies linked IL-6 and IL-8 pathways to delirium, Kalantar et al. (2018) found that IL-6 may in fact be more closely correlated with an increased inflammatory response than with delirium itself, especially after controlling for SIRS in the analysis when the association with IL-6 was not significant. This raises the question whether IL-8, which was found to be highest prior to delirium development, might be better explaining the correlation between UTI and delirium. In further research, it would be interesting to explore the role of interleukin-8 in delirium, UTI and asymptomatic bacteriuria.

Lastly, since the mechanisms behind the neuroinflammation hypothesis remain largely unknown, it would be very interesting to further investigate this. IL-6 is extensively studied as a potential marker of delirium, yet there has been a lot of heterogeneity among studies in demonstrating this association (Dunne et al., 2021). Some studies show elevated preoperative IL-6 levels preceding the onset delirium. However, not all studies adjusted for confounding variables, as independent factors like infection, cognitive dysfunction, and age were also

previously associated with IL-6. Adjusting for these factors may strongly influence significance of an IL-6 association with delirium. Moreover, the lack of attention for comorbidities, such as depression and/or pre-existing cognitive dysfunction/dementia, may add to the heterogeneity observed in IL-6 research. Given its associations with several factors, including age and inflammation, the use of IL-6 as a reliable marker for delirium remains challenging.

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