Enhancing a multi-purpose artificial urine for culture and gene expression studies of uropathogenic *Escherichia coli* strains

Patricia T. Rimbi[1], Nicky O'Boyle[2], Gillian R. Douce[1], Mariagrazia Pizza[3], Roberto Rosini[4], Andrew J. Roe[1*]

[1] School of Infection and Immunity, University of Glasgow, Glasgow, G12 8TA, United Kingdom

[2] School of Microbiology, University College Cork, National University of Ireland, Cork, T12 K8AF, Ireland

[3] Centre for Bacterial Resistance Biology, Imperial College London, London, SW7 2AZ, United Kingdom

[4] GSK Vaccines, Siena, Italy

*__Corresponding author:__ Professor Andrew Roe. Address: B214, Sir Graeme Davies Building, 120 University Place, Glasgow, G12 8TA, United Kingdom. Email: andrew.roe@glasgow.ac.uk

Running title: Enhanced artificial urine

Abstract

Aims: The main objective of this study was to modify a recently reported multi-purpose artificial urine (MP-AU) for culture and gene expression studies of uropathogenic *Escherichia coli* (UPEC) strains.

Methods and results: We used liquid chromatography mass spectrometry (LC-MS) to identify and adjust the metabolic profile of MP-AU closer to that of pooled human urine (PHU). Modification in this way facilitated growth of UPEC strains with growth rates similar to those obtained in PHU. Transcriptomic analysis of UPEC strains cultured in enhanced artificial urine (enhanced AU) and PHU showed that the gene expression profiles are similar, with less than 7% of genes differentially expressed between the two conditions.

Conclusions: Enhancing an MP-AU with metabolites identified in PHU allows the enhanced AU to be used as a substitute for the culture and *in vitro* gene expression studies of UPEC strains.

Impact statement: The data support this enhanced AU as a robust medium to study aspects of UPEC physiology *in vitro.*

Keywords: artificial urine, uropathogen, *E. coli,* transcriptome

Materials and methods

Bacterial growth conditions

All strains (Table 1) were cultured in lysogeny broth (LB) medium overnight at 37°C, 200 rpm. UPEC strains CFT073 and UTI89 were isolated from the blood of patients with pyelonephritis (Mobley *et al*., 1990) and cystitis (Hultgren *et al.*, 1986), respectively. The EC0 and EC1 bacteremia isolates were a gift from Professor Thomas Evans' laboratory and were isolated from the blood of hospitalized patients as described elsewhere (Goswami *et al*., 2018). For growth assays and growth for transcriptomic analysis, overnight cultures were diluted in culture medium (pooled human urine (PHU), MP-AU or enhanced AU), pre-warmed to 37°C, to an OD600 of 0.06. Cultures were incubated at 37°C, 200 rpm for 8 h growth assays, or until the desired OD600 was reached for RNA extraction and subsequent transcriptomic analysis. For growth assays, the optical density of 1 mL neat samples was measured at hourly intervals.

Pooled human urine (PHU)

The first morning urine from five healthy volunteers was pooled and filtered using a 0.22 µm vacuum filter (Sigma). Here, healthy describes the absence of infection. Volunteers were not asked to disclose other factors such as gender, diet, medication, or menstruation status. PHU was stored at 4°C for use within four days or aliquoted and stored at -20°C for later use. Frozen aliquots were thawed at 4°C overnight and brought to room temperature before use.

Multi-purpose artificial urine (MP-AU)

MP-AU was adapted from the literature (Sarigul *et al*., 2019). All components except uric acid were prepared as 1 mol L^{-1} or 0.5 mol L^{-1} stock solutions in distilled deionized water and filter sterilized (Table 2). Uric acid was added in powder form as it is not easily dissolved in

water. 1 mol L^{-1} urea was prepared on the day of use. In general, MP-AU without urea was prepared the day before use.

Enhanced AU

Supplement solutions were prepared in water and filter sterilized, with the exception of L-tyrosine, which was mixed in water but not filtered, due to its insolubility in water (Table S1). Supplements were added to MP-AU, with urea, at a final concentration of 5 mmol L^{-1} on the day of use.

Transcriptomic analysis

To directly compare the transcriptomic profile of UPEC strain CFT073 in MP-AU and PHU, CFT073 was cultured in triplicate in M9 minimal medium for 4 h. Cells were pelleted down by centrifugation at 2907 × *g* for 5 mins and resuspended in MP-AU or PHU. After 1 h culture in MP-AU or PHU, samples were taken and normalized to OD600 ~0.6. To compare the transcriptomic profile of CFT073 and UTI89 in enhanced AU and PHU, UPEC strains CFT073 and UTI89 were cultured in triplicate in enhanced AU or PHU until OD600 of >0.4 (mid-exponential phase). Cultures were normalized to OD600 1.0. For all transcriptomic analysis experiments, cells were centrifuged at 10000 × *g* for 1 min and the pellet was resuspended in RNAprotect Bacteria Reagent (Qiagen). The cells were incubated at room temperature for 10 mins and centrifuged as before. The supernatant was aspirated and the pellet was stored at -20°C until RNA extraction within four days. Total RNA was isolated as per manufacturer's instructions using the PureLink RNA Mini Kit (Invitrogen). DNA was removed using TURBO DNase (Invitrogen). RNA was further extracted using phenol:chloroform:isoamyl alcohol (Invitrogen) and was precipitated using 100% ethanol (Fisher Scientific). The concentration and purity of the extracted RNA was quantified using a spectrophotometer (DeNovix). The integrity of RNA was assessed by running gel

electrophoresis to examine 23S and 16S rRNA bands. The presence of residual or contaminant DNA was determined by polymerase chain reaction to amplify *fimA*. Samples were sent to Glasgow Polyomics for ribosomal depletion using a QIAseq FastSelect 5S/16S/23S Kit (Qiagen). A cDNA library was prepared using a TruSeq Stranded Total RNA Library Prep Gold kit (Illumina). RNA sequencing was performed to a depth of 10 million 75 bp or 100 bp single end reads using a NextSeq500 or NextSeq2000 sequencing system (Illumina). Data were analyzed using CLC Genomics Workbench version 21.0.5 (Qiagen). Reads were mapped to the CFT073 and UTI89 reference genomes (NCBI accession numbers NC_004431.1 and NC_007946.1, respectively) using default parameters. Differential expression was calculated using the Differential Expression for RNA-seq tool in CLC Genomics Workbench. Genes were taken as significantly differentially expressed with a fold change of ≥1.5 or ≤-1.5 and a false-discovery rate (FDR)-corrected *P* value of ≤0.05. Volcano plots were generated using Prism version 9.5.1 (GraphPad). Functional categories of the differentially expressed genes (DEGs) were assigned using the UniProtKB gene ontology (GO) biological process annotation in the first instance, or the GO molecular function where the GO biological process annotation was not available (Bateman *et al*., 2021). Raw sequencing data have been deposited in the European Nucleotide Archive under the project accession number PRJEB55151.

Metabolomics analysis

Metabolites were extracted from PHU or MP-AU using an extraction solvent of chloroform:methanol:water in a 1:3:1 v/v/v ratio. The components for the extraction solvent were all high-performance liquid chromatography (HPLC) grade and supplied by Fisher Scientific. Extracted metabolites were analyzed by LC-MS by Glasgow Polyomics using a Dionex UltiMate 3000 RSLC system and an Q Exactive Orbitrap (Thermo Fisher Scientific).

Data were analyzed using the Glasgow Polyomics Integrated Metabolomics Pipeline (PiMP) software (Gloaguen *et al*., 2017). Statistical analysis, the Venn diagram and bar charts were generated using Prism version 9.5.1 (GraphPad).

Iron quantification

ICP-MS of acidified PHU, MP-AU and enhanced AU containing final concentration 1 mmol L^{-1} iron (II) sulfate heptahydrate (Sigma-Aldrich) was performed by Dr Lorna Eades at the University of Edinburgh using the 8900 Triple Quadrupole ICP-MS instrument (Agilent). The samples were acidified with trace metal grade nitric acid (Fisher Scientific) and analytical reagent grade hydrochloric acid (Fisher Scientific).

Introduction

Uropathogenic *Escherichia coli* (UPEC) strains are the leading cause of urinary tract infections, which have associated morbidities, mortality, and economic burden. UPEC strains disseminate from the gastrointestinal tract and colonize the periurethral area, from which they can invade and colonize the urinary tract. Hence, urine is arguably the most appropriate growth medium to culture UPEC *in vitro* and for studying gene expression, however there are many hurdles associated with the use of urine. These challenges include variations in composition (Sarigul *et al*., 2019), the need for donors, and the stability of urine over time. Therefore, to better understand UPEC physiology *in vitro* we need a growth medium that is physiologically relevant and highly reproducible. Artificial urine has been used for different purposes in the literature, for example to examine the expression of virulence genes in UPEC strain CFT073 during cell adhesion (Sarshar *et al*., 2022). Certain

formulations have components that are not normally found in healthy human urine, such as bicarbonate (Brooks and Keevil, 1997) and therefore are not the most accurate representation of the chemical profile of healthy human urine.

Analytical techniques such as mass spectrometry, nuclear magnetic resonance, and liquid chromatography mass spectrometry (LC-MS) have been combined to demonstrate that human urine is a complex medium, with thousands of components (Sarigul *et al.*, 2019). Based on this analysis, a recent publication formulated an artificial urine that tried to replicate healthy human urine (Sarigul *et al.*, 2019). The authors compared their multi-purpose artificial urine (MP-AU) to urine collected from 28 individuals by using attenuated total reflection-Fourier transform infrared spectroscopy (Sarigul *et al.*, 2019). The 13 components of MP-AU were shown to be at more physiologically relevant levels compared to the two other artificial urine formulations (Brooks and Keevil, 1997; Chutipongtanate and Thongboonkerd, 2010; Sarigul *et al.*, 2019). In this study, we aimed to determine whether Sarigul's MP-AU could be used as an alternative to human urine in *in vitro* studies of UPEC strains. We have taken the pre-existing MP-AU and enhanced it with specific metabolites present in human urine to support the growth of UPEC strains *in vitro*.

Results

MP-AU does not support the growth of UPEC strains in vitro

To determine whether MP-AU is suitable for the culture of UPEC strains *in vitro*, we performed growth assays of prototypic UPEC strains CFT073 and UTI89 in this medium. The growth rates of these strains in MP-AU were compared to those obtained for growth in PHU. Sarigul's MP-AU did not support the optimal growth of UPEC strains CFT073 or UTI89 (Fig. 1a). The optical density of CFT073 and UTI89 in MP-AU decreased in the first hour of growth, suggesting that there was some lysis of the bacterial cells. Sarigul *et al.* suggests adding peptone and yeast extract to MP-AU for bacterial growth studies (Sarigul *et al*., 2019), however, these were omitted here due to their absence in healthy human urine and subsequent unsuitability for gene expression studies against PHU. To allow for transcriptomic analysis of CFT073 in MP-AU or PHU, bacteria were first cultured in M9 minimal medium before being resuspended in either MP-AU or PHU (Fig. 1b). There were vast differences in the transcriptomic profile following 1 h exposure to MP-AU or PHU, with 3284 differentially expressed genes in MP-AU compared to PHU (Fig. 1c). This corresponds to a shift in 66.9% of the CFT073 coding sequences. 1607 of these genes were upregulated, while 1677 were downregulated. The ten most significantly upregulated genes include those involved in arginine catabolism and carboxylic acid catabolism (*astABCDE, fadB*), as well as *argT*, which encodes a lysine/arginine/ornithine ABC transporter substrate-binding protein, *C_RS19130* (previously *c4034* or *yhdW*), and *yhdX* and *ytfT*, which encode ABC transporter permeases. The ten most significantly downregulated genes include *glnA*, *cyuA*, *gcvT*, *gcvH*, *lacY*, *rplC*, *lacA*, *rplD*, *sitC,* and *chuY*.

Metabolomic analysis of PHU

Sarigul *et al.* demonstrated that the levels of components in their MP-AU are similar to healthy human urine (Sarigul *et al*., 2019). However, as the MP-AU did not facilitate the growth of UPEC strains *in vitro*, we hypothesized that there were components in healthy human urine that could be used to supplement MP-AU and support the growth of UPEC strains. LC-MS analysis was used to identify and compare the relative abundance of metabolites extracted from MP-AU and healthy human urine samples. Untargeted LC-MS identified 68 metabolites across all samples and putatively annotated a further 5703 metabolites, however as many of these shared a peak ID and could not be further distinguished by LC-MS, there were 65 true identified metabolites and 1289 true annotated metabolites (Table S2). 53 identified metabolites were present in both human urine and MP-AU, while 10 metabolites were only present in human urine, and two were only present in MP-AU (Fig. 2a). Of the 53 identified metabolites in common between the two media, only orthophosphate and nicotinamide were more abundant in MP-AU compared to human urine. The difference was statistically significant for orthophosphate ($P \leq 0.05$) but not for nicotinamide. The remaining 51 metabolites were more abundant in human urine. There was no statistical significance in the different average abundances of creatinine or oxalate between human urine and MP-AU. The increased abundance in human urine compared to MP-AU was statistically significant ($P \leq 0.05$) for six metabolites, highly significant ($P \leq 0.01$) for 26 metabolites and very highly significant ($P \leq 0.001$) for 18 metabolites. We chose to focus only on the identified metabolites, rather than annotated metabolites. The ratio of metabolites in MP-AU relative to human urine is shown in Fig. 2b.

Modifying MP-AU

Metabolites were added to MP-AU based on their relative abundance in PHU samples over MP-AU, or studies published in the literature. Where LC-MS identified metabolites that were synthesized as by-products of another metabolite's degradation or synthesis, the by-product was not included in the enhanced AU formulation. For example, LC-MS analysis showed that L-kynurenine is present in PHU. L-kynurenine is a by-product of tryptophan catabolism (van der Leek *et al*., 2017) and therefore L-tryptophan was used as a supplement instead of L-kynurenine. The majority of amino acids added were L-enantiomers, due to their physiological relevance in protein synthesis (Suzuki *et al*., 2021). There have also been reports of endogenous D-amino acids eukaryotes, such as D-glutamate (Katane *et al*., 2020), D-cysteine (Roychaudhuri and Snyder, 2022) and D-serine (Anfora *et al*., 2008; Cava *et al*., 2011), hence these were also added. Although it was not in our LC-MS dataset, D-sorbitol is listed on the Urine Metabolome Database with detected quantities in the urine of adults at 3.5-9.9 µmol $mmol^{-1}$ creatinine (Bouatra *et al*., 2013) and was included as it is suggested to be an important carbon source for UPEC strains in the urinary tract (Mann *et al*., 2017). The components were added to MP-AU at a final concentration of 0.5 mol L^{-1}, and this was sufficient to support the growth of CFT073 and UTI89 (Fig. 3a). Both strains achieved the same OD600 after 8 h in enhanced AU and the OD600 of CFT073 was the same as that in PHU. Later experiments examined the viability of CFT073 and UTI89 when grown in enhanced AU compared to LB medium, M9 minimal medium, MP-AU, and MP-AU supplemented with peptone and yeast extract (Fig. S1a). The average numbers of CFT073 and UTI89, measured as colony forming units (CFU) mL^{-1}, do not increase during 8 h in MP-AU (Fig. S1b). Iron is an important metal for the survival of UPEC strains in the urinary tract, with UPEC strains encoding a number of proteins that acquire, scavenge, increase the

uptake of iron from urine. Sarigul's MP-AU does not include iron but Brooks and Keevil's artificial urine formulation contains small amounts of iron (II) sulfate (Brooks and Keevil, 1997; Sarigul *et al*., 2019). To assess iron levels in PHU, we used inductively coupled plasma mass spectrometry (ICP-MS) to compare iron levels in PHU with enhanced AU supplemented with iron (II) sulfate at 1 mmol L^{-1}. ICP-MS revealed that the levels of iron in PHU was negligible, while the levels in our enhanced AU were sixty-fold higher (Fig. S2). As a result, iron (II) sulfate was omitted from subsequent formulations.

Transcriptomic analysis in EnAU

Transcriptomics provides a global insight into how the bacterial cell responds to its environment. Subtle changes in the nutrient content of the growth medium are reflected in distinct patterns of gene expression. Moreover, if enhanced AU is to be used to study UPEC *in vitro*, it is important to understand if key virulence genes are expressed to the same extent. To determine whether this was the case, transcriptomic analysis was performed on RNA extracted from CFT073 and UTI89 cultured in enhanced AU or PHU. Samples for RNA extraction were taken during the mid-exponential phase to better capture the transcriptomic profile during growth in either condition. In contrast to the original MP-AU formulation where thousands of genes were affected, in this enhanced AU, 6.9% of the CFT073 coding sequences (340 genes) and 3.8% of the UTI89 coding sequences (189 genes) were significantly differentially expressed compared to PHU. There were 151 significant upregulated genes in CFT073 in enhanced AU compared to PHU, and 189 significant downregulated genes (Fig. 3b). In UTI89, there were 91 upregulated and 98 downregulated genes, including the hypothetical protein-encoding gene *UTI89_RS29285* carried on the UTI89 plasmid (Fig. 3c). The 50 most significant differentially expressed genes in CFT073 and UTI89 are shown in Tables S3 and S4, respectively.

Expression of virulence factors in EnAU

Promisingly, a number of genes associated with virulence were similarly expressed (not significantly up or downregulated) in CFT073 or UTI89 in EnAU and PHU. We examined genes known to be associated with virulence, such as those encoding toxins, adhesins, siderophores and iron uptake systems.

Almost half of all UPEC strains secrete the pore-forming toxin hemolysin (Nhu *et al*., 2019). Hemolysin is encoded for by *hlyA*, which is similarly expressed in CFT073 and UTI89 in EnAU and PHU. Another toxin, cytotoxic necrotizing factor 1 or CNF1, is an important toxin expressed by some UPEC strains, including UTI89, but it is not encoded for by the CFT073 genome (Smith *et al*., 2008). Here, there was a similar level of expression of *cnf1* in UTI89 in both EnAU and PHU. Unlike UTI89, CFT073 produces the serine protease autotransporter toxins Pic and Sat. These genes (*pic* and *sat,* respectively) are similarly expressed in both EnAU and in PHU. The colibactin genotoxin is expressed during UTI in humans, and is associated with DNA damage in the urothelial cells in a mouse model of UTI (Chagneau *et al*., 2021). 17 CFT073 *clb* genes and 15 UTI89 *clb* genes, which encode colibactin, were similarly expressed in EnAU and PHU.

Adherence to the uroepithelium through the expression of adhesins is a critical step in uropathogenesis. Six genes in the type 1 fimbriae operon (*fimC*, *fimD, fimF, fimG, fimH* and *fimI*) were significantly upregulated in CFT073 in EnAU compared to in PHU. In contrast, only three type 1 fimbriae genes (*fimF, fimG* and *fimH*) in UTI89 were significantly upregulated in EnAU compared to PHU. The adhesin FimH was significantly upregulated, 3-fold in CFT073 and 3.5-fold in UTI89 when cultured in EnAU compared with PHU.

Perhaps this is unsurprising, as Greene *et al.* found that culture in filtered human urine induces the phase OFF phase of UTI89 *fimS* (Greene *et al*., 2015). While *fim* operon

expression is upregulated in EnAU compared to PHU, this is still an advantage for the further study of type 1 fimbriae expression and function under different conditions. The pyelonephritis-associated pili (pap) or P fimbriae, thought to be associated with adhesion in the kidney, are similarly expressed in CFT073 in EnAU and PHU. Of all the *pap* operon genes in CFT073, only *papH_1* is significantly differentially expressed in EnAU relative to PHU (2.6-fold increase). *CsgBA* are the minor and major subunits of the curli fibers associated with adhesion and are similarly expressed in CFT073 in both EnAU and in PHU, however c*sgC* is significantly downregulated. In UTI89, the *csgBAC* operon is similarly expressed in both the media. The S and F1C fimbriae are encoded for by the *sfa* and *foc* genes, which are also similarly expressed by CFT073 in EnAU and in PHU.

Survival and growth in the urinary tract requires the uptake of iron from a nutritionally-poor environment via iron-uptake systems and siderophores that are secreted by UPEC strains (Frick-Cheng *et al*., 2022). The *sitABCD* genes, which encode an iron/manganese ABC transporter, are similarly expressed in CFT073 and UTI89 in EnAU and PHU, as well as the genes encoding TonB-dependent heme receptors ChuA (*chuA*) and Hma (*C_RS11765, UTI89_C2234*).

Siderophores aerobactin, enterobactin, salmochelin and yersiniabactin are encoded by UPEC strains to bind to iron sequestered by the host, for example in hemoglobin (Frick-Cheng *et al*., 2022). Aerobactin genes *iucABCD* are similarly expressed in CFT073 in EnAU and in PHU, so too is the gene encoding its receptor *iutA*. In CFT073 and UTI89, enterobactin genes *entCEBAH*, *entD* and *entS*, *fepA* (encoding the enterobactin receptor), salmochelin genes *iroBCDE* and *iroN* (encoding the salmochelin receptor) were similarly expressed in EnAU and PHU. Of the yersiniabactin genes, only *ybtX*, which encodes the yersiniabactin-associated zinc MSF transporter YbtX, is significantly upregulated in CFT073 in EnAU

compared to in PHU. The other yersiniabactin genes are similarly expressed. None of the UTI89 *ybt* genes were significantly differentially expressed in EnAU compared to PHU.

Suitability of enhanced AU to study other UPEC strains

While CFT073 and UTI89 are prototypic model strains for UPEC study, there is great diversity in the range of isolates associated with UTIs. A versatile artificial urine must be robust enough to support the growth of a variety of strains. To determine the usefulness of our enhanced AU, growth assays were performed on isolates taken from patients who had been catheterized, and subsequently developed blood infections. The isolates were varied in serotype but all from the B2 phylogroup that is strongly associated with UPEC infections. Enhanced AU supported the growth of these clinical isolates demonstrating that it is useful in supporting the growth of other UPEC strains, not just the prototypical laboratory strains (Fig. 4).

Discussion

Advances in formulation of artificial urine have meant that there are a number of different formulations proposed in the literature or available commercially. There are many examples of artificial urine facilitating research on UPEC in the past, with a number of studies using the Brooks and Keevil artificial urine medium for different purposes. Examples include the studying UPEC strains and other urinary pathogens (Juarez and Galván, 2018; Psotta *et al*., 2023), studying ethanolamine metabolism during UPEC culture (Dadswell *et al*., 2019), examining the effect of probiotic Lactobacillus strains on biofilm formation (Carvalho *et al*., 2021), and in investigating the use of metabolites to boost the effect of the broad-spectrum antimicrobial nitrofurantoin (Aedo *et al.*, 2021). In addition to the Brooks and Keevil artificial urine medium, commercially available artificial urine (LCTech GmbH) has been used in the to study virulence mechanisms in CFT073 cultured in LB medium compared to artificial urine (Sarshar et al., 2022). The use of artificial urine in existing studies is evidence of its importance. Some artificial urine formulations used to culture bacterial strains *in vitro* contain components not normally present in healthy human urine. Here, we attempted to modify MP-AU to better resemble PHU in terms of the ability to support growth of UPEC strains and to induce a similar transcriptomic profile during growth. MP-AU described in the literature was not suitable for the culture of UPEC strains CFT073 and UTI89 when peptone and yeast extract were omitted. Peptone and yeast extract used in some artificial urine formulations act as sources of amino acids, short peptides, trace elements and nucleic acids that may be normally present in human urine (Brooks and Keevil, 1997). As the quantities of components in commercially available reagents such as peptone and yeast extract are not always available, our approach of supplementing MP-AU with specific quantities of amino acids and metabolites aims to generate a more defined and reproducible medium. The

complexity of urine suggests that there are certain factors in the urine that allow for growth and survival of those UPEC strains *in vitro*. As revealed by LC-MS, there were 65 identified metabolites that varied in abundance between PHU and MP-AU. Unfortunately, LC-MS can indicate relative abundances but cannot be used to precisely quantify the metabolites present in a sample. Additionally, LC-MS cannot identify all of the metabolites present in a sample. LC-MS was used here only to identify metabolites in PHU that could be used to supplement MP-AU. The supplementation of MP-AU with amino acids and other metabolites identified in PHU to generate the enhanced AU provides sources of carbon and nitrogen that are required for the growth of UPEC strains. Nutritional selection and UPEC metabolism is reviewed elsewhere (Chan and Lewis, 2022). It is worth noting that these studies were all performed on filtered urine. Filter sterilization may result in the loss of components that contribute to bacterial growth and/or gene expression *in vivo*. Furthermore, *in vivo*, there is constant voiding and replenishing of urine in the bladder, whereas in our *in vitro* experiments, the medium is not replenished. Nevertheless, similar levels of transcription between the two media suggests that EnAU does in fact mimic conditions of PHU and we have generated an artificial urine that is consistent for growth of multiple UPEC strains for studying expression of virulence genes *in vitro*.

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Conflict of interest

No conflict of interest declared.

Data availability

Raw sequencing data have been deposited in the European Nucleotide Archive under the project accession number PRJEB55151.

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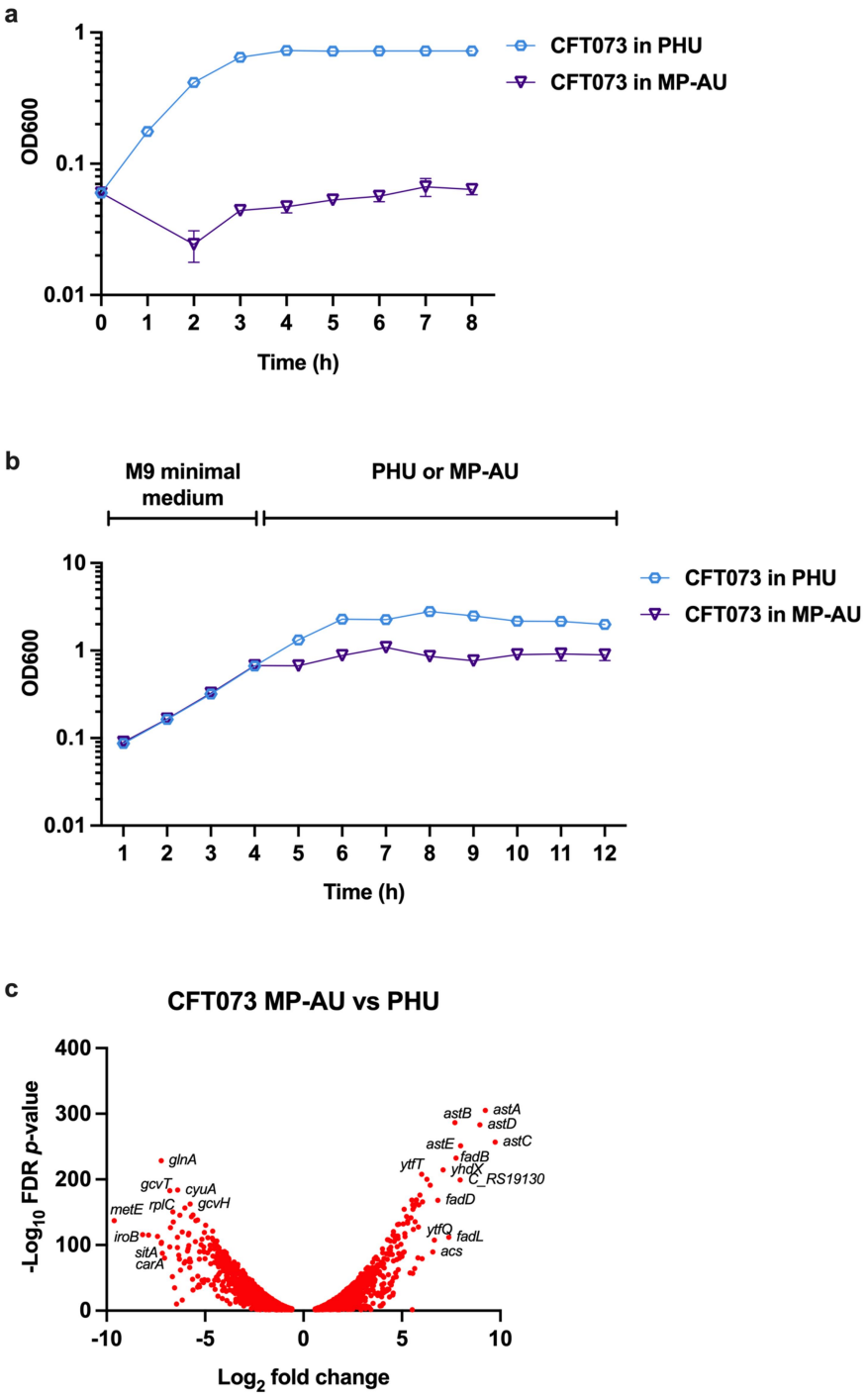
Author contributions

Patricia Rimbi (Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing) Nicky O'Boyle (Investigation, Methodology, Writing – review & editing), Gillian R. Douce (Conceptualization, Funding acquisition, Methodology, Project administration Supervision), Mariagrazia Pizza (Funding acquisition, Supervision), Roberto Rosini (Supervision), and Andrew J. Roe (Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing).



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442 **Fig 1. Assessing the suitability of multi-purpose artificial urine for *in vitro* culture of**
443 **uropathogenic *E. coli* strains. a)** Growth profile of wild-type CFT073 when overnight
444 cultures were inoculated into pre-warmed filtered pooled human urine (PHU, blue

hexagons) or multi-purpose artificial urine (MP-AU, purple triangles). Error bars represent the standard error of the mean of three biological replicates. **b) Growth profile of wild-type** CFT073 when overnight cultures were inoculated into pre-warmed M9 minimal medium and after 4 h, cultures were pelleted by centrifugation and resuspended in PHU (blue hexagons) or MP-AU (purple triangles). Error bars represent the standard error of the mean of three biological replicates. **c)** Transcriptomic profile of wild-type CFT073 when grown in PHU and MP-AU. Volcano plot represents significant differentially expressed genes in MP-AU relative to PHU. FDR $P \leq 0.05$, fold change ≥ 1.5 or ≤ -1.5.

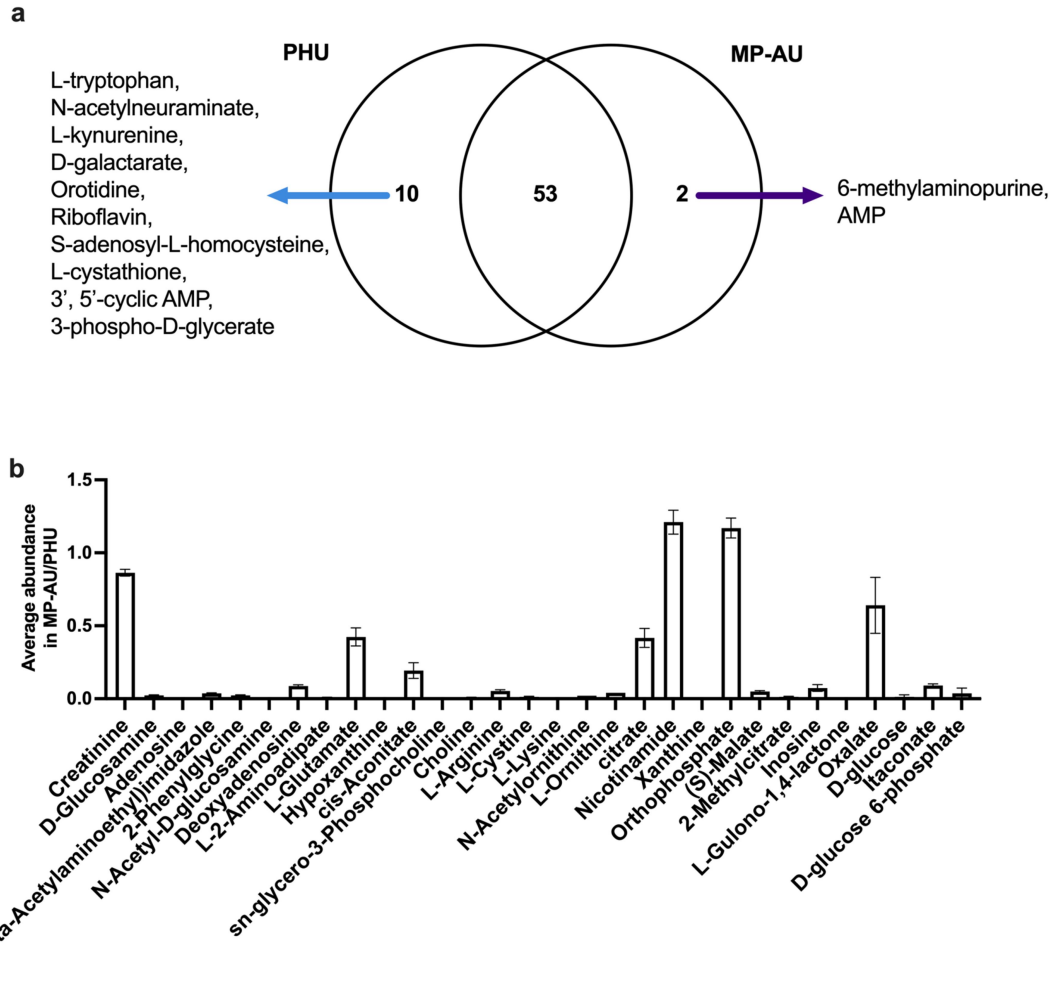

Fig 2. Determining the metabolites present in pooled human urine and multi-purpose artificial urine by liquid chromatography mass spectrometry. a) Venn diagram displaying the distribution of identified metabolites between pooled human urine (PHU) and multi-purpose artificial urine (MP-AU). **b**) The ratio of average abundance of identified metabolites in MP-AU relative to PHU. Metabolites with a ratio of average abundance of zero are excluded. Error bars represent the standard error of the mean of three technical replicates.

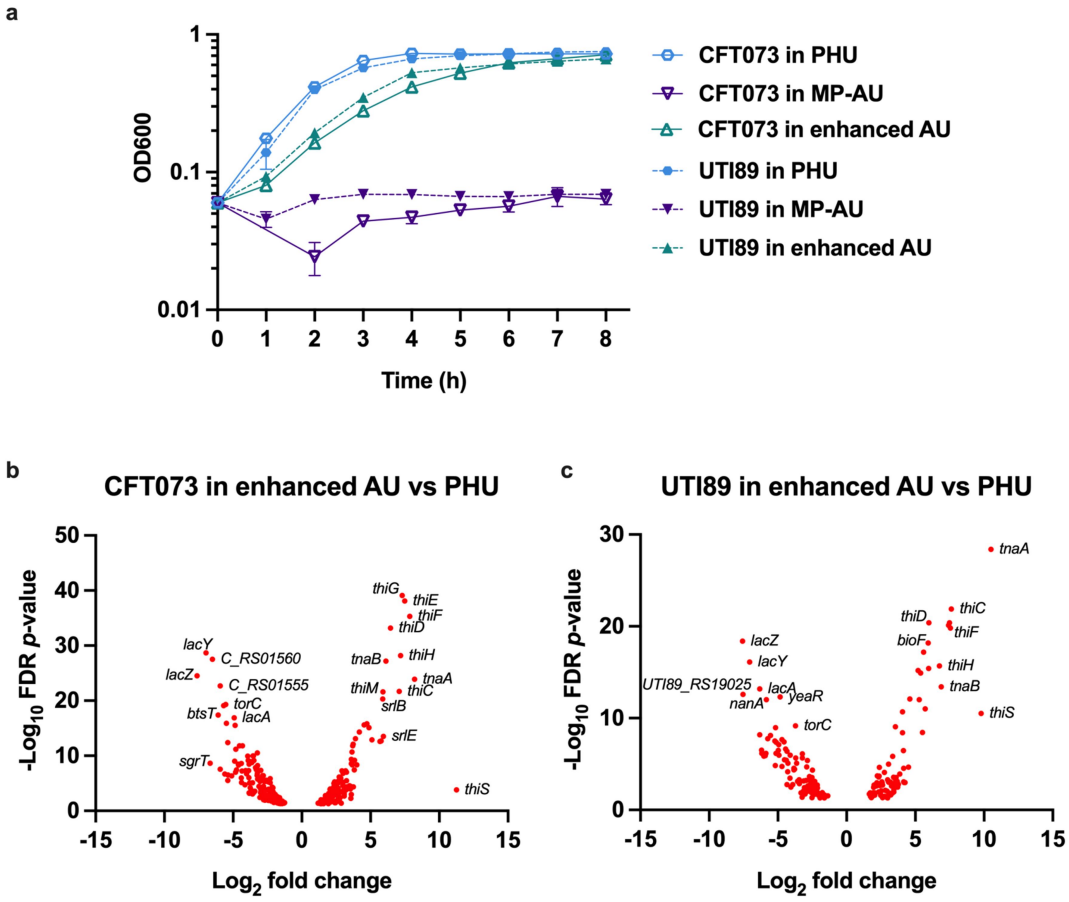

Fig 3. Growth and transcriptomic profiles of model UPEC strains in enhanced AU vs PHU and MP-AU. **a)** Growth profile of wild-type CFT073 and UTI89 when grown in pooled human urine (PHU, blue hexagons), multi-purpose artificial urine (MP-AU, purple triangles) or enhanced artificial urine (enhanced AU, green triangles). Error bars represent the standard error of the mean of three biological replicates. **b and c)** Transcriptomic profile of CFT073 and UTI89 when grown in enhanced AU or PHU. Volcano plots represent significant differentially expressed genes in enhanced AU relative to PHU. FDR $P \leq 0.05$, fold change ≥ 1.5 or ≤ -1.5. Three biological replicates were performed.

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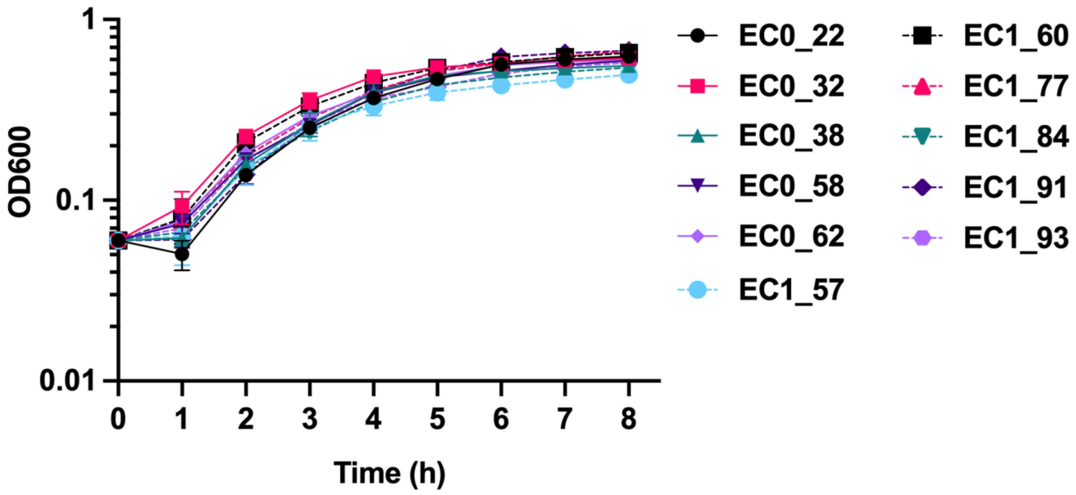

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478 **Fig 4. Growth of non-model UPEC strains in enhanced AU**. Growth profile of bacteremia

479 isolates when grown in enhanced artificial urine. Error bars represent the standard error of

480 the mean of three biological replicates.

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483 **Table 1.** Strains used in this study.

Name	Description	Phylogroup	Serotype	Reference
CFT073	Uropathogenic *E. coli* strain CFT073	B2	O6:H1	(Mobley *et al*., 1990)
UTI89	Uropathogenic *E. coli* strain UTI89		O18:H7	(Hultgren *et al*., 1986)
EC0_22	*E. coli* isolate EC0_22		O6:H1	(Goswami *et al*., 2018)
EC0_32	*E. coli* isolate EC0_32		O1:H7	
EC0_38	*E. coli* isolate EC0_38		O25:H4	
EC0_58	*E. coli* isolate EC0_58		O18:H7	
EC0_62	*E. coli* isolate EC0_62		O2:H7	
EC1_57	*E. coli* isolate EC1_57		O18:H31	
EC1_60	*E. coli* isolate EC1_60		O22:H1	
EC1_77	*E. coli* isolate EC1_77		O16:H5	
EC1_84	*E. coli* isolate EC1_84		O4:H1	
EC1_91	*E. coli* isolate EC1_91		O18:H1	
EC1_93	*E. coli* isolate EC1_93		O6:H31	

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488 **Table 2.** MP-AU components. The desired volume was made up with distilled deionized

489 water.

Component	Concentration in MP-AU	Supplier
Sodium sulfate decahydrate	11.965 mmol L^{-1}	Sigma-Aldrich
Trisodium citrate	2.450 mmol L^{-1}	Sigma-Aldrich
Creatinine	7.791 mmol L^{-1}	Sigma-Aldrich
Urea	249.750 mmol L^{-1}	Fisher Scientific
Uric acid	0.25 g L^{-1}	Sigma-Aldrich
Potassium chloride	30.953 mmol L^{-1}	Sigma-Aldrich
Sodium chloride	30.953 mmol L^{-1}	Sigma-Aldrich
Calcium chloride	1.663 mmol L^{-1}	Sigma-Aldrich
Ammonium chloride	23.667 mmol L^{-1}	Sigma-Aldrich
Potassium oxalate monohydrate	0.190 mmol L^{-1}	Sigma-Aldrich
Magnesium sulfate heptahydrate	4.389 mmol L^{-1}	Sigma-Aldrich
Sodium phosphate monobasic dihydrate	18.667 mmol L^{-1}	Sigma-Aldrich
Sodium phosphate dibasic dihydrate	4.667 mmol L^{-1}	Sigma-Aldrich

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