

Evaluation of phenotypic and genotypic methods for the identification and characterisation of bacterial isolates recovered from catheter-associated urinary tract infections

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Running title: Characterisation of CAUTI isolates

Abstract

Aims Urinary tract infections are the most common hospital-acquired infection, 80% are associated with catheterisation. Diagnostic methods may influence the reported identities of these pathogens, and phenotypic testing under laboratory conditions may not reflect infection phenotypes. This study aimed to evaluate the efficacy of diagnostic methods and whether medium composition alters phenotypes by characterizing catheter-associated urinary tract infection isolates from a UK hospital.

Methods and Results We compared five bacterial identification methods, including biochemical testing, MALDI biotyping, and genome sequencing, finding differences in genus or species level identifications. Antibiotic susceptibility comparisons between phenotypic assays and genomic predictions showed high agreement only in multidrug-resistant strains. To determine whether growth

rate and biofilm formation were affected by medium composition, strains were grown in both planktonic and biofilm states. Low planktonic growth and significant biofilm formation were observed in artificial urine compared to rich laboratory media, underscoring the importance of assay design.

Conclusions This study highlights the risks of relying on a single diagnostic method for species identification, advocating for whole-genome sequencing for accuracy. It emphasizes the continued importance of phenotypic methods in understanding antibiotic resistance in clinical settings and the need for characterization conditions that mirror those encountered by pathogens in the body.

Impact statement

Not all methodologies are able to correctly identify catheter associated urinary tract infections to species level, which has significant impact on national and global reporting. In the present study, we identified that only whole genome sequencing was able to correctly identify strains to species level.

There is an unmet clinical need detailed in the 2019-2024 UK AMR National Action Plan to reduce urinary tract infections. However, the failing antimicrobial pipeline currently produces insufficient novel antibiotics that will treat pandrug-resistant strains. A major contributing factor to development of new antimicrobials is creating fit for purpose testing methodologies; data presented herein outlines the importance of choosing the correct conditions for characterisation of pathogens to inform drug development in terms of resistances and species identification to ensure novel drugs are tested against the correct species.

Keywords Species identification, biofilm formation, urinary tract infection, catheter, antimicrobial resistance, genotypic identification.

48 **Introduction**

49 The Centers for Disease Control and Prevention recognise four top causes of hospital acquired
50 infections, these include, catheter-associated urinary tract infections (CAUTI), central line-associated
51 bloodstream infections, ventilator associated pneumonia and surgical site infections (Haque *et al.*,
52 2018). Within the UK healthcare setting, urinary tract infections account for approximately 17% of all
53 hospital associated infections with 70-80% of those being catheter associated, making catheterisation
54 a major risk factor for infections (Gokula *et al.*, 2004; Klevens *et al.*, 2007; Lo *et al.*, 2014; Kuriyama
55 *et al.*, 2017). The greatest risk factor for CAUTI development in catheterised patients is time, with
56 daily infection risks ranging from 3-7% (Maki, 2001; Lo *et al.*, 2014). Beyond 28 days of
57 catheterisation, the likelihood of contracting an infection approaches certainty (Cortese *et al.*, 2018).
58 Although mortality rates for CAUTI are relatively low amongst hospital acquired infections
59 (estimated at ~2.3%), they are the leading cause of secondary nosocomial bloodstream infections;
60 with approximately 17% of hospital acquired bacteraemias being of urinary origin, and thereby
61 contributing to the mortality of ~10% for bloodstream infections (Weinstein *et al.*, 1997).

62 Proportions of causative organisms in reported CAUTIs vary significantly between studies and
63 geographical location (Sabir *et al.*, 2017). The Centers for Disease Control and Prevention report that
64 *Escherichia coli* is responsible for approximately 21% of CAUTIs. Other significant contributors to
65 catheter-associated infections include *Candida* spp., *Enterococcus* spp., *Pseudomonas aeruginosa*,
66 *Klebsiella pneumoniae*, and *Enterobacter* spp. (Centers for Disease Control and Prevention, 2024).
67 The variety of causative organisms is likely due to the catheter environment, where the catheter
68 provides a scaffold for microbial adherence and proliferation prior to an infection. Another factor is
69 the varying health of patients during hospitalisation, with long-term catheterised patients often being
70 intensive care patients that are more vulnerable to infections.

71 Once microbes adhere to host cell surfaces or the catheter, their phenotype changes, allowing biofilm
72 formation to occur through production of an extracellular matrix. This affords the microbial
73 community within the biofilm additional protection from antimicrobials and host defences. Biofilms
74 pose a significant risk to patient health with resistance to antibiotic treatment increasing up to 1,000-

fold in this state. Biofilm formation also aids in evasion of the immune response creating resistance to phagocytosis and removal by physical forces. An additional complication of biofilm formation is the potential for physical blockage of the tubing that can lead to life-threatening urine retention, which backflows to the kidneys (Feneley, Hopley and Wells, 2015). *Klebsiella* spp. and *Pseudomonas* spp. often cause blockage due to high mucoid production, although *Proteus* spp. pose the greatest risk due to urease activity leading to crystallised biofilms that can become impossible to remove without surgery (Stickler, 2008).

Identification and the antibiotic susceptibility profile of microorganisms responsible for causing CAUTIs are vital to the well-being of the patient and for successful treatment. Identification of CAUTI isolates is therefore undertaken in both clinical and research settings, to aid in the treatment of patients and to better understand the characteristics of pathogens to support future treatment options and antimicrobial drug / device development. Identification of these isolates varies between the two settings, with research laboratories typically utilising a range of identification techniques including biochemical tests such as analytical profile index (API), sequence-based approaches including 16S rRNA gene sequencing, or whole-genome sequencing with comparisons to a reference genome using Average Nucleotide Identity (Bosshard *et al.*, 2006; Abayasekara *et al.*, 2017; Brumfield *et al.*, 2020; Janda and Abbott, 2002a). By contrast, the UK standards for microbiology investigation for examination of urine are followed by National Health Service (NHS) clinical laboratories (The Royal College of Pathologists, 2024). However, the implementation of these standard procedures allows for local variations, particularly in the choice of culture techniques used. Despite some variations, it is common practice for culture and sensitivity testing to be performed on positive urine cultures identified via flow cytometry. Isolates are initially cultured on Cystine Lactose Electrolyte Deficient (CLED) and / or chromogenic agar. CLED differentiates between lactose and non-lactose fermenting bacteria, whereas chromogenic agar allows for identification of common urinary tract infection pathogens at genus or species level depending on the organism and chromogenic agar used. Within the clinical setting the antibiotic profile of strains is often more critical than identification of isolates to species level and therefore species identification is not always necessary, for instance:

Enterobacteriaceae (excluding *Salmonella spp.*) are often identified as “coliforms”, even though *Klebsiella spp.* and *E. coli* are distinguishable if using chromogenic agar and *Pseudomonas spp.* (commonly referred to as pseudomonads) are identified only to genus level as are enterococci.

Agar culture methods remain simple, cheap and the gold standard for antibiotic susceptibility testing within the clinical setting, however, more advanced techniques are available for species identification including MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) or the VITEK 2 system (bioMérieux Ltd.), which uses biochemical testing including antibiotic sensitivity testing. These methods, while faster, are more costly and often reserved for situations where rapid results are required, for example in at-risk groups such as patients in intensive care units or patients with potential sepsis.

Three of the four major causes of healthcare associated infections involve medical devices and biofilm formation. Given the significant changes to phenotype and susceptibility to antimicrobials in biofilms compared to planktonically growing bacteria, there is a well-documented and urgent need to better understand biofilm formation on innate surfaces (Olsen, 2015). Less well documented but still of pivotal importance is to characterise pathogens in experimental conditions that mimic the clinical environment more closely to ensure the applicability of research to the real world (Chen *et al.*, 2020; Liang *et al.*, 2020). *In vivo* studies provide conditions most closely related to clinical infections; however, this is often unfeasible, particularly in the early stages of research or drug development due to ethical concerns. In these circumstances *in vitro* testing is required, however there is significant variation in practice for these assay types, which can generate misleading data.

In this study, we aimed to determine the most effective genotypic and phenotypic methods for identifying bacterial (CA)UTI pathogens. Additionally, we investigated whether genomic data alone can reliably indicate antibiotic resistance phenotypes. We further evaluated phenotypic properties of the isolates in different media compositions to examine the effects of altering these experimental parameters on planktonic growth and biofilm formation.

Materials and methods

All chemicals and media were purchased from Merck Life Science UK Limited, Gillingham, UK, unless otherwise stated.

Bacterial strains and culture conditions

Strains used in this study are listed in Table 1 and were obtained from the Nottingham University Hospitals Trust Pathogen Bank or the University of Sheffield (Forde *et al.*, 2014). Strains were typically plated onto Mueller-Hinton agar as required and liquid cultures were grown in Mueller-Hinton broth, defined minimal medium containing glucose (Smitten *et al.*, 2019), artificial urine medium (Brooks and Keevil, 1997) or plasma-like medium (Ackermann and Tardito, 2019; Voorde *et al.*, 2019). For growth analysis; 100 µL of each desired medium was added to wells of a 96-well plate and overnight culture was added to the plates at a final concentration of 1%. Plates were incubated with shaking at 37°C in a BioTek Cytation 3 plate reader (Agilent Technologies Inc, Stockport, UK) with turbidity measured at 600 nm every 15 min for 24 h.

Phenotypic and biochemical identification of bacterial isolates

Bacterial isolates were first identified by the Nottingham University Hospitals Trust Pathogen Bank using an in-house MALDI Biotyper by comparison against reference library entries following identification standard method 1.1 (Panda *et al.*, 2014). For phenotypic identification, strains were plated onto UTI ChromoSelect agar made as per manufacturer's instructions and incubated overnight at 37°C. Strains were also identified using the API 20E kit commercially available from bioMérieux UK Ltd. (Basingstoke, UK) according to manufacturer's instructions using freshly streaked bacterial cultures from Mueller-Hinton agar plates. Identification of strains was confirmed using APIWEB™.

DNA extraction, whole genome sequencing and bioinformatic analysis for bacterial isolate identification

Genomic DNA was extracted from cell pellets using the GenElute™ Bacterial Genomic DNA kit as per manufacturer's instructions. DNA quality was checked using a NanoDrop microvolume spectrophotometer (Thermo Fisher Scientific, Loughborough, UK) and quantified using a Qubit 4 fluorometer (Thermo Fisher Scientific, Loughborough, UK) high sensitivity dsDNA assay kit. A

hybrid genome sequencing approach was used to generate data via Oxford Nanopore Technologies' MinION and an Illumina MiSeq to achieve six closed genomes and one draft sequence. The six CAUTI isolates were sequenced in-house using the Nextera XT Library Prep Kit and a V2 2x150 cycles reagent kit on the MiSeq platform (Illumina Inc, Cambridge, UK). *E. coli* EC958 was sequenced on the Illumina NovaSeq6000 platform by MicrobesNG (Birmingham, UK). Illumina reads were combined with in-house Oxford Nanopore sequencing for genome assembly. Adapter sequences were trimmed from Illumina sequencing reads using Trimmomatic v0.39 (Bolger, Lohse and Usadel, 2014). Sickle v1.33 used a sliding window approach to trim reads to an average Phred quality score of Q20 (Joshi and Fass, 2011). Trimmed illumina reads for strain 19Y000094 were assembled using SPAdes v3.15.5 (Prjibelski *et al.*, 2020). Oxford Nanopore sequencing was completed on the Mk1B MinION (Oxford Nanopore Technologies plc, Oxford, UK). The native barcoding kit 24 V14 SQK-NBD114.24 was used for library preparation including multiplexing. Libraries were loaded onto a MinION R10.4 flow cell and run for 48 h.

All software used for genome assembly and error correction were run using default parameters unless otherwise stated. Oxford Nanopore raw fast5 data was basecalled using the super high accuracy (SUP) model of Guppy v6.5.7, and demultiplexed. Porechop v0.2.4 was used to trim adapter sequences, with middle- and end-threshold values of 85% and 95%, respectively. Filtlong v0.2.1 was used to trim reads by quality and by length, keeping reads with a minimum length of 1 kb. Overlapping Nanopore sequencing reads were *de novo* assembled using Flye v2.9.1 (Kolmogorov *et al.*, 2019). Genome assemblies were first polished using Nanopore reads, with four iterations of Racon v1.5.0 (Vaser *et al.*, 2017) (with parameters -m 2 -g -2 -u), followed by Medaka v1.7.2 (Vaser *et al.*, 2017).

Assemblies were further polished using trimmed Illumina data with Polypolish v0.5.0 (Wick and Holt, 2022), POLCA from the MaSuRCA v4.0.9 package (Zimin and Salzberg, 2020) and Nextpolish v1.4.1 (Hu *et al.*, 2020). Whole genome sequences are available under BioProject: PRJNA1034027.

Genome completeness and contamination were assessed with CheckM v1.2.1 (Parks *et al.*, 2015). FastANI v1.32 was used to confirm the species of each CAUTI isolate, by calculating average nucleotide identity compared to type strains of the presumptive species' genus (accession numbers

available in supplementary)(Jain *et al.*, 2018). Bakta v1.8.1 (database v5.0) was used for annotating genes within genomes (Schwengers *et al.*, 2021). Bakta annotations associated with this article are available via [figshare](#).

16S rRNA gene sequences were extracted *in silico* from Bakta annotations and each individual 16S rRNA gene copy was analysed through EZBioCloud and the top result taken. Where different copies provided different results, all were represented.

Bioinformatic characterisation of bacterial whole genome sequences

Virulence genes were identified using VFanalyser in the Virulence Factor Database (VFDB) (Chen *et al.*, 2004). Whole genome sequencing data was compared to the genus database for each strain, or to family databases where genus was not available. Phage elements were predicted using Phastest (Zhou *et al.*, 2011; Arndt *et al.*, 2016). Antimicrobial resistance markers were identified using the Resistance Gene Identifier (RGI) v6.0.0 tool of the Comprehensive Antibiotic Resistance Database (CARD) v3.2.5 (Alcock *et al.*, 2023). Only resistance genes that showed a perfect or strict match with coverage for a given gene and that achieved $\geq 90\%$ identity and read length in the database were included in this study.

Disk diffusion assays

Disk diffusion assays were performed in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (The European Committee on Antimicrobial Susceptibility Testing, 2022). Bacterial suspensions diluted to McFarland turbidity standard of 0.5 were swabbed evenly across Mueller-Hinton agar plates. Antibiotic-containing disks purchased from Thermo fisher Scientific (Loughborough, UK), were applied and plates incubated for 18 h at 37°C prior to measuring the zone of inhibition (mm). Sensitivity and resistance were determined by comparison to EUCAST 2023 breakpoints (The European Committee on Antimicrobial Susceptibility Testing, 2023). EUCAST control *E. coli* strain ATCC 25922 was included to verify the results.

Characterization of phenotypic and genomic concordance/discordance for antibiotic susceptibility and resistance

Whole-genome sequencing (WGS) was used to predict antimicrobial resistance genes using CARD (as described above). Isolates were classified as resistant to an antibiotic when at least one antibiotic resistance gene was identified by CARD and allocated as the mechanism of resistance to that antibiotic. Isolates were classified as susceptible to an antibiotic by whole genome sequencing analysis when no resistance gene targeting that antibiotic was identified after the CARD exclusion criteria were applied. These predictions were compared with phenotypic resistance profiles obtained via EUCAST disk diffusion assays (DDA, n=140 total combinations). Concordance and discordance of the data was determined as previously described (Rebelo *et al.*, 2022; Eladawy *et al.*, 2023; Vanstokstraeten *et al.*, 2023); briefly:

Concordance classification:

Resistant concordance (WGS-R/DDA-R): both whole genome sequencing and DDA classified the isolate as resistant. Susceptible concordance (WGS-S/DDA-S): both whole genome sequencing and DDA classified the isolate as susceptible.

Discordance classification:

Phenotypic sensitivity discordance (WGS-R/DDA-S): whole genome sequencing predicted resistance, but DDA indicated a phenotypically sensitive profile. Phenotypic resistance discordance (WGS-S/DDA-R): whole genome sequencing did not predict resistance, but DDA produced a phenotypically resistant profile.

96-well microtiter biofilm assays

Biofilm assays were performed in six different media, Tryptic Soy broth (TSB), Mueller-Hinton broth (MHB), Lysogeny broth (LB), plasma-like medium (PLM), defined minimal medium containing glucose (gDMM) and artificial urine medium (AUM). Overnight cultures grown in Mueller-Hinton broth were diluted to a turbidity of $OD_{600nm} = 0.05$ in each of the different fresh media, which was then added in 100 μ L volumes to the wells of 96-well plates and incubated statically at 37°C for 24 h. After incubation, microtiter plates were washed twice with 100 μ L of phosphate buffered saline. Biofilms were heat fixed at 60°C for 60 min prior to addition of 125 μ L of 0.1% crystal violet to each

well and incubation at room temperature for 20 min. Wells were washed three times with 150 μ L phosphate buffered saline then inverted and left to dry overnight. Subsequently, 150 μ L of acetic acid (33%) was added to each well and the plate was incubated with gentle shaking for 10 min before the absorbance was measured at OD_{540nm} using a BioTek Cytation 3 plate reader.

Results

Identification of (CA)UTI isolates by MALDI, 16S rRNA sequencing, whole genome sequencing, UTI chromogenic agar, and API 20E testing shows varying agreement between methodologies

CAUTI isolates were initially identified by MALDI analysis, with one *Escherichia coli*, two *Klebsiella pneumoniae* and three *Enterobacter spp.* identified. 16S rRNA gene sequencing agreed with MALDI analysis to genus level, however there was notable disagreement at species level (Table 1). Illumina and Oxford Nanopore hybrid genome assemblies resulted in six closed and one draft sequence. Isolates were identified as *E. coli*, *Klebsiella quasipneumoniae*, *K. pneumoniae*, *Enterobacter roggenkampii*, and *Enterobacter hormaechei* by ANI analysis against the type strain genome sequence for each respective species (>95-99% ANI (Chun et al., 2018), Table 1). Whole genome sequencing represents the most comprehensive identification of strains within this study and provided further genotypic detail. Isolates carried between one and six plasmids, ranging in size from 3632 - 274,807 bp. General features of the isolates are summarised in Table S1. Presence of prophage within genomes of the isolates were determined by Phastest (Table S2) and virulence factors identified via the Virulence Factor Database VFAnalyzer identified the presence of multiple virulence genes within the isolates, highlighting their pathogenic potential (Table S3).

Routine identification of (CA)UTI infection isolates in clinical settings often involves the use of UTI chromogenic agar. This agar contains various proprietary chromogenic substrates that when metabolised by common urinary tract infection strains produce different colours according to the metabolic pathways they possess, which can be utilised for species identification. We compared the presumptive chromogenic agar identification of the (CA)UTI isolates to other identification methods

to understand how reliable this common methodology was for these isolates (Table 1). When the whole genome sequence identification was compared to identification on chromogenic agar, just two of the seven bacterial isolates tested had the expected chromogenic colour according to manufacturer guidance; these were *E. coli* EC958 and *K. pneumoniae* 18Y001710. Strains 18Y000138, 18Y001710, 18Y001733, and 19Y000373 were identified as, *K. quasipneumoniae*, *K. pneumoniae*, *E. roggenskampii*, and *E. hormaechei* respectively by whole genome sequencing however, all four isolates produced large blue mucoid colonies on chromogenic agar suggesting the presence of *K. pneumoniae* (Fig. S1). *E. hormaechei* 19Y000094 showed pink colonies and would therefore likely be misidentified as *E. coli* during routine clinical identification tests and *E. coli* 20Y000092 also displayed a non-conventional colour on chromogenic agar, with no colour change, which would also lead to misidentification (Table 1). Of the tests performed, whole genome sequencing allows for the most precise species identification; this shows that chromogenic agar misidentified five of the seven strains.

The (CA)UTI isolates were also identified using the analytical profile index (API) 20E testing kit from bioMérieux UK Ltd, a common method for bacterial identification involving biochemical tests. Identification via this method only agreed with the whole genome sequencing data to species level for the *E. coli* isolates. However, API testing was generally able to correctly identify strains to genus level, with the exception of *K. pneumoniae* 18Y001710, being identified by API testing as *Pantoea spp*, compared to its identification as *K. pneumoniae* by whole genome sequencing.

Comparison of identification methods mostly showed agreement to genus level for all methods except chromogenic UTI agar. At species level there was no clear agreement for isolate identification between methods tested herein, with whole genome sequencing being required to reliably identify bacterial isolates (Table 1). All species names used hereafter refer to the species names as determined by whole genome sequencing.

Divergence between genotypic and phenotypic antibiotic resistance profiles in (CA)UTI isolates underscores the limitations of only relying on genomic methods to infer antibiotic resistance

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion assay remains the gold standard method for antibiotic susceptibility testing to establish clinical resistance or sensitivity to a wide range of antibiotics and ensuring the accurate prescription of antibiotic therapy. The (CA)UTI isolates in this study were tested against 21 antibiotics across 11 antibiotic classes to determine their antibiotic resistance profiles utilising EUCAST methodology (EUCAST, 2023). These antibiotic classes encompass the main treatment options for urinary tract infections set by the National Institute for Health and Care Excellence and include nitrofurantoin, trimethoprim and ampicillin (inferring amoxicillin sensitivity / resistance), which are the UK's first line antibiotic treatments against urinary tract infections (National Institute for Health and Care Excellence, 2018a). The USA's first line catheter-associated urinary tract infection antibiotic treatment options prior to sensitivity testing, cephalosporins, carbapenems, and fluoroquinolones, were also tested.

All strains except *E. coli* 20Y000092 were classified as multidrug resistant, being resistant to at least one antibiotic in three separate classes (Magiorakos *et al.*, 2012). The six multidrug resistant isolates were highly resistant, averaging resistance against 15 of the 21 antibiotics tested. *K. pneumoniae* 18Y001710 was only sensitive to gentamycin and tigecycline and *E. hormaechei* 19Y000094 was only sensitive to chloramphenicol and nitrofurantoin (Table 2). EUCAST does not provide breakpoints for colistin via disk diffusion assays due to the antibiotic's difficulty diffusing through agar causing poor reproducibility, therefore this antibiotic was not tested by this method. However, resistance was inferred genotypically for colistin via the mechanisms of efflux and antibiotic target alteration (Table 3). Two of the seven strains showed resistance to nitrofurantoin, four to trimethoprim and six to ampicillin, highlighting the increasing emergence of resistance to first line antibiotic treatments within the UK.

Genomic analysis of the (CA)UTI isolates using the Comprehensive Antibiotic Resistance Database (CARD) predicted 113 antibiotic resistance genes present within the seven isolates, which is unsurprising due to the resistant nature of urinary tract infection causing pathogens and that the isolates are from three separate genera. Across the isolates, antibiotic resistance mechanisms included antibiotic efflux, antibiotic inactivation, target alteration, target replacement, target protection and

reduced drug permeability. Resistances to all major classes of antibiotic were predicted as well as generalised efflux mechanisms for resistance to antibiotics, disinfecting agents and antiseptics (Table 3). The greatest number of efflux pumps were found in the *E. coli* isolates, EC958 was phenotypically resistant to 15 of the 21 antibiotics tested, whereas *E. coli* 20Y000092 also contained a significant number of efflux pumps but was clinically sensitive to all antibiotics tested, suggesting that presence of efflux pump genes is not a reliable indicator of phenotypic resistance.

In our investigation of how predicted genomic resistance profiles aligned with antibiotic resistance data from disk diffusion assays, we found a 63.6% agreement (concordance) between the two datasets, with a discordance rate of 36.4% observed within this panel (Fig. 1). The only type of discordance observed was phenotypic sensitivity discordance, where genome sequencing predicted resistance, but the disk diffusion assay showed clinical sensitivity. Concordance also significantly varied between the isolates with *E. hormaechei* 19Y000094 and *K. pneumoniae* 18Y001710 showing concordance at 90%, whilst the relatively antibiotic sensitive *E. coli* 20Y000092 showed just 10% concordance between genomic and phenotypic datasets. When the data was categorised by antibiotic; concordance varied between 28-85% with no obvious correlation between target antibiotic class nor mechanism of action and agreement of genomic and phenotypic datasets (Fig. S2).

Incubation of (CA)UTI strains in differing media conditions demonstrates varying metabolic capacity amongst the isolates

When characterising bacterial isolate phenotypes, it is typical to use a rich medium like Mueller-Hinton broth. However, this can result in misleading phenotypes if the isolates are adapted to different conditions. We analysed growth of the (CA)UTI isolates in various media to understand how they react to different chemical environments. We compared a rich laboratory medium (MHB), a defined minimal medium containing glucose (gDMM), and two physiologically relevant media to assess growth under conditions that better mimic nutrient availability in the human body. Artificial urine medium (AUM) represented the setting of a (catheter-associated) urinary tract infection, while a plasma-like medium helped us understand the strains responses to components typically found in human plasma and interstitial fluid.

Five of the seven strains exhibited significantly higher final carrying capacities when incubated in complex nutrient-rich Mueller-Hinton broth, compared to other media types tested ($p < 0.05$, Fig. 2). Five strains also achieved the highest growth rates in Mueller-Hinton broth (18Y001710, 18Y001733, 19Y000094, 19Y000373, 20Y000092). Two of the strains tested were unable to grow in defined minimal medium (18Y001710, 20Y000092), indicating a possible lack of essential nutrients found in the other media types but absent in this nutrient-sparse medium. All strains grew comparably well in the physiologically relevant plasma-like medium. However, artificial urine medium, which contains urea, citric, boric, and uric acids and is held at a lower pH of 6.4, resulted in significantly lower final carrying capacities ($p < 0.001$) and reduced maximum specific growth rates for all strains tested (Fig. S3).

Biofilm formation is significantly altered in different media types and between different strains

Catheter-associated urinary tract infections, often develop through biofilm formation within the catheter and urinary tract, making it crucial to understand the biofilm-forming capabilities of these isolates. Typically, studies of biofilms, including those on clinical strains, employ high-throughput assays in nutrient-rich media such as Mueller-Hinton broth, Tryptic Soy broth, and Lysogeny broth to encourage robust growth and strong biofilm formation. Such conditions provide comparative data amongst strains but may not replicate the biofilm dynamics observed *in vivo* (Vyas, Xia and Mai-Prochnow, 2022). Although some research incorporates physiologically relevant media such as artificial urine, particularly for uropathogen studies (Hancock, Ferriñeres and Klemm, 2007), this practice is not widespread, potentially due to ethical and cost considerations. Our study tested biofilm formation in these common rich media and a defined minimal medium to assess biofilm production under nutrient-limited conditions. Additionally, we explored two physiologically relevant media; plasma-like medium and artificial urine medium to investigate changes in biofilm biomass in conditions more closely resembling those of true infections.

Comparison of the different media types showed that defined minimal medium (gDMM) supported formation of the lowest levels of biofilm biomass across all strains (Fig. 3). For other media types, biofilm formation was strain dependent. Despite artificial urine medium supporting a significantly

reduced final carrying capacity in planktonic growth for all strains tested (Fig. 2) it supported the formation of greater biofilm biomass across strains (OD_{540nm} ranging from 0.3 – 0.9), including in *E. coli* 20Y000092 where significantly increased biofilm formation was observed ($p < 0.01$, Fig. S4).

Discussion

Urinary tract infections account for around 40% of hospital acquired infections making them the leading cause of hospital acquired infections (Liu, Xiao and Shi, 2018). With catheterisation in up to 80% of the UTIs reported in healthcare settings it is clear that catheterisation is a major risk factor for healthcare associated infections (Liu *et al.*, 2018). It is therefore important to study the causative agents of these infections and also the manner in which they adhere to and proliferate on innate surfaces prior to causing symptomatic bacteriuria. Herein, a hybrid whole genome sequencing approach was taken to gain a deeper insight into catheter-associated urinary tract infection strain genotypes from a UK hospital. This sequencing data was also utilised to identify isolates by 16S rRNA gene sequence. Additionally, we utilized other identification methods including MALDI, UTI chromogenic agar differentiation, and API 20E testing. Our aim was to ascertain whether these widely used approaches in clinical laboratories worldwide yielded consistent species identification and whether such identifications concurred with those from whole genome sequencing methods.

In this study, culturing on UTI ChromoSelect agar classified *Enterobacter* isolates as *K. pneumoniae* or *E. coli*. Furthermore *E. coli* 20Y000092, showed no colour on the chromogenic agar making presumptive identification by this method impossible. The expected pink colour for this species is a result of β -D-galactosidase activity. However, non-lactose fermenting and slow lactose fermenting *E. coli* account for 10-20% of *E. coli* isolates (Yaratha *et al.*, 2017; Mazumder *et al.*, 2022), which are often more sensitive to cephalosporins, agreeing with antibiotic sensitivity data (Table 2). Non-lactose fermenting *E. coli* strains are more likely to be community strains but this feature cannot be used as a predictive factor for infections as it does not affect the capability to cause disease compared with strains that are lactose fermenters (Yaratha *et al.*, 2017; Mazumder *et al.*, 2022)). This phenotype suggests that *E. coli* 20Y000092 is an opportunistic urinary pathogen, with whole genome sequencing confirming the presence of numerous virulence factors but a lack of those that would be present in

different pathovars of *E. coli* such as enteroaggregative or enteroinvasive (Table S3). *E. hormaechei* 19Y000094 colonies were pink when grown on UTI ChromoSelect agar. According to manufacturer guidance, the identities of all pink colonies should be confirmed by an indole test, since some *Salmonella*, *Shigella*, *Citrobacter* and *Enterobacter* strains may also appear pink, however this is at the discretion of the testing laboratory and may not always be undertaken to confirm identity (Lo *et al.*, 2014). Both *Klebsiella spp.* showed a typical blue colony appearance, and were readily distinguishable from *Enterococcus spp.* due to colony size. The lack of differentiation for *Enterobacter spp.* on chromogenic agar as well as relying on traditional biochemical markers highlights the dangers of relying on phenotypic testing to identify pathogens, with literature identification only correct to genus level 50% of the time (Sachu and Samuel, 2022). This issue is particularly critical given that the range of causative organisms is more diverse in CAUTIs compared to uncomplicated UTIs, further complicating accurate diagnosis and effective treatment.

Within the research literature, causative organisms of catheter-associated urinary tract infections have been identified via multiple methods, however it is clear that appearance and chromogenic UTI profile is a key factor in identification as it is within the NHS, likely due to cost, leading to misrepresentation of species within the literature and clinical setting. Although, this is unlikely to affect treatment as sensitivity testing is also carried out prior to prescription of antibiotics where possible and critical infections are identified with additional methods, it could potentially skew medical literature altering the proposed significance of a species within the clinical space (Janda and Abbott, 2002b). This is potentially true with identification of *E. coli*, which within the literature is recorded to account for 75-90% and 21% of all UTI and CAUTI respectively (Ronald, 2002; Flores-Mireles *et al.*, 2015). However, if identification is based purely on UTI chromogenic agar, any Enterobacteriaceae with the correct biochemical profile could contribute to this statistic despite the potential of the true identity being a different genus.

Within this study, we determined the antibiotic sensitivity of the bacterial strains both phenotypically and predicated genomically by the Comprehensive Antibiotic Resistance Database (CARD). The high levels of antibiotic resistance observed phenotypically across the (CA)UTI strains was unsurprising

with the current state of global resistance. *K. pneumoniae* 18Y001710 was of particular concern being resistant to 19 of the 21 antibiotics tested, with potential colistin resistance mechanisms predicted via CARD (Table 3). Global resistance within *Klebsiella spp.* to colistin has been observed and is often a result of changes in cell permeability encoded on the chromosome. *K. pneumoniae* 18Y001710 carries *arnT* and *eptB*, the products of which are involved in lipopolysaccharide modification that causes resistance to polymyxins (Capone *et al.*, 2013; Olaitan *et al.*, 2014; Jayol *et al.*, 2015; Li *et al.*, 2020). *E. coli* 20Y000092 was atypical of CAUTI causing pathogens, being sensitive to all antibiotics tested. However, antibiotic resistance genes identified from the genome sequence are suggestive of a resistance profile far greater than shown phenotypically. This raises interesting questions around the use of genomic data to infer antibiotic resistance. Further investigation will be required to determine the cause of discordance between the genomic and phenotypic antibiotic resistance data for this isolate.

Sensitivity to one of the first line antibiotic treatments for urinary tract infection strains in the UK, nitrofurantoin, was observed in five of seven bacterial isolates tested (National Institute for Health and Care Excellence, 2018b, 2018a). However, both *Klebsiella* isolates were resistant to this antibiotic, likely due to the predicted presence of OqxA efflux pumps that are known to contribute to nitrofurantoin resistance (Xu *et al.*, 2019).

As next generation sequencing is increasingly cost effective and readily available, and with new bioinformatic tools published that allow for basic genome annotation and interrogation with minimal bioinformatics expertise, it is tempting to utilise these technological advances to move to a more genomic predictive system in place of phenotypic and biochemical testing. However, as demonstrated herein and elsewhere, it is clear that further development is required to reliably predict phenotype from genotype with a high degree of confidence (Rebelo *et al.*, 2022; Eladawy *et al.*, 2023; Vanstokstraeten *et al.*, 2023). Care should be taken when using genomic tools to predict antimicrobial susceptibility. Using predictive tools alone could skew resistance data sets when comparing large numbers of strains and lead to incorrect treatment of a patient if used clinically without phenotypic testing. Although these tools are extremely useful for identification and tracking of resistance genes,

phenotypic data should continue to be used to confirm the accuracy of predictive tools, especially when susceptibility testing remains both fast and cost effective.

Characterisation of bacterial pathogens provides researchers with new avenues of investigation to prevent and / or treat infections. However, these studies are often performed using rich laboratory media containing many nutrients that the strains will not have access to during infections, or at significantly higher concentrations than found *in vivo*, which can alter bacterial metabolic processes and phenotype. The (CA)UTI isolates were phenotypically characterised utilising various media to determine the effects of physiologically relevant chemical environments and improve understanding of how bacteria might respond during infections.

Growth and biofilm assays were performed using a range of media compositions including rich laboratory media, a defined minimal medium and two physiologically relevant media (artificial urine and plasma-like medium). Differences in media composition including between different types of rich medium resulted in differences in biofilm formation. Glucose often promotes biofilm formation (Seneviratne *et al.*, 2013; Waldrop *et al.*, 2014) however, as the medium used in this study was a minimal medium, biofilm formation across the strains was low. Within *Staphylococcus* spp. Tryptic Soy broth generally acts a suitable medium for strong biofilm formation, especially when supplemented with glucose (Lade *et al.*, 2019), however Mueller-Hinton broth and Tryptic Soy broth are comparably suitable biofilm formation media for *Pseudomonas* spp. (A. Alkafaween and Bakar Mohd Hilmi, 2021) highlighting the differing responses of bacterial species to differing media components. Mueller-Hinton broth is a rich medium containing beef extract, hydrolysed casein, and starch, which can be broken down easily by bacteria to provide amino acids, carbon, vitamins, nitrogen, and other important nutrients. Therefore, it is not suitable for use in clinically relevant catheter-associated urinary tract infection assays as many of these nutrients will not be available, or will be severely limited in human urine. In biofilm assays and models, researchers often use these rich laboratory media at 50% of the recommended recipe strength to reduce the concentration of nutrients present, however this is still inconsistent with the chemical composition found in a clinical environment. Artificial urine medium is much closer to the chemical composition of human urine and

is able to promote strong biofilm formation for species including *P. aeruginosa*, particularly as part of a mixed biofilm (Nickel *et al.*, 1985; Cerqueira *et al.*, 2013). Human urine would theoretically be the most appropriate medium for characterisation of urinary pathogens, however it was not used in this study for multiple reasons; firstly it requires ethical approval and incurs significant cost to acquire. Secondly, patient urine will vary greatly with factors including host gender, age, diet and disease conditions such as cancer or pregnancy. Even pooled urine suffers from batch-to-batch variation based on donor profile (Shearman, 1845). Therefore, to support standardisation and reproducibility of data in a medium that better mimics the infection environment, artificial urine is an appropriate medium that replicates *in vivo* conditions in a consistent manner (Liang *et al.*, 2020).

The data present within this study underscores the need for caution when identifying strains to species level solely based on phenotypic or biochemical testing. It further highlights the need to study catheter-associated urinary tract infection isolated strains in clinically relevant conditions to more accurately understand how these pathogens will act *in vivo*, which would better support drug development in early pre-clinical phases. The data presented also exemplifies the antimicrobial resistance crisis, demonstrating on a small scale the significant resistance emerging in pathogens isolated within healthcare settings with all but one strain classified as multidrug resistant. Overall, this work demonstrates the need for a combination of phenotypic testing and genomic analysis to accurately identify and characterise clinically isolated bacterial pathogens so that we may overcome this threat to global health.

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Conflict of interest

None declared.

Authors contribution statement

Adam M. Varney (conceptualization, formal analysis, investigation, methodology, visualisation, writing – review & editing), Eden Mannix-Fisher (conceptualization, investigation, methodology, visualisation, writing – review & editing), Jonathan C. Thomas (formal analysis, methodology, supervision, writing – review & editing), Samantha McLean (conceptualization, formal analysis, funding acquisition, methodology, project administration, supervision, visualisation, writing – original draft, writing – review & editing).

Data availability

The genomic data generated during the current study are available in the National Center for Biotechnology Information BioProject: PRJNA1034027. BioSample accession numbers: SAMN38049403-9. SRR raw Illumina data: SRR26595501-7. SRR raw Nanopore data: SRR26624650-5. Annotated genomes are available in [figshare](#).

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718 **Supporting information**

719 **FastANI v1.32 accession numbers**

720 **Table S1** Summary information for genomes generated from the (CA)UTI isolates described in this
721 study

722 **Table S2** Predicted prophages present in the isolated genomes using PHASTEST

723 **Table S3A** Virulence factors predicted by VFAnalyzer using the Virulence Factor Database
724 (Adherence)

725 **Table S3B** Virulence factors predicted by VFAnalyzer using the Virulence Factor Database (Iron
726 Uptake)

727 **Table S3C** Virulence factors predicted by VFAnalyzer using the Virulence Factor Database (Secretion
728 System)

729 **Table S3D** Virulence factors predicted by VFAnalyzer using the Virulence Factor Database
730 (Miscellaneous)

731 **Table S3E** Virulence factors predicted by VFAnalyzer using the Virulence Factor Database
732 (Miscellaneous)

733

734 **Fig. S1** CAUTI isolates plated onto UTI ChromoSelect agar

735 Strains plated out onto UTI ChromoSelect agar as per manufacturer's guidance and incubated at 37°C
736 overnight. Top row: *E. coli* EC958, middle row (left to right): *E. roggenskampii* 18Y001733, *K.*
737 *pneumoniae* 18Y001710, *K. quasipneumoniae* 18Y000138, bottom row (left to right): *E. coli*
738 20Y000092, *E. hormaechei* 19Y000373, *E. hormaechei* 19Y000094.

739 **Fig. S2** Concordance and discordance between genotypic and phenotypic datasets of antimicrobial
740 resistance for seven (CA)UTI isolates.

Genotypic prediction of antibiotic resistances determined by evaluation of whole genome sequences using the Comprehensive Antibiotic Resistance Database were compared to disk diffusion assay results according to EUCAST guidelines for the (CA)UTI strains. Concordance (coloured in green) occurred when genotypic predictions of antibiotic susceptibility agreed with disk diffusion assay results. Discordance (coloured in orange) occurred when genotypic predictions disagreed with disk diffusion assay data.

Fig. S3 Artificial urine medium shows a significantly lower growth rate ($\mu_{\max}$) when comparing growth rate averages

Maximum growth rates of bacterial cultures were measured in four different growth media: Mueller-Hinton Broth (MHB) (Black), Glucose-Defined Minimal Medium (GDDM) (Pink), Plasma-like medium (PLM), and Artificial Urine Medium (AUM). Data points represent $\mu_{\max}$ values for individual bacterial species, with the horizontal line indicating the median of each group. One-way ANOVA with Tukey's HSD was performed. Significance levels are denoted as follows: not significant (ns, $p \geq 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).

Fig. S4 Biofilm formation capacity is significantly altered by media type and increased in the presence of artificial urine

Biofilms were grown statically in 96-well plates for 24 h at 37°C with an initial inoculation of $OD_{600} = 0.05$. *P. aeruginosa* PA01 was used as a positive control for biofilm formation. $N = 3 \pm SE$.

Figures

Figure 1. Genotype-phenotype antimicrobial resistance concordance shows high variation in (CA)UTI isolates.

Genotypic prediction of antibiotic resistances determined by evaluation of whole genome sequences using the Comprehensive Antibiotic Resistance Database were compared to disk diffusion assay results according to EUCAST guidelines for the (CA)UTI strains. Concordance (coloured in green) occurred when genotypic predictions of antibiotic susceptibility agreed with disk diffusion assay results. Discordance (coloured in orange) occurred when genotypic predictions disagreed with disk diffusion assay data.

Figure 2. Growth of (CA)UTI bacterial isolates in different media show differing growth profiles. Cultures were grown in 96-well plates in Mueller-Hinton broth (MHB, black), defined minimal medium (gDMM, pink), plasma-like medium (PLM, green), or artificial urine medium (AUM, purple), from a 1% overnight culture inoculum of the same medium. Plates were incubated at 37 °C with shaking for 24 h with OD₆₀₀ measured every 10 min. N = 4 with SD range 0.0004-0.1684. (A) *E. coli* ST131 EC958, (B) *K. quasipneumoniae* 18Y000138, (C) *K. pneumoniae* 18Y001710, (D) *E. roggenkampii* 18Y001733, (E) *E. hormaechei* 19Y000094, (F) *E. hormaechei* 19Y000373, and (G) *E. coli* 20Y000092. (H) Maximum specific growth rates were calculated for each isolate and medium combination. One-way ANOVA performed on final turbidity with Tukey's HSD. Significance levels: not significant (ns, $p \geq 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).

Figure 3. Biofilm formation capacity is significantly altered by media type and increased in the presence of artificial urine. Biofilms were grown statically in 96-well plates for 24 h at 37°C with an initial inoculation of OD₆₀₀ = 0.05. *P. aeruginosa* PA01 was used as a positive control for biofilm formation. N = 4 ± SE.

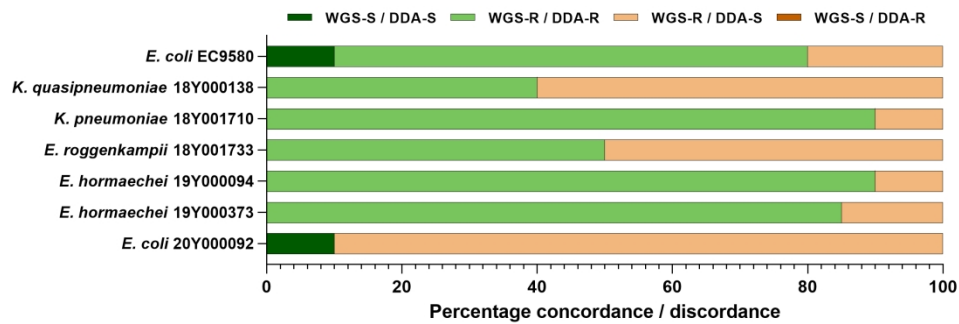


Figure 1. Genotype-phenotype antimicrobial resistance concordance shows high variation in (CA)UTI isolates.

Concordance (green) is marked by alignment of genotypic predictions with phenotypic disk diffusion results. Discordance (orange) is noted when genotypic predictions contradict phenotypic outcomes.

221x76mm (300 x 300 DPI)

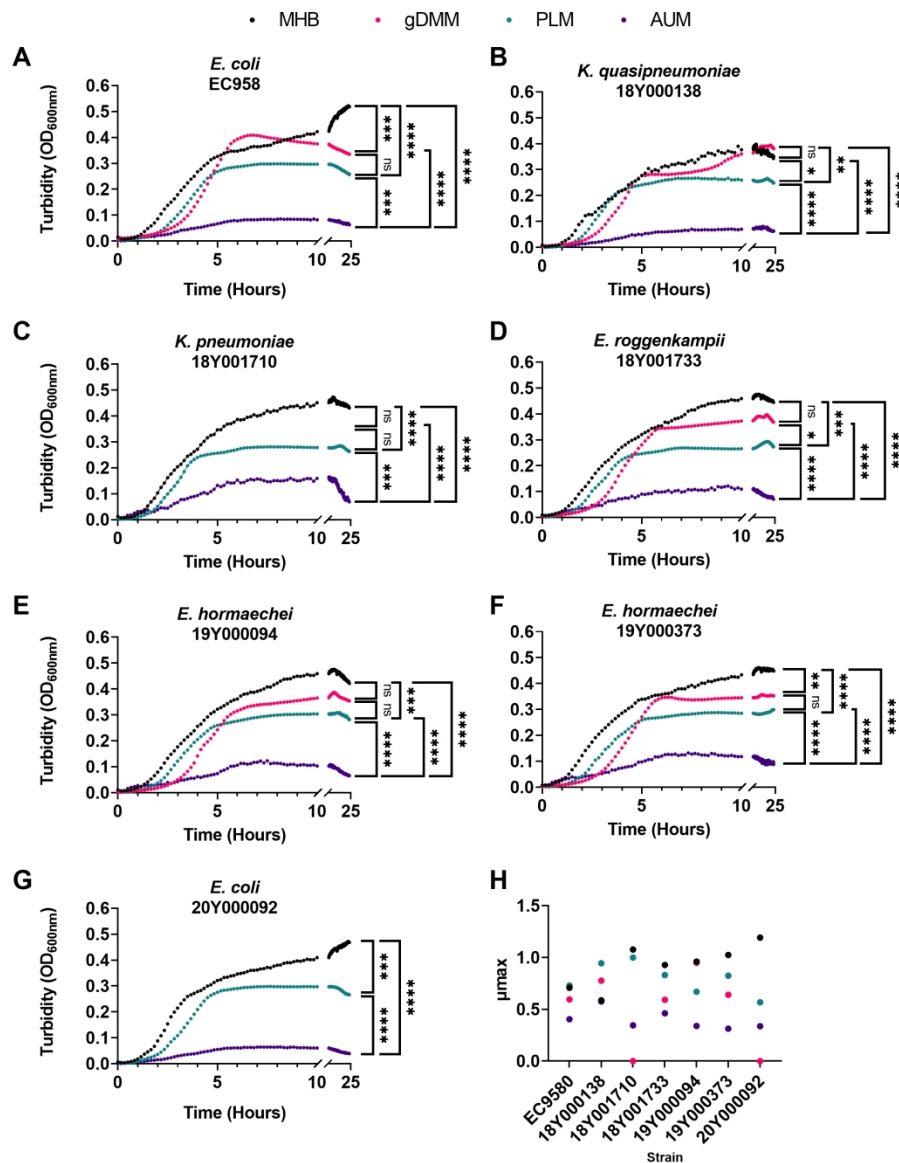


Figure 2. Growth of (CA)UTI bacterial isolates in different media show differing growth profiles. Cultures were grown in 96-well plates in Mueller-Hinton broth (MHB, black), defined minimal medium (gDMM, pink), plasma-like medium (PLM, green), or artificial urine medium (AUM, purple), from a 1% overnight culture inoculum of the same medium. Plates were incubated at 37 °C with shaking for 24 h with OD600 measured every 10 min. N = 4 with SD range 0.0004-0.1684. (A) *E. coli* ST131 EC958, (B) *K. quasipneumoniae* 18Y000138, (C) *K. pneumoniae* 18Y001710, (D) *E. roggenkampii* 18Y001733, (E) *E. hormaechei* 19Y000094, (F) *E. hormaechei* 19Y000373, and (G) *E. coli* 20Y000092. (H) Maximum specific growth rates were calculated for each isolate and medium combination. One-way ANOVA performed on final turbidity with Tukey's HSD. Significance levels: not significant (ns, $p \geq 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).

202x258mm (300 x 300 DPI)

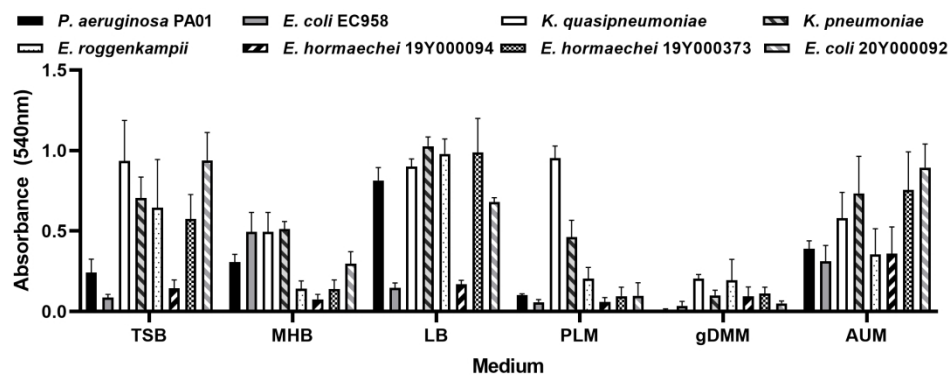


Figure 3. Biofilm formation capacity is significantly altered by media type and increased in the presence of artificial urine. Biofilms were grown statically in 96-well plates for 24 h at 37°C with an initial inoculation of OD₆₀₀ = 0.05. *P. aeruginosa* PA01 was used as a positive control for biofilm formation. N = 4 ± SE.

210x84mm (300 x 300 DPI)

801 **Tables**802 **Table 1.** A summary comparison of phenotypic and genotypic identification of seven (CA)UTI bacterial isolates

Strain origin /designation	Species identification by MALDI	Score Value	16S rRNA identification	Species identification by WGS	ANI (%)	Colour on UTI agar – presumptive identification	API 20E identification and code (% ID, T)
ST131 EC958 (Forde et al., 2014; Totsika et al., 2011) Human urine	N/A	N/A	<i>Escherichia fergusonii</i> / <i>Shigella flexneri</i>	<i>E. coli</i>	98.34	Pink – <i>E. coli</i>	5104572 <i>E. coli</i> (99.5, 0.96)
NUH: 18Y000138 Urinary catheter	<i>Klebsiella pneumoniae</i>	2.22	<i>K. quasipneumoniae</i>	<i>K. quasipneumoniae</i>	96.61	Blue – <i>K. pneumoniae</i>	1215773 <i>K. pneumoniae</i> (98.2, 0.93)
NUH: 18Y001710 Urinary catheter	<i>Klebsiella pneumoniae</i>	2.26	<i>K. quasipneumoniae</i>	<i>K. pneumoniae</i>	98.88	Blue – <i>K. pneumoniae</i>	1205773 <i>Pantoea</i> spp. 2 (46.1, 0.95)
NUH: 18Y001733 Urinary catheter	<i>Enterobacter bugandensis</i>	2.27	<i>E. - quasiroggenkampii</i> / <i>roggenkampii</i> / <i>pseudoroggenkampii</i> / <i>quasimori</i> /	<i>E. roggenkampii</i>	98.59	Blue – <i>K. pneumoniae</i>	3301563 <i>E. cloacae</i> (99.6, 0.59)
NUH: 19Y000094 Urinary catheter	<i>Enterobacter cloacae</i>	2.30	<i>E. hormaechei</i>	<i>E. hormaechei</i> subsp. <i>hoffmannii</i>	99.03	Pink - <i>E. coli</i>	3305532 <i>E. cloacae</i> (99.2, 0.56)
NUH: 19Y000373 Urinary catheter	<i>Enterobacter cloacae</i>	2.32	<i>E. hormaechei</i>	<i>E. hormaechei</i> subsp. <i>xiangfangensis</i>	95.69	Blue – <i>K. pneumoniae</i>	3205572 <i>E. cloacae</i> (92.6, 0.55)
NUH: 20Y000092 Urinary catheter	<i>Escherichia coli</i>	2.47	<i>E. fergusonii</i>	<i>E. coli</i>	98.78	None - unknown	4144512 <i>E. coli</i> (69.5, 0.77)

803 This table summarises the identification of each strain by five different methods. NUH: Nottingham University Hospitals Trust Pathogen Bank, Nottingham,
804 UK. MALDI scores: 0.00-1.69: No organism identification possible, 1.70-1.99: low-confidence identification, 2.00-3.00: high-confidence identification.
805 Identification for UTI ChromoSelect Agar (16636) was determined following Merck, UK guidelines. For API 20E; identification was performed using
806 APIWEB™. The 16S rRNA genes were used for identification using Bakta annotated genomes via EZBioCloud.

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809 **Table 2.** All tested (CA)UTI bacterial isolates were multidrug resistant by phenotypic testing

Bacterial strain and disk diffusion zone of inhibition size in mm (sensitivity / resistance)								
Antibiotic group	Antibiotic	<i>E. coli</i> EC958	<i>K. quasipneumoniae</i> 18Y000138	<i>K. pneumoniae</i> 18Y001710	<i>E. roggenkampii</i> 18Y001733	<i>E. hormaechei</i> 19Y000094	<i>E. hormaechei</i> 19Y000373	<i>E. coli</i> 20Y000092
Penams	Ampicillin	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	17 (S)
	Piperacillin	7 (R)	18 (R)	0 (R)	12.5 (R)	0 (R)	3.5 (R)	22.5 (S)
	Amox + Clav	6 (R)	21.5 (S)	7 (R)	3.5 (R)	0 (R)	0 (R)	19.5 (S)
Cephameycins	Cefoxitin	13 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	25 (S)
	Cefixime (3 rd)	0 (R)	22 (S)	0 (R)	0 (R)	0 (R)	0 (R)	21 (S)
Cephalosporins	Ceftriaxone (3 rd)	13 (R)	26 (S)	0 (R)	11.5 (R)	0 (R)	3.5 (R)	28.5 (S)
	Cefotaxime (3 rd)	0 (R)	21.5 (S)	0 (R)	0 (R)	0 (R)	0 (R)	26 (S)
	Ceftazidime (3 rd)	11.5 (R)	25 (S)	0 (R)	0 (R)	0 (R)	0 (R)	24.5 (S)
	Cefepime (4 th)	25.5 (R)	27.5 (S)	12.5 (R)	29 (S)	12 (R)	20 (R)	31.5 (S)
Carbapenems	Meropenem	31 (S)	24 (S)	14 (R)	23 (S)	13 (R)	25.5 (S)	29.5 (S)
	Doripenem	30 (S)	26 (S)	14.5 (R)	23.5 (R)	14 (R)	25 (S)	28 (S)
Monobactams	Aztreonam	20 (R)	29 (S)	9 (R)	15 (R)	23.5 (R)	11 (R)	29 (S)
Fluoroquinolones	Ciprofloxacin	0 (R)	22 (R)	16.5 (R)	26.5 (S)	14 (R)	21.5 (R)	30.5 (S)
	Norfloxacin	0 (R)	17.5 (R)	15 (R)	24 (S)	9 (R)	21 (R)	26 (S)
Aminoglycosides	Amikacin	15.5 (R)	19.5 (S)	14 (R)	18 (S)	0 (R)	19 (S)	20 (S)
	Gentamicin	17 (S)	18.5 (S)	17.5 (S)	17 (S)	0 (R)	13 (R)	18 (S)
	Tobramycin	7 (R)	18 (S)	8 (R)	17 (S)	0 (R)	14 (R)	16.5 (S)
Glycylcyclines	Tigecycline	20 (S)	15.5 (R)	19.5 (S)	17.5 (R)	0 (R)	19 (S)	21 (S)
Diaminopyrimidines	Trimethoprim	0 (R)	17 (S)	0 (R)	23.5 (S)	0 (R)	0 (R)	23 (S)
Phenicol	Chloramphenicol	22.5 (S)	15 (R)	0 (R)	17 (S)	19 (S)	0 (R)	21.5 (S)
Nitrofurans	Nitrofurantoin	20.5 (S)	8 (R)	10.5 (R)	12 (S)	14.5 (S)	14.5 (S)	16 (S)

810 Disk diffusion assays were performed in accordance with EUCAST standard methodology. (R) indicates resistance to the antibiotic and (S) indicates
811 sensitivity to the antibiotic in accordance with EUCAST guidelines (v13.1) (The European Committee on Antimicrobial Susceptibility Testing, 2023). For
812 cephalosporins, the numbers in parentheses indicate generation of cephalosporin. All data was collected in triplicate.

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815 **Table 3.** Predicted AMR genes encoded within genomes of the seven (CA)UTI isolates

Isolate	Resistance to drug class					
	B-lactams	Fluoroquinolones	Aminoglycosides	Peptides	Misc.	Efflux pumps
<i>E. coli</i> ^{EC958}	OXA-1, CTX-M-15, CMY-23, TEM-1,	<i>gyrA</i> mutants, <i>parC</i> mutants, AAC(6')-Ib-cr6	aadA5	BcA, EptA, PmrF, Ugd	EF-Tu mutation, MphA, Mrx, <i>glpT</i> mutation, <i>uhpT</i> mutation, Sul1, DfrA17	MdtABC-TolC, AcrD, QacEdelta1, EmrAB-TolC, MdtA, AcrEF-TolC, AcrAB, MdtM, EmrE, MdtEF-TolC, MsbA, MdtNOP, YojI, MdtG, EmrKY-TolC, TetA, MdfA
<i>K. quasipneumoniae</i> ^{18Y000138}	OKP-B-5, OmpK37			ArnT, EptB, OmpA	EF-Tu mutation, <i>uhpT</i> mutation, FosA6	KpnEF, KpnGH-TolC, MsbA, LptD
<i>K. pneumoniae</i> ^{18Y001710}	NDM-1, OXA-1, OXA-9, SHV-1, CTX-M-15, OmpK37, TEM-1	QnrS1, AAC(6')-Ib-cr6	AAC(6')-Ib10, aadA, aadA5, APH(3')-Ia, APH(3'')-Ib, APH(3')-Via, APH(6)-Id,	ArnT, EptB, OmpA	EF-Tu mutants, MphA, Mrx, CatII, <i>uhpT</i> mutation, FosA6, Sul1, Sul2, DfrA14, DfrA17	QacEdelta1, KpnEF, KpnGH-TolC, MsbA, LptD, TetD
<i>E. roggenkampii</i> ^{18Y001733}	MIR-7				EF-Tu mutation, <i>uhpT</i> mutation, FosA2	MsbA
<i>E. hormaechei</i> ^{19Y000094}	ACT-24, NDM-1, TEM-1	<i>gyrA</i> mutants	aadA2, RmtB,		<i>uhpT</i> mutation, FosA2, Sul1, DfrA12	QacEdelta1, AcrAB-TolC*, MsbA, EmrB, OxaA
<i>E. hormaechei</i> ^{19Y000373}	ACT-84, CTX-M-9	<i>gyrA</i> mutants	aadA, aadA2, ANT(2'')-Ia	MCR-9.1	EF-Tu mutation, SAT-2, CatI, <i>uhpT</i> mutation, FosA2, Sul1, DfrA1	QacEdelta1, AcrAB-TolC*, MsbA, TetA
<i>E. coli</i> ^{20Y000092}	EC-5			BacA, EptA, PmrF	EF-Tu mutation, <i>glpT</i> mutation,	MdtABC-TolC, AcrD, EmrAB-TolC, MdtA, AcrEF-TolC, AcrAB-TolC*, EmrE, MdtEF-TolC, MsbA, MdtNOP, YojI, MdtG, EmrKY-TolC, MdfA