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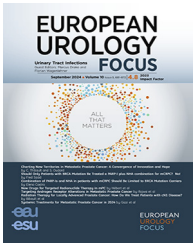

Infections

Influence of Kidney Environment Parameters on Antibiotic Efficacy Against Uropathogenic *Escherichia coli*

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Abstract

Background and objective: Urinary tract infections (UTIs) are common infections affecting the urinary system, predominantly caused by bacterial pathogens, with *Escherichia coli* being the most frequent pathogen. Infections of the kidney (eg, pyelonephritis) are severe and challenging to treat, due to the specific tissue microenvironment. In this study, the influence of different parameters mimicking the kidney environment on the effectiveness of antibiotics prescribed for pyelonephritis on the growth of uropathogenic strains was analyzed.

Methods: To investigate the influence of different factors mimicking the kidney environment, we tested the effect of different kidney-representative concentrations of sodium chloride and urea, and different pH values on the efficacy of ertapenem, levofloxacin, and ceftriaxone. The effectiveness was assessed by determining the minimal inhibitory concentrations (MICs) against various *E. coli* strains.

Key findings and limitations: The study revealed that pH significantly influences the MIC values of levofloxacin. Acidification of the pH led to an increase of the MIC values, while an alkaline pH had the opposite effect. The influence of sodium chloride and urea concentrations was strain and antibiotic specific. Since three different antibiotics were tested in this study, further research with additional antibiotics is warranted.

Conclusions and clinical implications: These results suggest that the physicochemical conditions within the kidney can substantially influence the success of antibiotic therapy for pyelonephritis. Therefore, it is crucial for clinicians to consider these factors when selecting and dosing antibiotics. Further research is needed to evaluate a broader range of antibiotics and additional environmental parameters, to develop a more comprehensive understanding of how the kidney environment affects antimicrobial activity. This knowledge will be vital in optimizing treatment strategies for pyelonephritis, ultimately improving patient outcomes.

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Patient summary: The physicochemical conditions within the kidney influence the success of antibiotic therapy for pyelonephritis. Our findings are vital in optimizing treatment strategies and will ultimately improve patient outcomes.

1. Introduction

Urinary tract infections (UTIs) are among the most prevalent microbial diseases, affecting individuals of all ages and sexes, and placing a substantial financial burden on society. UTIs account for over 100 000 hospital admissions annually, most frequently due to pyelonephritis [1–3].

Pyelonephritis, a severe ascending infection, predominantly affects the kidney medulla due to the diminished antibacterial activity of granulocytes in the hypertonic environment of the medulla [4,5]. Recent findings indicate the presence and formation of extracellular DNA traps of granulocytic and monocytic origin in the kidneys of pyelonephritis patients, particularly accumulating in the medulla [6].

In uncomplicated UTIs, *Escherichia coli* is the most common pathogen, isolated in over 80% of outpatients with acute uncomplicated cystitis worldwide [1,2,7]. In complicated UTIs, the bacterial spectrum is broader, including nonfermenter and Gram-positive organisms, although *E. coli* remains the predominant species [8].

To establish pyelonephritis, uropathogenic *E. coli* must ascend the ureters and invade the collecting tubules, which consist of principal cells (PCs) and intercalated cells (ICs). PCs are critical for ion and water transport, while ICs regulate acid-base homeostasis [9–13].

Antimicrobials are the cornerstone of UTI treatment, with choices tailored based on patient risk factors, infection localization and severity, and culture results including antimicrobial sensitivities.

There has been a notable increase in antimicrobial resistance (AMR) in *E. coli* from community-onset uncomplicated UTIs over the past 2 decades. For instance, a study from 18 European countries in 2018 found resistance to trimethoprim-sulfamethoxazole in 32.7% (range 23.1–56.2%) of *E. coli* isolates, with fluoroquinolone resistance exceeding 20% [14,15].

The kidneys play a central role in maintaining water and electrolyte balance, including blood pH/acid-base balance and the regulation of sodium chloride (NaCl), potassium, calcium, magnesium, hydrogen ions, and phosphate. The extreme milieu of the kidney medulla, characterized by high concentrations of these elements, may influence the antibacterial activity of antibiotics used to treat pyelonephritis [16,17].

This study examined the efficacy of ceftriaxone (CRX; a cephalosporin), ertapenem (ERT; a carbapenem), and levofloxacin (LVX; a fluoroquinolone). We assessed the influence of various kidney environment mimicking parameters, including different concentrations of glucose, urea, NaCl, and pH values, on bacterial growth and antibiotic effectiveness. The efficacy was measured by minimal inhibitory concentrations (MICs), defined as the lowest concentration of an antimicrobial that inhibits visible microorganism growth after overnight incubation [18].

2. Patients and methods

2.1. Bacterial strains

Clinical *E. coli* strains were originally isolated from urine or wound infection (Table 1). The seven bacterial strains used were stored at –80°C in a solution, which consisted of 200 µl glycerol (Carl Roth, Karlsruhe, Germany) and 800 µl tryptic soy broth (Oxoid Ltd., Hampshire, UK).

2.2. Bacterial growth

For the overnight culture, one colony was picked from an agar plate. This colony was transferred with a one-time inoculator to 5 ml of tryptic soy broth (Oxoid Ltd.). The bacterial cultures were grown for 16 h at 37°C at 180 rpm in a shaking incubator (IKA, Staufen, Germany). Of the bacterial overnight cultures, 100 µl was diluted 1:200 in Dulbecco's phosphate-buffered saline (Capricorn Scientific GmbH, Ebsdorfergrund, Germany).

2.3. MIC determination

For the determination of the MIC, serial dilutions of the antibiotics ERT (Sigma-Aldrich, St. Louis, MO, USA), LVX (Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany), and CRX (Melford Laboratories Ltd, Ipswich, UK) were tested for bacterial *E. coli* strains. The tested media were artificial urine (AU) and Müller-Hinton (MH) broth (Merck KGaA, Darmstadt, Germany). The AU used in this study was based on the formulation by Stickler et al [19]. The medium was adjusted to pH 6.1 and sterilized through a 0.22-µm pore filter (Thermo Fisher Scientific, Waltham, MA, USA). Tryptic soy broth (Oxoid Ltd.) was sterilized separately by autoclaving and was then added to the sterile basal medium to a final concentration of 10.0 g/l [19]. Each strain was tested with each antibiotic in triplicate.

MIC testing was performed with a two-fold drug dilution on a 96-well plate. The concentration ranges for AU were from 0.0156 to 512 µg/ml for ERT, from 0.125 to 470 µg/ml for LVX, and from 0.03125 to 940 µg/ml for CRX. For MH broth, the concentration ranged from

Table 1 – *E. coli* strains used in this study

Name	Source of isolation	Accession numbers	Previously published
CFT073	Urine	CP158446	[41]
536	Urine	CP158445	[41,42]
ATCC 25922	Clinical isolate	CP158442-CP158444	[43]
S115	Urine	CP158437-CP158441	[43]
ATCC BAA-2469	Urine	CP158432-CP158436	[44]
CDF6	Urine	CP158428-CP158431	[45]
NRZ14408	Wound swab	CP158424-CP158427	[43]

0.0313 to 102.4 μg/ml for ERT, from 0.016 to 58.8 μg/ml for LVX, and from 0.016 to 940 μg/ml for CRX. Two positive controls, which consisted of media and *E. coli* strain, were tested per strain. The inocula from the overnight culture was confirmed by plating and ranged from 2.36 to 6.65 × 10^7 CFU/ml per well. The optical density was measured at 600 nm photometrically after incubation at 37°C for 22 ± 2 h, and the MIC was defined as the lowest concentration inhibiting visible growth.

2.4. Influence of pH

To test the influence of pH on the MIC of selected antibiotics, the AU and MH broth were tested at unadjusted pH 6.3 and 7.2, respectively, and adjusted with hydrochloric acid to pH 5.5 or with sodium hydroxide to pH 7.0 and pH 8.0, respectively. The MIC determination tests were done as described above.

2.5. Influence of urea and NaCl

Various concentrations of NaCl (VWR, Leuven, Belgium) and urea (Sigma-Aldrich) were examined, resembling concentrations in the kidney [20]. The concentrations of NaCl tested were 40, 120, and 240 mM, while the urea concentrations tested were 80, 240, and 480 mM. To investigate the potential alteration of MIC, 10 μl of the 40, 120, and 240 mM of NaCl was added to the MIC determination test.

2.6. Influence of glucose

The influence of glucose on the MIC of antibiotics was tested in AU and in M63 minimal medium (Bio Basic Inc., Markham, Ontario, Canada) according to the manufacturer's instructions. The L(−) glucose (Sigma-Aldrich) concentration was adjusted to 1, 5, and 25 mM, and the MIC was determined as described above.

2.7. Osmolality

Osmolality was measured using an osmometer (Vogel MedTec GmbH, Fernwald, Germany). The kidney can reach osmotic pressures of 290 mOsm/kg H_2O in the cortex and 1200 mOsm/kg H_2O in the inner medulla [21,22]. To achieve these levels of osmotic pressure, different concentrations of NaCl (40, 120, and 240 mM) and urea (80, 240, and 480 mM) were added to AU and MH broth, and their osmotic pressures were determined.

2.8. Analysis of antibiotic resistance

The seven *E. coli* strains were grown as overnight cultures on an LB agar plate at 37°C in an incubator (VWR Inkuline Prime; Avantor, Radnor, PA, USA). After 16 h of incubation, one single colony was used for the analysis of antibiotic resistances with a Vitek 2 AST-N428 card (Biomerieux, Craponne, France) according to the manufacturer's instructions.

2.9. Calculation of MIC values

The calculation of MIC values for each strain and each antibiotic uses the measured optical density (OD_{sample}) at 600 nm and the optical density of the blank (OD_{blank}), as follows:

$$OD_{sample} - OD_{blank} = MIC_{sample} \text{ if } OD < 0.05$$

2.10. E. coli genome sequencing and bioinformatics

In short, bacterial genomic DNA was extracted using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) as recommended by the manufacturer. For the determination of the *E. coli* genomes, long-read sequencing from Oxford Nanopore Technologies (Oxford, UK) was used. For DNA library preparation, Native Barcoding Kit SQK-NBD114.24 (Oxford Nanopore Technologies, Oxford, UK) and corresponding supplemental materials (as advised by the manufacturer) were applied. Actual genome sequencing of the libraries was conducted with MinION Mk1B and Mk1c devices on MinION Flow Cell (FLO-MIN114, R10.4.1) as advised by Oxford Nanopore Technologies. Super high accuracy basecalling and demultiplexing were performed via Dorado (version 0.7.0) [23]. Read-set filtering was conducted with Filtlong (version 0.2.1) [24] and assembly with Flye (version 2.9.4) [25]. For genome sequence polishing, we used Medaka (version 1.12.0) [26] and reoriented genomic sequence contigs with Dnaapler (version 0.7.0) [27]. The AMR genes were detected with AMRFinderPlus (version 3.12.8) [28].

2.11. Data availability statement

Genome sequencing data have been submitted to the National Center for Biotechnology Information repository under the BioProject PRJNA1124524; the accession numbers of the *E. coli* strains are CP158446 for CFT073, CP158445 for 536, CP158442-CP158444 for ATCC 25922, CP158437-CP158441 for S115, CP158432-CP158436 for ATCC BAA-2469, CP158428-CP158431 for NRZ14408, and CP158424-CP158427XYZ for CDF6 (Table 1).

2.12. Statistical analysis

Statistical analyses were performed by Graph Pad Prism (GraphPad Prism version 10.2.3 for Windows; GraphPad Software, Boston, MA, USA; www.graphpad.com) with one-way analysis of variance or two-sided unpaired *t* test statistical test for the comparison of the individual MICs for each antibiotic and growth medium.

3. Results

The MICs of the *E. coli* strains CFT073, ATCC 25922, S115, 536, ATCC BAA 2496, CDF6, and NRZ14408 (Table 1) were investigated in AU, MH, or M63 medium.

The influence of various kidney environment mimicking parameters on the MIC values was evaluated. MIC values were assessed at different pH levels using both AU and MH broths. The impact of varying concentrations of NaCl (40, 120, and 240 mM), urea (80, 240, and 480 mM), and glucose (1, 5, and 25 mM) on MIC values was investigated. The MICs for ERT, LVX, and CRX were measured and compared (Table 2).

3.1. Influence of pH

The modulation of pH values altered the MIC for different antibiotics and *E. coli* strains (Fig. 1A). For example, at an acidic pH of 5.5, the MIC decreased for the *E. coli* strain S155 when treated with ERT in both AU and MH media (Fig. 1A). Conversely, the pH value of 8.0 led to an increase in the MIC, as was observed for the *E. coli* strain CFT073 for the antibiotic LVX in MH broth (Fig. 1A). Furthermore, a strain-specific response to the change of pH value was observed. A significant change in the MIC value for ERT within the different pH values was visible for the strain CFT073 in AU. For the strain 536, however, no significant change in the MIC for ERT was visible within the different pH values (Fig. 1A).

Significant differences with $p < 0.0001$ were found in AU (pH 6.3) for different pH conditions (pH 5.5, 7.0, and 8.0) for the bacterial *E. coli* strains ATCC 25922, S115, 536, and CDF6

Table 2 – Minimal inhibitory concentrations for the antibiotics ertapenem (ERT), levofloxacin (LVX), and ceftriaxone (CRX) in artificial urine and Müller-Hinton broth

Antibiotic	MIC (µg/mL)													
	Artificial urine							Müller-Hinton broth						
	E. coli ATCC 25922	*E. coli* CFT073	*E. coli* S115	*E. coli* 536	*E. coli* NRZ14408	*E. coli* ATCC BAA - 2469	*E. coli* CDF6	*E. coli* ATCC 25922	*E. coli* CFT073	*E. coli* S115	*E. coli* 536	*E. coli* NRZ14408	*E. coli* ATCC BAA-2469	*E. coli* CDF6
ERT	0.03	0.0156	0.5	0.0156	512	8	0.031	0.0625	0.0313	0.25	0.0313	102.4	4	0.063
LVX	0.25	0.125	470	0.125	64	96	58.75	0.016	0.0313	58.8	0.0313	8	8	3.67
CRX	0.06	0.0625	32	0.03125	235	64	940	0.0313	0.0156	8	0.0156	940	102.4	940

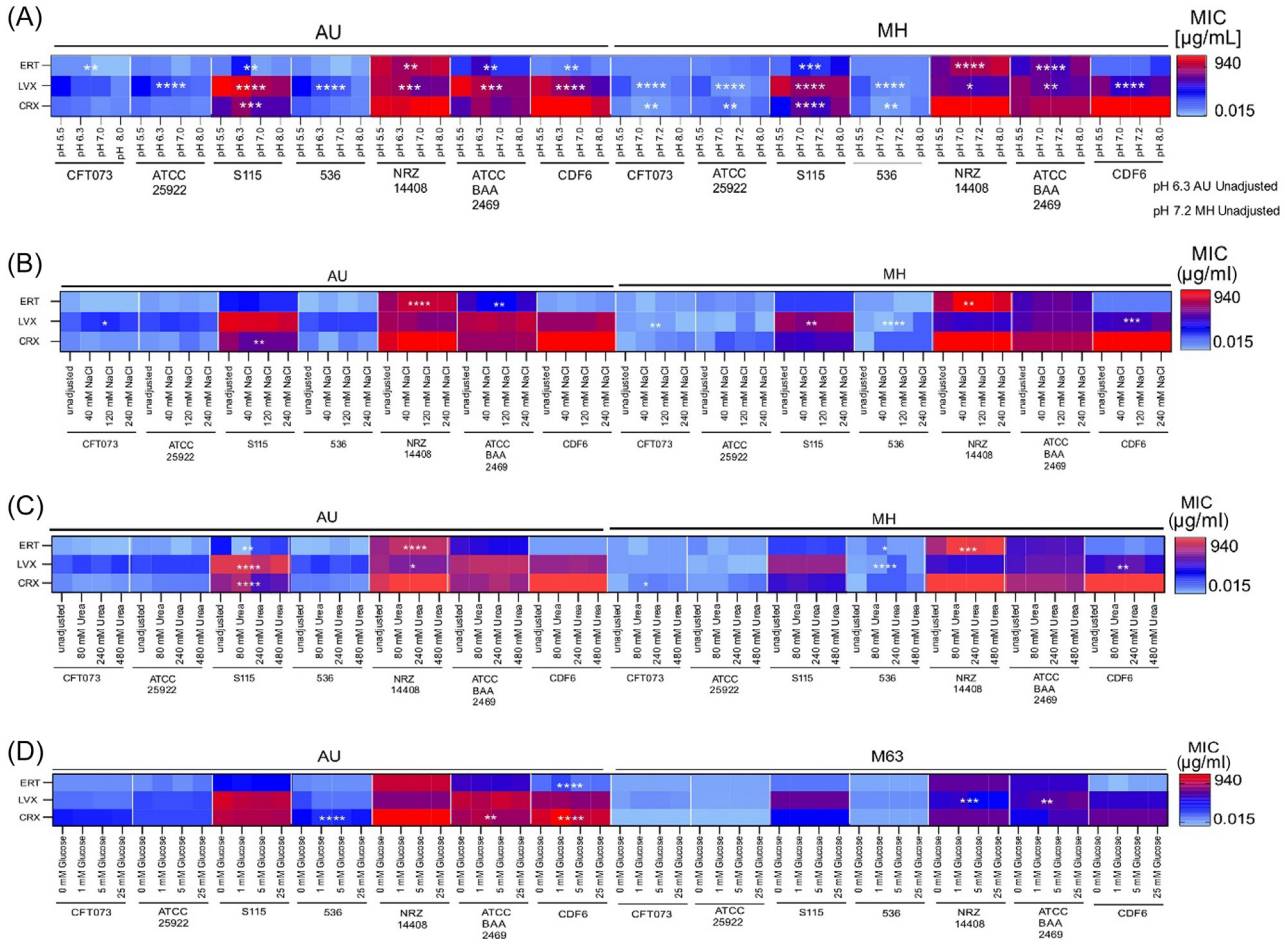

Fig. 1 – Influence of different parameters mimicking the kidney environment on the efficiency of antibiotics for *E. coli* strains in artificial urine (AU), Müller-Hinton (MH) broth, and minimal medium M63 (M63). The heatmaps display the MIC values of (A) different pH values, (B) different NaCl concentrations, (C) different urea concentrations, and (D) different glucose concentrations in AU and MH broth and M63 for different *E. coli* strains for ERT, LVX, and CRX. The darker the red color, the higher the MIC value. The lighter the blue color, the lower the MIC value. MIC values are transformed and projected in the heatmap as log10 values. CRX = ceftriaxone; ERT = ertapenem; LVX = levofloxacin; MIC = minimal inhibitory concentration; NaCl = sodium chloride. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

for the antibiotic LVX. Significant differences ($p < 0.001$) in AU with different pH values were found for the *E. coli* strains S115 for CRX, and NRZ14408 and ATCC BAA-2469 for LVX. Significant differences ($p < 0.01$) for different pH conditions in AU were found for the strains CFT073, S115, NRZ14408, ATCC BAA-2469, and CDF6 for ERT (Fig. 1A).

In MH broth, significant differences were observed for different pH conditions. Significant differences ($p < 0.0001$) were found between the unadjusted medium (pH 7.2) and MH broth with the pH values 5.5, 7.0, and 8.0 for the *E. coli* strains CFT073, ATCC 25922, 536, and CDF6 for LVX; NRZ14408 and ATCC BAA-2469 for ERT; and S115 for LVX and CRX (Fig. 1A). For ERT, significant differences ($p < 0.001$) were found for *E. coli* S115. Significant differences ($p < 0.01$) were found for the strains CFT073, ATCC 25922, and 536 for CRX, and for *E. coli* ATCC BAA-

2469 for LVX. For LVX, a significant difference ($p < 0.05$) was observed for the strain NRZ14408 (Fig. 1A).

The comparison between the individual conditions showed the antibiotic-specific differences in the influence of the nonadapted media AU (pH 6.3) and MH (pH 7.2) on the MIC for certain *E. coli* strains (Supplementary Fig. 1). For the antibiotic ERT, no significant difference between the two media is observable (Supplementary Fig. 1A). A significant difference is observable for LVX for the *E. coli* strains ATCC 25922, 536, NRZ14408, ATCC BAA2469, and CDF6 (Supplementary Fig. 1B). For CRX, a significant difference between the media AU and MH is observable for the *E. coli* strain S115 (Supplementary Fig. 1C).

3.2. Influence of NaCl and urea

The addition of NaCl and urea at different concentrations had the opposite effect on the MIC to than on the nonadapted medium alone (Fig. 1B and 1C). ERT showed a reduced MIC in AU when NaCl or urea was added, as seen for the *E. coli* strains CFT073, ATCC 25922, S115, and 536. For LVX, a strain-specific response to the addition of NaCl and urea was observed. For example, for NaCl in AU and MH broth, the strain CFT073 showed a significant difference between the MIC values for AU and MH with different pH values. When urea was added to the MIC test, a strain-specific response was visible for LVX. For example, for LVX in AU, a significantly different change of the MIC values was observed for the strains S115 and NRZ14408. For the antibiotic CRX, a strain-specific response was observed. For example, for the strain S115, a significant difference was observed in AU for the addition of urea and NaCl. For the strains NRZ14408 and CDF6, a high concentration of 940 µg/ml of CRX was measured. The addition of urea and NaCl did not change the MIC values for these two *E. coli* strains in AU and MH broth (Fig. 1B and 1C).

The addition of 240 mM NaCl to MH broth decreased the MIC value of *E. coli* NRZ14408 compared with the other NaCl additions. For LVX, a significant difference was observed for the strains CFT073, S115, 536, and CDF6 (Fig. 1B).

The comparison of the MIC values with the single addition of different concentrations of NaCl to AU showed significant differences for the strains NRZ14408 and ATCC BAA-2469 for the antibiotic ERT, for *E. coli* CFT073 for LVX, and for the strain S115 for CRX. For MH broth, significant differences were determined for the antibiotic ERT for *E. coli* NRZ14408. For NRZ14408, the addition of 40 and 120 mM NaCl to the unadjusted MH led to an increase in the MIC (Fig. 1B).

With the addition of urea to AU, significant MIC differences were observed for ERT and LVX for the strains S115 and NRZ14408. For CRX in AU, significant differences were observed for *E. coli* S115. In MH broth, significant differences were found for ERT in the *E. coli* strains ATCC 25922, 536, and NRZ14408. In LVX, significant differences were found for *E. coli* 536 and CDF6. In MH with CRX, significant differences were found for CFT073 (Fig. 1C).

3.3. Influence of glucose

The influence of glucose concentrations on the MIC in AU and M63 medium showed antibiotic- and strain-depending effects (Fig. 1D). In AU, significant differences between the MIC values were observed for ERT for the strain CDF6, and for CRX for the strains 536, ATCC BAA-2469, and CDF6. In AU, no significant difference was observed for the antibiotic LVX. In M63 medium, significant differences were observed for the antibiotic LVX for the strains NRZ14408 and ATCC BAA-2469. In M63, no significant differences were observed for ERT or CRX (Fig. 1D).

Regarding the MIC values at different glucose concentrations, significant differences were found for AU when ERT was added for *E. coli* CDF6 and when CRX was added for the strains 536, ATCC BAA-2469, and CDF6. For M63 medium, significant differences were found between the MIC values for LVX for the *E. coli* strains NRZ14408 and ATCC BAA-2469 (Fig. 1D).

3.4. Osmolality

The measured osmolality of AU was 900 mOsm/kg H_2O. The addition of different concentrations of NaCl and urea led only to a slight, but not significant, change in the osmolality to around 1000 mOsm/kg H_2O (Fig. 2). The osmolality of MH broth was approximately 300 mOsm/kg H_2O. The addition of NaCl and urea in different concentrations led only to a slight, but not significant, change in the osmolality of the medium (Fig. 2).

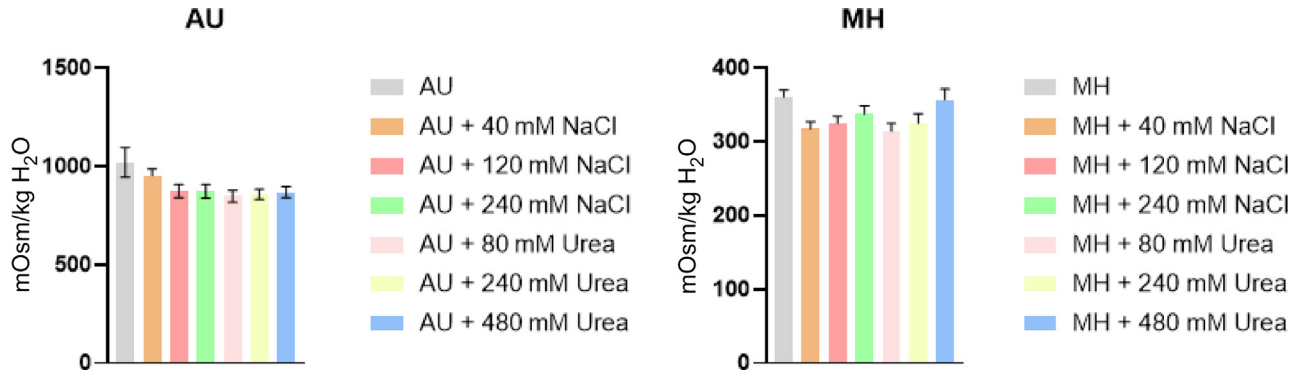

Fig. 2 – Osmolality for artificial urine (AU) and Müller-Hinton (MH) broth, and the effect of different concentrations of NaCl and urea. NaCl = sodium chloride.

Table 3 – Antibiotic resistances performed with the Vitek 2 AST for the seven *E. coli* strains

E. coli strain	ESBL	Ampicillin	Ampicillin/sulbactam	Piperacillin	Piperacillin/tazobactam	Cefuroxime	Cefuroxime-axetil	Cefotaxime	Ceftazidime	Ceftriaxone	Ertapenem	Imipenem	Meropenem	Gentamicin	Ciprofloxacin	Tigecycline	Trimethoprim/sulfamethoxazole
ATCC 25922	Neg	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
CFT073	Neg	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
S115	Neg	R	R	R	R	R	R	R	R	R	R	I	R	R	R	S	R
536	Neg	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
NRZ14408	Pos	R	R	R	R	R	R	R	I	R	R	R	R	R	R	S	S
ATCC BAA-2469	Neg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R
CDF6	Pos	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	R

ESBL = extended-spectrum beta-lactamase; I = intermediate; Neg = negative; Pos = positive; R = resistant; S = susceptible.

3.5. Analysis of antibiotic resistance

The antibiotic resistance test revealed resistances for the *E. coli* strains NRZ14408, ATCC BAA-2469, CDF6, and S115 against ampicillin, piperacillin, cefotaxime, meropenem, and gentamicin. For the antibiotic ERT, the strains S115, NRZ14408, and ATCC BAA-2469 showed resistances, while CDF6 showed susceptibility in this test. The *E. coli* strains CFT073, ATCC 25922, and 536 were shown to be susceptible to the abovementioned antibiotics including ERT and CRX (Table 3).

3.6. Genome sequencing analysis

To compare the observed antibiotic resistance phenotypes with the genotypes of the seven *E. coli* isolates, all the *E. coli* strains utilized in this study were subjected to nanopore DNA sequencing, which resulted in the complete DNA sequence of all seven isolates (Table 1).

The four LVX-resistant *E. coli* species ATCC BAA-2469, CDF6, NRZ14408, and S115 harbor mutations in the quinolone resistance–determining regions of *gyrA*, *parC*, and *parE*. Further, we found quinolone resistance genes on the plasmids of three *E. coli* species in this study. The ERT-resistant strains NRZ14408 and ATCC BAA-2469 have the plasmid borne antibiotic AMR genes for the β-lactamase blaKPC-2 and the metallo-β-lactamase blaNDM-1, respectively. The metallo-β-lactamase blaVIM-1 was present in *E. coli* S115. As resistance mechanism against cephalosporin antibiotics, we found extended-spectrum beta-lactamase blaTEM-1 for the strains NRZ14408 and S115, and broad-spectrum beta-lactamase blaCTX-M-1 for the strain CDF6. The oxacillin-hydrolyzing beta-lactamase blaOXA-1 was present in ATCC BAA-2469 and NRZ14408 (Supplementary Table 1).

4. Discussion

This study investigated seven *E. coli* strains isolated from patients' urine and wound infections to determine the MICs of three antibiotics: ERT, LVX, and CRX. The influence of different concentrations of NaCl, urea, and glucose on the antibiotics' MIC values was examined. Changes in the pH value of the medium produced heterogeneous results, depending on the *E. coli* strain and the antibiotic used. Previous studies have shown that pH can influence the activity of certain antibiotics [29,30]. For instance, a decrease in MIC for ERT at a pH value of 8.0 was demonstrated by Wagenlehner et al and Lemaire et al [30,31]. Similarly, Ordaz et al [32] reported a significant decrease in MIC and higher susceptibility of *E. coli* strains at alkaline pH for LVX. The pH of urine can influence antibiotic activity and bacterial growth, thereby affecting the therapeutic response and the choice of antibiotics for treatment [33]. Our study confirmed that pH significantly affects the MIC and effectiveness of the tested antibiotics.

Urine's high concentrations of inorganic ions and urea impose osmotic and denaturing stresses on bacterial cells [34]. The addition of NaCl (40, 120, and 240 mM) and urea (80, 240, and 480 mM) showed varied effects on MIC,

depending on the antibiotic and *E. coli* strain. NaCl and urea mimic the physicochemical environment of the kidney medulla. Michon et al [35] demonstrated that high NaCl concentrations inhibited biofilm formation and bacterial motility in *Pseudomonas aeruginosa*. Li et al [36] found that high NaCl concentrations inhibited *E. coli* growth and virulence phenotypes, such as biofilm formation, oxidative resistance, and motility, and led to reduced glucose consumption and glycogen accumulation, while increasing trehalose production . Withman et al [34] showed that osmotic stress induced by NaCl led to significant transcriptional network remodeling in *E. coli* CFT073, involving genes related to anaerobic metabolism.

Withman et al [34] found that urea addition induced the expression of genes involved in capsule formation, type 1 fimbriae, outer membrane porins, molecular chaperones, drug resistance, and ABC transporters in *E. coli* CFT073. Conversely, genes involved in acid stress and sulfur metabolism were downregulated.

The addition of glucose to AU and M63 media produced contrasting effects on the MIC of ERT, LVX, and CRX, depending on the media. This was reflected in significantly different MICs for the *E. coli* strains S115, 536, and CDF6 in AU media compared with NRZ14408 and ATCC BAA-2469 in M63 media. Glucose plays a crucial role in kidney function, including gluconeogenesis and glucose homeostasis [37]. Jiang et al [38] investigated the mechanisms of resistance in antibiotic-susceptible strains and found that glucose abundance decreased as ampicillin-sensitive strains acquired resistance. This was due to targeting the pts promoter and pyruvate dehydrogenase, promoting glucose transport and inhibiting glycolysis. These findings could explain the varying effects of ERT, LVX, and CRX on bacterial growth in the presence of glucose.

The *E. coli* strain NRZ14408 showed the greatest variation among all tested strains, which were also completely genome sequenced for investigating the antibiotic resistance gene profile. This strain, originally isolated from a wound swab, may not be as well adapted to the urinary tract's changing environmental conditions as the other urine-isolated strains. Additionally, CRX resistance was observed in the *E. coli* strains NRZ14408, CDF6, and ATCC BAA-2469. A comparative genome analysis revealed that NRZ14408 carries the *bla*KPC-2 gene, which encodes the carbapenemase KPC-2 [39,40].

These findings underscore the importance of considering kidney environmental parameters when selecting and dosing antibiotics for pyelonephritis treatment. Further research should explore additional antibiotics and environmental factors to optimize treatment strategies and improve patient outcomes.

5. Conclusions

This study highlights the significant impact of kidney environment parameters, particularly pH, on the efficacy of antibiotics against uropathogenic *E. coli* strains in the treatment of pyelonephritis. Additionally, the effects of NaCl and urea concentrations on antibiotic efficacy are both strain and antibiotic specific, underscoring the complexity of treating infections in such a variable environment. These results suggest that the physicochemical conditions within the kidney can substantially influence the success of antibiotic therapy for pyelonephritis. Therefore, it is crucial for clinicians to consider these factors when selecting and dosing antibiotics. Further research is needed to evaluate a broader range of antibiotics and additional environmental parameters, to develop a more comprehensive understanding of how the kidney environment affects antimicrobial activity. This knowledge will be vital in optimizing treatment strategies for pyelonephritis, ultimately improving patient outcomes.

***Author contributions*:** Anne-Christine Aust had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Wagenlehner.
Acquisition of data: Aust, Weigel.
Analysis and interpretation of data: Aust, Weigel.
Drafting of the manuscript: All authors.
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Appendix A. Supplementary material

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