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Effects of Urine Matrix and pH on the Potency of Delafloxacin and Ciprofloxacin against Urogenic *E. coli* (EC) and *K. pneumoniae* (KP)

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ABSTRACT

Background: Delafloxacin (DLX) is a novel fluoroquinolone (FQ) with broad spectrum *in vitro* activity against Gram-positive (GP) and Gram-negative (GN) organisms. Contrasting to the observations in other FQs, DLX has shown enhanced potency in acidic environment against GP and GN organisms. Herein, we aimed to describe the effects of the urine matrix and its varying pH on the potency of DLX and ciprofloxacin (CIP) against EC and KP, two frequently occurring GN pathogens causing urinary tract infections (UTI).

Methods: MICs in broth (MIC_{broth}) were determined for 16 urogenic strains (9 EC and 7 KP) using reference broth microdilution methods. Urine samples from patients with suspected UTIs based on urinalysis were considered for inclusion. All urines were sterile filtered, screened for antibacterial activity and assessed for pH. Corresponding MICs in 80% urine (MIC_{urine}) were determined using microdilution techniques. Change in potency was calculated as the difference in broth and urine MICs.

Results: Against highly-CIP-resistant strains (CIP MIC_{broth} ≥ 32 mg/L), DLX MIC_{broth} (mg/L) were 2 (n=1), 4 (n=3), 8 (n=3) and 16 (n=2) for EC; 8 (n=1), 16 (n=4) and 32 (n=2) for KP. Across the 143 urines collected, pH ranged from 4.7 to 9.0 with 71% at pH ≤ 6.5, 13% at pH 6.6-7.0 and 16% at pH ≥7.1. DLX MIC_{urine} (mg/L), measured in 100 unique patient samples (pH 5.0-8.3) were ≤2 (18 %), 4(23 %), 8 (21 %), 16 (23 %) and ≥32 (15 %) for *E. coli* and ≤2 (0.8 %), 4(6 %), 8 (18 %), 16 (33 %) and ≥32 (42 %) for *K. pneumoniae*. In patients with urine pH ≤5.5 (25% of frequency) and 5.6-6.0 (24% of frequency), compared with MIC_{broth}, DLX MIC_{urine} decreased by 1-3 fold doubling dilutions in 81% (48/59) and 44% (85/194) for EC; 100% (52/52) and 58% (95/164) for KP. At pH >6.0 (52% of frequency), DLX MIC_{urine} was unchanged (36% for EC and 39% for KP) or increased by 1-3 fold doubling dilutions (50% for EC and 47% for KP). In contrast, CIP MIC_{urine} remained ≥ 32 mg/L (99% for both EC and KP) in varying urine pH.

Conclusions: DLX MIC_{broth} were 2 to ≥5 fold doubling dilutions lower than CIP against these 16 urogenic Enterobacteriaceae. The majority of the urines collected from patients had pH ≤ 6.5. In contrast to CIP, DLX potency was enhanced in this acidic environment commonly observed in the UTI. Despite elevated DLX MIC_{broth}, these data suggested that the effective MIC in the biological urine matrix may be considerably lower. Clinical data are required to fully assess the impact of these observations.

INTRODUCTION

- Urinary tract infections (UTIs) are the most common bacterial infections with their clinical and economic burdens impacting both community and hospital settings. In the United States, UTIs accounted for nearly 7 million office visits and 1 million emergency department visits, which necessitated 100,000 hospitalizations annually.[1,2]
- Whereas various pathogens are responsible for UTIs, *Escherichia coli* is most commonly isolated in both uncomplicated and complicated UTIs along with other Enterobacteriaceae.[3,4]
- Delafloxacin, a novel fluoroquinolone with broad spectrum *in vitro* activity against Gram-positive and Gram-negative organisms,[5] has shown enhanced potency in acidic environment. [6]
- Given that urine pH can vary among patients with UTIs, understanding the impact of this observation on the activity of delafloxacin may provide insights about clinical implications of utilizing delafloxacin in UTI.

OBJECTIVES

- We aimed to evaluate the pharmacokinetic (PK) We aimed to describe the effects of the urine matrix and its varying pH on the potency of delafloxacin and ciprofloxacin against *E. coli* and *K. pneumoniae*.

MATERIALS and METHODS

Study design

- These were *in vitro* susceptibility studies of delafloxacin and ciprofloxacin in human urine collected from patients at Hartford Hospital, Hartford, CT. between Mar, 2014-May, 2014.
- The study was approved by the Hartford Hospital Institutional Review Board.
  - Informed consent was waived since data were collected from urine samples previously obtained as part of the standard clinical practice.

Study Population.

- All adult patients whose urine samples were collected for urinalysis and sent to the Chemistry Department as part of their clinical care were considered.
  - From this cohort, patients whose urinalyses were sent to the microbiology department for suspected infection (i.e. positive nitrite, ≥5 WBCs, or ≥ small (≥100) leukocyte esterase) were selected.

Procedure development studies

- ***Freeze-thaw-pH study***
  - To measure the freeze-thaw effect on pH, original urine sample pH of 10 patients, ranging from 4.9-7.8, were measured at the time of processing and rechecked two more times when thawed after being stored for 1 to 5 days in the -80ºC freezer.
- ***Time-pH study***
  - To assess the effect of time between the sample collection and pH measurement, original sample pH of 5 patients, ranging from 5.2-6.1, were measured at the time of processing and remeasured at 3h, 8h, 12h and 24h post-processing both at room temperature (25ºC) and under refrigeration (2-8 ºC).
- ***Broth-pH study***
  - To evaluate the magnitude of pH changes after the addition of Cation-adjusted Mueller Hinton broth (pH = 7.2; BD, Sparks, MD) to a urine sample, 12 urines with various pH (range 5.0-7.5) were selected, then their pH were remeasured after adding broth at ratios (urine:broth) of 9:1, 8:2 and 5:5.
- ***Growth study of urine and broth combination***
  - To examine if the mixture of urine to broth ratios of 9:1, 8:2 and 5:5 would support the growth of organisms, 8 Enterobacteriaceae (4 *E. coli* and 4 *K. pneumoniae)* were tested for growth in 90%, 80% and 50% urine. Three patient urines (one of pH 4.8 and two of pH 5.3) were used at each combination.
- ***Effect of pH changes by ± 0.5 on minimum inhibitory concentration (MIC)***
  - To measure the effect of small pH changes by ± 0.5 on MIC shift, delafloxacin urine MIC against 1 *E. coli* and 3 *K. pneumoniae* (delafloxacin broth MIC ranging 0.06-32 mg/L and ciprofloxacin broth MIC 1 to >16 mg/L) were measured at pH 5.5 and 5.5 ±0.5; pH were adjusted by 0.1 N hydrochloric acid or 0.1 N Sodium bicarbonate.

Urine sample processing

- Clinical samples collected during the evening and night shift (3 pm-7 am) were refrigerated (temperature 2-8ºC) until processed.
- Samples were filter sterilized, reassessed for sterility via culture, antibacterial effects, and pH as follows.
  - Filtering: samples were centrifuged at 1,000 x g for 10min at 25ºC; then, nonsterile-filtered first with 0.45 µm PVDF syringe filter (Millex, Merck Millipore Ltd, Tullagreen, Carrigtwohill, Co. Cork, IRL); secondly, sterile-filtered with 0.22 µm PVDF syringe filter (Millex-GV, Merck Millipore Ltd, Tullagreen, Carrigtwohill, Co. Cork, IRL).
  - To assure samples were adequately sterilized, 10 µl of the sample was plated on trypticase soy agar with 5% sheep blood (BAP, BD BioSciences).
  - To assess if there is antibacterial effect of the urine sample 10 µl was plated on BAP after lawn-plating *E. coli* ATCC 25922 of 1x10[8] CFU/ml.
  - All plates were incubated at 37ºC for 16-20 hours.
  - pH of the urine was checked using a pH meter (PerpHecT LogR Orion pH meter, Model 320, Thermo Fisher Scientific, Beverly, MA) and a gel-filled pH electrode (Model 911600, Thermo Fisher Scientific, Beverly, MA) according to the manufacturer's instructions.[7]
  - All samples were stored at -80ºC freezer.
- Urine samples that had insufficient volume, antibacterial effects, or did not support bacterial growth were not utilized for MIC testing.

Data collection

- Patients' demographics including: age, gender, location, and the results of urinalysis were collected from the medical record.
- On the day of urine MIC testing, the pH of 80% urine (urine:broth=8:2) was recorded for data analysis.

Antimicrobial test agents

- Analytical grade delafloxacin meglumine (ScinoPharm Taiwan, Ltd, Lot: 71263AA002, Shan-Hua, Tainan, Taiwan) was provided by Melinta Therapeutics (New Haven, CT, USA).
- Analytical grade ciprofloxacin was purchased from Sigma-Aldrich (Lot: P500044, Laramie, WY, USA).
- Analytical powders were weighed in a quantity sufficient to achieve the desired concentrations and reconstituted immediately prior to making MIC trays.

Bacterial isolates

- A total of 16 urogenic Enterobacteriaceae (5 *E. coli* and 7 *K. pneumoniae)* were studied; 4 of the *E. coli* isolates were collected during the study period from the Microbiology Department at Hartford Hospital.

Susceptibility testing in broth

- The broth MICs of delafloxacin and ciprofloxacin were determined in triplicate using the broth microdilution methodology as outlined by the Clinical and Laboratory Standards Institute (CLSI).[8]
- Modal MICs from all experiments were reported.

Susceptibility testing in urine

- The urine MICs of delafloxacin and ciprofloxacin were determined using the microdilution methodology per CLSI;[8] the trays were made of 80 µl of patient specific urine and inocula were made in 20 µl of broth.
- For each urine MIC study, 4 to 7 unique patients' urines became a set and broth MICs were repeated with each set to establish baseline MICs at pH 7.2.

Data Analyses

- The MIC change from broth to urine for delafloxacin and ciprofloxacin were calculated as {Log_2(MIC in urine) – Log_2(MIC in broth)}.
- Linear regression analysis (SigmaPlot 12.5, Systat Software, Inc., San Jose, CA) was performed to characterize the relationship between pH vs MIC change for all *E. coli* and all *K. pneumoniae*.

RESULTS

Study Population.

- Across the 143 urines collected, measured pH ranged from 4.7 to 9.0 with 71% at pH ≤ 6.5, 13% at pH 6.6-7.0 and 16% at pH ≥7.1 (Figure 1-2). 77% of measured pH were within ±0.5 from the pH reported from chemistry lab.

Protocol development studies

- ***Freeze-thaw-pH study***
  - Two out of 10 patients had ± 0.2 change in pH, but the rest had no greater than ± 0.1 change in pH, suggesting freeze-thaw has little effect on pH.
- ***Time-pH study***
  - Either at room temperature or refrigerated, all measured pH remained ± 0.1 from original sample pH over 24h.
- ***Broth-pH study***
  - Across 12 patient urines tested, the changes in pH in respective urine to broth combination were as follows: ≤ 0.2 at 9:1, ≤ 0.3 at 8:2, ≤ 0.5 at 7:2 and ≤ 0.8 at 5:5, respectively.
- ***Growth study of urine and broth combination***
  - Based on broth-pH study, growth experiments were performed in 80% and 90% urine to minimize the shift in pH as well as in 50% as a comparison. In 50% urine, all 8 Enterobacteriaceae tested grew in all 3 urines. However, in 9:1 and 8:2 urine to broth combination, positive growth rate reduced to 66% and 79%, respectively. Based on the results from broth-pH and growth studies, urine MIC studies were done in 80% urine.
- ***Effect of pH change by ± 0.5 on MIC study***
  - Across 4 Enterobacteriaceae tested, 87.5% showed no shift in MICs over the change in pH by ± 0.5. The remaining 12.5% showed 1 double-dilution shift. Therefore, during MIC testing in 80% urine, pH was not readjusted back to original 100% urine pH.

Bacterial isolates.

- Broth MICs of the 16 urogenic Enterobacteriaceae (9 *E. coli* and 7 *K. pneumoniae*) are summarized in Table 1.

Table 1. Broth MICs (mg/L) of 16 Enterobacteriaceae

Isolate number	Delafloxacin	Ciprofloxacin	Known Resistance Mechanism
E. coli 443*	2	64	ESBL
E. coli 348	4	>64	ND
E. coli 429*	4	64	ESBL
E. coli 435*	4	64	ND
E. coli 310	8	>64	ESBL
E. coli 350*	8	>64	ND
E. coli 432*	8	64	ESBL
E. coli 130	16	64	ESBL
E. coli 133	16	64	ESBL
*K. pneumoniae*368*	8	32	KPC
K. pneumoniae 331A	16	>64	KPC
*K. pneumoniae*350B*	16	>64	KPC
*K. pneumoniae*369*	16	>64	KPC
K. pneumoniae 378	16	>64	KPC
K. pneumoniae 320	32	>64	ESBL
K. pneumoniae 390	32	>64	KPC

ESBL: extended spectrum beta lactamases; KPC: *Klebsiella pneumoniae* carbapenemases; *isolates used when urine volume <40 ml; ND: no data

- 92 patients with urine volume > 40 ml were tested for 9 *E. coli* and 7 *K. pneumoniae* while 8 patients with urine volume 20-40 ml were tested for 5 *E. coli* and 3 *K. pneumoniae*.

Susceptibility testing in urine

- Demographic data for the 100 patients included in the urine MIC studies are presented in Table 2.

Table 2. Patient Characteristics (n=100)

Age	Median (Range, years)	58 (18->89)
Gender	Female (n)	83
Location	ICU, Inpatient (n)	9
	Non-ICU, Inpatient (n)	57
	ED (n)	34
Urinalysis (Criteria for reflex to culture)	Positive Nitrite (n)	26
	≥5 WBC in the microscopic exam (n)	90
	≥100 (≥small) Leukocyte esterase (n)	88

ICU: intensive care unit; ED: emergency department; WBC: white blood cell

- The shift in MICs from broth to urine for delafloxacin and ciprofloxacin are displayed in Figure 1 for all *E. coli* (A: delafloxacin, B: ciprofloxacin) and in Figure 2 for all *K. pneumoniae* (A: delafloxacin, B: ciprofloxacin).
- The magnitudes of MIC change were similar between *E. coli* and *K. pneumoniae*.
- Linear regression shows r^2=0.554 (p< 0.0001) with all *E. coli* and r^2=0.495 (p<0.0001) with all *K. pneumoniae* for delafloxacin; r^2=0.085 (p< 0.0001) with all *E. coli* and r^2=0.066 (p<0.0001) with all *K. pneumoniae* for ciprofloxacin.
- Delafloxacin urine MICs (mg/L), measured in 100 unique patient samples (pH 5.0-8.3) were ≤2 (18 %), 4(23 %), 8 (21 %), 16 (23 %) and ≥32 (15 %) for *E. coli* and ≤2 (0.8 %), 4(6 %), 8 (18 %), 16 (33 %) and ≥32 (42 %) for *K. pneumoniae*.
- In contrast, ciprofloxacin urine MICs (mg/L) mostly remained ≥32 (99%) for both *E. coli* and *K. pneumoniae*.

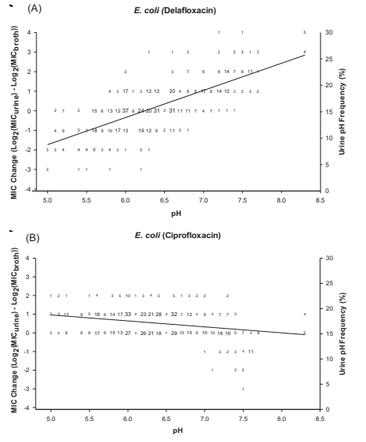

Figure 1. Influence of urine matrix on delafloxacin (A) and ciprofloxacin (B) MICs. Shaded area represents frequency of urinary pH. Numerical values denote the number of observations from 9 *E. coli* tested in 100 urines of patients with linear regression line.
(A: MIC Change=-8.67+1.39*pH, r^2=0.554; B: MIC Change=2.56-0.32*pH, r^2=0.085)

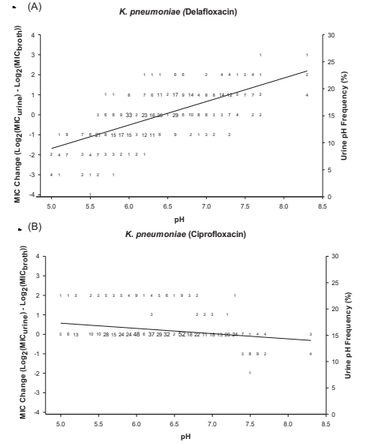

Figure 2. Influence of urine matrix on delafloxacin (A) and ciprofloxacin (B) MICs. Shaded area represents frequency of urinary pH. Numerical values denote the number of observations from 7 *K. pneumoniae* tested in 100 urines of patients with linear regression line.
(A: MIC Change=-7.56+1.18*pH, r^2=0.495; B: MIC Change=1.91-0.27*pH, r^2=0.066)

CONCLUSIONS

- Using standard MIC methods, delafloxacin broth MICs were 2 to ≥5 fold doubling dilutions lower than ciprofloxacin against these 16 urogenic Enterobacteriaceae.
- The majority of urines collected from patients suspected of infection had pH ≤ 6.5.
- In pH ≤ 6.0, delafloxacin potency was enhanced by median of one doubling dilution for both *E. coli* and *K. pneumoniae*.
- In contrast, ciprofloxacin potency remained relatively unchanged in varying urine pH.
- Despite elevated delafloxacin broth MIC, these data suggested that the effective MIC in the biological urine matrix may be considerably lower. Clinical data are required to fully assess the impact of these observations.

ACKNOWLEDGEMENTS

We thank Mary Anne Banevicius, Henry Christensen, Jennifer Hull, Lucinda Lamb, Sara Robinson, Debora Santini, Christina Sutherland and Kim Greenwood for their assistance with the urine sample processing and *in vitro* MIC testing. Chemistry Department and Microbiology department in Hartford hospital are thanked for collecting urine samples and clinical urogenic isolates, respectively.

This study was supported by Melinta Therapeutics (New Haven, CT, USA).

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