

Evaluation of Quinolone Resistance in *Escherichia coli* Isolates Recovered from Urine and Feces of Patients with Acute or Recurrent Urinary Tract Infection

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ABSTRACT

Introduction: Antibiotic resistance, especially in Gram-negative uropathogens such as *Escherichia coli*, is the main barrier to treat urinary tract infection (UTI). In recent years, the dramatically increased resistance of *E. coli* to quinolones, a group of widely used antibiotics, has become a significant concern. **Methods:** In this descriptive cross-sectional study, we collected 261 *E. coli* isolates from the urine and stool samples of patients, referred to or hospitalized at Loghman hospital in Tehran, Iran, with either acute or recurrent UTI. The susceptibility testing for quinolones was performed by the disk diffusion method according to the recent protocols. **Results:** The frequency of resistant *E. coli* isolates was higher against nalidixic acid than ciprofloxacin and norfloxacin (67.8% vs. 48.7% and 44.1% respectively). When comparing acute and recurrent phases of UTI, in the urine samples, no significant difference was seen in the frequency of resistant isolates against nalidixic acid and norfloxacin, while this frequency against ciprofloxacin was significantly higher in recurrent UTI (68% vs. 48.2%). However, in the stool samples, the frequency of resistant isolates against nalidixic acid was higher in recurrent UTI (77.1% vs. 55.7%), while no significant difference was seen against ciprofloxacin and norfloxacin in these phases. **Conclusion:** Regarding the antibiotic type and frequency of the administration, the resistance pattern of *E. coli* to quinolones seems to differ in acute and recurrent phases of UTI.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections worldwide [1], and uropathogenic *Escherichia coli* (UPEC) is the primary pathogen responsible for it [2]. Currently, according to the World Health Organization (WHO), the prevalence of antibiotic resistance among Gram-negative bacilli is dramatically on the increase [3]. This increase has also been observed in UPEC isolates, which in turn has led to increased mortality and morbidity rates among UTI patients [4], and has caused serious health problems, especially in the care units of the hospitals [5]. Quinolones are one of these antibiotics that resistance against them has increased since the 1990s, mostly due to excessive use of them [6]. These combinations are one of the most crucial groups of antibiotics used in the treatment of various infections, such as septicemia [7], and resistance to them poses severe clinical consequences.

Quinolones are broad-spectrum antibiotics that affect a wide range of bacteria and are used both in humans and animals. The high serum level, high feed uptake, relatively low side effects, and ease of use are the factors that put these antibiotics as the first-line treatment in many infections, especially UTIs [3]. George Lashar and colleagues (1962)

first introduced quinolones [8], and nalidixic acid was the first quinolone used for the treatment of uncomplicated UTI caused by enteric bacteria in 1962. However, a rapid resistance to this antibiotic [8], led to an interruption in the use of these compounds until the early 1980s, when the second generation came into the market. The new drugs displayed a considerably improved activity against gyrase and greater penetration into Gram-positive organisms [8, 9].

The fundamental changes to the quinolone skeleton led to the production of fluoroquinolones (norfloxacin in 1986 and ciprofloxacin in 1987). Having a broad spectrum of activity against both Gram-negative and positive bacteria, these antibiotics are widely used to treat clinical infections worldwide. They have very attractive *in vitro* activities against the community and nosocomial pathogens, as well as improved pharmacokinetics and pharmacodynamics properties [10-13]. Oral administration is another advantage of these antibiotics [14]. However, there are still restrictions for using norfloxacin for the treatment of UTI and sexually transmitted diseases due to low serum levels and poor tissue penetration [10-13]. Ciprofloxacin is used in the empirical treatment of UTI, especially when *E. coli* is the causative

pathogen. However, the resistance rate of *E. coli* isolates to ciprofloxacin has increased in recent years, e.g., the resistant rate has increased from 0.85% to 10.2% in different regions of Iran [15-18], and from 14.8% to 59.4% in other countries [19-21].

The basis for the proper treatment of UTI is the selection of appropriate antibiotics with high efficacy. Antibiotic resistance in uropathogens (especially Gram-negative bacteria such as UPEC) is one of the main barriers to the definitive treatment of UTI [22]. However, the UTI phase (acute or recurrent) as well as the source of infection (intestinal or urinary system), especially in the recurrent phase, plays an essential role in the antibiotic resistance pattern of uropathogens, which in turn defines the proper antibiotic [23]. Identification of this pattern against antibiotics such as quinolones in different phases of UTI is of high importance.

In the present study, we investigated the resistance patterns of quinolones in *E. coli* isolates originated from urine and stools samples of patients with either acute or recurrent UTI.

MATERIAL AND METHODS

Sample collection. In this cross-sectional study, *E. coli* isolates from patients with acute/recurrent UTI attending Loghman Hospital of Tehran from April 2016 to May 2017 were investigated. The Ethics Committee of Research Center of Pasteur Institute of Iran approved this study. Informed written consent was obtained from the patients or their guardians, and a questionnaire, including the information such as age, gender, and history of UTIs and antibiotic administration was filled in for each patient.

Fresh midstream urine samples from patients with signs and symptoms of UTI (dysuria, frequency, and urgency, as well as fever) were cultured on MacConkey and Blood agar (Merck, Germany) media and incubated at 37°C for 24 h. The samples with a colony count equal to or greater than 10⁵ CFU/ml of urine were considered a positive culture. Then, the stool samples of the patients with positive urine culture were cultured on Trypticase soy agar (Merck, Germany) with 5% sheep blood and MacConkey agar media. The cell and colony morphology and Gram stain reaction were examined along with different biochemical tests such as SIM, MRVP, Simmons' citrate, urease, and TSI. The standard strains of *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used as positive and negative controls, respectively. After verification of the *E. coli* isolates, 2-3 dominant colonies and all morphologically different colonies (a total of 10 colonies) from each plate of urine and stool samples were isolated according to previous studies [24]

Antimicrobial susceptibility test. Antibiotic susceptibility test for three antimicrobial agents was carried out by Kirby-Bauer disc diffusion method (Mast Disc, United Kingdom) on selected *E. coli* isolates according to the Clinical and Laboratory Standards Institute 2017 guideline, using nalidixic acid (NA, 30 mg/ml), ciprofloxacin (CIP, 5 mg/ml), norfloxacin (NOR, 10 mg/ml). The strain *E. coli* ATCC 25922 was used as quality control.

Statistical analysis. The SPSS software (version 23) was utilized for statistical analysis. Results were considered significant at $\alpha=0.05$. Chi-square and Fisher's exact tests were used to compare resistance patterns of different quinolones between the acute and recurrent phases of UTI in either urine or stool samples.

RESULTS

Clinical data. A total number of 56 UTI patients (27 acute and 29 recurrent cases) were selected in whom UPEC was the causative agent. The patients included both genders with the female/male ratio of 3 and the mean age of 64 ± 17.5 years. The ratio of acute to recurrent cases in patients under 70 years old was 0.7 (12 vs. 17), while in patients in their seventh decade of life was 1 (5 vs. 5) and in older patients was 1.4 (10 vs. 7). While female patients were mostly in the recurrent phase of UTI (62% of cases), most men were in their acute phase (79% of cases). Seventy percent of the cases were out-patients. Around 65% (11 out of 17) of in-patients aged more than 80 years old (Table 1).

Antibiotic resistance pattern. Based on the previous studies, 261 *E. coli* isolates from colonies of both urine and stool cultures of each patient were selected. The distribution of antibiotic resistance among the isolates, concerning the patients' gender and type of the hospital admission, revealed more resistance among the isolates from females and out-patients compared to ones from males and in-patients (Table 2).

In general, the isolates were mostly resistant to nalidixic acid (67.8%), followed by ciprofloxacin (48.7%) and norfloxacin (44.1%; Table 3). When the isolates were grouped based on the source of the samples (urine and stool) or the phase of UTI (acute and recurrent), the same order was observed. As expected, the resistance rates against all three quinolones in the isolates obtained from urine samples were higher against nalidixic acid (74.5%), ciprofloxacin (57.5%) and norfloxacin (53.8%) compared to stool samples (Table 3). Similarly, the frequency of resistant isolates, especially against nalidixic acid, was higher in patients with recurrent UTI rather than patients with acute UTI (72.2% vs. 63.7%; Table 3).

On the other hand, subgrouping the isolates based on the source (urine or stool) in the acute or recurrent UTI groups showed that in both groups, the isolates from urine were more resistant to all three quinolones than those from stool (Fig. 1). Moreover, concerning acute and recurrent UTI cases, the resistance rate against nalidixic acid was similar in the UPEC isolates derived from urine samples (75% vs. 74%), but higher in recurrent phase in the *E. coli* isolates obtained from stool samples (71.1% vs. 55.7%, Fig. 1A). However, the same comparison in the case of ciprofloxacin showed an opposite pattern, i.e., while the resistance rate in the UPEC isolates of urine samples was significantly higher in recurrent UTI (68% vs. 48.2%), in the *E. coli* isolates of stool samples, there was no significant difference between these two phases (Fig. 1B). Interestingly, the frequency of the intermediate state was significantly more in ciprofloxacin than two other antibiotics (Fig. 1). Moreover, when considering norfloxacin, there was no significant difference

between different phases in the resistance rate of the *E. coli* isolates derived from either urine or stool samples (Fig. 1C). The isolates were significantly more susceptible to norfloxacin than the other two antibiotics (Fig. 1).

Finally, when comparing the frequency of the isolates

based on the resistance to none, one, two, or all the quinolones (Table 4), the most common pattern was resistance to all three antibiotics (~ 40% of isolates). On the other hand, the resistance pattern, whatever it was, had a uniform distribution among the isolates from different phases.

Table 1. Distribution of patients according to different characteristics

Category	Sub-category	No. (%)	
Phase of UTI			
Acute		27 (48%)	
	Female	16 (59%)	
	Male	11 (41%)	
	Out-patient	20 (74%)	
	In-patient	7 (26%)	
	< 70 y	12 (44%)	
	70-79 y	5 (19%)	
Recurrent	≥ 80 y	10 (37%)	
		29 (52%)	
	Female	26 (90%)	
	Male	3 (10%)	
	Out-patient	19 (66%)	
	In-patient	10 (34%)	
	< 70 y	17 (59%)	
70-79 y	5 (17%)		
≥ 80 y	7 (24%)		
Gender			
Female		42 (75%)	
	Acute	16 (38%)	
	Recurrent	26 (62%)	
	Out-patient	31 (74%)	
Male	In-patient	11 (26%)	
		14 (25%)	
	Acute	11 (79%)	
	Recurrent	3 (21%)	
Out-patient		8 (57%)	
	In-patient	6 (43%)	
	Age		
	20-39 y		7 (13%)
Out-patient		5 (71%)	
40-59 y	In-patient	2 (29%)	
		15 (27%)	
60-79 y	Out-patient	14 (93%)	
	In-patient	1 (7%)	
> 80 y		17 (30%)	
	Out-patient	14 (82%)	
In-patient		3 (18%)	
		17 (30%)	
Out-patient		6 (35%)	
	In-patient	11 (65%)	
Type of hospital admission			
Out-patient		39 (70%)	
In-patient		17 (30%)	

Table 2. Number and percentage of the isolates resistant to each antibiotic based on characteristics of patients

Antibiotic	Number (%) of isolates	Gender		In/out patient	
		Female	Male	In-patient	Out-patient
Nalidixic acid	177 (76.8%)	126 (71.2%)	51 (28.8%)	63 (35.6%)	114 (64.4%)
Ciprofloxacin	127 (48.7%)	87 (68.5%)	40 (31.5%)	50 (39.4%)	77 (60.6%)
Norfloxacin	114 (43.7%)	72 (63.2%)	42 (36.8%)	50 (43.9%)	64 (56.1%)
Total	261 (100%)				

Table 3. Distribution of the isolates in different sources and different phases of UTI based on their resistance to each antibiotic

Sources of isolates/Phases of UTI		Antibiotics			Total
		Nalidixic acid	Ciprofloxacin	Norfloxacin	
Sources	Urine	79 (74.5%)	61 (57.5%)	57 (53.8%)	106
	Stool	98 (63.2%)	66 (42.6%)	58 (37.4%)	
UTI phase	Acute	86 (63.7%)	61 (45.2%)	58 (43%)	135
	Recurrent	91 (72.2%)	66 (52.4%)	57 (45.2%)	
Total		177 (67.8%)	127 (48.7%)	115 (44.1%)	261

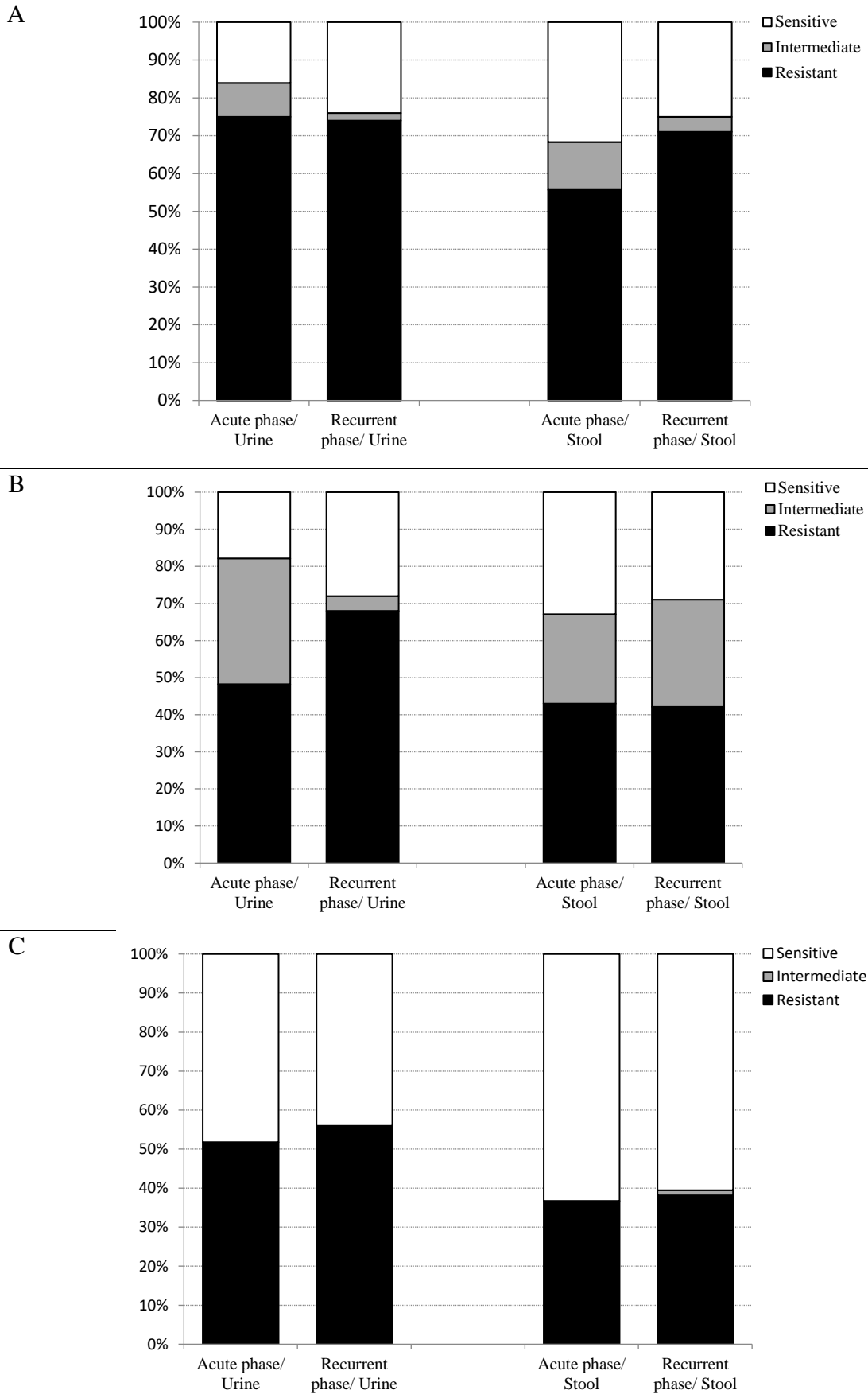


Fig. 1. Comparison between antibiotic resistance patterns in different phases of UTI among the isolates obtained from urine and stool. The patterns are illustrated separately for each antibiotic. A. nalidixic acid, B. ciprofloxacin, C. norfloxacin.

Table 4. Distribution of the isolates based on the number and pattern of the antibiotics they were resistant to, in different phases of UTI

Number of Resistance and Name of Antibiotics	Total Number of isolates	Recurrent			Acute		
		Total	Urine	Stool	Total	Urine	Stool
0	48 (18.4%)	26 (54.2%)	8 (30.8%)	18 (69.2%)	22 (45.8%)	7 (31.8%)	15 (68.2%)
1	67 (25.7%)	33 (49.3%)	13 (39.4%)	20 (60.6%)	34 (50.7%)	12 (35.3%)	22 (64.7%)
NA	59 (88.1%)	30 (90.9%)	13 (43.3%)	17 (56.7%)	29 (85.3%)	8 (27.6%)	21 (72.4%)
CIP	7 (10.4%)	2 (6.1%)	0	2 (100%)	5 (14.7%)	4 (80%)	1 (20%)
NOR	1 (1.5%)	1 (3%)	0	1 (100%)	0	0	0
2	19 (7.3%)	11 (57.9%)	2 (18.2%)	9 (81.8%)	8 (42.1%)	3 (37.5%)	5 (62.5%)
NA/CIP	9 (47.4%)	4 (36.4%)	0	4 (100%)	5 (62.5%)	2 (40%)	3 (60%)
NA/NOR	4 (21%)	2 (18.2%)	2 (100%)	0	2 (25%)	0	2 (100%)
CIP/NOR	6 (31.6%)	5 (45.4%)	0	5 (100%)	1 (12.5%)	1 (100%)	0
3 (NA/CIP/NOR)	104 (39.8%)	Total	27 (54%)	23 (46%)	54 (51.9%)	27 (50%)	27 (50%)

DISCUSSION

UTI is one of the most common infections in both hospitals and communities, making it a significant cause of mortality, morbidity, and treatment costs. UPEC is the most common cause of UTI [25]. Due to the high resistance to the beta-lactam antibiotics family, quinolones have become one of the most commonly prescribed groups used to treat UPEC strains in UTI. However, in recent years, excessive use of either oral or parenteral fluoroquinolones for UTIs and other infections has increased the resistance rate to them which has made treatment decisions difficult and, in some cases, alternative antibiotics are prescribed [26, 27]. In this study, we evaluated the resistance of *E. coli* isolates from urine and feces of patients with either acute or recurrent UTI against quinolones, the antibiotics commonly used in Iran in recent years.

In general, resistance against nalidixic acid was higher than two other fluoroquinolones, which is in line with many previous studies in Iran and other countries [21, 28-32]. In Shiraz, south of Iran, 75% of *E. coli* isolates, causing UTI in children, were resistant to nalidixic acid [33]. Similar results are available from different parts of the country. The nalidixic acid resistance has been on the increase in recent years, e.g., in Hamedan, western part of Iran, between 2010 and 2011 the resistance rate was 40.9% [34], while in 2017 in Rasht, north of Iran, the resistance rate among *E. coli* isolates had increased to 61.9% [35]. Such an increased resistance might be a consequence of extreme use of this antibiotic in recent decades especially for the treatment of UTI [15-18], a problem that has led to the limitation of its prescription as UTI antibiotic therapy in some regions of the world.

Considering the resistance rate against nalidixic acid, the isolates derived from urine and stool showed two different patterns when comparing acute with recurrent UTI. The similarity of the resistance rate in urine might be explained by the fact that due to excessive use of this antibiotic, UPEC isolates have already become resistant, regardless of the UTI phase [36]. Meanwhile, two different hypotheses might come to the mind for explaining the higher resistance rate in the recurrent phase among the *E. coli* isolates obtained from stool; 1) the *E. coli* isolates in gut microbiota might have become resistant to the antibiotic following the first prescription during acute phase of UTI [4], 2) the resistant UPEC isolates that remain intact in urinary tract as a

reservoir for the recurrent infection might have reached gut through blood circulation and transferred the resistance genes to the susceptible strains [37].

In the present study, the resistance to fluoroquinolones (ciprofloxacin and norfloxacin) was significantly lower than nalidixic acid. [34, 38], while in our previous study, the UPEC isolates from the urinary catheters of the patients without UTI were highly resistant to ciprofloxacin [39]. This result is in agreement with the previous reports from Iran; two studies in 2014 and 2015 from Qazvin and Zanjan, north and northwest of Iran reported 56% and 55.5% of resistance to ciprofloxacin and norfloxacin, respectively among *E. coli* isolates obtained from the clinical sample of UTI patients [40]. The results from other parts of Iran exhibited almost similar rates, with an increasing trend in the resistance rate. For instance, in the Sedighi and colleagues study (2010-11) the resistance rate was 15% for both antibiotics, which is significantly lower than the recent studies [34].

Ciprofloxacin was almost in contrast with nalidixic acid according to its resistance pattern in urine and stool when comparing acute with the recurrent phase of UTI. In urine samples, the higher frequency of resistant isolates in the recurrent phase could be explained by this hypothesis that the isolates, which are not resistant to ciprofloxacin in acute UTI, would become resistant to it after its administration and before acting as a reservoir for recurrent infection, especially more in relapse rather than re-infection [41]. This hypothesis is supported by this fact that in our study, the *E. coli* isolates were in the intermediate state to ciprofloxacin significantly more than the other two antibiotics, which means they are shifting from the susceptible state, but not reaching yet to the resistant state.

Compared to the other quinolones, norfloxacin showed a different resistance pattern among the isolates from different sources and phases. This difference might be explained by the fact that norfloxacin is not a drug of choice for the treatment of UTI in the clinic [36]. Though, there were predictably more susceptible isolates in stool than urine. Finally, the high frequency of the isolates which were resistant to all three quinolones shows that when the resistance occurs, it might be mainly against all the quinolones [36].

According to our knowledge, this study is the first one in the region that has collected, at the same time, both urine and

stool samples of patients suffering from either acute or recurrent UTI. In general, our results are in the line with previous studies, showing that the resistance to antibiotics, especially these selected ones, is growing. Our finding emphasizes the concern on the use, overuse, and abuse of antibiotics, specifically quinolones, which have been drugs of choice for the treatment of some significant infections. Moreover, our results show that each antibiotic has a unique resistance pattern in different phases of infection like UTI which should be concerned as well.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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