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Prevalence, Phenotypic Characterization and Antibiogram of Uro-pathogenic *Escherichia coli* among Patients attending a Tertiary Institution Teaching Hospital in Katsina Metropolis, Nigeria

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ABSTRACT: The objective of this paper was to investigate the prevalence, phenotypic characterization and antibiogram of uro-pathogenic *Escherichia coli* (UPEC) among patients attending a Tertiary Institution Teaching Hospital in Katsina Metropolis, Nigeria using appropriate standard methods with one hundred and fifty (150) urine samples. Data obtained reveals that 78(52%) yielded growth with 25(16.67%) UPEC prevalence from the study population. The highest prevalence based on age was observed in the age group 31-40 years (35.9%). Based on gender, females were observed to have high UTIs prevalence (55.55%) than males. UPEC isolates were found to be highly susceptible to Nitrofurantoin and Imipenem while notable UPEC resistance were observed with ERY, CAZ, CRX, and AMP. Multiple drug resistant index (MARI) ranged from 0.3 to 0.7 in FTHK patients indicating the existence of high multi-drug resistance UPEC. Routine surveillance on UPEC among UTI patients is recommended.

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A common bacterial infection, urinary tract infections (UTIs) are estimated to cause 150 million cases globally (Da Cruz Campos, 2020) and 400 million cases with 230,000 fatalities (Whelan *et al.*, 2023). One of the main causes of UTIs is Uro-pathogenic *Escherichia coli* (UPEC) (Islam *et al.*, 2024). Between 70 and 90 % of UTIs are caused by Uro-pathogenic Escherichia coli (UPEC), which presents a serious healthcare challenge because of the rise in antibiotic resistance (Larramendy *et al.*, 2020). The use of antibiotics is essential to reducing infection-related mortality and morbidity (Adenipekun *et al.*, 2016). In recent times, there has been a notable rise in antimicrobial resistance in Uropathogenic *Escherichia coli* (UPEC) (Gibreel, 2011). According to a 2019 Nigerian study by Onifade and Agunloye, the number of infections brought on by bacteria resistant to several drugs is rising, posing a severe risk that requires immediate attention. If antibiotic resistance is not addressed, it is estimated that its global economic impact will reach approximately $1 trillion by 2050 (Schoepp *et al.*, 2017).

Countries known as MINT (Mexico, Indonesia, Nigeria, and Turkey) and BRIC (Brazil, Russia,

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India, and China) will be particularly affected (Tomy *et al.,* 2020). Antibiotic stewardship, better patient outcomes, and quick assessment of antibiotic susceptibility, according to experts, may help combat the threat (Schoepp *et al.,* 2017). The emergence of multi-drug resistant bacteria from a variety of sources, including humans and animals, has been the main focus of numerous recent studies (Islam *et al.,* 2024). The kidneys, ureters, bladder, and urethra comprise the urinary tract (Katarzyna, 2017). Tiny tubules known as ureters carry urine from the kidney to the bladder. The bladder is a balloon-like organ that sits above the prostate gland in men and in the pelvis of women. The urethra is the tube that permits urine to exit the bladder (Katarzyna, 2017). UTIs are divided into two categories based on the anatomical classification of the urinary tract. According to Smelov *et al.* (2016) the first group comprises the lower urinary tract, which includes the bladder (cystitis) and urethra (urethritis). The kidney (pyelonephritis) and ureter (ureteritis) are included in the second category, which is the upper urinary tract (Smelov *et al.,* 2016). The symptoms of upper and lower urinary tract infections differ. Fever, backache, and nausea are common symptoms of pyelonephritis, whereas dysuria, urgency in voiding, nocturia, suprapubic pain, and hematuria are common symptoms of cystitis (Kumar *et al.,* 2015). Additionally, the prostate (prostatitis) or the epididymis (epididymitis) may be affected in some cases of lower UTIs in men (Adolfsson *et al.,* 2018). *Escherichia coli*, a common resident of the mammalian intestinal tract, is commonly the cause of bacteremia, urinary tract infections (UTIs), and intestinal infections in people of all ages (Adenipekun *et al.,* 2016). Antimicrobials like aminoglycosides, beta-lactams, cephalosporins, fluoroquinolones, sulfonamides, tetracycline, and trimethoprim are among those to which *E. coli* is resistant (Adenipekun *et al.,* 2016). UPEC is one of the most commonly isolated pathogens in clinical practice and is believed to be a major source of genes encoding antibiotic resistance. The ability of bacteria to horizontally transfer antibiotic resistance genes to other bacteria has been documented in numerous studies, necessitating an immediate assessment of their prevalence and control (Chinyere *et al.,* 2020). Numerous factors, including the amount of antibiotics used, can contribute to the development and spread of antibiotic resistance. Hence, the objective of this paper is to investigate the prevalence, phenotypic characterization and antibiogram of uro-pathogenic *Escherichia coli* (UPEC) among patients attending a Tertiary Institution Teaching Hospital in Katsina Metropolis, Nigeria.

MATERIALS AND METHODS

Study Area: With a land area of approximately 24,194 Km², Katsina State is situated between longitudes $6^{O}52'$, $9^{O}20'$E and latitudes $11^{O}8'$, $13^{O}22'$N. The National Population Commission (2006) estimates that the State has 5,800,672 inhabitants, of which 2,947,639 are men and 2,853,033 are women. The major occupation in Katsina State are farming, cattle rearing, trading, fishing, hunting, and crafts, among other things. Herbalists, crafts people, and traditionalists are few. The State has two distinct seasons: wet and dry. According to Dauda *et al.* (2016) the Sahel region experiences 300–400 mm of annual rainfall, the Sudan 600–800 mm, and the Northern Guinea Savannah 900–1100 mm of annual rainfall. The mean daily temperature ranges between 16^{0}C and 40^{0}C (Dauda *et al.,* 2016).

Study Population: In this investigation, purposive sampling was employed. One hundred and fifty (150) clinical urine samples made up the sample population. Both male and female patient samples are included in the group. The patients' age ranged from ≥20 to 81 years. Clinical urine samples were obtained from patients at General Hospital Katsina who had been diagnosed with UTIs (Nas *et al.,* 2019).

Sample Size Determination: The sample size of 150 was arrived using the equation (1)

$$n = Z2\,p\frac{1-p}{d2} \quad (1)$$

Where; Z= Z-score for 95% confidence interval = 1.96, p = previous prevalence in North West Nigeria= 10.8% and d = acceptable error (5%). The prevalence of UTIs among patients attending selected hospitals in Northern Nigeria was used (Chinyere *et al.*, 2020).

$$n = \frac{(1.96)^2 \times (0.108) \times (1-0.108)}{(0.05)^2} = 1.48.03 \quad (2)$$

The value 148.03 was rounded up to the nearest 100 which gave 150, therefore, 150 urine samples was assessed in this study (Ali and Abdallah, 2019).

Ethical Approval: The study was approved ethically prior to its initiation by the research ethics committee of the Katsina State Ministry of Health, using the Health Research Ethical Review Committee (HREC) assigned number FTHKTHREC:REG.24/06/22C/111. Prior to the collection of samples, informed consent

was acquired from each patient (Abdulkadir and Aisha, 2018; Abdu *et al.,* 2018).

Sample Collection and Handling: Three hundred (150) urine samples were collected in sterile, labeled universal container from the patients in the selected hospitals. The patients provided early morning clean catch mid-stream urine. The specimens were labeled and analyzed within 2 hours of collection at the microbiology laboratory of GHK and FTHK. All the patients were instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra (Ado *et al.,* 2019; Abdu *et al.,* 2018).

Isolation of Bacteria (UPEC) from Urine Sample: A sterile wire loop was dipped into the sediments of a 10-milliliter urine sample from each patient, which was centrifuged (Model-800-1, USA) for five minutes at 2000 rpm. The sediments were then streaked onto a surface that had been prepared with Cysteine-Lactose Deficient Agar (CLED). All of the samples underwent the same process, and the plates were incubated for 24 hours at 37 °C. To obtain pure UPEC colonies, the discrete yellowish colonies on CLED of the bacteria from each plate were further sub-cultured on EMB. The pure isolates of the bacteria were kept on Nutrient Agar slants for future use (Nas *et al.,* 2019).

Gram Stain: On a clean, labeled glass slide, a 24-hour bacterial culture of the isolates was emulsified to create a thin smear using sterilized wire-loop. The smear was heat-fixed after air drying. The slide was flooded for 60 seconds with crystal violet primary stain, and it was then cleaned with distilled water. The smear was then flooded with Lugol's iodine for 60 seconds and then rinsed with distilled water. Acetone was used to decolorize the slide for 30 seconds and immediately rinsed with distilled water. Safranin was used as counterstain for 60 seconds then giving it a quick rinse with distilled water. It was examined using a microscope (Carl Zeiss Gmbh: Model-37081, GERMANY) with oil immersion objective lens (×100) (Nas *et al.,* 2019).

Indole Test: After inoculating the organism into peptone water and incubating for 24 hours at 37°C, Kovac's reagent was added in drops. According to Nas *et al.* (2019) a positive outcome was indicated by the pink ring that forms in the center of the tube.

Citrate Utilization Test: UPEC colonies that were well isolated following overnight incubation were emulsified in citrate media using a sterilized wire-loop and incubated (TT-9052 Techmel and Techmel, USA) at 37°C for 24hrs. No changes in color indicated positive results (Nas *et al.,* 2019).

Triple Sugar Iron (TSI) Agar: A discrete colony was taken from a pure overnight culture and stabbed through the middle of the medium to the bottom (butt), then streaked on the slant of the medium. It was then incubated at 37^{O}C for a 24hrs. The procedure was done using a sterile wire-loop. When any sugar is fermented, acid is produced, as seen by a color shift in the phenyl red indicator from red to yellow in both the butt and the slant (Gladys, 2019).

Methyl Red-Voges Proskauer Test: Methyl red indicator is used in this test to identify acidity that results from glucose fermentation. Colonies from the overnight culture are picked using a sterile wire loop and inoculated into a labeled tube containing MR broth; an un-inoculated tube was kept as a control. The two tubes were incubated for 24 to 48 hours at 37^{0}C. Both tubes were filled with 3 drops of MR indicator, thoroughly mixed, and the color change was monitored. The red color that surfaced denotes Positive test (Gladys, 2019).

Voges-Prokauer Test: Using a sterilized wire loop, separate colonies were selected from an overnight culture and injected into the VP broth. The tube and control were incubated for 24 to 48 hours at 37 ^{O}C. Barrett's reagent B was added to both tubes in three (3 ml) doses. The caps were removed and the tubes were gently shaken for 30 seconds to expose the media to oxygen. Positive outcomes are indicated by the color crimson red when it appears (Gladys, 2019).

Standardization of the Inoculum: The inoculum was standardized by sub-culturing the isolates on fresh NA using sterilized wire followed by incubating at 37°C for 24 hours. Following incubation, discrete colonies were picked using sterile wire-loop and placed into 5 mL sterilized distilled water in a test tube. The size of the inoculums for the corresponding organisms was then compared with McFarland scale of 0.5 or 1.5×10^{8} cfu/mL (Oyeleke and Manga, 2008).

Antibiotic Susceptibility Testing and Multiple Antibiotic Resistant Index Determination: The antibiotic susceptibility testing followed the modified single disc diffusion techniques, interpreted accordance to Clinical and Laboratory Standard Institute (CLSI, 2017) guidelines. Statistical

significance was determined using Pearson's Chi-square test with $p<0.05$ was considered significant. Standardized overnight culture of each isolate (containing approximately 10^6 cfu/ml) equivalent to 0.5 McFarland Standard was used to flood the surface of Mueller Hinton agar plates and allowed to dry while the petri dish lid was in place. The following standard antimicrobial discs namely; Ciprofloxacin (CIP, 5µg), Erythromycin (ERY, 15µg), Gentimisin (GEN, 10µg), Imipenem (IMI, 10µg), Amoxicillin (AMP, 20µg), Ceftazidime (CAZ, 30µg), Cefuroxime (CRX, 30µg), Ofloxacin (OFL, 5 µg), Nitrofurantoin (NIT, 50µg), and Amoxicillin-Clavulanate (AUG, 30µg) obtained from MASTDISCS^{R}AST, UK, were aseptically placed at reasonable equidistance. Plates were then incubated at 37^0C for 18-24 hours. The diameter of the zone of inhibition produced by each antibiotic disc were measured with a graduated ruler in millimeters. Breakpoints and interpretative for susceptibility/resistance was based on the CSLI (2023) criteria (Abdu *et al.,* 2018).

Data Analysis: Data obtained were analyzed using descriptive statistics, Pearson correlation and Chi-square test. SPSS statistical software version 20 was used for the analysis (Mutonga *et al.,* 2019).

RESULTS AND DISCUSSION

Out of the 150 urine samples that were analyzed from FTHK, 78 (52 %) had positive growth, and 25 (16.67 %) had been confirmed to be UPEC. Similarly, females have the highest prevalence rate of UPEC 13(52%) than males 12(48%) with 31-40 years age group being the most affected. Age group 31-40 have the highest percentage rate of UTI 28(35.90%) in FTHK patients and this agrees with the work of Abdu *et al.* (2018) who reported higher UTI rates of 62(41.33%) and 37(24.67%) among patients' age group 20-39 years. Similarly, age group 71-80 years have the least UTIs 1(1.28%) which agrees with the report of Nas *et al.* (2019). Observed UTIs ranging from 20-30 and 41-70 years in this study corresponds with a study reported by Hassan *et al.,* (2018) who showed highest UTI in the 21-40 year age group (42.3%) followed by 41-60 years age group (27.6%), 61-80 years age group (8.5%), and above 80 year age group (5.6%). Ezugwu *et al.* (2021) also showed the highest UTI rate falls between 36-40 years, followed by 31-35 years, 26-30 years, respectively. These age brackets consist of teenagers, adolescents, young people and the elderly people. This may be due to increased sexual activity and use of spermicide which predisposes to UTIs.

UPEC growth of 78(52%) from FTHK patients in this study corresponds with the work of Islam *et al.* (2024) who reported 94(71.21%) UPEC isolates from urine samples in patients attending Popular Diagnostic Centre in Northern Bangladesh. UPEC prevalence in males 12(48%) is lesser compared to females 13(52%) among FTHK patients in this study. Report of Bhargava *et al.* (2022) also indicated higher prevalence of UTIs in females (60.7%) than males (39.3%).

Table 1: Association of UTIs with Socio-Demographic Information of Participants at Federal Teaching Hospital Katsina (FTHK) (n=150)

Variables	**Bacteriological Growth (%)**			
	Positive Growth= 78(52%)	**Negative Growth= 72(48%)**	**Pearson Chi-square**	**p-value**
Age (years)	**FTHK**	**FTHK**		
20-30	19(24.36)	22(30.56)		
31-40	28(35.90)	19(26.39)	X^2=24.00	0.242
41-50	18(23.08)	19(26.39)		
51-60	8(10.26)	6(8.33)		
61-70	4(5.13)	5(6.94)		
71-80	1(1.28)	1(1.39)		
Gender				
Male	35(44.87)	29(40.28)	X^2=2.00	0.157
Female	43(55.13)	43(59.72)		

KEY: FTHK= Federal Teaching Hospital Katsina.

Table 2: Prevalence of UPEC Isolates at FTHK

Hospitals	**No. of Urine Samples Examined (n=150)**	**No. of Positive Growth N (%)**	**No. of UPEC Positive Growth N (%)**	**No. of Males (%)**	**No. of Females (%)**
FTHK	150	78(52%)	25(16.67%)	12(48%)	13(52%)

KEY: FTHK= Federal Teaching Hospital Katsina.

UPEC prevalence of 26.67% is closed to 23.5% observed in Minna metropolis, Niger State, Nigeria by Iseghohi *et al.* (2020). Higher prevalence contrary to our findings were the reports of Idakwo *et al.*

(2015) and Oladeinde *et al.* (2011) who recorded 32.5% in Minna metropolis and 39.7% in Okada, Niger and Edo States respectively. Chinyere *et al.* (2020) also reported a higher prevalence of 35% among pregnant women attending two major hospitals in Jigawa State, Nigeria. Lower UPEC prevalence of 15.8% from Kano metropolis, Northwest Nigeria was also reported by Ali and Abdallah (2019). Lower rates of UTIs reported by Iseghohi *et al.* (2020) in Minna metropolis as compared with this study's findings showed age ranges of 21-30 (26.5%) and 31-40 (25.3%). The outcome of this study is contrary to the report of Abdulkadir and Aisha (2018) who indicated *Staphylococcus aureus* to be the most prevalent organism (40%), followed by coagulase negative *Staphylococcus* (32%) and the least is *Escherichia coli* (4%) among pregnant women attending Federal Teaching Hospital Katsina. Varying sanitary conditions among the patients and personal hygiene might be the cause of this prevalence in the study population.

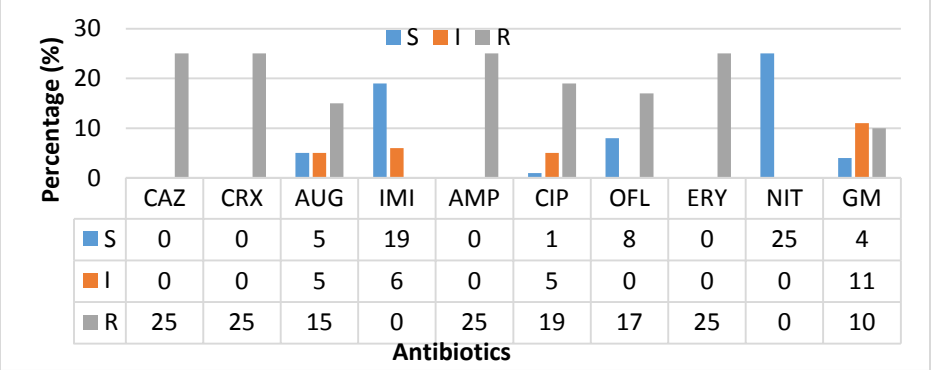

Fig. 1: Showing UPEC Antibiotic Susceptibility Profile for FTHK Patients

KEY: *CAZ= Ceftazidime, CRX= Cefuroxime, AUG= Amoxicillin-Clavulanate, IMI= Imipenem, AMP= Ampicillin, CIP= Ciprofloxacin, OFL= Ofloxacin, ERY= Erythromycin, NIT= Nitrofurantoin, GM= Gentimicin, S= Sensitive, I=.Intermediate, R= Resistance*

Fig.1 shows that AUG, IMI, CIP, and GM have moderate effect against the UPEC isolates, whereas CAZ, CRX, AMP, and ERY demonstrated 100% resistance against the FTHK-tested UPEC isolates. IMI, NIT, and GM demonstrated a noticeable susceptibility. Antibiotic resistance and UPEC isolates were significantly correlated, according to chi-square results (X^2=50, p<0.05). Table 4 above displayed different UPEC at different MARI levels, such as 0.3, 0.4, 0.5, 0.6, and 0.7, that were resistant to the antibiotics CAZ, CRX, GEN, CIP, OFL, AMP, ERY, and AUG. UPEC with 0.7 MARI was found to indicate high resistance, followed by 0.6, 0.5, 0.4, and 0.3

Plate 1: UPEC Antibiotic Susceptibility Test

Table 3: Antibiotics Resistance Pattern of UPEC Isolates among Patients attending FTHK

No. of Antibiotic Combination	No. of Isolate	Name of Antibiotics							MARI
3	2	CAZ	CRX	AMP					**0.3**
4	4	CAZ	CRX	AMP	ERY				**0.4**
4	1	CAZ	CRX	CIP	AMP				**0.4**
5	1	CAZ	CRX	GEN	AMP	ERY			**0.5**
5	2	CAZ	CRX	OFL	AUG	AMP			**0.5**
6	4	CAZ	CRX	CIP	OFL	AMP	ERY		**0.6**
6	1	CAZ	CRX	GEN	CIP	OFL	AMP		**0.6**
6	5	CAZ	CRX	CIP	OFL	AUG	AMP		**0.6**
7	4	CAZ	CRX	GEN	CIP	OFL	AMP	ERY	**0.7**
7	1	CAZ	CRX	CIP	OFL	AUG	AMP	ERY	**0.7**

KEY: CAZ= Ceftazidime, CRX= Cefuroxime, AUG= Amoxicillin-Clavulanate, IMI= Imipenem, AMP= Ampicillin, CIP= Ciprofloxacin, OFL= Ofloxacin, ERY= Erythromycin, NIT= Nitrofurantoin, GM= Gentimicin, MARI= Multiple Antibiotics Resistance Index.

UPEC isolates showed high varying susceptibility to IMI, NIT, GM, OFL and AUG. This is in concordance with the work of Kubone *et al.* (2020) who reported 100% UPEC susceptibility to

Imipenem in patients attending some hospitals in South Africa. Abdu *et al.* (2018) reported Nitrofurantoin to be the most sensitive 117(78%) against UPEC isolated from patients attending a tertiary care hospital in Maiduguri, North Eastern Nigeria and this is correlates with the findings of this study. Iseghohi *et al.* (2020) also reported UPEC susceptibility to Gentimicin and Ofloxacin and this further agrees with our findings. However, UPEC equally showed some level of resistance to AUG, OFL, CIP and GEN in in FTHK patients. Iseghohi *et al.* (2020); Adekanmbi *et al.* (2020) reported similar UPEC resistance from patients in various hospitals. The majority of UPEC isolates showed resistance to drugs commonly used to treat UTIs in this study. The reason for resistance to these antibiotics might be due to their availability in the market and easiness to obtain and abuse in the course of usage. Chi-square test showed a statistically significant variation ($p<0.05$) between the resistant and sensitive variables ($X=50.0$; $p<0.05$). Variations in susceptibility may be due to the prescription habits in different localities as inappropriate exposure to antibiotics drives development of resistance.

Table 4: Pearson Correlation Coefficients for UPEC Multi-drug Antibiotic Resistance in Relation to Gender and Age of Patients at FTHK

Correlations

		ANTIBIOTICS	AGE	GENDER
ANTIBIOTICS	Pearson Correlation	1	.713**	.571**
	Sig. (2-tailed)		.000	.003
	N	25	25	25
AGE	Pearson Correlation	.713**	1	.850**
	Sig. (2-tailed)	.000		.000
	N	25	25	25
GENDER	Pearson Correlation	.571**	.850**	1
	Sig. (2-tailed)	.003	.000	
	N	25	25	25

**. Correlation is significant at the 0.01 level (2-tailed).

Some of the isolates in this study were resistant to more than 3 classes of antibiotics, hence are multi-drug resistance. Multiple antibiotics resistance index (MARI) in this study ranged from (0.3-0.9) which is in agreement with the report of Iseghohi *et al.* (2020) who showed 90% of UPEC with a MARI of ≥0.5. This agrees with the work of Abdu *et al.* (2018) who showed 99% of UPEC isolates to be multi-drug resistant with 24.24% of the isolates having antibiotic resistance index of 0.3-0.8 each. Furthermore, Adenipekun *et al.* (2016) reported fifty-three (53) resistance patterns observed among all *E. coli* isolates where 70% of the isolates (172/247) were MDR, exhibiting resistance ranging from two to sixteen (2-16) antimicrobials and two to five (2-5) antimicrobial classes.

A strong positive linear relationship between UPEC multi-drug antibiotic resistant pathogens and patient age is suggested by a positive correlation of .713 among the 25 UPEC isolates evaluated, with a p-value of 0.00 at the 0.01 level of significance. That is, the degree of multi-drug antibiotic resistance found in the study is probably significantly influenced by age. At the 0.01 level of significance, a strong positive correlation between gender and UPEC multi-drug resistance was demonstrated by a .571 correlation and a p-value of 0.003. Notably, gender and age have an impact on multidrug antibiotic resistance.

Plate 2: Showing UPEC Antibiotic Resistance

Conclusion: Females were found to be mostly affected than males in terms of UTI caused by UPEC. The age group 31-40 years were observed to have

high rate of UPEC infection followed by >41 years. The results also showed high rates of UPEC antibiotic sensitivity to Nitrofurantoin and Imipenem followed by Gentimisin, Ofloxacin and Ciprofloxacin in GHK isolates. However, alarming rate of multi-drug resistance were observed from CAZ, CRX, AMP, ERY, and OFL antibiotics, posing a significant public health concern and limiting treatment options. These findings emphasize the widespread issue of antibiotic resistance in the study population.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors.

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