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Research Article

A Study of the Antibiotic Susceptibility Profile of Bacterial Uropathogens from School Age Pupils in Wukari, Taraba State, Nigeria

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Abstract

Background: Globally, uropathogens when encountered in paediatric investigations, particularly in developing regions are major health concerns as they are the leading causes of bacterial infections and illness especially when they are asymptomatic, non-specific or with medical findings that make their management using antibiotics difficult. Objective: This study aimed to investigate the presence of bacterial uropathogens in 100 pupils of Islamiya Primary School in Wukari, Taraba State, Nigeria, and to evaluate their antimicrobial susceptibility patterns. *Method*: Midstream urine samples were aseptically collected from pupils whose ages were between 5 and 15 years, with preponderance of males (80%) over females (74.5%). Samples were cultured on Cystine Lactose Electrolyte Deficient Agar and Eosin Methylene Blue Agar. Bacterial isolates were identified using morphological, Gram staining, and biochemical methods. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method, with bacterial isolates standardized to the 0.5 McFarland turbidity standard. *Result and conclusion*: Out of 100 samples, 77 yielded significant bacterial growth, with a total of 93 isolates identified. Bacteriuria was highest (100%) in pupils aged 6-15 years, and least in those between the ages of 1 and 5 years. The predominant pathogen was *Escherichia coli* (20%) while *Salmonella* species was the least (6.0%) occurring pathogen. All the organisms were resistant to amoxycillin, azithromycin and rifampicin. Notably, *Staphylococcus* species exhibited multidrug resistance. The study revealed a high prevalence of uropathogens among school-aged children in Wukari, with *E. coli* and *S. aureus* as leading pathogens. The observed antimicrobial resistance patterns highlight the urgent need for antibiotic stewardship and hygiene-based interventions. Routine screening and public health education in schools are essential to mitigate the burden of paediatric UTIs in the region.

Keywords

Isolation, Identification, Bacteriuria, School Age Pupils, Wukari, Nigeria

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1. Introduction

Urinary tract infection (UTI), defined as the significant growth of a single microbial species in the urine typically exceeding 10^5 colony-forming units per milliliter (CFU/mL), is a term generally applied to variety of clinical infections ranging from asymptomatic bacteriuria to severe kidney damage and sepsis [1-4]. Urinary tract infections (UTIs) are frequently reported as major health concerns in infants and young children, representing one of the leading causes of bacterial illness in this age group [4, 5].

The occurrence of UTIs in children has been shown to vary with age and gender [6]. Multiple risk factors contribute to the development of UTIs in this population, encompassing anatomical, physiological, and behavioural aspects [7]. Girls are generally at greater risk while male infants especially those who are not circumcised tend to have a higher incidence during their first year of life [8]. Inadequate hygiene of the perineal area, particularly in young females, significantly raises the risk of infection by increasing the chances of bacterial transfer from the anus to the urinary tract. Additionally, congenital or acquired abnormalities in the urinary system, such as vesico-ureteral reflux (VUR), neurogenic bladder, or posterior urethral valves, can cause urine retention and lead to repeated infections [8, 9].

Incidence in children however is difficult to estimate, particularly because young children with UTI may only have non-specific symptoms or signs and symptoms, that can vary depending on the child's age group, such as fever vomiting, pyuria, haematuria, abdominal discomfort, foul-smelling urine, poor appetite and persistent irritability and, in newborns, jaundice [10-12].

In Nigeria, UTIs among children are a growing public health concern, particularly in rural and underserved areas. Poor hygiene practices, limited access to healthcare services, and the use of contaminated water sources are prevalent issues that exacerbate the risk of infection in these communities [13]. UTIs are primarily acquired through the ascending haematogenous route when bacteria from the gastrointestinal tract colonize the urethra and migrate upward into the urinary tract. This colonization is often facilitated by disruptions in the normal microbial flora due to prolonged antibiotic use, or the introduction of transient microorganisms from environmental sources or contaminated medical devices such as invasive catheters [14, 15]. Regardless of age, females consistently exhibit a higher incidence and susceptibility to UTIs compared to males likely due to due to their shorter urethra and anatomical positioning of the female urinary tract, which is closer to the external environment, making it more prone to bacterial invasion [16].

Most infections however are caused by facultative anaerobes of the enterobacteriaceae group [10, 17]. Studies have shown that *Escherichia coli* is the prominently isolated urinary tract pathogen [4, 5, 10]. The common gram-positive species are Staphylococcus and beta-haemolyticstreptococci [18]. The prognosis of UTI is usually favourable, but it relies on timely administration of appropriate treatment. Despite the advances in diagnosis and prophylaxis of infections, many incidence of UTI may remain unnoticed due to mild or asymptomatic manifestations. That is why treatment of identified cases of bacteriuria in all young children is advised in addition to preventing permanent renal scarring due to reflux [8].

A precise and dependable diagnosis of UTIs in children is crucial, particularly in those under two years of age, where symptoms are often vague and non-specific while its management typically begins with empirical antibiotic therapy, based on local resistance patterns of urinary pathogens [19].

Therefore, this study aims to isolate and identify bacterial uropathogens among school children in Wukari Local Government Area of Taraba State.

2. Materials and Methods

2.1. Study Area

The study was carried out in Wukari town located in Wukari Local Government Area of southern Taraba State, Nigeria. Wukari lies within latitude 7°51N of the equator and 9°47E of the meridian with a land area of approximately 4,308km^2. Wukari with a total population of 238,283 people [20, 21], is made up of ten (10) political wards namely: Akwana, Avyi, Bantaje, Chonku, Hospital, Jibu, Kente, Puje, RafinKada and Tsokundi.

2.2. Study Population

This was apparently healthy children attending primary 1 and 6 classes at Islamiya primary school in Wukari whose ages were between 5 and 16 years. Pupils who participated in the study were selected at random. The inclusion criterion was children attending primary schools only, while children below or above these ages, who were on antibiotics medication for at least three days before collection, and who took large quantities of fluids less than an hour before coming to school, were excluded from the study.

2.3. Ethical Approval

This study was conducted in accordance with the principles outlined in the declaration of Helsinki. Approval to carry out the research was granted by the Research Project Committee of the Federal University Wukari. Ethical clearance was also obtained from the Taraba State Ministry of Health and the management of Islamiya Primary School, Wukari. All sample collection and diagnostic procedures were performed with the full understanding and informed consent of the participants or their guardians.

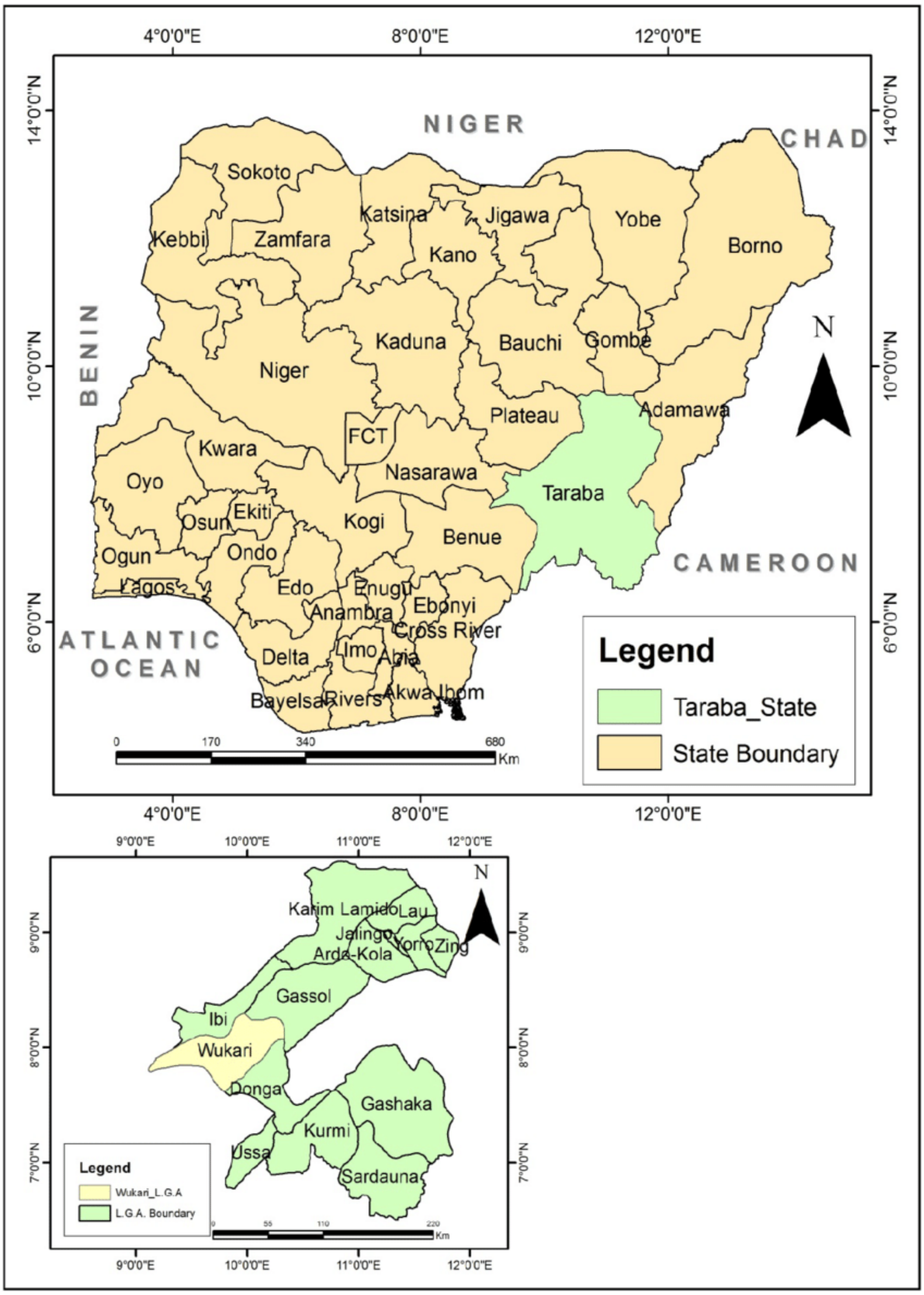

Figure 1. Wukari, Taraba State Map; Source: [21].

3. Materials

3.1. Sample Collection

A clean voided mid-stream urine sample was collected in 20 mL calibration sterilized screw-capped generic containers by the most recommended method of urine sampling in children above the age of three and labeled appropriately. Before collection, all participants were given detailed instructions on how to take the specimen aseptically to prevent contamination and were supervised accordingly to ensure sample integrity. After collection, the clean catch mid-stream urine sample in sterile containers was transported immediately to the laboratory for analysis. Samples were stored in refrigerator at 4 ℃ prior to analysis [22].

3.2. Sterilization of Media and Materials

The media used were Eosin Methylene blue (EMB) and Cystine Lactose Electrolyte Deficient Agar (CLED). All glassware were washed with detergent and rinsed with water, then allowed to dry. The glassware were later wrapped in aluminum foil and sterilized in a hot air oven at 160 ℃ for 3 hours. Media were sterilized by autoclaving at 121 Ib g^{-1} for 15 minutes.

3.3. Isolation of Bacteria

A loopful of each urine sample was streaked on CLED (Cystine Lactose Electrolyte Deficient) and EMB (Eosin Methylene blue) plate and incubated for 24 hours at 37 ℃ to isolate the bacteria present in the urine. After incubation, plates with growth were selected and distinct colonies were sub-cultured on nutrient agar to obtain pure culture for subsequent analysis. Bacterial identification on pure culture was based on colonial morphology [22, 23].

3.4. Bacterial Identification

Bacterial isolates were subjected to gram staining reaction and biochemical tests that included the catalase, indole, oxidase, and triple sugar iron tests using standard protocols [22, 23].

3.5. Antibiotic Susceptibility Test

3.5.1. Preparation of McFarland 0.5 Standards

Approximately 85 ml of 1% sulphuric acid was added to a 100ml volumetric flask. This was followed by the addition of 0.5 ml of 1.175% barium chloride using a 0.5ml volumetric pipette and water drop-wise to the sulphuric acid while constantly swirling the flask. The flask was placed on a magnetic stirrer for approximately 3 minutes [23, 24].

3.5.2. Standardization of Inoculum

A twenty-four-hour culture of the isolate was suspended into a test tube of sterile normal saline using sterile wire loop to form turbidity that corresponded with 0.5 scale of McFarland's standard (1.5×10^8 cells/ml) [24].

3.5.3. Antibiotic Sensitivity Test (Kirby-Bauer Disc Diffusion Assay)

Antibiotic susceptibility testing was conducted following the Kirby-Bauer disc-diffusion test [25]. Briefly, 3mL of sterile normal saline was used to emulsify an inoculum of each pure bacterial isolate and its density adjusted to 0.5 McFarland standards. The mixture was inoculated onto the Mueller-Hilton Agar (MHA) plates (Oxoid, England) using a sterile cotton swab dipped into the standardized suspension of bacterial cultures after which the plates were left to air dry. Antibiotic discs were then placed onto MHA plates using sterile forceps. The plates were incubated aerobically at 37 ℃ for 24 hours and the zone of inhibition on each plate, measured in millimeters.

4. Results

The bacteria isolated, characterized and identified included *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus*, *Salmonella* and *Klebsiella*species. Their characteristics (morphology, Gram reaction, catalase, oxidase, coagulase) are presented in Table 1.

Table 1. *Morphological characterization and biochemical identification of isolates.*

S/N	Colony characteristics	Gram reaction	Catalase	Oxidase	Coagulase	Probable isolate
1	Large, elevated, yellow, opaque	- cocci in cluster	+	-	-	*E. coli*
2	Green coloured with matted surfaces and rough peripheries	- rods	+	+	-	*P. aeruginosa*
3	Translucent, blue coloured	- rod in pairs	+	-	-	*Proteus* species
4	Round, convex, deep yellow coloured	+ cocci in cluster	+	-	+	*S. aureus*
5	Light pink coloured, slightly raised, round, smooth, opaque and shiny	+ cocci in cluster	+	-	-	*S. epidermidis*
6	Flat, blue coloured	- cocci in cluster	+	-	+	*Salmonella* species
7	Very mucoid, whitish coloured	- rods	+	-	-	*Klebsiella* species

Key: + = Positive, - = Negative, S. aureus = Staphylococcus aureus, E. coli = Escherichia coli

The recovery rate of bacteria in all urine samples with respect to sex is shown in Table 2. The rate was higher in the males (80%) than in the females (74.5%).

Table 2. *Gender-associated occurrence of bacteria from urine samples.*

Sex	Number examined	Number positive	Percentage (%)
Male	45	36	80.0
Female	55	41	74.5
Total	100	77	77.0

Pupils in the 6-10 years and 11-15 years age group had the highest (100%) occurrence of bacteriuria while those in the 1-5 years age group had the least (76.0%) occurrence (Table 3).

Table 3. *Age-related occurrence of bacteria from urine samples.*

Age group (years)	Number examined	Number positive	Percentage (%)
1-5	25	19	76.0
6-10	30	30	100
11-15	20	20	100
>15	25	21	84.0
Total	100	90	90.0

Table 4 shows the distribution of each bacterial isolates in the two genders. Among the seventy-seven positive samples, there was an equal distribution of bacteria in male (50.5%) and female (49.5%) pupils. *E. coli* was the most commonly isolated organism (21.5%) and it was slightly more prevalent in males (55.0%) than in females (45.0%). In contrast, *Salmonella* species was the least isolated bacterial uropathogen (6.54%), accounting for 83.3% of the recovered isolates. *S. aureus* and *S. epidermidis* showed a relatively balanced distribution amongst the genders.

Table 4. *Distribution of bacterial uropathogens in Islamiya primary school pupils.*

Bacterial isolate	Occurrence (%)		
	Male	Female	Total
Staphylococcus aureus	9 (47.4)	10 (52.6)	19 (20.4)
Staphylococcus epidermidis	8 (47.1)	9 (52.9)	17 (18.3)
Escherichia coli	11 (55.0)	9 (45.0)	20 (21.5)
Pseudomonas aeruginosa	3 (30.0)	7 (70.0)	10 (10.8)
Salmonella species	1 (16.6)	5 (83.3)	6 (6.54)
Proteus species	10 (76.9)	3 (23.1)	13 (14.0)
*Klebsiella*species	5 (62.5)	3 (37.5)	8 (8.60)
Total	47 (50.5)	46 (49.5)	93

Figures in parentheses represent percentages.

Table 5 shows the antimicrobial susceptibility patterns of various bacterial isolates against a panel of fourteen (14) commonly used antibiotics. *E. coli* exhibited a broad antibiotic susceptibility with the highest (24 mm) and least (16 mm) zone of inhibition produced by ciprofloxacin and chloramphenicol respectively. The organism was not susceptible to trimethoprim, erythromycin, amoxycillin, azithromycin, and rifampicin.

S. aureus and *S. epidermidis* displayed marked decrease in antibiotic susceptibilities, with *S. aureus* being susceptible to ciprofloxacin (24 mm) and pefloxacin (16 mm). With the exception of erythromycin that produced a clearance zone of 16 mm against it, *S. epidermidis* was not susceptible to the other tested antibiotics. Overall, all the bacteria but *S. epidermidis*, were susceptible to ciprofloxacin and pefloxacin.

Table 5. *Antimicrobial sensitivities of bacterial isolates.*

Bacteria isolate	CPX	S	SXT	E	PEF	CN	APX	Z	AM	R	AU	OFX	CH	SP
Zone of inhibition (mm)														
E. coli	24	21	0	0	20	20	0	0	18	0	19	19	16	21
Proteus species	15	18	16	0	15	15	0	0	19	0	18	16	19	22
S. aureus	24	0	0	0	16	0	0	0	0	0	0	0	0	0
S. epidermidis	0	0	0	16	0	0	0	0	0	0	0	0	0	0
Salmonella species	19	16	20	0	20	21	0	0	16	0	21	19	18	21
P. aeruginosa	20	20	0	0	16	17	0	0	17	0	16	20	19	16
Klebsiella species	25	25	14	0	20	19	0	0	0	0	15	20	19	17

Key: CPX = Ciprofloxacin, S = Spiramycin, SXT = Trimethoprim, E = Erythromycin, PEF = Pefloxacin, CN = Cefalexin, APX = Amoxycillin, Z = Azithromycin, AM = Ampicillin, R = Rifampicin, AU = Augmentin, OFX = Ofloxacin, CH = Chloramphenicol, SP = Spiramycin.

5. Discussion and Conclusions

The findings of this study offer critical insights into the bacterial profile and antimicrobial susceptibility patterns among school age pupils. The presence of both Gram positive and Gram negative bacteria indicates a wide range of potential uropathogens in the study population. The present findings implicating *E. coli* and *S. aureus* as major uropathogens in children are in concurrence with previous reports [10, 26]. For instance, among the children attending Pilot Central Primary School in Bokkos, Plateau State in Nigeria, *E. coli* was the most frequently isolated organism, accounting for 59.2% of the total isolates, followed by *Klebsiella* (17.5%) and *Staphylococcus* (15.8%) species [10]. Similarly, among primary school pupils in Yola North, Adamawa State, *E. coli* was the main isolate (41.9%) trailed by *S. aureus* (29.0%) [26]. The similarities in pathogen distribution not only underline the role of *E. coli* as the leading causative agent of paediatric UTIs across different regions of Nigeria but also has important health implications. *E. coli* infection if left untreated, may progress to kidney infections and lead to complications like renal scarring and long term kidney damage [27]. Additionally, the significant detection of *S. epidermidis* and *Proteus* species in this current study reinforces the need to consider both skin flora and enteric bacteria in the differential diagnosis of paediatric UTIs. *S. aureus*, though commonly found on the skin, can cause infections when it enters sterile body sites and, may contribute to UTIs, and, in severe cases, invasive diseases such as septicemia particularly in children with underlying health conditions [28].

Our current finding of an age-related occurrence of uropathogens in children between the ages of 5 and 15 years higher than those previously reported [10, 26] is striking. Predisposition of children to these bacteria may possibly be due to their increased physical activity, inadequate personal hygiene, or developing immune systems. This reinforces the need for targeted interventions within this age group, as they appear to be at greater risk of acquiring urinary tract infections due to both behavioral and physiological factors.

This present study's report of a slight predominance of UTI in male rather than female children contrasts with the global trend, which generally shows higher prevalence in females [29]. There was however, a consistency in the results obtained in this present study with previous reports of the isolation of *Proteus* and *Klebsiella* more in males than in females. For example, *Klebsiellapneumoniae* was more frequently isolated from male (7.5%) than female (2.5%) paediatric patients in Pakistan [6]. In another instance, a higher prevalence of UTI was recorded in female (43.3%) compared to male (25.8%) primary school children in Plateau State of Nigeria [10]. Similarly, *Proteus* species was also more commonly found in male patients than in female patients at a private multi-specialty healthcare facility in Abuja, Nigeria [30]. The contrasting pattern in the male predisposition to UTIs over females, as reported in this present study, has broader epidemiological implications. These disparities may be attributable to local factors such as hygiene practices, socio-cultural behaviors, or environmental conditions unique to the study population. Therefore, while the present findings reflect regional variations, they also emphasize the importance of considering both local data and global trends in the development of prevention and intervention strategies.

The antimicrobial susceptibility profiles observed in this study provide important insights into both effective and ineffective treatment strategies for UTIs among school age pupils. While all isolates were insensitive to amoxycillin and azithromycin, all but for *Staphylococcus* species were susceptible to ciprofloxacin, erythromycin and pefloxacin. These antibiotics act by inhibiting bacterial DNA gyrase and topoisomerase IV enzymes essential for DNA replication and transcription [31]. This mode of action is highly effective against a wide range of Gram negative and some Gram positive bacteria, including *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella* and *Salmonella* species. Their strong bactericidal activity, high tissue penetration, and prolonged post antibiotic effects might be contributory to their clinical effectiveness [32].

These susceptibility patterns point to possible antibiotic misuse and raise concerns about the continued effectiveness of third and fourth line therapies. Consequently, the study underscores the importance of antibiotic stewardship, routine microbial surveillance, and targeted therapy guided by susceptibility testing to effectively manage infections and combat the rise of antimicrobial resistance in the community.

This study revealed a high prevalence of uropathogens among school age children in Wukari, with *E. coli* and *S. aureus* as leading pathogens. The observed antimicrobial susceptibility patterns underscore the urgent need for antibiotic stewardship and hygiene based interventions. Routine screening is not only essential to ensure early detection and treatment, the rational use of antibiotics after medical diagnosis should be guided by current local susceptibility test patterns. Whilst the establishment of local surveillance systems to monitor the prevalence and antimicrobial susceptibility patterns of uropathogens is critical, comprehensive health education campaigns focusing on personal hygiene and proper toilet practices should be implemented for parents, pupils and teachers to mitigate the burden of paediatric UTIs in the region.

Abbreviations

UTI	Urinary Tract Infection
CFU/ml	Colony Forming Unit Per Milliliter
VUR	Vesico-Uretheral Reflux
°N	Degree North
°E	Degree East
Km^2	Square Kilometer
°C	Degree Celcius
EMB	Eosin Methylene Blue
CLED	Cysteine Lactose Electrolyte Deficiency
Ibg^{-1}	Pounds Per Gram
MHA	Mueller Hilton Agar
DNA	Deoxyribonucleic Acid

Author Contributions

Chinedu Nkem Awujo: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing, Data curation

Ladan Auwalu: Resources, Investigation, Validation, Writing – original draft

Emmanuel Gaina: Visualization, Validation, Formal Analysis

Patience Kwache Kolo: Visualization, Validation, Formal Analysis

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Conflicts of Interest

The authors declare no conflicts of interest.

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