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A Cross-Sectional Study of Antimicrobial Resistance Pattern Across Two States in Nigeria

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ABSTRACT

Antimicrobial resistance has been declared by the World Health Organization as a threat to countries and across various sectors. This study aimed to analyze antimicrobial resistance patterns of the common pathogens occurring in the Federal Capital Territory and Lagos states of Nigeria. A retrospective analysis of microbiological culture results from five private and public laboratories between January and April 2016 in Lagos and the Federal Capital Territory (FCT). A total of 544 isolates were obtained from the five laboratories. Urine 137 (25.2%), was the most prominent specimen, followed by Stool 64 (11.7%). Out of 374 specimens that yielded growth after culture, gram-negative isolates were the most prevalent bacteria 169 (56%). There were significantly more samples from FCT and by gender (P=0.03) compared to Lagos (P=0.0003). There was no significant association between the type of bacteria isolate and location. The predominant gram-positive bacteria were *Staphylococcus aureus* 94 (70%), while gram-negative bacteria were *Klebsiella* species 67 (40%). Multi-drug resistance was observed in 202 (62%) of isolated urogenital pathogens. Maximum resistance was observed with commonly used first-line antimicrobials such as co-trimoxazole, tetracycline, second-generation cephalosporins -Cefotetan, and third generation Cephalosporins -Ceftriaxone. *Staphylococcus aureus* had the highest antimicrobial resistance expression (93.6%) within the gram positives, with multiple drug resistance to all the antibiotics. Our survey revealed a rising rate of antimicrobial resistance to commonly used antibiotics. We recommend the prudent use of antibiotics to limit the spread of antimicrobial resistance.

Keywords: Antimicrobial resistance; Nigeria; Antimicrobial susceptibility; Urogenital; *Staphylococcus aureus*

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INTRODUCTION

The advent of antibiotic-resistant strains has threatened to render existing treatments ineffective against many infectious diseases [1]. Globally, a projected 700,000 deaths will be attributed to antimicrobial resistance with an estimated 10 million deaths in 2050 [1]. The inappropriate use, overuse, sub-standard or counterfeit antibiotics have accelerated the emergence of antimicrobial resistance [2]. The emergence of multidrug resistance to several classes of antibacterial is a serious cause of concern. There is a notable increase in multi-drug resistant bacteria jeopardizing the effectiveness of antibiotic, unfortunately since the last major discovery of antibiotics in 1987, there have been too few drugs in development [2]. Multi-resistance strains of pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, and *Salmonella* Typhi have shown increasing resistance to first and second-generation antibiotics [3]. Urinary tract infections (UTIs) are considered the most common and most pathological health conditions acquired in hospitals and communities[4]. An estimated 50 percent of women have been infected with UTIs in their lifetimes than men. Treatment of UTIs varies according to age, sex, causative agent, and region of the urinary tract affected. However, drug resistance has been reported among bacteria causing UTIs [5]. Due to the growing emergence of antimicrobial resistance (AMR), the World Health Assembly adopted the Global Action Plan (GAP) on Antimicrobial Resistance in May 2015. There is increasing drug resistance reported for HIV programs and several pathogens in Africa. To ascertain the burden of AMR, one of the strategic objectives of GAP includes “to strengthen the knowledge and evidence base on AMR through surveillance and research” [6], .

Moreover, several initiatives have been set up to conduct studies on the burden of AMR such as ReACT and global antimicrobial resistance partnership, WHO’s Global action plan to develop and implement national action plans on AMR, and Africa CDC Anti-microbial Resistance Surveillance Network (AMRSNET) on AMR surveillance and control for Africa. As of 2016, Nigeria had no AMR surveillance network nor Antimicrobial Resistance Surveillance (AMRS) national data [7]. In line with the GAP, the country committed itself to establishing a national AMR surveillance system [8]. To understand the AMR situation in the country, the Federal ministries of health, agriculture, and environment conducted a situational analysis of antimicrobial use and resistance in certain geographic areas of the countries and found an alarming score of 70% - 100% resistance rate to some antibiotics [9]. Furthermore, UTIs’ accounted for a 3.4-88.5% prevalence rate with associated resistance organisms such as *Pseudomonas, E.coli, Klebsiella*, and *Staphylococcus* [8,10]. The Nigerian surveillance program is expected to monitor the susceptibility pattern of bacteria over time for improved decision-making. The surveillance program was set up with an initial network of ten clinical and public health laboratories that could conduct testing for antibiotic sensitivity. However, the quality management system was recognized as key in antimicrobial and sensitivity testing (AST), amongst several common challenges such as the lack of standardized operating procedures across the laboratories for susceptibility testing, quality assurance, data management, and reporting [11]. This lack of concordance has a huge implication on the data quality reported. To address this, Nigeria in conjunction with stakeholders developed a guideline for antimicrobial resistance laboratory surveillance in 2018 with the aim of providing a framework for AMR surveillance implementation and using standardized Standard Operating Procedure (SOP) within the participating network of labs. Nigeria like most countries has reported growing antimicrobial resistance requiring continuous monitoring of susceptibility patterns [12, 13, 14]. The exact prevalence of AMR in uro-vaginal microorganisms of public health importance in Nigeria is inadequate. This study was undertaken as part of a larger surveillance program to provide supporting data on antimicrobial resistance patterns of common pathogens with an emphasis on urogenital pathogens within Lagos state and the Federal Capital Territory of Nigeria.

METHODOLOGY

Study Area and Sites

This study was conducted in Federal Capital Territory and Lagos, Nigeria. Lagos and Federal Capital Territory are considered the economic hubs of the country with 20 and 6 local government and area councils respectively and according to the Health facility registry of the federal ministry of health, they both have about 2333 and 757 public and private registered health facilities respectively [15]. The laboratory departments of the public and private facilities receive patients from all socioeconomic strata.

Study Design and Population

The cross-sectional study using retrospective data was conducted in three health facilities (1 public health laboratory and 2 public health facilities) in Lagos and two health facilities (1 public health facility and 1 private health facility) in Abuja in March 2016. Microbiological culture logs were pulled from the facility registrar that is 3 months prior to the date of review specifically data from

January to March 2016. The facilities in Lagos and Abuja were purposively sampled after meeting the minimum criteria following the initial gap assessment.

Study Inclusion
The majority of facilities selected that met minimum criteria included those that perform microbial, culture, and sensitivity tests and had records of the following information such as age, sex, bacteria isolates, and culture results.

Data Collection Procedures
A structured checklist adapted from World Health Organization (WHO) laboratory assessment tool was used to assess over 40 facilities on several functionalities and capabilities ranging from availability of laboratory equipment, staffing, culture, and sensitivity testing among others [16]. A subset of these facilities (5) was purposively selected for AMRS assessment. After securing approval from the hospital administrations of purposively selected facilities, a structured data collection tool was used to collect AMR data from hospital records. Records of patients who presented for microscopy, culture, and sensitivity tests from the period of January to March 2016 were included in the study. Most of the laboratories sampled had variations in standardized operating procedures for antimicrobial susceptibility testing, data management, and reporting.

Facilities sampled in this study collected urine, pus, sputum, and vagina including high vagina swabs, urethral swabs, ear swabs, and semen. As of 2016, there were no national standard guidelines for collecting, processing, identifying, characterizing, and reporting antimicrobial resistance organisms in Nigeria. However, some of the laboratories performed similar microscopic identification as described by Cheesbrough in terms of media preparation and identification of isolates [17].

Culture and identification of isolates followed the standard operating procedures of the laboratory. Blood agar, MacConkey agar, and chocolate agar were used to culture and isolate the organisms. Gram staining and colony characteristics were used for presumptive identification. Enzymatic and biochemical confirmatory tests were performed on pure colonies following culture on nutrient agar for 24 hours at 37^0C.

Antibiotic Susceptibility Testing
Each microbiological bench in the laboratory's health facility visited performed lab susceptibility tests. The modified Kirby-Bauer disk diffusion method was employed to obtain antimicrobial susceptibility testing (AST) results according to the guidelines by Clinical Laboratory Standard Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST). This method involves aseptically inoculating bacteria samples on Mueller Hinton Agar using the spread method. Antibiotics discs are placed on the agar and incubated for 24 hrs at 35^OC. Zones of inhibition which are circular areas around the antibiotic discs are observed on the surface of the agar after incubation. A larger zone of inhibition indicates a stronger sensitivity while areas with no growth indicates total resistance. Laboratories used different antibiotic disks with their concentrations in micrograms produced by various manufacturers available in the market. In this study, multidrug-resistant isolates are classified as resistant to two or more antimicrobial classes. The classes of antibiotics studied were Cephalosporins (cefuroxime, ceftriaxone, cefotaxime), Penicillin (Amoxicillin, amoxicillin/clavulanic acid, ampicillin), Nitrofuran (Nitrofurantoin), Macrolides (Erythromycin), Aminoglycosides (gentamicin), Quinolones (Ofloxacin, ciprofloxacin), and tetracyclines (tetracycline).

Data Analysis
Unrelated sample data on age, sex, bacteria isolates, and culture results were pooled on the sample type, pathogen isolated, and the antimicrobial susceptibility results. Data were entered into a structured checklist using Excel and Descriptive analysis was conducted. Results were presented as absolute numbers and percentages. Chi square tests were used to compare the association between the type of bacteria isolates, specimen type and location at a significance level of 0.05. Antibiotic resistance pattern per listed antibiotic was calculated as the percentage of isolate resistant to specific antibiotic over the total number of resistant isolates.

Ethical Consideration
This is a retrospective study wherein de-identified patient-level data was collected and provided as part of a larger surveillance program. Hospital management of all health facilities that participated in this study also provided approval for data to be collected from facility registers.

Results
A total of 544 bacterial and fungal samples were collected during the study period. There were 102 males and 268 females and 174 (32.0%) missing records. Male to female ratio was 1:2.6. The majority, 71 (13.2%) of patients were between 31-45 years of age. However, about 177 (32.9%) of the age records were missing and 163 (29.9%) did not have age defined (specified as adult and child). Overall, age ranged from 5 months to 70

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years with a mean of 29.9 years (Table 1). Out of 544 samples collected, Urine 137 (25.2%), was the most prominent specimen followed by Stool 64 (11.7%). However, about 229 (42%) were missing records for the type of specimen collected. Of the 137 specimens collected from Urine, the most frequent pathogen isolated was *Staphylococcus aureus* 19 (13.87%), followed by Coliform sp 15 (10.95%) (Figure 1).

A total of 374 of the 544 specimens yielded growth after culture. Among those with positive bacteria growth, within the specified age groups, female 86% (16-49 years) were most affected. Moreover, of the 374 which yielded growth after culture*, Staphylococcus aureus* 94 (25%) was the most prominent isolated organism affecting both genders, with females 47 (60.3%) having a higher proportion than men. Moreover, there were more women reported with *Staphylococcus aureus* 44 (62%) and *E.coli* 23 (85%) in Abuja than in Lagos 4(66%). This is not significant (P=0.75 and P=0.52 respectively). Also, facilities in Abuja presented the highest proportion 70 (90%) of *Staphylococcus aureus* than Lagos followed by *E. coli* with majority of cases reported from facilities within Abuja 27 (77%). (Table 2). There were significantly more samples from FCT and by gender (P=0.03) compared to Lagos (P=0.0003). There was no significant association between the type of bacteria isolate and location. (Table 3).

Out of the samples that yielded growth (374), Gram-negative isolates were the most prevalent bacteria 169 (56%) and among the gram negatives, *Klebsiella* species 67 (40%) was the most detected while *Haemophilus influenza* 2 (1.2%) was the least isolate detected. Of the 134 (44%) Gram-positive isolates found, *Staphylococcus aureus* accounted for more than 94 (70%) of the isolates detected. (Table 4).

Staphylococcus aureus isolate was resistant to Ceftazidime (98%), and Ciprofloxacin (98%), while low resistance was observed for Nalidixic acid (32%). *Klebsiella* species and *E. coli* were most resistant to imipenem, ceftazidime, and ceftriaxone at 99% and 98%. The overall resistance rate was 97.6%, 98%, and 93.6% for *Klebsiella* species*, E.coli,* and *Staphylococcus aureus* respectively (Table 5).

On Multiple antibiotic resistance, *Klebsiella* species, *E. coli,* and *Staphylococcus aureus* were resistant to almost all the antibiotics tested. These isolates made up 202 (62%) of the total bacteria isolates. Overall, *Staphylococcus aureus* showed reduced sensitivity for all antibiotics tested (Table 6).

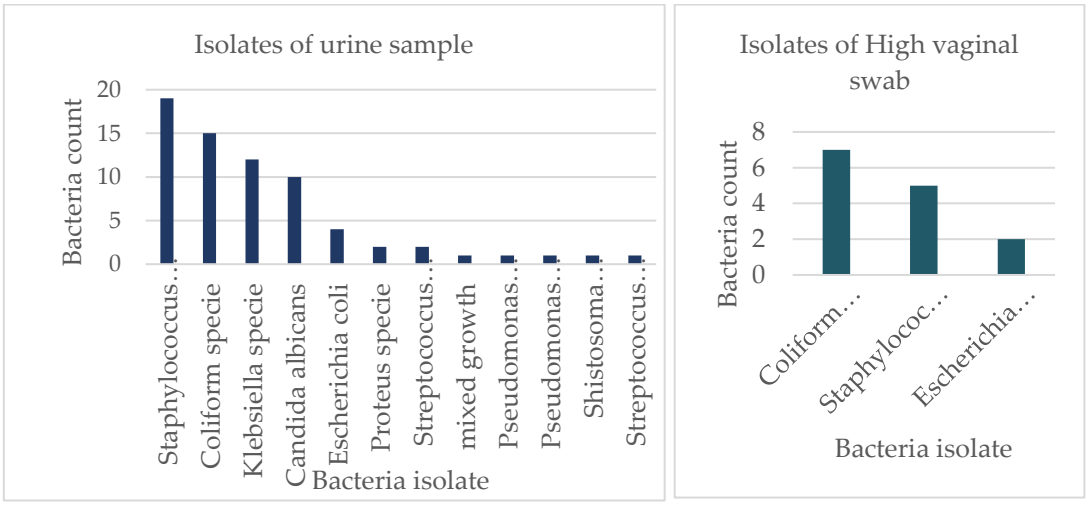

Figure 1: Distribution of isolates from urine and high vaginal samples

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Table 1: Sociodemographic variables

Variable	Frequency (%)
Gender	
Male	102 (18.8)
Female	268 (49.3)
Missing	174 (32.0)
Facility	149 (27.3)
Private facility	373 (68.5)
Public facility	22 (4)
Central Laboratory	
Clinical samples	60(11)
High vaginal swabs	137 (25.2)
Mid-stream urine	26 (4.8)
Sputum	64(11.8)
Stool	28(5.1)
Others	229(42.1)
Missing	
AGE	68(12.6)
1-15	40(7.5)
16-30	71(13.2)
31-45	20(3.7)
46-70	161(29.9)
Adults	177(32.9)
Missing	

Table 2: Association between some bacteria isolates and location

Bacteria Isolates	Frequency (n)	Location (n)		Test statistic	P Value
		Abuja	Lagos		
Klebsiella spp.	27	11	16	1.17	0.28
Females		9	10		
Males		2	6		
E. coli	35	27	8	0.42	0.52
Female		23	0		
Male		4	0		
S. aureus	77	70	7	0.1	0.75
Female		44	3		
Male		26	4		
Coliform Spp	18	16	2	0.31	0.58
Female		11	2		
Male		5	0		
Total	157	124	33		

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Table 3: Buvariate analysis of bacteria isolates and gender by location

Variable	FCT (n)	Lagos (n)	Test statistics	P Value
Bacteria agents				
Coliform spp	24	2	163.76	5.411
Enterobacter spp	3	0		
Escherichia coli	29	12		
Haemophilus spp	1	1		
Klebsiella spp	11	56		
mixed growth	1	0		
Neisseria gonorrhoea	3	28		
Proteus spp	7	2		
Pseudomonas spp	3	4		
Salmonalla species	6	0		
Staphylococcus spp	122	12		
Total	210	117		
Specimen type				
Aspirate	3	0	32.70	0.0003*
Ear Swab	3	4		
Eye swab	1	0		
High vagina swab	25	35		
Semen	6	0		
Sputum	7	19		
Stool	49	15		
Urethral swab	3	0		
Urine	77	60		
Vaginal swab	0	1		
Wound swab	2	5		
Total	176	139		
Gender				
Male	160	106	4.37	0.036*
Female	74	29		
Total	234	135		

*Significant

Table 4: Distribution of bacteria isolates

Variable	Number (%)
Bacteria agents	
Escherichia coli	41(10.9)
Klebsiella species	67(17.9)
Neisseria gonorrhea	31(8.2)
Staphylococcus aureus	94(25.1)
Coliform species	26 (6.95)
Enterobacter species	3(0.8)
Haemophilus species	2(0.53)
Proteus species	9(2.4)
Pseudomonas species	7(1.8)
Salmonella species	6(1.60)
Streptococcus Species	40 (10.69)
Fungal agent	
Candida albica	43(11.47)

Table 5: Antibiogram showing the resistant profile of the bacteria isolates to different antibiotics

Organisms Bacteria	Number of Isolates (No)	% Resistant														
		Gentamycin	Ceftazidime	Amoxicillin	Ceftriaxone	Erythromycin	Cefuroxime	Amoxocillin_Clavulunate	Levofloxacin	Imipenem	Nalidixic acid	Norfloxacin	Nitrofurantoin	Tetracycline	Ciprofloxacin	Co-trimoxazole
Gram-Negative																
Klebsiella spp.	67	99	99	99	99	99	99	99	99	99	78	99	99	99	99	99
E. coli	41	98	98	98	98	98	98	98	98	98	-	98	98	98	98	98
Gram-Positive																
Staphylococcus aureus	93	98	98	98	98	98	98	98	98	98	32	98	98	98	98	98

Table 6: Antibiogram showing the susceptibility profile of the bacteria isolates to different antibiotics

Organisms Bacteria	Number of Isolates (No)	% Susceptible								
		Gentamycin	Ciprofloxacin	Amoxicillin+Clavuluate	Amoxicillin	Ofloxacin	Tetracycline	Nitrofurantoin	Ceftazidime	Nalidixic Acid
Klebsiella spp.	67	82	82	69	78	82	52	82	75	82
Staphylococcus aureus	93	32	32	32	32	32	32	32	33	32

DISCUSSION

Urovaginal pathogens have been responsible for the majority of infections, and this is exacerbated by the high cost of treatment. There is increasing antimicrobial resistance among urinary tract infections (UTIs). *E. coli* is documented as the predominant cause of symptomatic and non-symptomatic UTI. A higher proportion of females 23 (65%) than males were reported with *E. coli* in this study. The majority of *E. coli* cases were reported from facilities within Abuja 27 (77%) than in Lagos 8 (22%). The high prevalence of UTI among the female population in this study was similar to reports in Nigeria [18], including biological plausibility of occurrence due to decreased normal vagina flora and poor hygienic conditions. *Staphylococcus aureus* is the leading major cause of infection in health facilities. They are the most predominant gram-positive organisms consisting of about (25%) of bacterial isolates which is similar to what was reported in Lagos, Nigeria [12]. In this study, facilities in Abuja presented a significantly higher proportion 70 (90%) of *Staphylococcus aureus* than in Lagos 7 (0.09%). This is similar to a study conducted in Nigeria with 54.1% of *Staphylococcus aureus* identified in the North Central and 2.5% in the south-south region [19]. Overall, there was no significant association between the type of bacteria isolate and location in this study. Antibiotic Resistance to *S.aureus* in this study ranged from 32-98%. Similarly, other studies have reported multi-drug resistance of *S.aureus* [12,13,14,18]. Both genders have been almost equally affected by *Staphylococcus aureus* infection (49%) followed by *E. coli* (23%). This is

contrary to findings in Nigeria [13,18]. This study found low sensitivity such as *Klebsiella* spp. and *Staphylococcus aureus* to several antibiotics which is in agreement with several published reports in Pakistan [20] and Indonesia [21]. There was a high level of resistance to major antibiotics which could be a result of self-medication and antibiotic abuse [22].

Cephalosporins are used to treat a variety of bacterial infections and are also useful in the empirical treatment of urinary tract infections. The third-generation antimicrobial (ceftazidime and ceftriaxone) Cephalosporins are useful against a broad range of bacteria including gram-negative bacteria[23]. In this study, the majority of the gram-negative bacteria such as *E. coli* were resistant to the 3rd generation cephalosporins (ceftazidime and ceftriaxone). The reduced susceptibility of these gram negatives has been reported in other studies [24,25]. Multidrug resistance was observed at 97.6%, 98%, and 93.6% for *Klebsiella* species*, E. coli*, and *Staphylococcus aureus* respectively. The observed multi-drug resistance is higher than the result reported in Lagos (86.7%, 84.2%, 83%) [12]. This high level of resistance observed for penicillin, co-trimoxazole, and tetracycline can be attributed to the inexpensive nature of these drugs, relatively broad spectrum in nature, and on the list of essential medicines frequently in use in developing countries [22].

During this study, investigators found poor data management and reporting practices among the selected laboratories. For example, Missing data from facility records such as age 177 (32.9%) and sex 174 (32.0%) were observed. Reporting was mostly paper based. In addition, there were variations in standard laboratory practices regarding identification, culture, resistance, and sensitivity testing. For example, laboratories in Lagos had different reporting systems for culture and microscopy results making quality management a challenge. Moreover, some laboratories reported the unavailability of selective and differential media. Quality testing of reagents and media was rarely done. The use of different antibiotic discs at the time of this survey was also observed in the majority of laboratories sampled. These challenges highlighted were acknowledged by the Nigerian Center for Disease Control in 2018 as an issue requiring a structured approach through the development of a national laboratory guideline to guide processes for isolation, identification, and AMR testing [10,11]. Regarding antimicrobial susceptibility protocol, there are GLASS recommendations for antibiotics testing and recommended antibacterial agents. Although allowance has been provided for testing according to local prescribing practices, this study found a lot of antibiotics testing conducted for bacterial isolates outside the GLASS recommendations. We recommend appropriate SOP be developed and used according to the most current guidelines of CSLI or EUCAST.

STUDY LIMITATION

Out of the facilities (40) that met the minimum criteria for microscopic culture and sensitivity testing capacity, only a subset of the facilities (5) provided permission to conduct this study. Quality assurance in laboratory procedures from specimen receipt, handling, testing, analysis, and reporting needs to be reliable to meet the appropriate regulatory requirement. In this study, we observed that several antibiotic testing and sensitivity (ATS) laboratory processes and procedures will need to be strengthened to assure the safety and effectiveness of the tests provided.

CONCLUSIONS

The most prevalent bacteria isolated in this study is *Staphylococcus aureus* from urine specimens. Although Gram-negative bacteria were the most isolated in terms of number, the *Staphylococcus aureus* –a Gram-positive bacterium were the most predominant isolate. Several bacterial isolates showed high resistance to first-line antimicrobials like amoxicillin and second-line antimicrobials like fluoroquinolones indicating the need for regular monitoring. Moreover, it is imperative that hospitals use microscopic culture and sensitivity (MCS) data to procure antibiotics. Facilities should consider the development of antibiograms for the clinical management process. Routine MCS testing can be valuable for AMR surveillance. There is an urgent need for Improvement in Microscopic sensitivity and culture sensitivity documentation through continuing education and congruent record-keeping practices across laboratories.

ABBREVIATIONS

AMR - Antimicrobial Resistance
AMRSNET - Anti-microbial Resistance Surveillance Network
AST - Antimicrobial and sensitivity testing
CLSI - Clinical and Laboratory Standards Institute
EUCAST - European Committee for Antimicrobial Susceptibility Testing
FCT - Federal Capital Territory
GAP - Global Action Plan
GLASS - Global Antimicrobial Resistance and Use Surveillance System
HIV - Human Immunodeficiency Virus
ReACT - Action on Antibiotic Resistance
SOP - Standard Operating Procedure
UTI - Urinary Tract Infections
WHO - World Health Organization

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS
HA, MN, BG, and TO participated in the design of the experiment, methodology, validation, and Analysis; KN, AI, JS, RI, WU, EM, AE, and SO participated in the validation, formal analysis, and investigation; HA AI, JS, KN, WU prepared the original draft; MN, RI, BG, SA, OO, EM, AE, SO review and edited the manuscript and visualization, MN, and BG supervised and provided overall project administration.

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