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Prevalence and molecular detection of ESBL genes in uropathogens isolated from children and adolescents in a General Hospital in Lagos, Nigeria

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Abstract:

Background: Urinary tract infections (UTIs) are a significant global health concern with rising antimicrobial resistance (AMR) posing a serious challenge to effective treatment. The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing uropathogens further complicates management, as these bacteria are resistant to third-generation cephalosporins and other β-lactam antibiotics. The objective of this study is to determine the prevalence and characterize ESBL genes in uropathogens isolated from children with UTIs in Ajeromi General Hospital, Ajegunle, Lagos, Nigeria.
Methodology: A total of 165 children and adolescents presenting with symptoms of UTI were consecutively recruited into the study. Socio-demographic and clinical characteristics were collected from the participants using a structured data collection developed for the study. Voided midstream urine sample was collected from each participant. Standard microbiological methods were used to isolate and identify uropathogens from the sample, followed by antibiotic susceptibility testing (AST) using the Kirby-Bauer disk diffusion method. ESBL production was phenotypically detected by the double-disk synergy test (DDST), and conventional polymerase chain reaction (PCR) assay was performed to detect *bla*$_{TEM}$ and *bla*$_{CTX-M1}$ ESBL genes.
Results: Of the 165 children and adolescents whose urine samples were collected, 49 (26.7%) tested positive for significant bacterial growth, with *Escherichia coli* (53.1%) and *Klebsiella pneumoniae* (16.3%) being the predominant bacterial isolates. The AST revealed high resistance rates to third-generation cephalosporins (with 76.9% to ceftriaxone) and carbapenem (imipenem). While the phenotypic DDST did not confirm ESBL production in any of the isolates, PCR detected ESBL genes in 37 (75.5%) isolates.
Conclusion: Our study highlights the widespread presence of ESBL-producing uropathogens, despite limitations in phenotypic detection methods, underscoring the need for routine molecular screening in AMR surveillance. The findings emphasize the critical role of antibiotic stewardship, infection control measures, empirical treatment guidelines, and promoting targeted molecular diagnostics in preventing the spread of multidrug-resistant (MDR) uropathogens.

Keywords: Urinary tract infections; ESBL; Multidrug-resistant; Molecular screening

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Prévalence et détection moléculaire des gènes BLSE chez des uropathogènes isolés chez des enfants et des adolescents dans un hôpital général de Lagos, au Nigéria

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Résumé:

Contexte: Les infections urinaires (IU) constituent un problème de santé mondial majeur, la résistance croissante aux antimicrobiens (RAM) posant un sérieux défi à l'efficacité de leur traitement. La prévalence croissante des uropathogènes producteurs de bêta-lactamases à spectre étendu (BLSE) complique encore davantage la prise en charge, car ces bactéries sont résistantes aux céphalosporines de troisième génération et à d'autres antibiotiques bêta-lactamines. L'objectif de cette étude est de déterminer la prévalence et de caractériser les gènes BLSE chez les uropathogènes isolés chez des enfants atteints d'IU à l'hôpital général d'Ajeromi, à Ajegunle, à Lagos, au Nigéria.

Méthodologie: Au total, 165 enfants et adolescents présentant des symptômes d'IU ont été recrutés consécutivement pour l'étude. Les caractéristiques sociodémographiques et cliniques des participants ont été recueillies à l'aide d'un recueil de données structuré développé pour l'étude. Un échantillon d'urine mictionnelle a été prélevé chez chaque participant. Français Des méthodes microbiologiques standard ont été utilisées pour isoler et identifier les uropathogènes de l'échantillon, suivies d'un test de sensibilité aux antibiotiques (AST) à l'aide de la méthode de diffusion sur disque de Kirby-Bauer. La production d'BLSE a été détectée phénotypiquement par le test de synergie à double disque (DDST), et un test de réaction en chaîne par polymérase (PCR) conventionnel a été réalisé pour détecter les gènes ESBL *bla*$_{TEM}$ et *bla*$_{CTX-M-1}$.

Résultats: Sur les 165 enfants et adolescents dont les échantillons d'urine ont été collectés, 49 (26,7%) ont été testés positifs pour une croissance bactérienne significative, *Escherichia coli* (53,1%) et *Klebsiella pneumoniae* (16,3%) étant les isolats bactériens prédominants. L'AST a révélé des taux de résistance élevés aux céphalosporines de troisième génération (avec 76,9% à la ceftriaxone) et aux carbapénèmes (imipénème). Bien que le test phénotypique de détection des antigènes de surface (DDST) n'ait confirmé la production de BLSE dans aucun des isolats, la PCR a détecté des gènes de BLSE dans 37 isolats (75,5%).

Conclusion: Notre étude met en évidence la présence généralisée d'uropathogènes producteurs de BLSE, malgré les limites des méthodes de détection phénotypique, soulignant la nécessité d'un dépistage moléculaire systématique dans la surveillance de la RAM. Les résultats soulignent le rôle crucial de la gestion des antibiotiques, des mesures de contrôle des infections, des recommandations thérapeutiques empiriques et de la promotion de diagnostics moléculaires ciblés dans la prévention de la propagation des uropathogènes multirésistants (MR).

Mots-clés: Infections urinaires; BLSE; Multirésistance; Dépistage moléculaire

Introduction:

Urinary tract infections (UTIs) are common medical conditions resulting from the invasion of pathogenic microorganisms into the urinary tract, leading to infections affecting various parts of the urinary system (1,2). The condition is more prevalent in certain populations, such as newborns, young children, sexually active women, and the elderly (3-6). Antimicrobial resistance (AMR) presents an increasing challenge in UTI management, particularly with uropathogenic bacteria exhibiting high resistance rates to multiple antibiotics (7,8). Reports have shown that resistance rates to ciprofloxacin, the most commonly prescribed antibiotic for UTIs, range from 8% to 93% for *Escherichia coli* and from 4% to 79% for *Klebsiella pneumoniae* (7). This increasing prevalence of multi-drug-resistant (MDR) organisms, particularly Gram-negative bacteria, complicates treatment, as they exhibit notable resistance to commonly prescribed antibiotics (9,10).

Studies have shown varying UTI prevalence rates among children, including 26.8% in Uganda (11), 20.65% in Tanzania (12), 11.0% in Nigeria (13), and 27.0% in Ethiopia (9). Also, recent studies have reported notable rise in extended-spectrum β-lactamase (ESBL)-producing *E. coli* in paediatric UTIs. In Duhok City in Iraq, 64.0% of *E. coli* isolates tested positive for ESBL production, with molecular analysis revealing that over 90% harbored *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes (14). A meta-analysis by Azzam et al., (15) found an overall ESBL prevalence rate of 62.0% among pediatric UTI cases, while Kibwana et al., (16) reported 97.0% prevalence of *bla*$_{CTX-M}$ genes in phenotypically detected ESBL-PE isolates in Tanzania.

These findings highlight the importance of thoroughly characterizing ESBL-resistant genes in uropathogens linked to UTIs in children and adolescents to guide treatment approaches and limit the spread of MDR infections. Nevertheless, there exists a scarcity of evidence-based research concerning antibiotic-resistant UTIs in children and adolescents in Nigeria. Consequently, this study determined the prevalence and antimicrobial susceptibility patterns, and detected ESBL genes in bacterial uropathogens involved in UTIs among clinically suspected children and adolescents in a General Hospital in Lagos, Nigeria.

Materials and method:

Clinical setting, study participants and ethical approval:

This was a descriptive cross-sectional

study of 165 consecutively selected children and adolescents with clinical symptoms of UTI attending the Ajeromi General Hospital, Ajegunle, Lagos, Nigeria. The study protocol was approved by the Health Research Ethics Committee of the University of Lagos College of Medicine (Approval Number: CMUL/HREC/01/24/1342). Informed consent was obtained from the parents/guardians/caregivers of the children and informed assent of adolescents.

Data collection:

Demographic and clinical information were obtained from each participant using a structured data collection form developed for the study. Trained healthcare personnel collected the data during routine clinical consultation at the pediatric outpatient department of the hospital. Clinical information collected included presenting symptoms (such as fever, dysuria, urinary frequency, urgency, suprapubic pain), history of previous UTI and patient location (inpatient or outpatient).

Sociodemographic variables collected included age, sex, type of school attended, history of use of school toilets, knowledge of participants toilet use and personal hygiene. These data were obtained directly from the caregivers/parents of the participants through interview.

Urine sample collection:

Voided mid-stream urine sample was collected from each participant by the parent/caregiver into sterile sample bottle, with clear instruction on how urine should be collected. The urine samples were transported immediately to the laboratory for analysis.

Culture isolation of bacteria from urine:

The urine samples were carefully inoculated on Blood and Cystine-Lactose-Electro lyte-Deficient (CLED) agar plates using the streak plate method. The culture plates were incubated at 37°C for 24 hours in aerobic incubator. Culture plates showing significant bacteriuria ($\geq 10^5$ colony-forming units/ml of a single uropathogen on culture plate) were characterized further and isolate identified based on colony morphology, hemolysis patterns on blood agar, lactose fermentation on CLED agar, and conventional biochemical test schemes, including indole, citrate, urease, methyl red (MR), and Voges Proskauer (VP) tests, in accordance with the protocol outlined in Cheesbrough's guidelines for diagnostic microbiology (17). Culture plates with mixed growths or with colony counts of less than 10^5 CFU/ml were excluded from further analysis.

Antibiotic susceptibility test:

The Kirby-Bauer disk diffusion method was used to determine the susceptibility of each isolate to 11 selected antibiotics (18). The antibiotic discs included ofloxacin (OFX, 5µg), cefixime (CFM, 5µg), nalidixic acid (NA, 30µg), ceftriaxone-sulbactam (CRO, 30/15µg), cefuroxime (CXM, 30µg), cefotaxime (CTX, 30µg), imipenem/cilastatin (IMP, 10/10µg), amoxicillin-clavulanic acid (AUG, 20/10µg), levofloxacin (LEV, 5µg), gentamicin (GN, 10 µg) and nitrofurantoin (NIT, 300µg).

The plates were incubated at 37°C in an aerobic environment for 18-24 hours. The zone diameter of inhibition around the disk was measured with a graduated ruler to the nearest millimetre, and interpreted as sensitive, intermediate or resistant according to the CLSI guideline (18).

Phenotypic ESBL screening and confirmation of Gram-negative bacteria:

Gram-negative bacterial isolates resistant to at least one third-generation cephalosporin (cefotaxime, ceftriaxone or cefixime) in the AST were suspected to be presumptive ESBL producers. These isolates were confirmed as ESBL producers using the double disk synergy test (DDST).

Inoculum of the bacterial isolates was standardized to 0.5 McFarland standard, which was then used to inoculate Mueller-Hinton agar plates. Amoxicillin-clavulanate disc was placed in the center of the plate, and cefotaxime and ceftriaxone discs were then placed 15mm away from the amoxicillin-clavulanate disc. After incubation, an ESBL-producing bacteria was confirmed if there was enhancement of inhibition zones of cefotaxime or ceftriaxone around the amoxicillin-clavulanate disc to produce the characteristic 'keyhole' or 'champagne cork' shape.

Genomic DNA extraction:

Fresh bacterial cultures were used for genomic DNA extraction using the boiling method, as described by Egwuatu et al., (19). The resulting supernatant, containing the extracted DNA, was then stored at -20°C for subsequent PCR analysis.

PCR amplification of ESBL genes:

The ESBL genes (bla_{TEM} and $bla_{CTX\text{-}M\text{-}1}$) were amplified by using gene-specific primers (Table 1). Each PCR reaction (12.5 µL) included a master mix of One Taq Quick-Load 2x Master Mix, forward and reverse primers, template DNA, and molecular-grade water. The mixture was amplified in a thermocycler with the following reaction cycle parameters; an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing (temperature specified in Table 1) for 1 minute, and extension at 72°C for 1 minute.

Electrophoresis of amplified products:

The amplified PCR products were analyzed using 2% agarose gel electrophoresis, run-

ning at 80V for 90 minutes. The DNA bands were visualized after ethidium bromide staining, and a 100 bp DNA ladder was used to determine the size of the amplified DNA fragments.

Results:

Prevalence of urinary tract infection with respect to age and gender:

A total of 165 children and adolescents with clinical features of UTI were recruited into the study, with 51.5% (n=85) males and 48.5% (n=80) females. The age group 11-18 years constitute the majority of the participants (63.6%, n=105), followed by age group 6-10 years (20. 0%, n=33) and age group 0-5 years (16.4%, n=27) (Table 2).

Urine samples of 49 participants showed significant bacterial (monomicrobial) growth, giving a 29.7% prevalence of UTI in the study (Table 2). The UTI prevalence of 32.5% in the females is higher than 27.1% in the males but this difference is not statistically significant (p= 0.5525). The prevalence of UTI among age group 0-5 years (29,6%), 6-10 years (33.3%) and 11-18 years (28.6%) was not significantly different (p=0.8725) (Table 2).

Bacterial isolates from urine specimens:

Of the 49 clinical isolates identified, 26 (53.1%) are *E. coli,* 8 (16.3%) *K. pneumoniae,* 6 (12.2%) *Proteus mirabilis,* 3 (6.1%) *P. aeruginosa* and 6 (12.2%) *S. aureus* (Table 3).

Table 1: Primers used for PCR assay of ESBL genes in the study

Target gene	Primer sequence (5' - 3')	Annealing temperature (°C)	Product size (bp)	References
$bla_{CTX\text{-}M\text{-}1}$	F-CGTCACGCTGTTGTTAGGAA R-ACGGCTTTCTGCCTTAGGTT	55	780	Zeynudin et al., (20)
bla_{TEM}	F-GTATCCGCTCATGAGACAATAACCCTG R-CCA ATG CTT AAT CAG TGA GGC ACC	62	918	Kaur and Aggarwal., (21)

Table 2: Prevalence of urinary tract infection with respect to gender and age of the study participants

Variable		Urine culture positive (n, %)	Urine culture negative (n, %)	x^2	OR (95% CI)	p value
Sex						
	Male (n=85)	23 (27.1)	62 (72.9)	0.3529	0.7705 (0.3945-1.505)	0.5525
	Female (n=80)	26 (32.5)	54 (67.5)			
	Total (n=165)	**49 (29.7)**	**116 (70.3)**			
Age group (years)						
	0–5 (n=27)	8 (29.6)	19 (70.3)	0.2728	NA	0.8725
	6–10 (n=33)	11 (33.3)	22 (66.7)			
	11–18 (n=105)	30 (28.6)	75 (71.4)			
	Total (n=165)	**49 (29.7)**	**116 (70.3)**			

OR = Odd ratio; CI= Confidence interval; NA = Not applicable

Table 3: Frequency of bacterial isolates recovered from urine samples of the study participants

Bacterial isolates	**Number of isolates**	**Frequency (%)**
Escherichia coli	26	53.1
Klebsiella pneumoniae	8	16.3
Proteus mirabilis	6	12.2
Pseudomonas aeruginosa	3	6.1
Staphylococcus aureus	6	12.2
Total	**49**	**100.0**

Table 4: Association of UTI prevalence with socio-demographic characteristics of the study participants

Variables	**No positive for UTI (%)**	**No negative for UTI (%)**	**x2**	**OR (95% CI)**	***p* value**
Type of school					
Public	39 (36.8)	67 (63.2)	13.988	NA	0.0009*
Private	8 (14.0)	49 (86.0)			
Home-based	2 (100.0)	0			
Use of school toilets					
Yes	49	112	0.5807	3.960	0.3194
No	0	4		(0.2090 - 75.02)	
Knowledge of personal hygiene for toilet use					
Yes	37	83	0.1092	1.226	0.7033
No	12	33		(0.5698 - 2.637)	
History of UTI					
Yes	6	14	0.001	1.017	1.000
No	43	102		(0.3663 - 2.822)	
Location					
Outpatient	49	102		14.01	0.0111
Inpatient	0	14		(0.818 - 239.76)	

UTI = urinary tract infection; OR = Odd ratio; CI = confidence interval; NA = Not applicable

Association of sociodemographic characteristics of the study participants with UTI prevalence:

The prevalence of UTI was significantly higher among children who attends home-based school (100.0%) compared to those who attend public (36.8%) or private school (14.0%) (p=0.0009) (Table 4). However, other factors such as children use of school toilets, knowledge of personal hygiene on the use of the toilets, and history of UTI were not significantly associated with UTI prevalence (p>0.05).

Antibiotic susceptibility results of bacterial isolates:

The antibiotic susceptibility test result for *E. coli* in Table 6 shows that all 26 isolates were 100% resistant to cefuroxime, cefotaxime, ceftriaxone, imipenem, amoxicillin-clavulanate and cefixime. The isolates were sensitive to ofloxacin (100.0%), levofloxacin (80.8%) and gentamicin (100.0%) (Table 5).

Klebsiella pneumoniae isolates was 100.0% sensitive to ofloxacin, levofloxacin and gentamicin but 100.0% resistant to cefotaxime, imipenem, cefuroxime, cefixime, ceftriaxone and amoxicillin-clavulanate. *Proteus mirabilis* isolates were 100.0% sensitive to ofloxacin, levofloxacin and gentamicin but 100.0% resistant to cefixime, cefuroxime, cefotaxime, nitrofurantoin, amoxicillin-clavulanate, ceftriaxone and imipenem (IMP). *Pseudomonas aeruginosa* isolates were 100.0% sensitive to ofloxacin, levofloxacin and gentamicin but 100.0% resistant to ceftriaxone, cefotaxime and cefuroxime and imipenem

Staphylococcus aureus isolates were sensitive to ofloxacin (100.0%), levofloxacin (83.3%), and gentamicin (83.3%) but 100.0% resistant to cefixime, cefuroxime, ceftriaxone, amoxicillin-clavulanate and cefotaxime (Table 6).

Table 5: Antibiotic susceptibility test results of Gram-negative bacterial isolates to selected antibiotics

Antibiotic/Bacteria isolate	*Escherichia coli* (%)		*Klebsiella pneumoniae* (%)		*Proteus mirabilis* (%)		*Pseudomonas aeruginosa* (%)	
	S	R	S	R	S	R	S	R
β-lactam- β-lactamase inhibitor combination								
Amoxicillin-clavulanic acid	0	26 (100.0)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)
Cephalosporins								
Cefotaxime (CTX)	0	26 (100)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)
Cefuroxime (CXM)	0	26 (100)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)
Ceftriaxone (CRO)	0	26 (100)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)
Cefixime (CFM)	0	26 (100)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)
Quinolones								
Ofloxacin (OFX)	26 (100.0)	0	8 (100)	0	6 (100.0)	0	3 (100.0)	0
Levofloxacin (LEV)	21 (80.8)	5 (19.2)	8 (100)	0	6 (100.0)	0	3 (100.0)	0
Nalidixic acid (NA)	5 (19.2)	21 (80.8)	1 (12.5)	7 (87.5)	2 (33.3)	4 (66.7)	2 (66.7)	1 (33.3)
Carbapenems								
Imipenem (IMP)	0	26 (100.0)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)
Nitrofurans								
Nitrofurantoin (NIT)	4 (15.4)	22 (84.6)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)
Aminoglycosides								
Gentamicin (GEN)	26 (100.0)	0	8 (100.0)	0	6 (100.0)	0	3 (100.0)	0

Table 6: Antibiotic susceptibility test results of *Staphylococcus aureus* isolate to selected antibiotics

Antibiotics/Bacterial isolate	*Staphylococcus aureus* (%)	
	S	R
β-lactam- β-lactamase inhibitor combination		
Amoxicillin-clavulanic acid (AUG)	0	6 (100.0)
Cephalosporins		
Cefotaxime (CTX)	0	6 (100.0)
Cefuroxime (CXM)	0	6 (100.0)
Ceftriaxone (CRO)	0	6 (100.0)
Cefixime (CFM)	0	6 (100.0)
Quinolones		
Ofloxacin (OFX)	6 (100.0)	0
Levofloxacin (LEV)	5 (83.3)	1 (16.7)
Nalidixic acid (NA)	1 (16.7)	5 (83.3)
Carbapenems		
Imipenem (IMP)	0	6 (100.0)
Nitrofurans		
Nitrofurantoin (NIT)	0	6 (100.0)
Aminoglycosides		
Gentamicin (GEN)	5 (83.3)	1 (16.7)

Phenotypic confirmation of ESBL production and ESBL genes:

Although all the 43 Gram-negative and 6 Gram-positive bacterial isolates were resistant to the 3rd generation cephalosporins used in the AST, none of the 43 Gram-negative isolates was positive for the confirmatory ESBL test with the double disc synergy test. However, 37 of the 43 (86.0%) Gram-negative isolates carried ESBL genes on PCR amplification. The most prevalent ESBL gene carried was the bla_{CTXM-1} harbored by 33 (89.2%) of the 37 ESBL Gram-negative

Discussion:

The global increase in bacterial resistance to extended-spectrum cephalosporins presents a major obstacle to effective antibiotic treatment. This rising resistance reduces the range of available therapeutic options, complicating the management and control of infections. In this study, the prevalence of bacterial UTI among the selected children and adolescents was 29.7%, which agrees with findings of studies conducted in Hawassa, Ethiopia, where a prevalence of 27.5% was reported (9), Uganda with 26.8% (11), and Tanzania with 20.65% (12).

However, the prevalence in our study is higher than those from other studies with same population range conducted in Bahirdar, Ethiopia with 16.7% (10), Abakaliki, Nigeria with 3% (22), Enugu, Nigeria with 11% (14), and Dar es Salaam, Tanzania with 16.7% (23). This difference in the prevalence may be due to variations in nutritional status of the population studied, immunity, and geographical location of the study (24).

Table 7: Frequency of ESBL genes carriage among the Gram-negative bacterial isolates

ESBL gene	*Escherichia coli*	*Klebsiella pneumonia*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
bla_{CTXM-1} only	16	0	5	3
bla_{TEM} only	2	2	0	0
bla_{TEM} + bla_{CTXM-1}	2	6	1	0
Total (%)	**20 (46.5)**	**8 (18.6)**	**6 (13.9)**	**3 (6.9)**

Although the age group 11-18 years constituted the majority of the children and adolescents (63.6%) with clinical symptoms of UTI recruited in this study, the prevalence of UTI by culture was highest among the age group 6-10 years (33.3%), although the UTI prevalence difference was not statistically significant with respect to age group of the participants (p=0.8725). However, the recruitment of high number of children with symptoms of UTI in this age group may not be unrelated to increased sexual activity being reported within this age demographic. A study in Benin, Nigeria reported that approximately 31.6% of the study respondents had had sexual intercourse before the age of 15 years (25), a predisposing factor to UTI. Another study in Nigeria reported mean age of onset of sexual activity of 11.68±1.98 years and prevalence of premature sexual activity of approximately 11.0% (26). Additionally, community-based studies in Ghana, Malawi, Uganda and Burkina Faso reported that 45.0% of young men and nearly 60.0% of young women in sub-Saharan Africa had had sexual intercourse before the age of 18 years (27).

In this study, the prevalence of UTI was higher in the females (32.5%) than males (27.1%) although this difference was not statistically significant (p=0.5525). However, it has long been established in literature that females are at higher risk of UTI than males due to the short urethra in females, and the close proximity of vaginal and anal canal with the urinary system, which facilitate entry of vaginal and rectal flora into the urinary tract.

Escherichia coli is the most predominant uropathogen in our study, responsible for over half of the UTI followed by *K. pneumoniae* (16.3%), which is consistent with trends reported globally, where *E. coli* is recognized as the leading cause of UTIs in paediatric populations, accounting for up to 90% of cases (28-30). The predominance of these two bacterial uropathogens also agrees with findings of other studies in Africa such as Ethiopia and Tanzania, which also reported a high prevalence of *E. coli* and *Klebsiella* in paediatric UTIs (9,12). The prevalence rate of 53.1% for *E. coli* UTI in our study is comparable with the rate of 48.6% reported in Hawassa, Ethiopia (9), 63.6% in Bahir Dar, Ethiopia (10), 71.7% in Iran (31), 31.8% in Enugu, Nigeria (13), 35.7% in Dar es Salaam, Tanzania (23), and 41.2% in Mwanza, Tanzania (12). However, our finding contrasts the study conducted in Abakaliki, Nigeria, where the most common bacterial uropathogen was *Klebsiella* spp with 24.5% (22), and in Uganda where *Proteus* spp was the predominant uropathogen with 39.5% (11). The possible explanation for these differing rates may be due to variations in sample size of the studies, specimen collection technique, host immunity system, and nutritional state (32,33).

Being a common gut commensal, *E. coli* is the leading cause of community-acquired UTI and its increased prevalence among pediatric UTI cases may be partly attributed to inadequate personal hygiene practices, which can facilitate faecal contamination of the periurethral area. However, this is considered alongside other contributory factors such as age, sex, and individual host susceptibility. Additionally, *E. coli* has a special structure that encourages urinary tract colonization and evades the immune system, as well as being able to move from the perineum area that is contaminated with faecal matter, invading the host tissues within the urinary tract (34). *Staphylococcus aureus* was isolated from 12.2% of the children which is unexpected because this pathogen has been implicated in predominantly nosocomial infection. However, recent epidemiological trends have shown increasing prevalence of community-acquired *S. aureus* infections including UTIs among individuals without prior hospitalization or known risk factors (35).

The prevalence of UTI was significantly higher among children who attended home-based school (100.0%) compared to those who attended public (36.8%) or private school (14.0%) (p=0.0009). This observation may however need to be cautiously interpreted in view of the few numbers of children attending home-based school in the study, which could affect the validity of the statistical analysis. Although regular urination and prompt bladder emptying are essential components of good toilet hygiene that help

prevent UTIs (36), our study found no significant association of children use of school toilets or knowledge of personal hygiene on the use of toilets and UTI prevalence. The prevalence of UTI among children from outpatient department was 100% compared to 0% among those from inpatient wards (p= 0.0111). This appears to suggest that community-associated paediatric UTI is significantly higher than nosocomial paediatric UTI, however, the prevalence of 100% reported is far higher than the global prevalence, that ranges between 13 and 33% (37). Our finding, which must be interpreted with caution, may be due to the small number of paediatric inpatients with symptoms of UTI recruited into the study.

Antibiotic resistance in microbial population have become one of the most pressing concerns in healthcare worldwide. The emergence and spread of antibiotic-resistant bacteria significantly complicate the treatment of infections and increase mortality, as infections become harder to treat with conventional antimicrobial therapies (38,39). In this study, we assessed the antibiotic resistance of the bacterial uropathogens to eleven selected antibiotics because of previous reports from the Global Antimicrobial Resistance and Use Surveillance System (GLASS), which indicate rising resistance levels among uropathogens, particularly to first-line antibiotics (7). Resistance rates were high to the antimicrobial agents tested, especially the 3rd generation cephalosporins, amoxicillin-clavulanate and carbapenem, to which all the bacterial isolates were 100.0% resistant. The observed high resistance rates may be attributed to the widespread misuse and over-use of antibiotics in our community which stemmed from lack of robust antibiotic stewardship programs in the region (40,41)

Although all the 43 Gram-negative uropathogens isolated in our study were resistant to 3rd generation cephalosporins in the susceptibility testing, making them to be presumptive ESBL producers, none of them were confirmed to produce ESBL by the double disc synergy test. However, molecular analysis by PCR showed that 37 (86.0%) of the 43 isolates harbored ESBL ($bla_{\text{CTX-M-1}}$ and bla_{TEM}) genes, which are well-documented genetic basis of resistance to 3rd generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime. This molecular finding aligns with the antibiotic susceptibility results, where 100.0% resistance rate was recorded against the cephalosporins, suggesting potential ESBL production as the major mechanism of resistance in the Gram-negative uropathogens. The multi-drug resistance patterns observed however suggest that some of the Gram-negative uropathogen harboring $bla_{\text{CTX-M-1}}$ and bla_{TEM} genes may in addition contain other resistance determinants, contributing to reduced efficacy of β-lactam/β-lactamase inhibitor combinations, and resistance to fluoroquinolones and aminoglycosides, which could be linked to co-selection pressure from the ESBL plasmids that often carry multiple resistance genes. This is clinically significant as fluoroquinolones and aminoglycosides are often used as alternative therapies for complicated UTIs.

Previous studies have reported rising trend of ESBL-producing pathogens not only in the hospital but also in community settings (42,43). The high carriage rate of $bla_{\text{CTX-M-1}}$ gene in particular aligns with the global reports that identify CTX-M as the most prevalent ESBL type in both community-acquired and healthcare-associated infections (44). The carriage rate of $bla_{\text{CTX-M-1}}$ was highest among *E. coli* isolates (n=18) in our study, which is consistent with previous findings that *E. coli* is a major producer of CTXM-1 enzyme, particularly in healthcare-associated infections. This was followed by *K. pneumoniae* (n=6), which is expected, as this organism is also known to harbour ESBL genes, including CTX-M-1. All the *P. aeruginosa* and *P. mirabilis* isolates in our study also harbored the CTX-M-1 genes. The highest carriage rate of bla_{TEM} gene was among *K. pneumoniae* (n=8), followed by among *E. coli* (n=4). This suggests that these organisms are capable of producing TEM-type beta-lactamases also, which are often encoded on plasmids and can be transferred between different species. *Proteus mirabilis* isolates have a much lower carriage rate of ESBL genes (n=1), indicating that this species may harbour TEM gene but in a smaller proportion of isolates compared to *E. coli* and *K. pneumoniae*.

Our study showed disparity in the phenotypic confirmatory ESBL detection test (double disc synergy test-DDST) and genotypic (PCR) method used. This emphasizes the necessity of more sensitive and precise techniques to detect these resistant species. The observed discrepancy could be caused by a combination of environmental factors, differences in ESBL expression levels, reduced sensitivity of phenotypic tests, and technical issues when performing the phenotypic confirmatory tests. This may compromise patient outcomes by resulting in incorrect diagnoses and ineffective therapy. To guarantee precise identification of ESBL-positive isolates and direct suitable antibiotic therapy, it is essential to integrate cutting-edge molecular approaches into routine susceptibility testing (45, 46).

There are a number of reasons why the DDST approach for ESBL detection may produce false negative results. Without necessarily engaging ESBL genes, resistance to all

β-lactam antibiotics can be conferred by the co-production of enzymes that hydrolyze carbapenem and aztreonam (42,47). Furthermore, phenotypic detection techniques may not be adequate due to the growing complexity of bacterial resistance mechanisms, which include the development of various β-lactamase types and the combination of ESBLs with AmpC β-lactamases. These drawbacks highlight the necessity of more advanced molecular methods to precisely identify bacteria that produce ESBLs and direct the proper course of antibiotic treatment.

Plasmid-mediated AmpC-type β-lactamases have been discovered in *K. pneumo niae* isolates in a number of studies. It has been discovered that some of these species contain both ESBLs and AmpC-type β-lactamases (42,48). The presence of both enzyme types in the same strain raises the minimum inhibitory concentration (MIC) for cephalosporins, but it can also lead to false-negative ESBL detection tests. It is possible that AmpC type β-lactamases obstruct the synergistic impact of clavulanate and cephalosporins against ESBLs because they are resistant to clavulanate inhibition. It is not known how much of one β-lactamase is needed in relation to the other to mask its presence.

Conclusion:

Our study results have significant clinical implications. The high resistance of *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa,* and *S. aureus* to antibiotics commonly used for treatment of UTI and other infections underscores the necessity of susceptibility testing to guide tailored treatment. The global rise of resistance to third-generation cephalosporins and fluoroquinolones in Gram-negative bacteria raises concerns about their long-term effectiveness. ESBL-producing *E. coli*, along with carbapenem-resistant *P. aeruginosa* and *K. pneumoniae,* pose major treatment difficulties, demanding the development of new antibiotics or alternative therapies.

These findings highlight the critical need to combat antibiotic resistance through enhanced diagnostics, personalized treatment plans, and robust antibiotic stewardship programs. Ongoing monitoring of resistance trends and the discovery of new antimicrobial agents are essential to combat resistant pathogens and preserve antibiotic efficacy for future use.

Contributions of authors:

ENF was involved is study conceptualization, project administration, investigations, methodology, resources and writing of the original draft; UMC was involved in supervision, validation of data, review and editing of the manuscript; AKC was involved in supervision, methodology, visualization, investigation, review and editing of manuscript; CCR was involved in supervision, investigation, cytology validation and review of manuscript; CHN was involved in the design, validation of research data and review of the manuscript; and OLC and MUC were involved in molecular analysis, methodology and data validation. All authors approved the manuscript for submission.

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