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Characterization of Extended Spectrum Beta-lactamase and Carbapenamase Producing Enterobacteriaceae Causing Urinary Tract Infection among Children in Kenya: A Cross-Sectional Study

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Abstract

Introduction: Enterobacteriaceae causing urinary tract infections (UTI) have developed resistance to the commonly used antibiotics due to emergence of Extended Spectrum Beta-Lactamases (ESBLs) and Carbapenamase producing Enterobactericeae which are a public health problem worldwide. This study aims to determine the prevalence and characterize ESBLs and carbapenamase producing Enterobactericeae. **Method:** A cross-sectional study was carried out in Gertrude's Children's Hospital, Nairobi. 238 urine samples were collected from patients with urinary symptoms attending the outpatient department within the period 2020-2021. The urine were examined macroscopically and microscopically. Identification and antimicrobial susceptibility testing were done using VITEK® 2 Compact system (BioMérieux). Double disc synergy test and modified hodge tests were done as confirmatory tests for ESBLs and Carbapenamase phenotypes respectively. Polymerase Chain Reaction was used for the detection of bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{KPC} and bla_{OXA-48} genes. **Results:** From the 238 children sampled the prevalence of UTI caused by Enterobactericeae was 22.3%. The Enterobacteriaceae species isolated were *Escherichia coli* (84.9%), *Klebsiella pneumoniae* (5.66%), *Proteus mirabillis* (5.66%), *Enterobacter aerogenes* (1.89%) and *Morganella morganii* (1.89%). The isolated species were resistant to ampicillin. Meropenem had the highest susceptibility. Only *E. coli* species had the ESBLs (26.4%) and carbapenamase (1.9%) phenotypes. 100% had Bla_{CTX-M} while 50% had bla_{TEM} resistant gene. There was a significant association ($p < 0.05$) between non-school going children and ESBLs related urinary tract infection. A significant association ($p < 0.001$) was also seen between presence of red blood cells in urine and ESBL UTI. **Conclusion:** Ampicillin resistance resulted to use of alternative drugs and Meropenem was the drug of choice where increased resistance to the recommended drugs was noted. Further research on resistant genes is recommended.

Keywords

Enterobactericeae, Urinary Tract Infection, Prevalence, Beta Lactamases, Polymerase Chain Reaction, Children, Outpatient, Antimicrobial Resistance, Phenotypes, *Escherichia coli*

1. Introduction

Enterobacteriaceae are gram negative bacteria that are residents of the gastrointestinal intestinal tract. They cause intestinal and extraintestinal infections like urinary tract infections (UTI), respiratory infections and bacteremia [1]. Urinary tract infections are among the most common infections in children [2]. Worldwide, they cause 2.6% - 7.5% of high fevers in children with females accounting for 89.2% of all urinary tract infections [3]. It is estimated 3% - 5% of girls compared to 1% of boys acquire the infection in their childhood and it is an infection of either the lower or upper urinary tract or both [4]. UTI's result from ascent of bacteria from the peri urethra area while others are through haematogenic route, especially in neonates [5]. Female children are at a higher risk of infection due to the proximity of the urethra to the anus while in male children, bacteria such as *Proteus mirabilis* colonizes the balanopreputial sac [6]. These infections are commonly treated using antibiotics in which resistance to commonly used antibiotics such as beta-lactams has been on the rise among gram-negative bacteria like Enterobacteriaceae [7]. Antimicrobial resistance is a major concern since it increases patient morbidity, hospital expenditure and results in limited treatment options due to resistance as a result of the bacteria producing hydrolyzing enzymes such as the extended spectrum beta-lactamases (ESBL), AmpC beta-lactamases and carbapenamase which hydrolyze the beta lactam rings of the antibiotics and open them up leaving them inactive [8]. Beta-lactamases are widespread and clinically important since they confer resistance to penicillin, cephalosporins and carbapenems and there are 4 classes of β-lactamases grouped according to the nature of the resistant genes A (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$), A Carbapenamase (bla_{KPC}), B (NDM, IMP, VIM)), C (AmpC), D ($bla_{OXA\text{-}48}$). Carbapenamase resistance encoding genes are acquired through horizontal gene transfer interceded by broad-host-range plasmids, transposons and insertion sequences [9] [10].

In de cruces Bilbao hospital in Bilbao, Spain, 15.7% of the children in a study population had UTI, the pathogen most frequently isolated was *E. coli* accounting for 91% of all pathogens, *Enterococcus spp.* 3%*, K. pneumoniae* 2%*, P. mirabilis* 1.4% and *Enterobacter spps* 1% [11]. In a study done in Siddhi Memorial Hospital, Bhaktapur, Nepal, 12.3% of the urine cultures showed a positive result with *E. coli* accounting for 57.8% followed by *Klebsiella spp*. 31.4% while *Proteus spp.*, *Citrobacter spp* and others represented 10.5% of the isolates. Further, the study showed 94.1% of *K. oxytoca* isolates were resistant to cephalexin*.* 71.9% and 77.8% of *E. coli* and *K. pneumoniae* isolates were resistant to ampicillin respectively. Multi drug resistance was observed in 61.9% of the isolates [12]. According to the global surveillance data (2020) on multi drug resistant organisms, the prevalence of UTI caused by carbapenamase producers is 1% - 5%. In a study done in Hawassa University Comprehensive Specialized Hospital, in Southern Ethiopia 75% of the isolates were Enterobacteriaceae with *E. coli* accounting for 44.4%, *K. pneumonia* 27.8%, *K. oxytoca* 8.33%, *Providencia spp*. 5.6%, *C. diversus* 4.16%, *E. cloacae* 2.8%, *P. mirabilis* 2.8%, and *K. ozaenae* 4.16% whereby 41.7% of the Enterobacteriaceae isolates were confirmed as extended beta-lactamase producers [13]. Another study done in Tikur Anbessa Specialized Hospital in Addis Ababa, Ethiopia the prevalence of ESBL and carbapenamase producing Enterobacteriaceae was 78.57% and 12.12%, respectively. Out of all the *K. pneumoniae*, 84.2%, were ESBL positive while *E. coli* and *K. oxytoca* were 100%, meaning all were positive for extended beta-lactamase. Double-disk synergy method showed 90.9% sensitivity, 66.7% specificity. Carbapenamase production was seen in 9.09% of *K. pneumoniae* and 3.03% [14].

In Kenya, there is limited data on ESBLs and carbapenamase related UTI among children therefore, Gertrude's Children's Hospital which a Level 5 Healthcare Facility and the most established pediatric hospital in Eastern and Central Africa, attending to over 300,000 outpatients annually was chosen as the study site. The aim of this study was to determine the prevalence of Enterobacteriaceae, the proportion of ESBL and Carbapenamase producing Enterobacteriaceae, the presence of $bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}, bla_{KPC} and $bla_{OXA\text{-}48}$ genes in ESBL producing Enterobacteriaceae and risk factors associated with ESBL and carbapenamase producing Enterobacteriaceae causing UTI in children in Gertrude's Children's Hospital.

This study will help to guide the clinicians on the resistance patterns among children which will ensure correct drugs are administered to avoid further resistance and used of commonly resistant drugs.

2. Methods

Study site: The study was done at Gertrude's Children's Hospital Nairobi between March 2021 and September 2022.

Study design: A cross-sectional study

Study population: Children taken for treatment at Gertrude's Children's Hospital outpatient department at the time of study. The children were between

6 months and 17 years who presented with urinary tract infection symptoms which are fever, dysuria, change in normal toilet habits such as bed wetting or wetting themselves, deliberately holding their urine, frequent urination, unpleasant smelling urine, lower abdominal pains and nausea. The distribution of sample collection did not vary across the study period.

Inclusion criteria: All children between 6 months and 17 years whose parents and guardians had consented and suspected of having UTI.

Exclusion criteria: Children suspected to have UTI but were already enrolled in the study. Children on antibiotics 3 days prior to sampling

Ethical Approval: Ethical approval was granted by Gertrude's Children's Hospital ethical review board reference number GCH094/2021. Research license was also granted by National Commission for Science, Technology and Innovation (NACOSTI) Research clearance committee; Ref: License No: NACOSTI/P/23/28051. Informed consent was obtained from the parents or guardians of the children involved in the study and confidentiality of the study participants was maintained by coding their names.

Sample size: Fisher's formula was used to calculate the sample size using the prevalence of UTI caused by Enterobacteriaceae of 19.1% Gertrude's Children's Hospital, Nairobi laboratory reports (2019-2020) leading to a sample size of 238.

Sample collection: Approximately 10 - 15 milliliters of urine was collected in a sterile urine container. A clean catch of mid-stream urine was collected from all the patients with the help of their guardians or parents after cleaning the perineum. For children under 3 years, a sterile plastic urine bag was placed around the perineum for urine collection after cleaning the area. The urine sample was placed into a biohazard bag and taken to Gertrude's Children's Hospital laboratory immediately at room temperature, ensuring not to exceed two hours after collection.

Laboratory procedures:

Urinalysis: The color of the urine and clarity or turbidity or cloudiness of the urine were checked. The urine was transferred into a urine centrifuge tube and dipstick test done to check the biochemical characteristics of the urine. The urine was then centrifuged and the deposit observed under the microscope for presence of red blood cell, leucocytes and bacteria.

Culture: Samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) media and incubated at 37˚C for 24 hours. The isolated colonies were gram stained and identified using biochemical tests on VITEK® 2 Compact system (BioMérieux) using GN card. Antimicrobial susceptibility testing was done using broth microdilution minimum inhibitory concentration technique on VITEK® 2 Compact system (BioMérieux) using AST GN83 card and interpretation done as per the CLSI 31st edition, 2020 guidelines.

Extended Spectrum Beta-Lactamases phenotype detection: Isolates that were resistant to cephalosporins were subjected to phenotypic detection using the double disc synergy test (DDST). A suspension of the test organism was prepared using 0.5 McFarland standard as the reference. The entire surface of Mueller Hinton agar (MHA) plate was inoculated with a sterile swab. Amoxicillin-Clavulanic acid (20/10 ug) was placed at the center of the inoculated plate, Ceftriaxone (30 ug), cefepime (30 ug) and Cefotaxime (30 ug) discs were placed at 15mm apart and the plate incubated for 24 hours at 37˚C. Appearance of an enlarged zone of inhibition from either of the antibiotics towards the Amoxicillin-Clavulanate disc signified presence of a potential ESBL producing organism. *E. coli* ATCC 25922 was used as a control.

Carbapenamase phenotype detection: The isolates that were resistant to carbapenems were subjected to phenotypic detection using Modified Hodge test (MHT). *E. coli* ATCC 25922 in a 1:10 dilution was evenly swabbed on Muller Hinton Agar media (MHA) and allowed to stand for 10 minutes at room temperature for drying to take place. Meropenem (10 ug) disk was placed on the swabbed MHA. Using 10ul loop, three straight lines were streaked on the surface of the swabbed media. One line of the test microbe, a second for a positive control *K. pneumoniae* ATCC 1705 and a third for a negative control *E. coli* ATCC 25,922 each starting from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35˚C for 24 hours. The plate was examined for growth around the test streak. Growth near the meropenem disc indicated that the organism was positive for carbapenamase production while no growth indicated that the test microbe was negative for carbapenamase production. This looked like a clove leaf.

DNA extraction: Colony PCR was carried out. Pure cultures were used in DNA preparation by picking two colonies from a fresh culture media plate using a sterile wire. It was suspended in 0.5 mL of distilled water and heated in a water bath at 100˚C and allowed to boil for 30 minutes. It was cooled down to room temperature and centrifuged at 15000 rpm. The supernatant which contained the suspended DNA preparation was then harvested and stored at −20˚C. The harvested DNA preparation was Purified using, Qiaquick purification kit (Qiagen) and the concentration checked using a Nanodrop 2000 spectrophotometer (Themo Fisher Scientific -US).

Molecular detection of resistant genes: Multiplex PCR was used to detect presence of resistant genes. 25 µL of the reaction mix was prepared by adding 12.5 ul of PCR master-mix (Taq polymerase 0.05 U/µl, 0.4 mM of each dNTP and 4 mMMgCl$_2$) (Biolabs Inc) to specific forward and reverse primers (10pmol) 0.5 µL each (**Table 1**) and 2.5 µl DNA template.

The PCR was run by using a thermocycler (Biorad iCycleriQ ^{TM}) in 0.2ml sterile Eppendorf tubes. Amplification was done using an optimized program set at 94˚C for initial heating for 6 minutes followed by thirty denaturation cycles at 95˚C for a minute. Annealing was done at specific primer temperatures (**Table 1**) for one minute, followed by two extension cycles at 70˚C for one minute and 70˚C for ten minutes. PCR products were stored at −20˚C awaiting gel electrophoresis. The PCR products were visualized on 1.5% agarose gel (ThermoFisher Scientific) prepared with 0.03% ethidium bromide stain. The amplicons were identified by matching to a molecular marker (100 bp DNA ladder, England Biolab) that was run alongside the samples and the controls. The negative control used was *E. coli* 25922. The positive controls were *E. coli* ATCC 35218 for Bla$_{TEM}$, *K. pneumoniae* ATCC 700603 for Bla$_{SHV}$ and BLA$_{CTX-M,}$ *K. pneumoniae* ATCC 1705 for Bla$_{KPC}$ and *E. coli* ATCC 2523 for Bla$_{OXA-48.}$

Table 1. Primer sequence of selected resistant genes.

	Target Gene	Primer sequence	Annealing temperature (˚C)	Bp size
Extended spectrum beta-lactamase genes	bla$_{CTX-M}$	**F:** ATG TGC AGY ACC AGT AAR GTK ATG GC **R:** TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	55	593
	bla$_{TEM}$	**F:** CGC CGC ATA CAC TAT TCT CAG AAT GA **R:**A CG CTC ACC GGC TCC AGA TTT AT	55	445
	bla$_{SHV}$	**F:** CTT TAT CGG CCC TCA CTC AA **R:**AGG TGC TCA TCA TGG GAA AG	55	392
Carbapenamase genes	bla$_{KPC}$	**F:** CGC CGT GCA ATA CAG TGA TA **R:** GCA GAG CCC AGT GTC AGT 7 TT	56	232
	bla$_{OXA-48}$	**F:** GCG TGG TTA AGG ATG AAC AC **R:** CAT CAA GTT CAA CCC AAC CG	52	438

Data analysis: Data was collected and entered into excel. It was then exported to R software (R Foundation for Statistical Computing, Vienna, Austria, version 4.1) where analysis was done. Chi square was used to analyze the differences in categorical variables associated with ESBL and carbapenamase phenotype causing UTI in children among children in Gertrude's Children's hospital and their statistical significance whereby a $p < 0.05$ was considered statistically significant. Charts, graphs and tables were used to display the result

3. Results

Demographic data of the study participants: Two hundred and thirty eight (238) participants were recruited in this study. Of these, 68.5% were female and 31.5% were males. According to age, 44.5% of the participants were aged below 5 years, 32.8% were aged 6 - 10 years and 22.7% were aged 11 - 18 year. Then 66.4% were school going children, 77.6% had history of previous UTI infection.

Urinalysis: This was carried out in all the 238 samples. Out of these, 58 were turbid, 20 were nitrite reduction positive, 58 were leucocytes esterase positive and 11 had red blood cells.

Prevalence of Enterobactericeae causing UTI among the children: Fifty eight (58) out of the 238 urine specimens had microbial growth. The prevalence of UTI caused by Enterobactericeae was 22.3% (53/238). Non-Enterobactericeae was 2.1% (5/238). Non-Enterobactericeae identified were *Staphylococcus aureus was* 1.7% (1/58), *Enterococcus faecalis* 1.7% (1/58), *Enterococcus avium* 1.7%

(1/58), *Pseudomonas steturi* 1.7% (1/58), and *Staphylococcus lentus* 1.7% (1/58).

Distribution of isolated Enterobactericeae species causing UTI in children: The isolated species of Enterobactericeae were as follows; *Escherichia coli* 84.9% (45/53), *P. mirabilis* 5.66% (3/53), *Klebsiella pneumoniae* 5.66% (3/53), *Enterobacter aerogenes* 1.89% (1/53) and *Morganella morganii* 1.89% (1/53).

Antimicrobial susceptibility pattern of the isolated Enterobactericeae causing UTI in children among children in Gertrude's Children's hospital

E. aerogenes, E. coli, K. pneumoniae, M. morganii, P. mirabillis isolates were all susceptible to Cefuroxime, Cefotaxime, Ciprofloxacin, Ceftriaxone, Gentamicin, Aztreonam, and Meropenem antibiotic while *E. aerogenes* and *P. mirabillis* isolates were all susceptible to Sulfamethoxazole Trimethoprim. All *E. aerogenes* and *M. morganii* isolates and 96% of *E. coli* isolates were susceptible to Nitrofurantoin.

E. coli showed resistance against Amoxicillin-Clavulanate (*27%)* Cefuroxime (38%), Cefotaxime (37%), Ciprofloxacin (20%), Ceftriaxone (31%), Aztreonam (33%), and Meropenem (2%). *E. aerogenes* was resistant to ampicillin (100%) and amoxicillin clavulanate (100%). *M. morganii* was resistant to ampicillin (100%), Trimethoprim-sulfamethoxazole (100%) and amoxicillin clavulanate (100%). *P. mirabillis* was resistant to nitrofurantoin (100%).

All microbes were susceptible to Meropenem except one *E. coli* isolate as shown in Table 2 below.

Table 2. Antimicrobial susceptibility pattern of the isolated Enterobactericeae causing UTI among children in Gertrude's Children's Hospital.

Antibiotic	*E. aerogenes* (n = 1)		E. coli (n = 45)		*K. pneumoniae* (n = 3)		*M. morganii* (n = 1)		*P. mirabillis* (n = 3)	
	R	S	R	S	R	S	R	S	R	S
Ampicillin	1 (100)	-	41 (91)	4 (9)	3 (100)		1 (100)	-	1 (33)	2 (67)
Amoxicillin-Clavulanate	1 (100)	-	12 (27)	33 (73)	1 (33)	2 (67)	1 (100)	-	-	3 (100)
Cefuroxime	-	1 (100)	17 (38)	28 (62)	-	3 (100)	-	1 (100)	-	3 (100)
Cefotaxime	-	1 (100)	16 (37)	29 (63)	-	3 (100)	-	1 (100)	-	3 (100)
Trimethoprim-sulfamethoxazole	-	1 (100)	37 (82)	8 (18)	1 (33)	2 (67)	1 (100)		-	3 (100)
Nitrofurantoin	-	1 (100)	2 (4)	43 (96)	1 (33)	2 (67)	-	1 (100)	3 (100)	-
Ciprofloxacin	-	1 (100)	9 (20)	36 (80)	-	3 (100)	-	1 (100)	-	3 (100)
Ceftriaxone	-	1 (100)	14 (31)	31 (69)	-	3 (100)	-	1 (100)	-	3 (100)
Gentamicin	-	1 (100)	4 (9)	41 (91)	-	3 (100)	-	1 (100)	-	3 (100)
Aztreom	-	1 (100)	15 (33)	30 (67)	-	3 (100)	-	1 (100)	-	3 (100)
Meropenem	-	1 (100)	1 (2)	45 (98)	-	3 (100)	-	1 (100)	-	3 (100)

Proportion of Enterobactericeae with ESBL and carbapenamase phenotype: ESBL and carbapenamase phenotypes were detected only in *E. coli* species. Out of the 53 isolated Enterobactericeae, 26.4% (14/53) showed resistance against cephalosporins and 1.9% (1/53) against carbapenem and were therefore classified as ESBL and carbapenamase phenotypes respectively. These were DDST positive (ESBL phenotype) and MHT positive (carbapenamase phenotype) respectively. The carbapenamase phenotype was present in only one isolate. The patient was a 1year old male, with a history of congenital hydronephrosis, reccurent fever, recurrent urinary tract infection, and hematuria.

Extended Beta-lactam and Carbapenem Resistant genes_causing UTI in children among children in Gertrude's Children's hospital: Out of the 14 extended spectrum beta-lactamase phenotype *E. coli* had; Bla $_{\text{CTX-M}}$ (14, 100%), Bla $_{\text{TEM}}$ (7, 50%) and Bla$_{\text{SHV}}$ (0%) as shown in Figure 1 below. The selected Carbapenamase resistant genes bla$_{\text{OXA-48}}$ and bla$_{\text{KPC}}$ were not detected from the isolated carbapenamase phenotype.

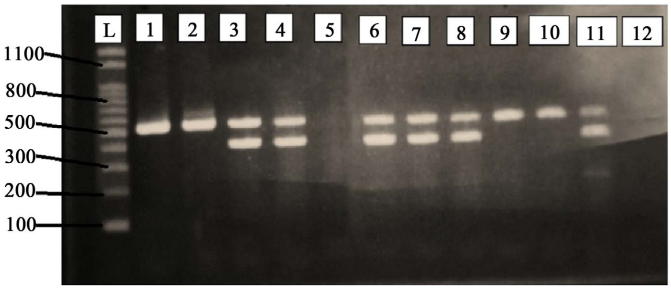

Figure 1. **ESBL resistant genes Gel electrophoresis:** From left, L: Ladder, Lane 1 - 2: Clinical isolates with Bla$_{\text{CTX-M}}$ (593 bp), Lane 3 - 4: Clinical isolates with Bla$_{\text{CTX-M}}$ (593 bp) and Bla$_{\text{TEM}}$ (445 bp); Lane 5: Negative isolate *E. coli* 25922, Lane 6 - 8: Clinical isolates with Bla$_{\text{CTX-M}}$ (593 bp) and Bla$_{\text{TEM}}$ (445 bp), Lane 9 - 10: clinical isolates with Bla$_{\text{CTX-M}}$ (593 bp), Lane 11 Positive controls for Bla$_{\text{TEM}}$ (*E. coli* ATCC 35218), Bla$_{\text{SHV}}$ and Bla$_{\text{CTX-M}}$ (*K. pneumoniae* ATCC 700603), Lane 12: Negative control (distilled water).

Risk factors associated with ESBL and carbapenamase phenotype causing UTI in children among children in Gertrude's Children's hospital: There was a significant association ($p < 0.05$) between non-school going and the UTI caused by ESBL phenotype *E. coli*. Also, a significant association ($p < 0.05$) between presence off blood in urine and UTI from ESBL *E. coli* was noted. Other variables like Sex, age and previous UTI treatment had no significant association with ESBL phenotype (Table 3). The *E. coli* carbapenamase phenotype was present in only one patient.

Table 3. Risk factors associated with ESBL phenotype causing UTI in children among children in Gertrude's Children's hospital.

Variables	Levels	ESBL Phenotype (E. coli) (n=14)	Chi-squared test	p-value
Sex	Female	7 (50%)	$\chi^2 = 3.76$, df = 1	0.052
	Male	7 (50%)		

Continued

Age	<5 years	10 (71%)	$\chi^2 = 2.02$, df = 2	0.364
	6 - 10 years	3 (21%)		
	11 - 17 years	1 (8%)		
School going	No	11 (79%)	$\chi^2 = 11.68$, df = 1	**0.001**
	Yes	3 (21%)		
Previous UTI treatment	No	8 (57%)	$\chi^2 = 1.13$, df = 1	0.287
	Yes	6 (43%)		
RBC in urine	Present	11 (79%)	$\chi^2 = 11.68$, df = 1	**0.001**
	Absent	3 (21%)		

4. Discussion

Pathogenic Enterobacteriaceae has been included by World Health Organization in the global priority pathogen list of antibiotics resistant bacteria because they are a global public health threat and more research is required so as to discover and develop new antibiotics specifically active against multidrug and extensively drug-resistant Gram-negative bacteria. Enterobactericeae are the most common isolated gram-negative bacteria causing UTI. This is because they are inhabitants of the gastrointestinal tract and children are infected due to poor hygiene practices such as improper wiping and poor hand hygiene whereby the Enterobactericeae ascend from the periurethral area, via the urethra to the bladder and potentially the upper urinary tract. UTIs caused by Enterobactericeae in this study was 22.3%, this could be due to poor toilet hygiene. This prevalence correlates with another study done in Hawassa University Comprehensive Specialized Hospital, Ethiopia where prevalence of UTI caused by Enterobactericeae was 25.4% [13]. Our study's prevalence was higher than in a study done in Webuye Sub-County Hospital, Kenya, where the prevalence was 11.9%, this was because non enterobactericeae species were frequently isolated [15].

Non-Enterobactericeae identified from this study were *Staphylococcus aureus* (0.01%), *Enterococcus faecalis* (0.01%), *Enterococcus avium* (0.01%), *Pseudomonas. steturi* (0.01%) and *Staphylococcus lentus* (0.01%). This disagrees with a study done in kenya where the prevalence was as follows; Staphylococcus aureus (4/31; 12.9%), Pseudomonas spp (2/31; 6.5%) making the prevalence from this study lower [16].

From the isolated Enterobactericeae species, *E. coli* (84.9%) was the most prevalent, followed by *P. mirabilis* (5.66%) and *K. pneumoniae* (5.66%), *E. aerogenes (*1.89%) and *M. morganii* (1.89%). These species are residents of the gastrointestinal tract. They cause infection by ascension of bacteria from the gastrointestinal tract to the urinary tract. *E. coli* is the commonest flora of the gastrointestinal tract and possesses well characterized virulence factors such as adhesins, toxins (alpha-hemolysin, cytotoxic necrotizing factor 1 and autotransporter toxins), iron/heme-acquisition systems, iron ion transport and fimbriae that help to colonize the urinary tract [17]. *K. pneumoniae* possess virulence factors that aid in colonization of the urinary tract such as capsules, exopolysaccharides associated with mucoviscosity, lipopolysaccharides (LPSs), adhesins and iron uptake systems [17]. *P. mirabilis* causes community acquired UTI and expresses virulence factors that help them cause UTI such as biofilm formation, production of enzymes and cytotoxins, motility and iron acquisition systems [18]. *M. morganii* is an opportunistic pathogen possessing virulence factors such as hemolysins, urease and lipopolysaccharides that help in penetrating the host barrier causing UTI [19]. *E. aerogenes produces toxins* that disrupt epithelial integrity and permit bacterial invasion causing UTI in children [20]. Our study's findings agree with a study done in Arba Minch, Southern Ethiopia where *E. coli* was the most frequently isolated organism causing UTI in children [15]. It also agrees with a study done among children with UTI attending Felege Hiwot Referral Hospital, Bahir Dar, Northwest Ethiopia where the most prevalent Enterobactericeae causing UTI was *E. coli* (67%) followed by *K. pneumoniae* (23.8%) and *E. aerogenes* [21].

All the isolated Enterobactericeae species, were resistant to ampicillin. Above 60% of the isolated species were susceptible to cefuroxime, cefotaxime, ciprofloxacin, ceftriaxone and gentamicin, with the highest susceptibility to meropenem except one *E. coli* species (2%). Ampicillin and cephalosporin inhibit bacterial cell wall synthesis leading to bacterial lysis. Overtime the bacteria produce beta lactamase enzymes that hydrolyze the beta lactam ring rendering the antibiotic inactive leading to emergence of resistance [22]. This is because when a certain antibiotic is used in the community regularly, the more resistant strains are selected and maintained in the environment resulting to resistance of that certain antibiotic [23]. This study's findings agree with a study done in Felege Hiwot Referral Hospital, Bahir Dar, Northwest Ethiopia where high resistance rate was observed against ampicillin (80%) and a high susceptibility rate in ciprofloxacin across all the Enterobactericeae isolated [21]. High susceptibility seen in meropenem could be due to low usage of carbapenems in the community. This agrees with a study done among small children of Garoua, Northern Cameroon where highest susceptibility was seen in carbapenems which was due to lack of availability of the carbapenems in the region [24].

In this study, the prevalence of ESBL producing Enterobactericeae was 26.4%. Our study's prevalence is lower than in a previous study done in Garoua Cameroon where the prevalence of ESBL producing Enterobactericeae was 55% [24]. In another study done in Hawassa University, Ethiopia, the prevalence rate of ESBL producing Enterobactericeae was 41.7%. This high prevalence was due to poor antimicrobial surveillance, self-medication, poor diagnosis of UTI, poor quality of antibiotics. Inadequate dose has all attributed to high prevalence of AMR in recent years [13]. In this study, ESBL phenotypes were all detected in *E. coli* species. This is because *E. coli* is the most common producer of ESBL. ESBL production is commonly associated with gastrointestinal normal flora in which *E. coli* forms the largest proportion. This agrees with a study done in Sydney Children's Hospital, Australia where *E. coli* was the most prevalent ESBL Enterobactericeae species [25]. This study's results disagree with a study done in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia where *K. pneumoniae* was the most isolated ESBL producing Enterobactericeae species among the children. *K. pneumoniae* can complicate treatment due to increased resistance that is caused by the acquisition of plasmids containing multiple antimicrobial resistances including genes coding for ESBL resistance [14].

The carbapenamase phenotype was present in only one *E. coli* isolate (1.9%). This findings agree with the global surveillance data (2020) on multi drug resistant organisms in which the prevalence of UTI caused by carbapenamase producers is 1% - 5%. Resistance to carbapenems among the *E. coli* is mostly due to the production of enzyme carbapenamase, which are β-lactamases with capacity to hydrolyze not only the carbapenems themselves but also all the other beta lactam agents. These carbapenems resistance is mediated by plasmids which carry the genes encoding carbapenamase production [15]. The presence of carbapenamase is due to the resistance of ESBL Enterobactericeae to Beta-Lactams which reduce therapeutic options maintaining a steady increase in the prescription of carbapenems [24].

From this study, $\text{Bla}_{\text{CTX-M}}$ and Bla_{TEM} resistant gene were detected only from *E. coli* isolates. None of the isolates had Bla_{SHV} encoding gene. $\text{Bla}_{\text{CTX-M}}$ and Bla_{TEM} mostly associated with cephalosporins resistance due to ease of insertion of genetic mobile elements, such as plasmids into the bacterial gene which facilitate their spread between bacterial species. This agrees with a study done in children attending three main hospitals of N'Djamena, Chad which showed that $\text{Bla}_{\text{CTX-M}}$ (96.7%) was the most common resistant gene detected. $\text{Bla}_{\text{CTX-M}}$ is now considered endemic and is rapidly disseminating among different Enterobacteriaceae species [26]. However, another study done in Duhok City, Kurdistan Region, Iraq among Children showed the prevalence of Bla_{TEM}, $\text{Bla}_{\text{CTX-M}}$ and Bla_{SHV} was 94%, 90%, 1.4% respectively [27]. In this study, the selected Bla_{KPC} and $\text{Bla}_{\text{OXA-48}}$ carbapenamase resistant genes were not detected.

There was a significant association ($p < 0.05$) between the non-school going participants and UTI caused by the ESBL phenotype. This may be attributed to children who are still on diaper care and are still being toilet trained. The risk of UTI or contamination maybe due to poor toilet hygiene. This study agrees with a study done among Children in Duhok City, Kurdistan Region, Iraq where small children were significantly at risk of ESBL related UTI. This was because of poor toilet and hand hygiene [27]. There was also a significant association between presence of blood in urine and ESBL, UTI. This is due to the colonization of the urinary tract *E. coli* leading to inflammation and phimosis. Underlying renal conditions such as reflux, bilateral hydronephrosis and hydronephrosis with uretic obstruction can also result to presence of blood in urine.

5. Conclusions

From this study, Enterobactericeae *E. coli, K. pneumoniae, P. mirabilis, E. aerogenes, M. morganii* were the bacterial pathogens causing UTI in children.

Further antibiotic screening showed high resistance to ampicillin which indicates misuse of the drug in the population. There was a high susceptibility to Meropenem.

Only *E. coli* showed carbapenamase and ESBL phenotypes.

Non-school going children and those with RBC in blood were at a risk of ESBL, UTI.

Besides using proper treatment regiments, medical doctors need to give guidance in management of UTI in children touching on proper hygiene, especially cleaning toddlers and pre-school children during toilet training to avoid UTIs.

Recommendations

Further research on extended spectrum beta lactamases and carbapenamase producing bacteria in children should be done to identify more resistant genes responsible for antimicrobial resistance.

Limitations

Limited research findings to use for review literature from Kenya on urinary tract infection caused by Extended Spectrum Beta Lactamase and carbapenamase producing enterobactericeae among children.

Authors' Contributions

Proposal writing—Rachael Wangeci Waithaka. Proposal review—Celestine Khalechi Makobe, Janet Kerubo Maranga. Sample collection, laboratory experiments and data analysis—Rachael Wangeci Waithaka. Supervision of work—Celestine Khalechi Makobe, Janet Kerubo Maranga. Manuscript writing—Rachael Wangeci Waithaka. Manuscript review—Celestine Khalechi Makobe, Janet Kerubo Maranga.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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