Research Article

Antibiogram and Prevalence of ESBL-producing Gram-negative Bacteria among Pediatric Patients in Abakaliki, Nigeria

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ABSTRACT

The emergence and dissemination of extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria among children is worrisome as this poses a serious threat in patient management. The cardinal objective of this study was to determine the antibiogram and prevalence of ESBL-producing *E. coli* and *Klebsiella* species among paediatric patients in three different healthcare institutions in Abakaliki, Ebonyi State. A total of 422 clinical samples [mid-stream urine (232) and feces (190)] were collected from pediatric patients. Isolates obtained were characterized and identified using standard microbiological techniques. Identified bacterial isolates were phenotypically screened for ESBL-production by double-disc synergy test. The antibiogram of ESBL-positive isolates was determined by the Kirby-Bauer disc diffusion technique. A total of 74 (66.7 %) bacterial isolates [*E. coli* (39), *Klebsiella* species (35)] were obtained from urine samples while 37 (33.3 %) isolates [*E. coli* (25), *Klebsiella* species (12)] were recovered from fecal samples. Exactly 27 (26.1 %) isolates [*E. coli* (15), *Klebsiella* species (12)] were confirmed to be ESBL producers. Antibiotic susceptibility test revealed that ESBL-producing *E. coli* and *Klebsiella* species were susceptible to meropenem, colistin, ciprofloxacin and gentamicin but exhibited high resistance frequencies to amikacin, kanamycin, tetracycline, sulphamethoxazole/trimethoprim, nitrofurantoin and chloramphenicol. Isolates also exhibited multiple antibiotic resistance index value ranging from 0.3 – 0.8. This study showed that multidrug-resistant ESBL-producing *E. coli* and *Klebsiella* species were highly prevalent in paediatric patients in Abakaliki. Thus, antibiotic regulatory policy should be implemented and monitored to curtail the spread of bacterial pathogens in hospitals and communities.

Keywords: ESBL, *E. coli*, *Klebsiella*, Antibiotic resistance, Pediatric patients, Antibiogram, Clinical samples.

INTRODUCTION

Antimicrobial resistance has been identified as one of the greatest threats to human health, and developing countries like Nigeria are the worst hit by this crisis[1]. The major cause of this crisis is the indiscriminate and widespread use of antibiotics, especially the beta-lactams (antibiotics containing beta-lactam ring), in prophylaxis and treatment of bacterial diseases[2]. This misuse and abuse of β-lactam antibiotics bought over the counter without doctor's prescription has led to antibiotic selective pressure and development of resistance to these drugs by most bacteria, particularly the *E. coli and Klebsiella* species of which β-lactamase production remains the most important contributing factor to this resistance[3]. Beta-lactamases are bacterial enzymes that inactivate the β-lactam antibiotics by hydrolyzing the β-lactam ring. One of the groups of β-lactamases called the extended-spectrum beta-lactamases (ESBLs) have the extended ability to hydrolyze and cause resistance to various types of the newer β-lactam antibiotics such as the 2nd and 3rd generation extended-spectrum cephalosporins[4]. ESBLs are chromosomally or plasmid-mediated and occur as a result of a spontaneous mutation that takes place in the active site of the wild-type beta-lactamase enzyme by adding 4-6 new amino acids, thereby extending their hydrolytic properties[5]. Extended-spectrum beta-lactamase enzymes are produced mostly by *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Shigella*, *Salmonella* and *Proteus* species that are prevalent causes of nosocomial and community-acquired infections globally. The rapid emergence of ESBL-producing bacteria has drawn global attention because they are important causative agents of hospital infections typically associated with pneumonia, urinary tract infections, bacteremia and other intra-abdominal infections[4]. The spread of these ESBLs poses a serious threat to good health including hindering effective treatment, prolonged hospitalization, and increased treatment costs[5]. These have necessitated the need for extensive peer review research on ESBLs and increased awareness campaigns on antimicrobial resistance, most especially in developing countries like Nigeria, where there is little or no surveillance activity and regulations guiding the use of antibiotics. Several studies have demonstrated the prevalence of ESBL-producing bacteria in many parts of the world[6] and several individual reports are available in Nigeria[2]. However, despite the studies carried out in different parts of Nigeria, there is a paucity of information on the prevalence of ESBL-producing bacteria in children. Therefore, this study was

designed to determine the antibiogram and prevalence of ESBL-producing *E. coli* and *Klebsiella* species among paediatric patients in Abakaliki, Ebonyi State.

MATERIALS AND METHODS

Study Area

This study was conducted in Abakaliki, Ebonyi State, Nigeria. Ebonyi state is in the Southeast geopolitical zone of Nigeria, and it was created from Abia and Enugu States on 1st October, 1996. It derived its name from the Ebonyi River and the state capital of Ebonyi state is Abakaliki. The 2006 population census conducted in Nigeria pegged the population of Ebonyi state at an estimated population of 4.3 million people comprising 1,064,156 males and 1,112,791 females. The land mass of Ebonyi state is 5,935 km^2. Approximately 75 % of the population of Ebonyi state dwell in rural areas and the state is known for its farming activities[7]. This study was carried out in the Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria from March, 2017 to May, 2018.

Sample collection

Clinical samples (mid-stream urine, n= 232 and feces, n= 190) were collected from children [male (294) and female (128)] visiting three different public hospitals; Federal Teaching Hospital (FETHA I), Mile four Hospital and Nwezenyi (FETHA Annex), respectively in Abakaliki, Ebonyi State, Nigeria. Samples were collected with clean containers, labeled appropriately, and immediately transported to the laboratory for analysis within two hours[8].

Bacteriological analysis of clinical samples

Mid-stream urine and fecal samples were aseptically streaked on MacConkey agar and incubated at 37 °C for 24 hours. After incubation, the plates were observed for typical *E. coli* and *Klebsiella* spp growth (red or pink colonies) on MacConkey agar. These suspected bacterial isolates were further characterized using standard microbiology techniques such as Gram-staining, motility test, and other biochemical tests such as indole, methyl red, Voges-Proskauer, citrate, motility, and urease test[9]. Pure cultures of isolates were further inoculated onto nutrient agar slants, incubated at 37 °C for 24 hrs and stored in a refrigerator at 4 °C for future use[10].

Ethical clearance

Ethical clearance was obtained from the ethical and research committee of the Federal Teaching Hospital (FETHA) and Mile 4 Hospital.

Antibiotic Susceptibility Test

Antibiotic susceptibility of the isolates was determined using the Kirby-Bauer disc diffusion method according to the recommendations of the Clinical and Laboratory Standard Institute CLSI[11]. The isolates were sub-cultured on nutrient agar, incubated at 37 °C for 18-24 hours. Then the colonies of each of the isolate were adjusted to 0.5 McFarland turbidity standard (equivalent to 1.5×10^8 cfu/ml) in sterile nutrient broth. The standardized broth culture was incubated for 10 minutes and using sterile a swab stick, the standardized broth culture of the isolates was inoculated onto Mueller-Hinton agar plates. The surface of the medium was streaked in four directions while the plates were rotated approximately 60° to ensure even distribution. The inoculated Mueller-Hinton agar plates were allowed to dry for a few minutes. The following standard antibiotic discs were used against the isolates; amikacin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), colistin sulfate (25 µg), gentamicin (10 µg), kanamycin (5 µg), meropenem (10 µg), nitrofurantoin (300 µg), tetracycline (30 µg) and sulphamethoxazole/trimethoprim (25 µg). Sterilized forceps were used to place the antibiotic discs evenly on the inoculated Mueller-Hinton agar so that the disc should be about 15mm from the edge of the plate and not closer than 25 mm from disc to disc. After 30 minutes, the plates were inverted and incubated for 24 hours. A ruler was used to measure the diameter of each zone of inhibition in mm on the underside of the plate. The inhibitory zone diameter was interpreted as susceptible, intermediate or resistant according to the criteria of CLSI[11].

Screening of bacterial isolates for ESBL Production

Screening the bacterial isolates for ESBL production was done by observing their sensitivities to 2nd and 3rd generation cephalosporins; such as aztreonam (30 µg), cefepime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg). These antibiotics were aseptically placed at a distance of 30 mm apart on Mueller-Hinton agar (Oxoid, UK) plate that was previously inoculated with standardized inocula of the test bacterium using a sterile swab stick to get a confluent growth. The plates were allowed to stand for about 30 minutes for pre-diffusion of the antibiotics and after which was incubated for 18-24 hours at 37 °C. After the incubation time, the zones of inhibition were measured in millimeters using a meter rule and results were interpreted according to Clinical and Laboratory Standard Institute (CLSI) chart. ESBL production was suspected if any of the test bacteria showed reduced susceptibility or is resistant to any of the antibiotics used for the screening studies according to the CLSI guidelines[11].

Confirmation of ESBL production by bacterial isolates using double-disk synergy test (DDST)

The bacterial isolates that exhibited reduced susceptibility to any of the 2nd and 3rd generation cephalosporins were phenotypically confirmed for ESBL production using the double-disc synergy test[2]. DDST was performed as a standard disc diffusion assay on Mueller-Hinton (MH) agar (Oxoid, UK) plates in line with CLSI criteria[11]. Sterile swab sticks were dipped into bacterial suspension(s) standardized to 0.5 McFarland turbidity standards; and was inoculated on MH agar plates. Antibiotic disc of amoxicillin/clavulanic acid (20/10 µg) was placed at the center of the MH agar plate and antibiotic discs containing cefotaxime (30 µg) and ceftazidime (30 µg) each was

placed at a distance of 15 mm (center to enter) from the central disc, amoxicillin/clavulanic acid (20/10 µg) and the plates were incubated at 37 °C for 18-24 hours. ESBL production was suspected phenotypically when the zones of inhibition of the cephalosporins (cefotaxime 30µg and ceftazidime 30 µg) increased in the presence of amoxicillin/clavulanic acid disk (20/10 µg). A ≥ 5mm increase in the inhibition zone diameter for either of the cephalosporins (cefotaxime and ceftazidime) tested in combination with amoxycillin-clavulanic acid versus its zone when tested alone confirmed ESBL production phenotypically[2].

Multiple antibiotic resistance (MAR) index

The multiple antibiotic resistance index was calculated as the ratio of the number of antibiotics to which the isolates were resistant /the total number of antibiotics against which the isolates were tested. MAR index values greater than 0.2 (20 %) were considered a high-risk source of contamination where antibiotics are often used[1, 12].

RESULTS

Exactly 111 (26.3 %) Gram-negative bacteria [*E. coli* 64(57.6 %) and *Klebsiella* species 47(42.3 %)] were isolated and phenotypically characterized from the 422 clinical samples [mid-stream urine (232) and feces (190)] collected from pediatric patients in Federal Teaching Hospital Abakaliki (FETHA), Mile 4 Hospital, and Nwaezenyi (FETHA Annex) Hospital (Tables 1 & 2).

In Federal Teaching Hospital Abakaliki (FETHA), 17(13.2 %) *E. coli* and 19(15.8 %) *Klebsiella* spp isolates were recovered from 128 urine samples while 11(10.4%) *E. coli* and 7(6.6%) *Klebsiella* spp isolates were recovered from 105 fecal samples. In Mile Four hospital, 15(25.8%) *E. coli* and 11(18.9%) *Klebsiella* spp isolates were recovered from 58 urine samples while 9(21.9%) *E. coli* and 2(4.8%) *Klebsiella* spp isolates were recovered from 47 fecal samples. In Nwezenyi (FETHA annex), 7(15.2%) *E. coli* and 5(6.5%) *Klebsiella* spp isolates were recovered from 46 urine samples while 5(14.2%) *E. coli* and 3(7.8%) *Klebsiella* spp isolates were recovered from 38 fecal samples (Tables 1 & 2). A total of 8 (28.6 %) *E. coli* and 9 (34.6 %) *Klebsiella* spp isolated from FETHA were positive for ESBL production while in Mile 4 hospital, 6 (25 %) *E. coli* and 3 (23.1 %) *Klebsiella* spp were ESBL producers. In Nwaezenyi (FETHA annex), 2(16.7 %) *E. coli* and 1(12.5 %) *Klebsiella* spp were positive for ESBL production (Tables 3-5, Figure 1).

Antibiotic susceptibility test result of ESBL-positive *Escherichia coli* isolates from paediatric patients in FETHA 1 showed that they were highly resistant (100 %) to tetracycline, sulphamethoxazole/trimethoprim, and kanamycin. Isolates also exhibited resistance to ciprofloxacin (87.5 %), chloramphenicol (75 %), gentamicin (75 %), nitrofurantoin (75 %), and amikacin (50 %). Isolates were highly susceptible to meropenem (100 %) and colistin (62.5%) (Table 6). The ESBL-positive *Klebsiella* species isolates in FETHA 1 also showed that they were highly resistant (100 %) to tetracycline, sulphamethoxazole / trimethoprim, and kanamycin. Isolates also showed resistance to nitrofurantoin (88.9 %), ciprofloxacin (77.8 %), chloramphenicol (66.7 %), gentamicin (66.7 %), and amikacin (66.7 %). Isolates were highly susceptible to meropenem (100 %) and colistin (55.5 %) (Table 6). Antibiotic susceptibility test result of ESBL-positive *Escherichia coli* isolates from pediatric patients in Mile 4 hospital showed that they were highly resistant (100 %) to tetracycline, sulphamethoxazole/trimethoprim, and kanamycin.

Isolates also exhibited resistance to ciprofloxacin (83.3 %), nitrofurantoin (66.7 %), amikacin (66.7 %), and chloramphenicol (50 %). Isolates were highly susceptible to meropenem (100 %), colistin (100 %), and gentamicin (83.3 %) (Table 7). Also, the ESBL-positive *Klebsiella* spp isolates were resistant (100 %) to kanamycin, nitrofurantoin, tetracycline and sulphamethoxazole/trimethoprim. Isolates were also resistant (66.7 %) to amikacin, chloramphenicol, and ciprofloxacin (Table 7). Isolates were also highly susceptible to meropenem (100 %), colistin (100 %), and gentamicin (66.7 %) (Table 7). Antibiotic susceptibility test result of ESBL-positive *Escherichia coli* isolates from pediatric patients in Nwaezenyi (FETHA Annex) showed that they were highly resistant (100 %) to tetracycline, sulphamethoxazole/trimethoprim, nitrofurantoin, ciprofloxacin, chloramphenicol, amikacin, and kanamycin. Isolates also exhibited resistance to gentamicin (50 %).

Isolates were highly susceptible (100 %) to meropenem and colistin (Table 8). The ESBL-positive *Klebsiella* spp isolates were highly resistant (100 %) to colistin, kanamycin, nitrofurantoin, tetracycline, sulphamethoxazole/trimethoprim, chloramphenicol and amikacin but were very susceptible (100 %) to meropenem, ciprofloxacin, and gentamicin (Table 8). All the ESBL-producing *E. coli* and *Klebsiella* spp. isolates from pediatric patients in this study had MARI values that ranged from 0.30 – 0.80 (Tables 9-11).

Table 1: Clinical Samples Collection and Bacterial Distribution

Location (participants)	Number of clinical samples collected		
	Urine n (%)	Faeces n (%)	Total n (%)
FETHA	128 (30)	105 (25)	233 (55)
Mile Four Hospital	58 (14)	47(11)	105 (25)
Nwezenyi (FETHA Annex)	46 (11)	38(9)	84 (20)

Table 2: Prevalence of *E. coli* and *Klebsiella* spp in clinical samples of Pediatric Patients in FETHA, Mile 4 Hospital, and Nwaezenyi (FETHA Annex)

Organisms	FETHA		MILE 4		Nwaezenyi	
	Urine	Feces	Urine	Feces	Urine	Feces
E. coli	17	11	15	9	7	5
Klebsiella spp	19	7	11	2	5	3
Total	36	18	24	12	12	8

Table 3: Preliminary Screening and Confirmatory test for ESBL-producing *E. coli* and *Klebsiella* spp Isolates from Pediatric Patients in FETHA

Isolate	ESBL Pre-screening (%)	ESBL Confirmation using DDST (%)
***Escherichia coli* (n = 28)**		
ESBL positive	10 (35.7)	8 (28.6)
ESBL negative	18 (64.3)	
***Klebsiella* spp (n = 26)**		
ESBL positive	11 (42.3)	9 (34.6)
ESBL negative	15 (57.7)	
Total positive		**17(31.4 %)**

Figure 1: Pictorial Representation of Double Disc Synergy Test of ESBL-producing Bacteria (*E. coli* and *Klebsiella* species).

Key: CAZ= Ceftazidime, CTX = Ceftriaxone, AMC = Amoxicillin/Clauvulanic acids

Table 4: Preliminary Screening and Confirmatory test for ESBL-producing *E. coli* and *Klebsiella* spp Isolates from Pediatric Patients in Mile 4 General Hospital

Isolate	ESBL Pre-screening (%)	ESBL Confirmation using DDST (%)
***Escherichia coli* (n = 24)**		
ESBL positive	8 (33.3)	6 (25)
ESBL negative	16 (66.7)	
***Klebsiella* spp (n = 13)**		
ESBL positive	5 (38.5)	3 (23.1)
ESBL negative	8 (61.5)	
Total positive		**9 (16.7)**

Table 5: Preliminary Screening and Confirmatory test for ESBL-producing *E. coli* and *Klebsiella* spp Isolates from Pediatric Patients in Nwaezenyi (FETHA Annex)

Isolate	ESBL Pre-screening (%)	ESBL Confirmation using DDST (%)
***Escherichia coli* (n = 12)**		
ESBL positive	4 (33.3%)	2 (16.7%)
ESBL negative	8 (66.7%)	
***Klebsiella* spp (n = 8)**		
ESBL positive	2 (25%)	1 (12.5%)
ESBL negative	6 (75%)	
Total positive		**3 (11.1%)**

Table 6: Antibiotic Susceptibility Frequency of ESBL-producing *E. coli and Klebsiella* spp Isolated from Pediatric Patients in FETHA 1

Antibiotics (potency)	Disc code	*Escherichia coli* (n = 8)		*Klebsiella* spp (n = 9)	
		% S	% R	% S	% R
Amikacin (30µg)	A	50	50	33.3	66.7
Chloramphenicol (30µg)	C	25	75	33.3	66.7
Ciprofloxacin (5µg)	CIP	12.5	87.5	22.2	77.8
Colistin sulphate (25µg)	CT	62.5	37.5	55.4	44.5
Gentamicin (30µg)	CN	25	75	33.3	66.7
Kanamycin (5µg)	K	0	100	0	100
Meropenem (10µg)	MEM	100	100	100	0
Nitrofurantoin (300µg)	F	25	75	11.1	88.9
Tetracycline (30µg)	T	0	100	0	100
Sulphamethoxazole /Trimethoprim (25 µg)	SXT	0	100	0	100

Key: n = Number of isolates, % S = Percentage susceptibility, % R = Percentage resistance

Table 7: Antibiotic Susceptibility Frequency of ESBL-producing *E. coli and Klebsiella* spp Isolated from Pediatric Patients in Mile 4 Hospital

Antibiotics (potency)	Disc code	*Escherichia coli* (n = 8)		*Klebsiella* spp (n = 9)	
		% S	% R	% S	% R
Amikacin (30µg)	A	33.3	66.7	33.3	66.7
Chloramphenicol (30µg)	C	50	50	33.3	66.7
Ciprofloxacin (5µg)	CIP	16.7	83.3	100	0
Colistin sulphate (25µg)	CT	100	0	66.7	33.7
Gentamicin (30µg)	CN	83.3	16.7	0	100
Kanamycin (5µg)	K	0	100	100	0
Meropenem (10µg)	MEM	100	0	0	100
Nitrofurantoin (300µg)	F	33.3	66.7	0	100
Tetracycline (30µg)	T	0	100	0	100
Sulphamethoxazole /Trimethoprim (25 µg)	SXT	0	100	0	100

Key: n = Number of isolates, % S = Percentage susceptibility, % R = Percentage resistance

Table 8: Antibiotic Susceptibility Frequency of ESBL-producing *E. coli and Klebsiella* spp Isolated from Pediatric Patients in Nwezenyi (FETHA Annex)

Antibiotics (potency)	Disc code	*Escherichia coli* (n = 8)		*Klebsiella* spp (n = 9)	
		% S	% R	% S	% R
Amikacin (30µg)	A	0	100	0	100
Chloramphenicol (30µg)	C	0	100	0	100
Ciprofloxacin (5µg)	CIP	0	100	100	0
Colistin sulphate (25µg)	CT	100	0	0	100
Gentamicin (30µg)	CN	50	50	100	0
Kanamycin (5µg)	K	0	100	0	100
Meropenem (10µg)	MEM	100	0	100	0
Nitrofurantoin (300µg)	F	0	100	0	100
Tetracycline (30µg)	T	0	100	0	100
Sulphamethoxazole /Trimethoprim (25 µg)	SXT	0	100	0	100

Key: n = Number of isolates, % S = Percentage susceptibility, % R = Percentage resistance

Table 9: Multiple Antibiotics Resistance index (MARI) of the ESBL-producing *E. coli* and *Klebsiella* Species Isolates from pediatric patients in FETHA

Isolates	MARI
***Escherichia coli* (n = 8)**	
1	0.70
2	0.70
3	0.30
4	0.80
5	0.70
6	0.60
7	0.60
8	0.50
***Klebsiella* spp. (n = 9)**	
1	0.80
2	0.60
3	0.60
4	0.70
5	0.80
6	0.40
7	0.70
8	0.60
9	0.60

Table 10: Multiple Antibiotics Resistance Index (MARI) of ESBL-producing *E. coli* and *Klebsiella* spp from pediatric patients in Mile Four Hospital

Isolates	MARI
***Escherichia coli* (n = 6)**	
1	0.40
2	0.50
3	0.60
4	0.40
5	0.70
6	0.60
***Klebsiella* spp. (n = 3)**	
1	0.50
2	0.60
3	0.70

Table 11: Multiple Antibiotic Resistance Index (MARI) of the ESBL-producing *E. coli* and *Klebsiella* spp isolates from pediatric patients in Nwezenyi (FETHA Annex)

Isolates	MARI
***Escherichia coli* (n = 2)**	
1	0.70
2	0.70
***Klebsiella* spp. (n = 1)**	
1	0.50

DISCUSSION

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae are increasingly common worldwide. They are usually multi-drug-resistant (MDR) and are recognized as important nosocomial and community-acquired pathogens[13]. This study showed that EBSL-producing Gram-negative bacteria, namely *E. coli* and *Klebsiella* spp are present in pediatric patients in Abakaliki. Our findings also showed that all the ESBL-producing *E. coli* and *Klebsiella* spp isolates obtained from the clinical samples of the pediatric patients in Abakaliki were multidrug-resistant. Interestingly, ESBL-producing *Klebsiella* spp were more prevalent than ESBL-producing *E. coli* in the three hospitals in our study. In our study, 111 (26.3 %) Gram-negative bacteria [*E. coli* 64(57.6 %) and *Klebsiella* species 47(42.3 %)] were isolated and phenotypically characterized from the 422 clinical samples [mid-stream urine (232) and feces (190)] collected from pediatric patients in Federal Teaching Hospital Abakaliki (FETHA), Mile 4 Hospital, and Nwaezenyi (FETHA Annex) Hospital. Several studies have reported *Escherichia coli* and *Klebsiella* species as the most frequently isolated bacterial pathogens from pediatric clinical samples[14]. Our study showed that the carriage rate of *E. coli* and *Klebsiella* spp was high among pediatric children in Abakaliki, and this may be as a result of higher prevalence and dissemination of ESBL-producing pathogens in hospital settings as reported in various studies[6]. This high carriage rate could pose a serious threat to pediatric healthcare in the future, hence there is a need for a multifunctional approach combining continued research, prudent use of antibiotics, effective ESBL-infections control measures, and rapid detection of ESBL-producing organisms in a routine clinical laboratory. Confirmatory screening of suspected ESBL producers by double-disc synergy test showed all the 9 *Klebsiella* spp and 8 *E. coli* isolates obtained from FETHA were ESBL-positive. In Mile Four hospital; 6 *E. coli* and 3 *Klebsiella* spp isolates were confirmed to be ESBL producers, while in Nwezenyi 2 *E. coli* and 1 *Klebsiella* spp isolates were confirmed to be ESBL producers. The observed prevalence frequency (31.4 %) of ESBL-producing Gram-negative bacteria among pediatric patients in FETHA is in agreement with the prevalence frequency of 31 % reported in hospitalized children in Niger and the 32.6 % recorded in Guinea-Bissau among children admitted to hospital[15,16]. However, the prevalence frequency (31.4 %) of ESBL-producing Gram-negative bacteria among pediatric patients in our study is greater than the 13 % prevalence reported among hospitalized children in Harare, Zimbabwe[17], but lower than the 45 %, 54.1 %, 58.3 %, 78.6 %, and 80.9 % prevalence frequencies recorded in hospitalized children due to diarrheal and other diseases in Madagascar, Tanzania, and Ethiopia respectively[18-20]. The variation in the prevalence of ESBL-producing Gram-negative bacteria among pediatric patients recorded in these studies could be due to differences in the beta-lactam antibiotics used and variations in ESBL detection methods. In Tanzania, a prevalence frequency of 11.2 % was reported among healthy community children while 13.4 % was recorded among healthy children in Libya[18,21]. However, a prevalence frequency of 2.7 % among healthy children was reported in Portugal while 2.9 % was recorded among healthy children in Sweden[22,23]. However, there was a report of 23 % prevalence among pre-school children in Lagos, and 24.8 % in healthy children in a Lebanese community[14,24]. Previous studies have shown that the prevalence of ESBL-producing Gram-negative bacteria among sick and healthy children was more in developing countries than in developed countries. This difference could be because antibiotics usage is strictly regulated in developed countries than in developing countries. The high prevalence of ESBL-producing *Klebsiella* spp in our study, when compared to *E. coli,* is in agreement with some other studies in Nigeria. The reason for this high prevalence of ESBL-producing *Klebsiella* spp could be because *Klebsiella* spp tends to be more associated with nosocomial infections than *E. coli*, hence it has more chance to acquire multi-drug resistance genes and disseminate in the hospital settings[2]. Also, *Klebsiella* species have been known to survive longer than other enteric bacteria on hands and environmental surfaces, facilitating the dissemination, and cross-infection from one hospital to another[4]. Antibiotic susceptibility studies in our study revealed that ESBL-producing *E. coli* and *Klebsiella* spp were multidrug-resistant and exhibited high resistance frequency to tetracycline, sulphamethoxazole/ trimethoprim, kanamycin, nitrofurantoin, ciprofloxacin, chloramphenicol, gentamicin, and amikacin. Interestingly, the isolates in our study were highly susceptible to meropenem and colistin. Our study is in agreement with a study that reported similar resistance frequency of ESBL-producing Enterobacteriaceae[17]. The high resistance frequency of ESBL-producing *E. coli* and *Klebsiella* spp to ciprofloxacin, as observed in this study, is worrisome as quinolones are considered as second-line antibiotics for the treatment of infection caused by multidrug-resistant bacteria. The complete susceptibility (100 %) of the ESBL-producing isolates in our study to meropenem showed that carbapenems remains the best last resort drug for the effective treatment of infections caused by ESBL-producing Enterobacteriaceae. This was also reported previously in another study[2]. The findings of this study also showed that all the ESBL-producing *E. coli* and *Klebsiella* spp isolates were multidrug-resistant as they were resistant to at least two different classes of antibiotics. This may be attributed to poor antibiotic policies, wrong prescription and high use or misuse of antibiotics, high rate of resistance plasmids transfer among bacterial species, clonal dissemination of ESBL-producing Enterobacteriaceae from patients to patients in the hospital settings and to healthy individuals in the community that serves as reservoirs of these pathogens, and the emergence of new strains of community-acquired ESBL-producing bacteria. Therefore, for effective treatment of infections caused by ESBL-producing bacteria, empirical antibiotic regimens should be selected based on antibiograms.

CONCLUSION

The findings from this study showed that EBSL-producing Gram-negative bacteria, namely *E. coli* and *Klebsiella* species were present among pediatric patients in Abakaliki. These isolates were also multidrug-resistant as they exhibited resistance to at least two different classes of antibiotics. The prevalence of ESBL-producing *E. coli* and *Klebsiella* spp among pediatric patients in this study was high and this may be a result of the high dissemination of ESBL-producing pathogens in hospitals settings which are easily acquired from hospital facilities or infected hospitalized patients. The high prevalence of EBSL-producing Enterobacteriaceae is a serious threat to public health as this could lead to the rapid spread of multidrug-resistant bacteria within hospitals and even communities.

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