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Prevalence of ESBL genes in *Klebsiella pneumoniae* from individuals with community-acquired urinary tract infection in rural communities of Enugu State, Nigeria

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Abstract

Background: The emergence and spread of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae is now a major challenge in treating urinary tract infections. This study was conducted to determine the Prevalence of ESBL genes in *Klebsiella pneumoniae* and the influence of these genes on the antimicrobial susceptibility patterns of the isolates, obtained from community acquired urinary tract infection in Enugu state rural communities.

Method: A total of 735 clean catch mid-stream urine samples were collected from February, 2021 to June, 2021. The urine samples were cultured and *Klebsiella pneumoniae* were identified morphologically, biochemically, and then typed down, using PCR, Gel electrophoresis, and Sanger Sequencing by Genewiz. Antibiotic susceptibility testing was done by modified Kirby-Bauer disc diffusion method and interpretation was done following Clinical and Laboratory Standard Institute (CLSI) guidelines. ESBL screening was done by the phenotypic method, using the standard disk diffusion method, whereas the phenotypic confirmation of ESBL producers was done by the double-disk synergy method, and interpreted according to CLSI guidelines. Multiplex PCR was used to detect the genes for SHV and CTX-M while conventional linear PCR was done for TEM and *blas* GES type ESBL genes.

Results: A total of 77 isolates were identified as *Klebsiella pneumoniae*, and the prevalence of positive ESBL in them were 10(12.9%). Of the 77 isolates that were tested for antibiogram, 29 isolates were multidrug-resistance (MDR). Out of the MDR isolates, 10 were ESBL positive, whereas 19 were not. Of this 29 MDR isolates, 6 were extensively-drug resistant (XDR). Of the 6 XDR isolates, 3 do not possess ESBL enzymes, whereas 3 of them do. Age range of 31-40 contributed the highest prevalence of ESBL genes (60%), whereas age range of 10-20, 21-30, and 51-70 did not produce any ESBL gene. *bla* TEM gene was the most prevalent ESBL resistant genes with a prevalence of 10 (100%), followed by SHV 9(90%), whereas *bla* GES gene and *bla* CTX-M have the least prevalence of 4 (40%) each.

Conclusion: Higher prevalence of MDR, XDR, and ESBL-producing *Klebsiella pneumoniae* were observed, thus the need for public health intervention for effective prevention and control of antimicrobial resistance, and proper treatment of UTIs.

Keywords: *Klebsiella pneumoniae*; UTI; ESBL; MDR; Antimicrobial resistance; Enugu

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1. Introduction

Urinary tract infections (UTIs) are microbial infections that attack different parts of the urinary system, causing urethritis (urethra), cystitis (bladder), and pyelonephritis (kidney), and hosts of associated pathologies, including septicemia. In poor-resourced and tropical countries, UTIs are still the major source of morbidity and death [1], with an estimated annual global incidence of at least 250 million in developing countries [2]. Urinary tract infections can be community acquired or nosocomial. Community-acquired UTI is the second most commonly encountered microbial infection in the community setting [3].

UTI is mostly caused by bacteria, though other microorganisms such as fungi and viruses are rare etiologic agents [4]. UTI caused by *Klebsiella species*, is of particular concern, as these bacteria possess substantially high levels of extended-spectrum beta-lactamases (ESBL) enzymes activity [5]. These enzymes have resistance mechanisms capable of hydrolyzing and causing resistance to various antibiotics, such as new β - lactamases, including third - generation cephalosporins of broad-spectrum activity (ceftriaxone, cefotaxime, ceftazidime) and monobactams (aztreonam) [6].

ESBLs are often plasmid-mediated enzymes and have various genotypes. So far, about 400 different types of ESBLs have been recognized around the world, among which *blas* TEM and *blas* SHV were more prevalent [7]. *Klebsiella* pneumoniae are known to produce high level of ESBLs genes, which confers high resistance to these bacteria, thereby making treatment of UTIs a mere impossibility, thus a need for special attention. This study was conducted to determine the Prevalence of ESBL resistant genes: *blas* CTX-M, *blas* SHV, *blas* TEM, and *blas* GES genes in *Klebsiella pneumonia* isolates and the influence of these genes on the antimicrobial susceptibility patterns of the isolates, obtained from community acquired urinary tract infection in Enugu state rural communities, where there is paucity of such data. The findings from this study will give insights in the epidemiology of ESBL genes and thus, the interventions necessary to check UTIs and antimicrobial resistance.

2. Material and methods

2.1. Study Area

This study was conducted in Enugu State University of Science and Technology Teaching Hospital, and the University of Nigerian Teaching Hospital, Ituku/Ozalla, all in Enugu state.

2.2. Sample size

This population-based, cross-sectional survey was conducted between February 2021 and, June, 2021 in Enugu state, Nigeria. A random sample of males and females aged 10-70 years residing in the selected communities within the three senatorial districts of Enugu state were selected. Simple random sampling technique was used to select three communities from the three senatorial zones in Enugu state, who participated in the study.

2.3. Study instruments

A well-structured questionnaire was used to collect data from the subjects on antibiotic usage, symptoms of UTI (pain or burning sensation at urinating; a feeling of urgency at urination; cramps or pain in the lower abdomen; the need to urinate more often than usual; urine that looks turbid and has foul smell; pain, pressure or tenderness in the area of the bladder and, back pain, chills, fever), physiological state, and some sociodemographic factors, like age and sex, in order to ascertain their suitability for either inclusion or exclusion. Screw tight sterile universal containers (with boric acid) was used to collect urine samples for bacterial isolation.

2.4. Inclusion and Exclusion criteria

Subjects who represented at least three of the symptoms of UTIs, as outlined in the questionnaire were recruited for the study. Also included were those who were neither on antibiotic therapy, nor have taken antibiotics within the last two weeks before the study, and women who were not in their menstrual period. Subjects excluded from the study were those who did not meet up with the criteria for inclusion and those who did not give their consent for participation.

2.5. Data/Sample collection

Questionnaires were administered as self or by interviewer for the literate or illiterate respondents respectively. Subjects who met the inclusion criteria were educated on how to collect clean catch mid-stream urine aseptically, and thus were given urine bottles for sampling. Using a sterile, wide mouthed, and leak proof boric acid universal containers,

a total of 740 urine samples were collected from selected communities. The samples were pre-stored in a cooler, maintained at +2 to +6°C, using an ice pack, before transportation to testing site.

2.6. Urine culture

A 10 μl (0.01 ml) well-mixed urine sample was inoculated on MacConkey agar (Oxoid, UK), and Chromagar Orientation Media (M6: Plasmatec Laboratories, United Kingdom), and incubated at 37°C for 24 hours. The colony count with at least 10^5 CFU/ml for single midstream urine was taken as positive urine culture**.** All the isolates were preliminarily screened by their colonial morphology, pigment production and Gram reactions. All Isolates showing the identity of Klebsiella were subjected to biochemical tests, using: methyl-red, citrate utilization, and motility tests. Isolates that gave Methyl-red negative, Citrate positive, and Motility negative were considered as Klebsiella. These isolates were subjected to molecular studies, where *Klebsiella pneumonia* was typed down, using PCR, carried out using Amplitaq® 360 Polymerase and ran in an Eppendorf Mastercycler [8], Gel electrophoresis [9] and Sanger Sequencing by Genewiz® [10]. Isolates typed down as *Klebsiella pneumoniae* were used for antimicrobial susceptibility testing. Isolates were temporally stored at +2 to +8°C in the nutrient broth for not more than 24 hours prior to antimicrobial sensitivity testing.

2.7. Antimicrobial susceptibility testing

An antibiotic susceptibility assay was performed using the Kirby Bauer disk diffusion method, of NCCLS [11], and resistance and sensitivity were interpreted according to the National Committee for Clinical Laboratory Standards criteria [12]. The antimicrobial discs used include gentamycin (10 µg), ofloxacin (30 µg), co-amoxiclav (30 µg), ciprofloxacin (5 µg), levofloxacin (10 µg), cefotaxime (30 µg) ceftazidime (30 µg), cefepime (30 µg), ceftriaxone (10 µg), and meropenem (10 µg).

2.8. ESBL Detection Methods

K. pneumonia were first screened for ESBL production by the Phenotypic method, using ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg) [Oxoid, UK]. The isolates with reduced susceptibility to cefotaxime (zone diameter of ≤27 mm), ceftazidime (zone diameter of ≤22 mm), and ceftriaxone (zone diameter of ≤25 mm) around the disks were suspected as ESBLs producers [13]. This was followed by Phenotypic confirmation of ESBL Producers, which was done by the double-disk approximation or double-disk synergy (DDS) method, using he following antibiotic discs: amoxicillin + clavulanic acid (20 µg), cefotaxime (30 µg) and ceftazidime (30 µg), in accordance with CLSI guidelines 2014 [13]. The isolates with expansion of inhibition zone of the oxyimino-β-lactam caused by the synergy of the clavulanate in the amoxicillin-clavulanate disk was interpreted as positive ESBL producer [13].

2.9. Molecular detection of ESBL genes

Bacterial DNA was extracted from all *K. pneumoniae* ESBLs producer isolates, using thermo scientific genejet genomic DNA purification kits (Promega, USA). DNA Amplification was performed by polymerase chain reaction (PCR), carried out using the one Taq Quick load 2X Master Mix with Standard Buffer (New England Biolabs, MA, U.S.A.), Multiplex PCR was used to detect the genes for SHV and CTX-M while conventional linear PCR was done for TEM type ESBL genes. The PCR products were analyzed on 1.5% Agarose gel stained with ethidium bromide (1µg/mL) and electrophoresis was carried out at 70 volts for 90 min and visualized/ illuminated under ultraviolet transilluminator. A 100 bp DNA ladder (Norgen Biotek Corp., Canada) was used as DNA molecular weight marker.

Table 1 Primer sequences for PCR detection of extended-spectrum beta-lactamases genes in K. Pneumoniae

Target Gene	Primer	Sequence (51 - 31)	PCR conditions	Amplicon Size (bp)	Reference
TEM	F R	GAGACAATAACCCTGGTAAAT AGAAGTAAGTTGGCAGCAGTC	Initial denaturation of 95^{0}C for 5 min; 35 cycles of denaturation of 94^{0}C for 45 S; annealing at 55^{0}C for 30 S; extension at 72^{0}C for 2 mins and final extension at 72^{0}C for 5 min.	459	[14]
SHV	F R	GTCAGCGAAAAACACCTTGCC GTCTTATCGGCGATAAACCAG	Initial denaturation of 95^{0}C for 5 min; 35 cycles of denaturation of 94^{0}C for 45 S; annealing at 60^{0}C for 30 S; extension at 72^{0}C for 3 mins and final extension at 72^{0}C for 5 min.	383	[14]
CTX- M	F R	GAAGGTCATCAAGAAGGTGCG GCATTGCCACGCTTTTCATAG	Initial denaturation of 95^{0}C for 5 min; 35 cycles of denaturation of 94^{0}C for 45 S; annealing at 55^{0}C for 30 S; extension at 72^{0}C for 2 mins and final extension at 72^{0}C for 5 min.	560	[14]
GES	F R	ATGCGCTTCATTCACGCAC CTATTTGTCCGTGCTCAGG	Initial denaturation of 94^{0}C for 4 min; 35 cycles of denaturation of 94^{0}C for 1 min; annealing at 50^{0}C for 1 min; extension at 72^{0}C for 1 mins and final extension at 72^{0}C for 5 min.	920	[14]

3. Results

Table 2 Age and sex distribution of participants whose isolates showed positive ESBL gene

S/N	Lab No	AGE	SEX	ESBL POSITIVE
1	001	40	F	Yes
2	541	35	F	Yes
3	187	65	F	Yes
4	008	37	M	Yes
5	286	49	F	Yes
6	314	43	M	Yes
7	642	37	F	Yes
8	238	41	F	Yes
9	217	39	M	Yes
10	690	35	F	Yes
			Pos: Male=3(30%) Pos: Female= 7(70%)	POSITIVE: 10(12.99%) NEGATIVE: 67 (87.01%)

Of the total number of 77 isolates screened for ESBL production, 10(12.99%) were positive, whereas 67 (87.01%) were negative. Of the 10 (12.99%) positive samples, 3 (30%) were from male respondents, whereas 7(70%) were from female respondents.

Table 3 Age of participants and prevalence of ESBL gene**.**

S/N	Age range	Sex		Frequency of ESBL gene	Prevalence of ESBL gene
		male	female		
1	10-20	nil	nil	NIL	NIL
2	21-30	nil	nil	NIL	NIL
3	31-40	1	5	6	60%
4	41-50	1	2	3	30%
5	51-60	nil	nil	nil	NIL
6	61-70	nil	1	1	10%
		2 (20%)	8 (80%)	10	100%

Age range of 31-40 contributed the highest prevalence of ESBL genes (60%), followed by age range of 41-50. The least production of ESBL was observed among age range of 65-70 (10%). Age range of 10-20, 21-30, and 51-70 did not produce any ESBL gene. Of the total ESBL genes produced, 80% came from females, whereas 20% came from males.

Table 4 Prevalence of ESBL Resistant genes

ESBL genes	Sample 008	Sample 009	Sample 010	Sample 011	Sample 012	Sample 013	Sample 014	Sample 015	Sample 016	Sample 017	Total	Prevalence (%)
CTX-M	NO	YES	YES	NO	NO	NO	YES	YES	NO	NO	4	40
SHV gene	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES	9	90
TEM	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	10	100
bla GES	NO	YES	YES	NO	NO	NO	NO	NO	YES	YES	4	40
TOTAL	2	4	3	2	2	2	3	3	3	3	27	

From the table above, it can be deduced that bla TEM is the most prevalent ESBL resistant genes among the 10 isolates tested, with a prevalence of 10 (100%), followed by SHV 9(90%), whereas bla GES gene and bla CTX-M have the least prevalence of 4 (40%) each.

Table 5 Klebsiella isolates that showed multidrug resistant to the antibiotics used

S/N	L/No	AGE	SEX	GEN	CoA	*LIV*	*CIP*	CefT	CefZ	CefP	CefTR	MERO	*OFX*	S	I	R
1	001	40	F	R(15)	R(10)	R(15)	S(20)	S(20)	S(22)	S(21)	R(16)	S(25)	S(20)	6	NIL	4
2	008	37	F	R(10)	R(9)	R(12)	R(14)	S(20)	I(18)	S(20)	R(16)	S(21)	R(15)	3	1	6
3	035	30	F	R(15)	R914)	R(14)	R(16)	I(18)	I(17)	I(17)	R(16)	S(20)	R(14)	1	3	6
4	045	26	M	R(16)	R(14)	R(15)	R(18)	R(16)	R(15)	R(16)	I(19)	I(19)	R(12)	NIL	2	8
5	065	57	M	R(15)	R(15)	R(14)	I(19)	R(13)	R(14)	R(16)	S(20)	S(21)	S(20)	3	1	6
6	073	32	F	R(16)	R(15)	R(18)	S(20)	S(21)	S(21)	I(18)	S(24)	S(28)	I(18)	5	2	3
7	128	50	M	I(17)	R(14)	R(15)	R(15)	R(16)	R(14)	R(16)	R(15)	I(17)	R(13)	NIL	2	8
8	143	37	F	R(16)	R(14)	R(15)	R(15)	R(14)	R(14)	R(14)	R(16)	R(16)	R(15)	NIL	NIL	10
9	175	22	M	S(20)	R(16)	R(16)	R(15)	R(16)	R(16)	R(16)	R(21)	I(19)	I(19)	1	2	7
10	187	65	F	R(12)	R(13)	R(13)	R(14)	R(13)	R(12)	R(10)	R(16)	R(14)	R(14)	NIL	NIL	10

11	193	48	F	I(19)	R(16)	R(14)	R(16)	I(18)	I(18)	R(15)	S(20)	I(19)	I(19)	1	5	4
12	217	39	M	R(14)	R(13)	R(15)	R(15)	R(15)	R(14)	R(14)	R(15)	R(15)	R(14)	NIL	NIL	10
13	238	41	F	I(19)	R(15)	I(18)	R(14)	R(15)	R(15)	R(15)	I(19)	S(20)	I(18)	1	4	5
14	286	49	F	R(15)	R(13)	I(18)	S(20)	I(18)	I(18)	I(18)	S(21)	S(22)	R(11)	3	4	3
15	314	43	M	R(14)	R(13)	R(13)	R(14)	R(15)	R(15)	R(13)	S(20)	S(20)	R(15)	2	NIL	8
16	323	55	F	I(19)	R(15)	S(20)	S(20)	S(22)	S(21)	R(13)	S(22)	S(28)	R(14)	6	1	3
17	348	20	F	I(18)	R(15	R(15)	R(14)	R(14)	R(14)	R(14)	I(18)	I(19)	R(13)	NIL	4	6
18	406	20	F	R(14)	R(15)	R(15)	R(15)	R(14)	R(15)	R(14)	R(15)	R(15)	R(12)	NIL	NIL	10
19	433	19	M	R(15)	I(18)	I(18)	I(19)	R(14)	R(15)	R(14)	S(22)	S(29)	R(12)	2	3	5
20	465	29	F	I(19)	R(14)	I(18)	I(18)	I(18)	R(15)	R(14)	I(19)	S(20)	R(13)	1	5	4
21	489	50	F	R(12)	R(09)	R(13)	R(13)	R(15)	R(13)	R(12)	R(15)	R(15)	R(12)	NIL	NIL	10
22	502	41	F	R(14)	R(15)	R(14)	R(14)	R(10)	S(20)	S(20)	S(22)	S(22)	R(15)	4	NIL	6
23	707	29	M	I(19)	R(14)	I(18)	I(19)	R(12)	R(13)	R(15)	I(18)	I(19)	R(14)	NIL	5	5
24	541	35	F	I(19)	R(14)	S(20)	S(22)	S(20)	I(18)	R(14)	S(21)	S(24)	R(15)	5	2	3
25	619	29	M	R(14)	R(15)	R(14)	I(19)	I(18)	I(18)	R(14)	I(19)	S(20)	R(15)	1	4	5
26	642	37	M	I(19)	R(15)	R(15)	R(14)	I(19)	R(14)	R(14)	I(18)	S(24)	R(14)	1	3	6
27	690	35	F	R(14)	R(07)	R(09)	R(10)	R(5)	R(07)	R(8)	R(15)	R(15)	R(11)	NIL	NIL	10
28	716	24	M	S(21)	R(12)	R(16)	R(15)	I(19)	I(18)	R(16)	S(20)	S(21)	I(19)	3	3	4
29	727	23	F	I(18)	R(13)	R(14)	R(13)	R(15)	R(14)	R(14)	I(19)	I(18)	R(12)	NIL	3	7
			M=11 F=18	S=2 I=10 R=17	S=nil I=1 R=28	S=2 I=5 R=22	S=4 I=5 R=20	S=5 I=7 R=17	S=4 I=7 R=18	S=3 I=2 24	S=10 I=8 R=11	S=17 I=7 R=5	S=2 I=5 R=22			

Keys: GEN (Gentamycin) CoA (co-amoxiclave) LIV (levofloxacin) CIP (ciprofloxacin) CefT (ceftazidime) CefZ (cefotaxime) CefP (cefipime) CefTR (ceftriaxone)MERO (meropenem) OFX (ofloxacin) S (sensitive) I (intermediate) R (resistant)

From the table above, a total of 29 isolates of the test organism showed multidrug resistant to the antibiotics used. Of this number, 11 (37.9%) were isolated from male participants, whereas 18(62.1%) came from female participants.

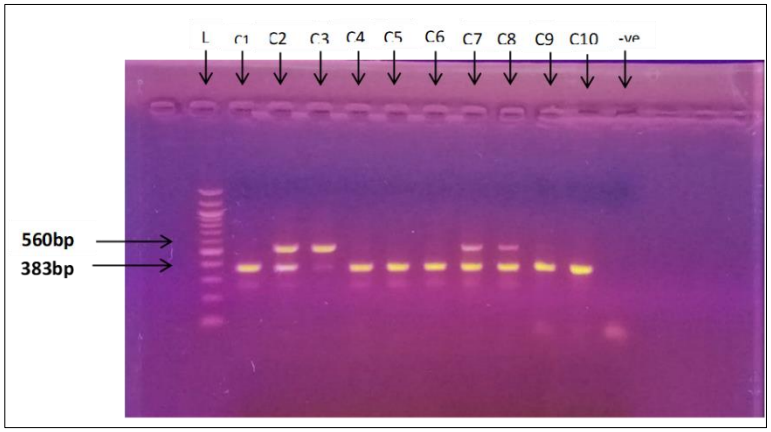
Table 6 Relationship between ESBL production and multidrug resistance

S/N	L/No	AGE	SEX	MDR	POSITIVE ESBL
1	001	40	F	4	YES
2	008	37	M	6	YES
3	035	30	F	6	NO
4	045	26	M	8	NO
5	065	57	M	6	NO
6	073	32	F	3	NO
7	128	50	M	8	NO
8	143	37	F	10	NO
9	175	22	M	7	NO

10	187	65	F	10	YES
11	193	48	F	4	NO
12	217	39	M	10	YES
13	238	41	F	5	YES
14	286	49	F	3	YES
15	314	43	M	8	YES
16	323	55	F	3	NO
17	348	20	F	6	NO
18	406	20	F	10	NO
19	433	19	M	5	NO
20	465	29	F	4	NO
21	489	50	F	10	NO
22	502	41	F	6	NO
23	707	29	M	5	NO
24	541	35	F	3	YES
25	619	29	M	5	NO
26	642	37	F	6	YES
27	690	35	F	10	YES
28	716	24	M	4	NO
29	727	23	F	7	NO
			M=11 F=18		ESBL pos= 10 MDR=29 XDR=6

Of the 29 isolates that were MDR, 10 of them were ESBL positive, whereas 19 were not. Out of this 29 MDR isolates, 6 were XDR. Of the 6 XDR isolates, 3 do not possess ESBL enzymes, whereas 3 of them do.

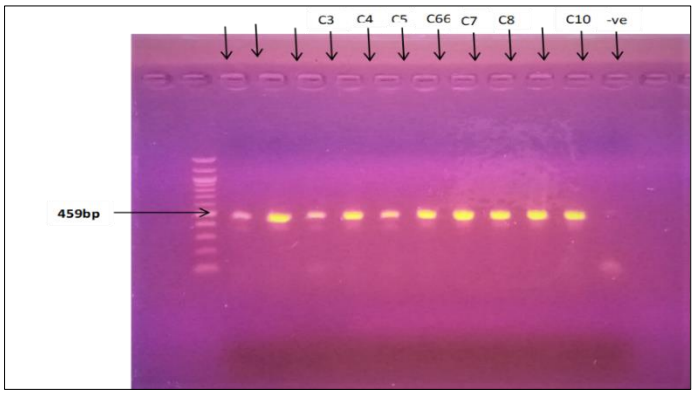
3.1. Gel electrophoresis for the CTX-M and SHV genes



Keys; C1= 8,C2=9, C3=10, C4=11, C5=12, C6=13, C7=14, C8=15, C9=16, C10=17**;** L= DNA Ladder -ve= Negative Control

Figure 1 CTX-M Gene (560bp) and SHV gene (383bp) gel image

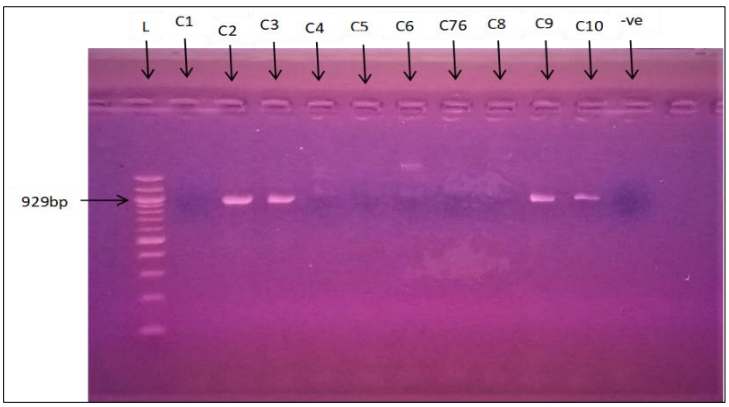
3.2. Gel electrophoresis for TEM gene



Keys; C1= 8, C2=9, C3=10, C4=11, C5=12, C6=13, C7=14, C8=15, C9=16, C10=17**;** L= DNA Ladder**;** -ve= Negative Control

Figure 2 TEM gene (459bp) gel image

3.3. Gel electrophoresis for the *bla* GES gene.



Keys; C1= 8, C2=9, C3=10, C4=11, C5=12, C6=13, C7=14, C8=15, C9=16, C10=17**;** L= DNA Ladder-ve= Negative Control

Figure 3 Bla GES gene (920bp) gel image

4. Discussion

In this study, the overall prevalence of samples showing positive ESBL genes among the participants was 10 (12.99%), of which 3 (30%) were from males, whereas 7(70%) were from females. This finding is lower than the report on a work done in Bahir Dar City, Northwest Ethiopia [15], which gave ESBL Klebsiella prevalence rate of 10 (26.3%), however, the findings of this study is slightly higher than a similar work done in community on-set UTI in China, where a 110 (6.3%) ESBL *Klebsiella pneumoniae* was obtained [16]. The variation in prevalent rate can be attributed to antibiotic usage and exposure to mobile pool of antimicrobial resistance genes (ESBL) in hospital settings, as pointed out by researchers [17], [18]. As reported in this study, the highest prevalence of ESBL genes was recorded among age range of 31-40 (60%). This varies with the findings of a similar study [19], where the highest Klebsiella ESBL isolates were obtained among age range of 16-30 (10/16.7%), with women contributing the highest ratio (83.3%).

These isolates were then tested by polymerase chain reaction (PCR) for the presence or absence of *bla* $_{TEM}$, *bla* $_{CTX-M}$, *bla* $_{SHV}$, and *blas* $_{GES}$ genes. The result showed that *bla* $_{TEM}$ gene was present in all isolates (100 %) followed by *bla* $_{SHV}$ (90%) and *bla* $_{CTX-M}$ /*bla* $_{GES}$ genes were the least represented, with a prevalence of 40% and 40% respectively. Thus, making *bla* $_{TEM}$ the most common ESBL genotype among our isolates, followed by *blas* $_{SHV}$. This is in line with research findings [20], [21], except for *blas* $_{SHV}$ gene, which was more common in these studies, instead of *blas* $_{TEM}$ gene as obtained in the present study. Of the 77 isolates tested for antimicrobial susceptibility testing, 29(37.7%) showed MDR, and 6 (7.8%) showed XDR, thus these 6 isolates resisted all the 10 antibiotics used, which were selected from 4 antibiotic classes: Aminoglycosides, B-lactams, Carbapenems, Fluoroquinolones, and Cephalosporins. However, out of the 29 MDR isolates, only 10 (34.5%) were ESBL positive, and out of the 6 XDR isolates, only 3 (50%) were ESBL positive. The findings of this study reflect similar findings in similar studies performed elsewhere [22], [23], [24], [25]. However, we report that apart from possession of ESBL genes, which are known to confer resistance to bacterial organisms, there are

other factors that are responsible to for drug resistance as observed in this study, which the present study could not determine.

5. Conclusion

Highest ESBL production was observed among the younger participants, with females contributing more than the males. TEM genes were the most prevalent, whereas *bla*-$_{CTX}$ and *blas* $_{GES}$ genes were the least prevalent, and about one quarter of the isolates showed MDR. Apart from ESBL possession, it is believed that there are other factors that contributed to drug resistance among the participants, since most non ESBL producers were both MDR and XDR. Therefore, there is need for public health interventions to check drug resistance and determine other factors that encourage drug resistance.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

CC is the principal investigator of the study and performed the design of the study, data management, study supervision, analysis, interpretation of data, and manuscript preparation JT, GA, and NO were involved in the designing, supervision, acquisition of data, and critical review of the manuscript. Finally, all authors read and approved the final manuscript for publication.

Data Availability

All the data supporting the conclusion of the study are included in the paper.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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