Int. j. adv. multidisc. res. stud. 2024; 4(3):1411-1415

Received: 01-05-2024
Accepted: 11-06-2024

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Pathotypes of Extended Spectrum Beta-Lactamase Producing *Escherichia Coli* Isolated from "Suya" Sold in Nasarawa State, Nigeria

[1] Danladi WD, [2] Ishaleku D, [3] Ngwai YB, [4] James AA, [5] Eko KO, [6] Anzaku S

[1, 5, 6] Department of Microbiology, Faculty of Natural and Applied Sciences, Nasarawa State University, PMB 1022, Keffi, Nigeria

[2, 3] Professor, Department of Microbiology, Faculty of Natural and Applied Sciences, Nasarawa State University, PMB 1022, Keffi, Nigeria

[1] Standards Organisation of Nigeria (SON), Corporate Headquarters, 52 Lome Crescent, Wuse Zone 7, Abuja, Nigeria

[4] Department of Zoology, Faculty of Natural and Applied Sciences, Nasarawa State University, PMB 1022, Keffi, Nigeria

Corresponding Author: **Danladi WD**

Abstract

Escherichia coli (*E. coli*) can be pathogenic and non-pathogenic. Pathogenic *E. coli*, like other pathogenic bacteria, expresses virulence genes. This study screened extended spectrum beta-lactamase producing *E. coli* (ESBL-EC) isolated from "Suya" sold in Nasarawa State, Nigeria for diarrhegenic *E. coli* (DEC) pathotypes. A total of fourteen (14) ESBL-ECs pathotypes were identified by means of the amplification of DEC genes using multiplex Polymerase Chain Reaction (mPCR). The prevalence of DEC pathotypes was: 5(35.7%) EPEC; 4(28.6%) EPEC/EHEC, 2(14.3%) EAEC; 2(14.3%) STEC/EHEC and 1(7.1%) EHEC. This study has established "Suya" as reservoir of diarrhegenic *E. coli* and a potential source of *E. coli* diarrheal infections.

Keywords: "Suya", *Escherichia coli*, Extended Spectrum Beta-Lactamase, Pathotypes

Introduction

Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli* are Gram-negative bacteria that produce enzymes that confer resistance to penicillin, 3rd generation cephalosporin (ceftazidime, cefotaxime, and ceftriaxone) and monobactam aztreonam, but not to cephamycin (cefoxitin and cefotetan) and carbapenems (Bonnet, 2004; Al-Muharrmi *et al*., 2008; Shaikh *et al*., 2015) [1, 2, 3] resulting to major public health issue (EFSA, 2011; ECDC, 2017). Food animals colonized with ESBL-producing bacteria can enhance the spread of bacteria at the community level (Bortolai, 2010) [4]. The ESBL pandemic in *E. coli* is primarily associated with *CTX-M* beta-lactamases, particularly *CTX-M*-15 (Pitout, 2012) [5], but additional enzymes may be in charge of inactivating beta-lactams. For instance, the *SHV* or *TEM* types accounted for the majority of ESBLs during the 1980s and 1990s. (Paterson, 2005) [6]. Antimicrobial-resistant *E. coli* asymptomatically colonizes the intestinal flora of food animals with a likelihood of becoming infectious to humans if consumed through the food chain (Lavilla, 2008) [7]. This infers that food animals can serve as a reservoir for ESBL-producing bacteria and their genes (Franco *et al*., 2011; Tyrrel *et al*., 2016) [9].

The concept of "Suya", a meat product with soaring demand for its protein content, vitamins and minerals has been observed to be implicated in the emergence of foodborne pathogens such as *E. coli* and disease outbreaks (Carnot *et al*., 2014) [10]. It is, a popular traditionally processed, ready-to eat meat product (NIS 604: 2008) [11] which is usually served or sold along the streets and served at hospitality industries such as social functions, club houses, picnics, restaurants and perceived and consumed wholesome and unadulterated by consumers with no attention paid to its safety; hence the possible occurrence of food borne diseases (Okonkwo *et al*., 2012; Nyenje *et al*.,2012) [12, 13] and the dissemination of pathogens bacteria (Nyenje *et al*., 2012) [13] such as *E. coli* and antibiotic resistance (Ngwai, 2016) [14].The presence of *E. coli* in foods that are ready for consumption in this study is indicative of poor hygiene and contamination which could have arisen from human or animal faecal sources (Adesoji *et al*., 2019) [15]. It has been reported that consumption of poorly processed and contaminated beef with EHEC has been a cause of bloody diarrhea that evolved into hemolytic-uremic syndrome (HUS) (Surendran-Nair, 2017) [16].

Meat has been implicated in numerous disease outbreaks caused by *E. coli* pathotypes (Sperandio & Nguyen, 2012) [17]. According to its pathogenesis and epidemiologic characteristics on a specified host, six pathotypes of *E. coli* are: Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E.coli* (EHEC) Enteroinvasive *E. coli* (EIEC), Enteroinvasive *E.coli* (EIEC) and Enteroaggregative *E.coli* (EAEC) (Sperandio & Nguyen, 2012) [17]. These pathotypes are delineated by series of challenges associated with meat safety with the pathogens having food producing animals as primary reservoir. Beef carcass and its derived cuts have been reported as the source of infection in many outbreaks caused by EHEC (Barham *et al.*, 2002) [18]. The first outbreak documented in the United States of America (USA) was in 1982, which was associated to beef, and up to day, it remains as the most common vehicle among disease outbreaks caused by *E. coli* pathotypes (Rangel *et al.*, 2005, Cardona-Lopez *et al*., 2020) [19, 20]. Various virulence factors determinants have been attributed to *E. coli* pathogenicity, including Shiga toxin-associated genes (*stx*1 and *stx*2), toxin production genes such as hemolysin (*hly*) and the *ast*A gene encoding enteroaggregative *E. coli*, heat-stable enterotoxin, intimin encoding gene (*eae*), and fimbrial H gene (*fim*H). In this context, the aim of this research was to determine the diarrhegenic pathotypes of ESBL producing *E. coli* from "Suya" sold in Nasarawa State, Nigeria.

Materials and Methods

Bacterial Isolates

Fourteen (14) extended spectrum beta-lactamase producing *E. coli* (ESBL-EC) isolated from "Suya" sold in Nasarawa State, Nigeria were obtained from previous study (Danladi *et al.*, 2024) [21]. The characteristics of the isolates are given in Table 1.

Table 1: Characteristics of extended spectrum beta-lactamase producing *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria

Isolate	ESBL Genes	Class of Antimicrobial Resistance	Antimicrobial Resistance Pattern	Source
L1	*blaSHV*	MDR	OFX, AMP, CIP, AMC,CN,S, CEF,SXT	Keana
EL4	*blaSHV*	MDR	AMP,CIP,AMC,S,CTX, SXT, CAZ	Nasarawa Eggon
SR3	*blaSHV*	MDR	OFX,CN,S,CTX, CEF,SXT	Karu
AKJ5	*blaSHV*	MDR	OFX,CIP,CN, CTX, CEF,SXT, CAZ	Akwanga
L3	*blaSHV*	MDR	OFX,CIP,CN, CTX, CEF,SXT, CAZ	Lafia
L6	*blaSHV*	XDR	OFX,CN,S,CTX, CEF,SXT	Lafia
PL1	*blaSHV*	PDR	OFX, AMP,CTX,SXT	Keffi
ncm1	*blaSHV*	MDR	OFX,CIP,AMC,CN,S,CTX, CEF, CA	Nasarawa
Mg4	*blaSHV*	MDR	OFX,CIP,AMC,CN,S,CTX, CEF, CAZ	Nasarawa
Kn3	*blaSHV*	XDR	OFX,CN,S,CTX, CEF,SXT	Karu
PL4	*blaSHV*	MDR,	OFX, CTX, CEF,SXT, CAZ	Keffi
AKJ2	*blaSHV*	MDR	OFX,CIP,CN, CTX, CEF,SXT, CAZ	Akwanga
nrpjr6	*blaSHV*	PDR	OFX, AMP,CTX,SXT	Keffi
nenn	*blaSHV*	MDR	AMP,CIP,AMC,S,CTX, SXT, CAZ	Nasarawa Eggon
L3	*BlaOXA-1*	MDR	OFX,CIP,CN, CTX, CEF,SXT, CAZ	Lafia
AKJ2	*BlaOXA-1*	MDR	OFX,CIP,CN, CTX, CEF,SXT, CAZ	Akwanga
L1	*blaCTX-M-4*, *blaCTX-M-9*	MDR	OFX, AMP,CIP,AMC,CN,S, CEF,SX	Keana
EL4	*blaTEM*	MDR	AMP,CIP,AMC,S,CTX, SXT, CAZ	Nasarawa Eggon
PL4	*blaTEM*	MDR	OFX, CTX, CEF,SXT, CAZ	Keffi
nrpjr6	*blaTEM*	PDR	OFX, AMP,CTX,SXT	Keffi

MDR = Multidrug Resistance; PDR = Pandrug Resistance; XDR = Extensive Drug Resistance

Detection of Diarrhgenic Pathotypes of Extended spectrum Beta-lactamase producing *Escherichia coli*

Genomic DNA of the ESBL-EC strains was extracted based on the protocol described by Abimiku *et al.* (2016) [22]. The DNA templates was subjected to multiplex polymerase chain reaction (mPCR) with specific primers (Table 2) for the detection of the following virulence markers: *eaeA* (structural gene for intimin of EHEC and EPEC), *bfpA* (structural gene for the bundle-forming pilus of EPEC), *vt1* and/or *vt2* (Shiga toxins 1 and 2 of EHEC), *eltB* and/or *estA* (enterotoxins of ETEC), *ial* (invasion-associated locus of the invasion plasmid found in EIEC and *Shigella*) and *pCVD* (the nucleotide sequence of the EcoRI-PstI DNA fragment of *pCVD432* of EAEC) as shown in Table 2.

The *E. coli* isolates were submitted to the mPCR in a 25µl reaction mixture containing 5 µl of template DNA, 0.2µl of 18x PCR buffer II, 1.6µl of a 1.25mM mixture of deoxynucleoside triphosphates, 1.6 µl of 25 mM $MgCl_2$, 0.1µl of 5U of AmpliTaq Gold DNA polymerase per µl and a 0.2µM concentration of each primer except primer VT1, which was used at a concentration of 0.4 µM. The thermocycling conditions used were as follows: 95°C for 5 min (Initial denaturation), 94°C for 20sec. (denaturation) 55°C for 30 sec. (Annealing) and 72°C for 30sec. (initial extension) for 30 cycles, with a final 7 min extension at 72°C (Abimiku *et al.*, 2016) [22]. The pathotypes were analyzed by comparing the sizes of the PCR products of the samples with 1kb molecular ladder then photographed and documented.

Table 2: Primers and amplicon sizes of Diarrhegenic *Escherichia coli* Pathotypes

E. coli Pathotypes	Primer	Target gene	Oligonucleotide sequence 5' → 3'	Amplicon size (bp)	Reference
EPEC/EHEC	EAE1	*eae*	F: AACAGGTGAAACTGTTGCC R: CTCTGCAGATTAACCTCTGC	490	Belete *et al*., 2022
STEC/EHEC	EAE2 EVS1	*stx1*	F: ATCAGTCGTCACTCACTGGT R: CTGCTGTCACAGTGACAAA	110	Belete *et al*., 2022
STEC/EHEC	EVC2 EVT1	*stx2*	F: CAACACTGGATGATCTCAGC R: CCCCCTCAACTGCTAATA	390	Belete *et al*., 2022
EHEC	EVT2 EHEC	*hly*A	F: ACGATGTGGTTTATTCTGGA R: CTTCACGTCACCATACATAT	167	Belete *et al*., 2022
EAEC	EAEC	*aat*A	F: CTGGCGAAAGACTGTATCAT R: CAATGTATAGAAATCCGCTGTT	630	Belete *et al*., 2022
EPEC	BFP	*bfp*A	F: AATGGTGCTTGCGCTTGCTGC R: GCCGCTTTATCCAACCTGGTA	494	Belete *et al*., 2022
ETEC	ST1 ST2	*St*	F:TTTATTTCTGTATTGTCTT R: GCAGGATTACAACACAATTC	294	Belete *et al*., 2022
ETEC	LT1 LT2	*Lt*	F: GCTCTATGTGCATACCGAGT R: CCATACTTGATTGCCGCAAT	696	Belete *et al*., 2022
EIEC	IAL 1 IAL 2	*Ial*	F: GAACGTTGGTTAATGTGGGGT R: TATTCACCGGTCGGTTATCAG	320	Belete *et al*., 2022
DAEC	daaE1 daaE2	*daaE*	F: GAACGTTGGTTAATGTGGGGT R: TATTCACCGGTCGGTTATCAG	542	Belete *et al*., 2022

ETEC= Enterotoxigenic *E. coli*; STEC= Shiga toxin *E. coli;* EHEC= Enterohemorrhagic *E. coli*; EIEC= Enteroinvasive *E. coli*; EPEC= Enteropathogenic *E. coli*; EAEC= Enteroaggregative *E. coli*; DAEC= Diffusely adherent *E. coli*.

Results and Discussion

Diarrheaginic pathotypes of the extended spectrum beta-lactamase producing *Escherichia coli*

Diarrhegenic pathotypes of ESBL-EC were detected as follows:EPEC 1(100.0%) in Akwanga followed by EPEC/EHEC 2(66.7%) in Karu then EAEC 1(50.0%) in Keffi, STEC/EHEC 1(50.0%) in Lafia while EHEC 1(50.0%) in Keana. No DEC pathotype (0.0%) was detected at Nasarawa Eggon and therefore did not belong to any pathotype.

Table 3: Distribution of Diarrhegenic Pathotypes of extended spectrum beta-lactamaseproducing *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria

DEC Pathotypes	No. (%) Pathotypes of ESBL *E. coli* (n = 14)							Total ESBL-EC DEC Pathotypes
	NW			NS		NN		
	Karu (n-3)	Keffi (n-2)	Nasarawa (n-4)	Lafia (n-2)	Keana (n-2)	Akwanga (n-1)	Nasarawa Eggon (n-0)	
EPEC/EHEC (*eae*)	2(66.7)	0(0.0)	1(25.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	4
EAEC (*aatA*)	0(0.0)	1(50.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2
EPEC (*bfpA*)	1(33.3)	0(0.0)	2(50.0)	1(50.0)	0(0.0)	1(100.0)	0(0.0)	5
STEC/EHEC (*ST1*)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	1
(*ST2*)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1
EHEC (*hlyA*)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	1
EIEC (*lal*)	0(0.0)	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0
ETEC *(lt*)	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0
DAEC (*daaE*)	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0(0.0	0
Total	**3(21.4)**	**2(14.3)**	**4(28.6)**	**2(14.3)**	**2(14.3)**	**1(7.1)**	**0(0.0)**	14

NW= Nasarawa West Senatorial District; NS= Nasarawa South Senatorial District; NN= Nasarawa North Senatorial District.
ETEC= Enterotoxigenic *E. coli*; STEC= Shiga toxin *E. coli;* EHEC= Enterohemorrhagic *E. coli*; EIEC= Enteroinvasive *E. coli*; EPEC= Enteropathogenic *E. coli*; EAEC= Enteroaggregative *E. coli*; DAEC= Diffusely adherent *E. coli.*

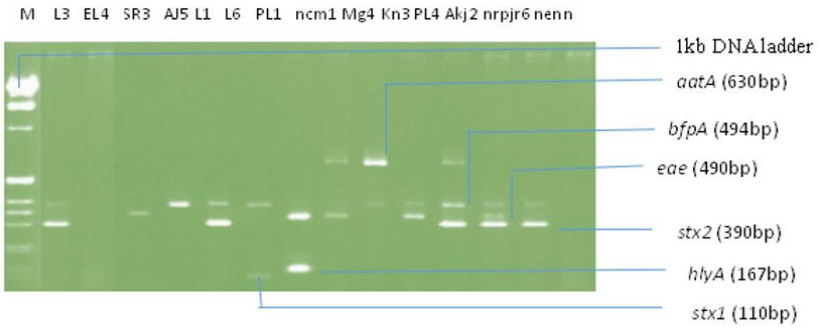

Plate 1: Agarose gel electrophoresis of the amplified pathotypes *gene* of *Escherichia coli*. Lane ncm1 and PL4 represent the expression of the *aatA* gene at 630bp; Lane SR3, Mg4, PL4, and Lane PL1 represent the expression of the *eae* gene (490bp).

Lane L3, L1, Mg4, AKJ2, and Kn3, represent the expression of the *bfpA* gene (494bp); Lane L6 represents the expression of the s*tx1* gene (110bp); Lane PL1 represents the expression of the *hlyA* gene (167bp) and Lane PL4, represent the expression of the s*tx2* gene at 390bp while Lane M represents 1kb DNA molecular ladder.

Key::*eae* = EPEC/EHEC; *stx*1 = STEC/EHEC; *stx*2 = STEC/EHEC; *hly*A = EHEC; *aat*A =EAEC; *bfp*A = EPEC; s*t* = ETEC; l*t* = ETEC; *Ial* = EIEC; *daa*E = DAEC.

Isolates = L3, EL4, SR3, Aj5, L1,L6, PL1, ncm1, Mg4, kn3, PL4, Akj2, nrpjr6 and nenn.

Location = L3, L6 = Lafia; EL4, nenn = Nasarawa Eggon; L1, PL1 = Keana; nrpjr6, PL4 = Keffi,; Akj2, Aj5 = Akwanga; ncm1,Mg4 =Nasarawa and SR3, Kn3 = Karu.

Discussion

The frequency of detection of ESBL *E. coli* pathotypes showed the occurrence rate of 9(64.3%) isolates in Nasarawa West Senatorial District, 4(28.6%) Nasarawa South Senatorial District and 1(7.1%) in Nasarawa North Senatorial District. This finding negates the occurrence of 7.2% ESBL *E. coli* pathotypes reported elsewhere by Nguyen *et al*., (2005). In the present study, 14 isolates were confirmed to be DEC and the most prevalent pathotype of DEC encountered was 2(66.7%) EPEC/EHEC amplified by *eae* gene *at* 490bp in Karu of Nasarawa West Senatorial District. This finding is significant and agreed with that of Nakhjavani *et al*., (2013) [24] who reported that EPEC/EHEC encoded by the *aea* gene was considered as a determinant for virulence factor to infect the host and cause disease. This result showed that EPEC/EHEC was the most common pathotype that existed in "Suya" in this study area hence could implicate in Traveller's diarrhea episodes.

The low frequency of detection of diarrheagenic *E. coli* was observed in Keana (EHEC: 1[50.0%] and Nasarawa (:EAEC 1[25.0%]) of Nasarawa South and West Senatorial Districts. The detection of EHEC in the study area aligned with the previous report of EHEC infections which was adjudged to occur both in developing and developed countries of the world - India (Samal *et al*., 2008) [25], Republic of Korea (Cho *et al*., 2008) [26] and Nigeria (Aworth *et al*., 2019) [27]. This suggest that a high contamination rate with DEC would infer poor hygiene which might pose societal health risks to the study population and hence routine surveillance is imperative in identifying outbreaks and help in determining various reservoirs and transmission routes (Odetoyin *et al*., 2015) [28].

All the isolates had at least one virulence gene, with *bfpA* 1(100.0%) and *eae*, 2(66.7%) being the most frequently identified genes and two (2) isolates encoded for *stx1*, *stx2* genes, a crucial factor for the pathogenicity of Shiga toxin producing *E. coli* (STEC). None of the pathotypes were detected at Nasarawa Eggon showing that the isolates probably did not belong to any of the pathotypes or isolation rate of different pathotypes of diarrheagenic *E. coli* might have different geographical areas. Similarly, ETEC, EIEC and DAEC were not detected in this study. This could probably be attributed to the primers that were used as multiplex PCR assay could have not identified the positive EAEC strains by virtue of *aatA* genes at 630bp.

Conclusion

The prevalence of diarrhegenic extended spectrum beta-lactamase producing *E. coli* pathotypes was: 5(35.7%) EPEC; 4(28.6%) EPEC/EHEC, 2(14.3%) EAEC; 2(14.3%) STEC/EHEC and 1(7.1%) EHEC. This study has established "Suya" from the study area as reservoir of diarrhegenic extended spectrum beta-lactamase producing *E. coli* and a potential source of *E. coli* diarrheal infections hence serotyping of the pathotypes recommended for further study.

Acknowledgement

Contributions made by Staff of Microbiology laboratory, Nasarawa State University Keffi, Nigeria and Molecular Laboratory, Institute of Human Virology, Plateau Specialist Hospital Jos, Nigeria towards the preparation, execution and completion of the analysis of the data is dully acknowledged.

References

1. Bonnet R. Growing group of extendedspectrum beta-lactamases: The CTX-M enzymes. Antimicrobial and Agents and Chemotherapy. 2004; 48(1):1-14.
2. Al-Muharrmi Z, Rafay A, Balkhair A, Jabri A. Antibiotic combination as empirical therapy for extended spectrum Beta-lactamase. Oman Medical Journal. 2008; 23(2):78-81.
3. Shaikh S, Fatima J, Shakil S, Mohd S, Rizvi D, Amjad MK. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi Journal of Biological Science. 2015; 22(1):90-101.
4. Bortolaia V, Guardabassi L, Bisgaard M, Larsen J, Bojesen AM. *Escherichia coli* producing CTX-M-1, -2, and -9 group -lactamases in organic chicken egg production. Antimicrobial Agents and Chemotherapy. 2010; 54(8):3527-3528.
5. Pitout JD. Extraintestinal pathogenic *Escherichia coli*: An update on antimicrobial resistance, laboratory diagnosis and treatment. Expert Review of Anti Infective Therapy. 2012; 10:1165-1176.
6. Paterson DL, Bonomo RA. Extended-Spectrum Beta-Lactamases: A Clinical Update. Clinical Microbiology Review. 2005; 18(4):657-686.
7. Lavilla S, Gonzalez-L JJ, Opez J, Mir EO. Dissemination of ´ extended-spectrum beta-lactamase-producing bacteria: The foodborne outbreak lesson. Journal of Antimicrobial Chemotherapy. 2008; 6(61):1244-1251.
8. Franco A, Leekitcharoenphon P, Feltrin F, Alba P, Cordaro G, Iurescia M, *et al*. Emergence of a clonal lineage of Multidrug-Resistant ESBL-Producing Salmonella Infantis transmitted from broilers and broiler meat to humans in Italy between 2011 and 2014. PLoS ONE. 2016; 10(12):e0144802.
9. Tyrrell JM, Wootton M, Toleman MA, Howe RA, Woodward M, Walsh TR. Genetic & virulence profiling of ESBL-positive *E. coli* from nosocomial and veterinary sources. Veterinary Microbiology. 2016; 186:37-43.
10. Carnot AJS, Guerra TS, Souza L, Carneiro C. Antimicrobial resistance and plasmid characterization of *Escherichia coli* isolated in natural water. American Journal of Drug Discovery and Development. 2014; 4:80-84.

11. Nigerian Industrial Standard for "Suya", NIS 604, 2008.
12. Okonkwo I, Sunday M, Emeka C. Microbiological Safety and Proximate Composition of *Suya* Stored at Ambient Temperature for Six Hours from Maiduguri, Northern Nigeria. Internet Journal of Food Safety. 2012; 14:11-16.
13. Nyenje ME, Odjadjare CE, Tanih NF, Green E, Ndip RN. Foodborne Pathogens Recovered from Ready-to-Eat Foods from Roadside Cafeterias and Retail Outlets in Alice, Eastern Cape Province, South Africa: Public Health Implications. International Journal of Environmental and Research Public Health. 2012; 9(8):2608-2619.
14. Ngwai YB. 13th Inaugural Lecture, Nasarawa State University, Keffi, Nigeria, 2016.
15. Adesoji AT, Onuh JP, Musa AO, Akinrosoye PF. Bacteriological qualities and antibiogram study of bacteria from. The Pan African Medical Journal. 2019; 33:219.
16. Surendran-Nair M. Controlling Enterohemorrhagic E. coli O157: H7 using Selenium and Rutin. Doctoral Dissertations. 2017; 1552:1. https://opencommons.uconn.edu/dissertations/1552/ [Last Checked: December 2019].
17. Sperandio V, Nguyen Y. Enterohemorrhagic E. coli (EHEC) pathogenesis. Frontiers in cellular and infection Microbiology. 2012; 2:90.
18. Barham AR, Barham BL, Johnson AK, Allen DM, Blanton JR, Jr. Miller MF. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and Salmonella spp. Journal of Food Protection. 2002; 65(2):280-283.
19. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia col*i O157:H7 outbreaks, United States, 1982-2002. Emerging Infectoius Diseases. 2005; 11(4):603-609.
20. Cardona-López MA, Padilla-Frausto JJ, MadrizElisondo AL, Hinojosa-Dávalos J, Navarro-Villarruel CL, Varela-Hernández JJ, *et al*. Identification of *Escherichia coli* pathotypes in ground beef from butcher shops of Guadalajara, Jalisco, Mexico. Revista Bio Ciencias. 2020; 7.
21. Danladi WD, Ishaleku D, Ngwai YB, Okunlola OI, Igbawua I. Antimicrobial resistance profile and phylogenetic grouping of *Escherichia coli* isolated from "Suya" sold in Nasarawa State, Nigeria. Journal of Emerging Technologies and Innovative Research. 2024; 6(11):2349-5162.
22. Abimiku RH, Ngwai YB, Nkene IH, Tatfeng YM. Molecular detection of diarrheagenic pathotypes of *Escherichia coli* from diarrheic patients in Keffi, Nigeria. Journal of Microbiology and Biomedical Research. 2016; 2(3):1-6.
23. Belete MA, Demlie TB, Chekole WS, Sisay TT. Molecular identification of diarrheagenic *Escherichia coli* pathotypes and their antibiotic resistance patterns among diarrheic children and in contact calves in Bahir Dar city, Northwest Ethiopia. PLoS ONE. 2022; 17:9.
24. Nakhjavani *et al*. Molecular analysis of typical and atypical enteropathogenic *Escherichia coli* (EPEC) isolated from children with diarrhoea. Journal of Medical Microbiology. 2013; 62(2):195-196.
25. Samal SK, Khuntia HK, Nanda PK, *et al*. Incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India. Japanese Journal of Infectious Diseases. 2008; (61)5:350-355.
26. Cho SH, Shin HH, Choi YH, Park MS, Lee BK. Enteric bacteria isolated from acute diarrheal patients in the Republic of Korea between the year 2004 and 2006. The Journal of Microbiology. 2008; 46(3):325-330.
27. Aworh MK, Kwaga J, Okolocha E, Mba N, Thakur S. Prevalence and risk factors formulti-drug resistant Escherichia coli among poultry workers in the Federal Capital Territory, Abuja, Nigeria. PLoS One. 2019; 11(14):225379.
28. Odetoyin BW, Hofmann J, Aboderin AO, Okeke IN. Diarrhoeagenic Escherichia coli in mother-child Pairs in IleIfe, South Western Nigeria. BMC Infectious Diseases. 2015; 16(1):28.