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ANTIMICROBIAL RESISTANCE PROFILE AND PHYLOGENETIC GROUPING OF *ESCHERICHIA COLI* ISOLATED FROM "SUYA" SOLD IN NASARAWA STATE, NIGERIA.

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Abstract

"Suya" product contaminated with *Escherichia coli* (*E. coli*) continue to be a food safety concern, economic losses and has the potential to dissemination infectious diseases and antimicrobial resistance.. This study was carried out to determine the antimicrobial resistance profile and phylogrouping of *E. coli* isolated from "Suya" sold in Nasarawa State, Nigeria. A total of two hundred and ten (210) "Suya" samples were collected and screened and *E. coli* was isolated and identified using standard microbiological techniques. The antibiotic susceptibility test was carried out using Kirby-Bauer disc diffusion method on Mueller-Hinton agar against ten antibiotics. Isolates were confirmed as ESBL producers phenotypically by Double Disc Synergy Test (DDST) while ESBL genes and phylogenetic grouping were determined by Polymerase Chain Reaction (PCR). Out of 210 samples collected, 70(33.3%) *E. coli* were isolated. Isolates were highly resistant to amoxicillin/clavulanic acid 7(77.7%) in Keffi and 6(75.0%) in Nasarawa of Nasarawa West Senatorial District. These isolates were also resistant to cefoxitin, streptomycin, and co-trimoxazole 6(75.0%) in Nasarawa, Karu and Nasarawa Eggon however, none of the isolates were resistance to gentamicin 0(0.0%) at Lafia in Nasarawa South Senatorial District. Most of the *E. coli* isolates had multiple resistance (MAR) to at least five antibiotics (MAR Index of = 0.5) and the most frequent MAR Index was 0.8 with 25.0% occurrence and all were Multi-Drug Resistance (MDR) strains.The ESBL encoding genes were found in 21 isolates with *blaSHV* detected in 14(66.7%), *blaTEM* 3(14.3%), *blaOXA-1* 2(9.5%) *blaCTX-M-9* and *blaCTX-M-4* 1(4.8% isolates each. 24 isolates were confirmed to phylogroups A, B1, B2, D with phylogroup B2 the most predominant 10(41.7%) then 7(29.2%) phylogroup D, 4(16.7%) B1 and 3(12.5%) A. This study has established "Suya" as potential reservoir of *E. coli* and the observable high degree of resistance of the isolates to antimicrobial agents gave clues to health practitioners on the best antibiotics of choice in treating *E. coli* infections.

Keywords:Antimicrobial resistance, public health, "Suya", *Escherichia coli*, Phylogenetic grouping.

1. INTRODUCTION

The overuse of antimicrobial in livestock industry as growth promoters and treatment of infection is possibly one of the most important factors that accelerated the selection, emergence and dissemination of antimicrobial-resistant microorganisms in both veterinary and human medicine, thus posed a global challenge (FAO, 2015 WHO, 2019). Both pathogenic bacterial strains such as ETEC, STEC and the endogenous flora of exposed individuals (animals and humans) or populations acquired virulence factors on plasmids or other mobile genetics element via horizontal gene transfer thus resulting in intestinal or extraintestinal disease (Reygaert, 2018). The United States Food and Drug Administration reported that .2.8 million people in the United States contracted an antimicrobial resistant infection, leading to over 35,000 deaths (USFDA, 2019).Thus the concept of One Health was conceived in view of animals, humans and the environment to attain optimal public health by preventing and controlling zoonotic diseases (OHITF, 2008, Bidaisee and Macpherson, 2014).

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). It is, a popular traditionally processed, ready-to eat meat product (NIS 604: 2008) 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) and the dissemination of pathogens bacteria (Nyenje *et al*., 2012) such as *E. coli* and antibiotic resistance (Ngwai, 2016).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). Most non-pathogenic but virulent strains of *E. coli* which causes several kinds of ailments, including food-borne infections have been identified (Yun *et al*., 2018). *Escherichia coli* strains that are widely resistant to commonly prescribed antibiotics have been reported (Eze *et al*., 2013, Tsaku *et al*., 2019). The property of multidrug resistance could be transferred through conjugation from resistant strains of *E. coli* to another by means of plasmid, which occur in cytoplasm of the donor bacterium and multiply independently of the chromosomal DNA

The *E. coli* is a member of the family Enterobacteriaceae and a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* commonly found in the intestinal tract of humans, farm animals, pests and wild animals including severe invasive disease such as bacteremia and sepsis (Marc *et al*., 2021) thus, deemed as one of the first facultative organism to colonise the human gut (Nataro & Kaper, 1998; Fanaro *et al*., 2003) causing about 80% of the cases which may either be asymptomatic or symptomatic (Marc *et al*., 2021).

As a commensal, *E. coli* consist of stable genetic isolates, which translates that each individual has only one phylogenetic group. The Phylogroup represents the study of ecological niches and lifestyles in pathogenic *E. coli* indicating that *E. coli* strains are not randomly dispersed but rather exhibit both host taxonomic and environmental components thereby providing epidemiological information (Coura *et al*., 2015). Four phylogenetic group (A, B1, B2 and D) are predominant in several humans and animal populations (Clermont *et al*., 2000).

It has been observed that antimicrobial susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Hassan, *et al*., 2011). This therefore demands for the screening of common bacterial pathogens for their antimicrobial resistance profile and phylogenetic grouping of *E. coli* isolates from "Suya" sold in Nasarawa State, Nigeria.

2. RESEARCH METHODOLOGY

2.1 Study Area

The study was carried out in Nasarawa State , Nigeria. The study site was in Keffi, Karu, Nasarawa of Nasarawa West Senatorial Districts, then Keana, Lafia of Nasarawa South Senatorial District and Akwanga, Nasarawa Eggon Local Government Areas of Nasarawa North Senatorial District respectively (Anudu *et al*., 2011). The state is centrally located in the Middle Belt Region of Nigeria. It lies between latitude 7° 45' and 9° 25' North of the equator and between longitude 7° and 9° 37' East of the Greenwich Meridian. Nasarawa state has a total land area of 27,137.8sqkm and is bounded to the east by the States of Taraba and Plateau, to the north by Kaduna State, to the south by the states of Kogi and Benue and to the West by the Federal Capital territory (Anudu *et al*., 2011).

2,2 Sample Collection

Two hundred and ten (210) "Suya" samples were randomly purchased at Karu, Nasarawa, Keffi, Akwanga, Nasarawa Eggon, Lafia and Keana (30 samples from each location and sampling was carried out from February to April, 2023 on different days within 15.30hrs to 17.30hrs based on the availability of the products). The samples were aseptically wrapped and labelled with date, time of sampling, area of sampling in a foil paper and transported to the Microbiology Laboratory of Nasarawa State University, Keffi, Nigeria for analysis.

2.3 Isolation and identification of *Escherichia coli*

Primary culture was prepared by aseptically inoculating 1 g of the "Suya" sample in 10 ml of nutrient broth and incubated at 37°C for 24 hrs. To obtain pure cultures, samples from the primary culture were sub-cultured on Levine Eosin Methylene Blue (EMB) Agar (HiMedia, India) plates by streaking and incubated at 37°C. The plates were observed after 24 hrs incubation; greenish metallic sheen indicates the presence of *E. coli* (Cheesbrough, 2006). API 20E (Biomerieux TM) kit was used for biochemical identification of *E. coli* following manufacturer´s instructions.

2.4 Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method was used to evaluate the isolates' antimicrobial susceptibility in accordance with the standards established by the Clinical and Laboratory Standards Institutes (CLSI) against ten antimicrobial agents- ampicillin (10μg), cefoxitin (30μg), amoxicillin/clavulanic acid (30μg), ciprofloxacin (5μg), streptomycin (30μg), gentamicin (10μg), co-trimoxazole (30μg), ofloxacin (30μg), ceftazidime (30μg) and cefotaxime (5μg) (Becton, Dickinson and Company, MD, USA). The antibiotic discs were firmly placed on the sterile Mueller Hinton Agar (MHA) plates, seeded with test organisms standardized to 0.5 McFarland´s turbidity and incubated at 37^{o}C for 24 hrs. Diameter of zones of inhibition was then measured to the nearest millimetre and reported in accordance with the antimicrobial susceptibility break point of CLSI (Patel, 2017).

2.5 Antimicrobial Resistance Phenotype

The pattern of antimicrobial resistance by each *E. coli* isolate was noted from the type of antibiotics the isolates were resistant to.

2.6 Determination of Multiple Antimicrobial Resistance (MAR) Index

The MAR Index was determined by dividing the number of antibiotics to which the E. coli isolates were resistant to by the total number of antibiotics to which the isolates were exposed to (x/y) (Ngwai *et al.*, 2014).

2.7 Classification of Antibiotic Resistance of the Isolates

Antimicrobial resistance in the isolates in this study was classified into: Multi-Drug Resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); Extensive Drug Resistance (XDR: non-susceptible to ≥1 agent in all but ≤ 2 antimicrobial categories); Pan Drug Resistance (PDR: non-susceptible to all to antimicrobial listed) (Magiorakos *et al.*, 2012; Sulleyman, *et al.*, 2018).

2.8 Detection of Extended –Spectrum Beta-Lactamase (ESBL) in the isolates

Isolates were confirmed as ESBL producers phenotypically by Double Disc Synergy Test (DDST) (CLSI, 2019) while ESBL genes and phylogrouping by Polymerase Chain Reaction (PCR).

2.9 Detection of Phylogenetic Group of the Extended Spectrum Beta-lactamase producing Isolates.

2.9.1 DNA Extraction and Quantification

DNA was extracted by boiling method as described by Abimiku *et al.*, (2016). The extracted genomic DNA was quantified by absorbance method using the Nanodrop 1000 spectrophotometer. A multiplex Polymerase Chain Reaction (mPCR) assay was conducted to amplify the *E. coli* housekeeping *uidA* gene. The PCR products were loaded onto 1% agarose gels and subjected to electrophoresis. A total of 14 ESBL *E. coli* isolates were confirmed by PCR targeting the *uidA gene*.The *E. coli* that was positive for the *uidA* PCR assay was further screened by 16SrRNA PCR to reconfirm the isolates using the forward (F) 5'-AAAACGGCAAGAAAAAGCAG-3' and reverse (R)5'-ACGCGTGGTTACAGTCTTGCG-3' primers (Zymo research, USA) on ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 µl for 25

cycles. The PCR amplification reaction conditions were set up as described by Abimiku *et al.*, (2016).The product was resolved on a 1% agarose gel and visualized on a UV transilluminator.

The confirmed 14 ESBL *E. coli* isolates were phylotyped using the primers *chu*A, *yja*A and the DNA fragments *tspE4.C2* genes which generated 279, 211 and 152bp fragments respectively (Table 2.1). Multiplex Polymerase Chain Reaction (mPCR) was employed in determining the phylogenetic grouping of the ESBL *E. coli* isolates (Tarazi *et al.*, 2023) in a reaction mixture of 45µl total volume using the ABI 9700 Applied Biosystems thermal cycler, containing 2.5µl MgCl2 (50 mM), 2.5µl 10 × buffer, 0.9µl of each primer (2.7µM), 8mM of dNTPs, 0.5µl Taq DNA polymerase (Zymo research, USA) and 5*µ*l of DNA template. The primers used were *chuA*, *yjaA* and *TspE4.C2* which generated 279, 211 and 152bp fragments respectively. Amplification was carried out in: an initial denaturation step at 95°C for 5 min, followed by 40 denaturation cycles at 94°C for 1 min, annealing at 55°C for 30s and extension at 68°C for 30s, and a final extension step at 68°C for 10minutes. The amplicons were electrophoresed on 1.5% (w/v) agarose gel containing ethidium bromide and visualized on a UV transilluminator. The size of the amplicons was determined by comparison with 1kb DNA molecular ladder (Thermo Scientific, Germany). The phylogroups were known by the presence or absence of three genetic sequences called *chuA*, (existing in B2, and D phylogroups, absent from B1 and A), *yjaA* (existing in B2, absent from D) and *tspE4-C2* (existing in B1, absent from group A) (Clermont *et al*., 2000).

Table 2.1: Primers and Oligonucleotide Sequences used in this study for the phylogenetic grouping

Target gene	Primer sequence 5'-3'	Amplicon size (bp)	Reference
ChuA	F-GAC GAA CCA ACG GTC AGG AT R-TGC CGC CAG TAC CAA AGA CA	279	Tarazi *et al*., 2023
YjaA	F-TGA AGT GTC AGG AGA CGC TG R-ATG GAG AAT GCG TTC CTC AAC	211	Tarazi *et al*., 2023
tspE4.C2	F-GAG TAA TGT CGG GGC ATT CA R-CGC GCC AAC AAA GTA TTA CG	152	Tarazi *et al*., 2023

2.10 Data Analysis

Data obtained was entered into Microsoft Excel TM 2016 and subsequently SPSS v25 for statistical computation. The difference in the number of the bacterial isolates was compared from the different locations using $p < 0.05$ as the threshold for statistical significance.

3. RESULTS

3.1 Isolation and identification of *Escherichia coli*

The overall isolation rate of *E.coli* from the 210 "Suya" samples was 70(33.3%). The isolation rate in relation to location within the study area is outlined in Table 3.1. The *E. coli* isolation rates exhibited no significant variation at *P*=0.0931 among the samples collected from the study area, which is higher than the significance level of 0.05. The highest isolation rate 12(40.0%) was detected at Akwanga and Nasarawa Eggon in Nasarawa North Senatorial Districts. This was followed by Keana 11(36.6%) and Lafia 11(33.3%) in Nasarawa South Senatorial District while that of Keffi 9(30.0%) with the least from Karu and Nasarawa 8(26.6%), all in Nasarawa West Senatorial District.

Table 3.1 : Occurrence of *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria

Senatorial Districts	Locations	No Sampled	n(%) n= 70	Chi Square (χ^2)
Nasarawa North	Akwanga	30	12(40.0)	
	Nasarawa Eggon	30	12(40.0)	
Nasarawa South	Lafia	30	10(33.3)	
	Keana	30	11(36.7)	
Nasarawa West	Karu	30	8(26.7)	0.857
	Keffi	30	9(30.0)	
	Nasarawa	30	8(26.7)	
Total		**210**	**70(33.3)**	

Degree of freedom = 2, Significance level =0.05, *p* =0.0931, n= Number of isolates, % = Percentage

3.2 Antimicrobial Resistance Profile

The antimicrobial resistance profile in this study showed high resistance to amoxicillin/clavulanic acid and was observed at Keffi 7(77.7%) and Nasarawa 6(75.0%) of Nasarawa West Senatorial District; 6(75.0%) to cefoxitin, streptomycin, and co-trimoxazole at Nasarawa, Karu and Nasarawa Eggon. However, none 0(0.0%) of the isolates were resistant to gentamicin at Lafia in Nasarawa South Senatorial District (Table 3.2)

3.3 Antimicrobial Resistance Phenotypes

The antimicrobial resistance phenotypes in the *E. coli* isolates from the "Suya" sold in Nasarawa State, Nigeria are as shown in Table 4. The commonest phenotype in Nasarawa West Senatorial District was OFX - CN - S - CTX - CEF - SXT 2(25.0%) recorded at Karu and that of Nasarawa South Senatorial District was AMP - AMC - CN - CTX- CEF 2(18.1%) Keana while Nasarawa North Senatorial District was AMP- CIP- AMC - S - CTX - SXT - CAZ 2(16.7%) Nasarawa Eggon. All the seventy (70) *E. coli* isolates were consistently resistant to CTX as shown in Table .3.3

Table 3.2: Antimicrobial resistance Profile of *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria

Antibiotics	Disc content (µg)	No. (%) Isolates (n =70)						
		NW			NS		NN	
		Karu (n-8)	Keffi (n-9)	Nasarawa (n-8)	Lafia (n-10)	Keana (n-11)	Akwanga (n-12)	Nasarawa Eggon (n-12)
Ofloxacin (OFL)	30	4 (50.0)	6 (66.7)	4(50.0)	4(40.0)	5(45.4)	3(25.0)	3(25.0)
Amoxicillin-Clavulanic acid (AMC)	30	5 (62.5)	7(77.8)	6(75.0)	5(50.0)	4(36.3)	6(50.0)	5(41.7)
Ceftazidime (CAZ)	30	3 (37.5)	4(44.4)	4(50.0)	6(60.0)	5(45.4)	3(25.0)	3(25.0)
Cefotaxime (CTX)	30	4 (50.0)	3(33.3)	4(50.0)	2(20.0)	3(27.2)	4(33.3)	5(41.7)
Cefoxitin (CEF)	30	5 (62.5)	6(66.7)	6(75.0)	4(40.0)	5(45.5)	4(33.3)	5(41.7)
Ciprofloxacin (CIP)	5	3 (37.5)	2(22.2)	3(37.5)	3(30.0)	4(36.3)	4(33.3)	4(33.3)
Gentamicin (CN)	10	2 (25.5)	3(33.3)	4(50.0)	0(0.0)	3(27.2)	2(16.7)	3(25.0)
Streptomycin (S)	30	6 (75.0)	5(55.5)	5(62.5)	6(60.0)	8(72.7)	7(58.3)	7(58.3)
Ampicillin (AMP)	30	4(50.0)	4(44.4)	4(50.0)	3(30.0)	3(27.2)	5(41.7)	5(41.7)
Co-trimoxazole (SXT)	30	5(62.5)	6(66.7)	5(62.5)	7(70.0)	7(63.7)	8(66.7)	9(75.0)

NW = Nasarawa West Senatorial District, NS = Nasarawa South Senatorial District, NN = Nasarawa North Senatorial District.

Table .3.3: Antimicrobial Resistance Phenotypes of *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria

Antimicrobial Resistance Phenotypes	No. (%) Isolates (n = 70)						
	NW			**NS**		**NN**	
	Karu (n-8)	Keffi (n-9)	Nasarawa (n-8)	Lafia (n-10)	Keana (n-11)	Akwanga (n-12)	Nasarawa Eggon (n-12)
CIP, CTX, CAZ	0(0.0)	1 (11.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CTX, CEF,SXT, CAZ	1(12.5)	0(0.0)	0(0.0)	1(10.0)	1 (9.0)	1(8.3)	1(8.3)
OFX, AMP,CTX,SXT	0(0.0)	1(11.1)	0(0.0)	1(10.0)	0(0.0)	1(8.3)	1(8.3)
AMC,S,CTX,SXT	0(0.0)	2(22.2)	0(0.0)	1(10.0)	1(9.0)	0(0.0)	1(8.3)
AMC,CN,CTX,SXT, CAZ	1(12.5)	0(0.0)	0(0.0)	0(0.0)	1(9.0)	1(8.3)	0(0.0)
AMP,CIP,AMC,S, CEF	0(0.0)	2(22.2)	0(0.0)	1(10.0)	1(9.0)	0(0.0)	0(0.0)
AMP,AMC,CN,CTX, CEF	1(12.5)	0(0.0)	0(0.0)	1(10.0)	2(18.1)	1(8.3)	1(8.3)
OFX, CTX, CEF,SXT, CAZ	1(12.5)	2(22.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(8.3)
OFX,CIP,S,CTX, CEF,SXT	0(0.0)	0(0.0)	1(12.5)	0(0.0)	1(9.0)	1(8.3)	1(8.3)
AMP,CIP,CN,CTX, CEF,SXT	0(0.0)	0(0.0)	1(12.5)	1(10.0)	1(9.0)	1(8.3)	0(0.0)
OFX,CN,S,CTX, CEF,SXT	2 (25.0)	0(0.0)	1(12.5)	1(10.0)	0(0.0)	0(0.0)	1(8.3)
AMP,AMC,CTX, CEF,SXT, CAZ	0(0.0)	1(11.1)	1(12.5)	0(0.0)	0(0.0)	1(8.3)	1(8.3)
AMP,CIP,CN,S,CTX,SXT, CAZ	0(0.0)	0(0.0)	1(12.5)	0(0.0)	1(9.0)	1(8.3)	1(8.3)
OFX, AMP,CIP,CN,CTX,SXT, CAZ	1(12.5)	0(0.0)	1(12.5)	1(10.0)	1(9.0)	0(0.0)	1(8.3)
AMP,CIP,AMC,S,CTX, SXT, CAZ	1(12.5)	0(0.0)	0(0.0)	1(10.0)	0(0.0)	1(8.3)	2(16.7)
OFX,CIP,CN, CTX, CEF,SXT, CAZ	0 (0.0)	0(0.0)	0(0.0)	1(10.0)	0(0.0)	1(8.3)	0(0.0)
OFX, AMP,CIP,AMC,CN,S, CEF,SXT	0(0.0)	0(0.0)	1(12.5)	0(0.0)	1(9.0)	0(0.0)	0(0.0)
OFX,CIP,AMC,CN,S,CTX, CEF, CAZ	0(0.0)	0(0.0)	1(12.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

NW = Nasarawa West Senatorial District, NS = Nasarawa South Senatorial District, NN= Nasarawa North Senatorial District

3.4 Multiple Antimicrobial Resistance index (MAR)

The MAR Index of the *E. coli* isolates obtained from the "Suya"samples is given in Table 3.4.

Table 3.4 : Multiple Antimicrobial Resistance (MAR) Index of *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria.

No. of Antibiotics Resistance to (a)	No. of Antibiotics Tested (b)	MAR Index (a/b)	No (%) MAR Isolates (n = 70)						
			NW			NS		NN	
			Karu (n-8)	Keffi (n-9)	Nasarawa (n-8)	Lafia (n-10)	Keana (n-11)	Akwanga (n-12)	Nasarawa Eggon(n-12)
10	10	1.0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
9	10	0.9	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
8	10	0.8	0(0.0)	0(0.0)	2(25.0)	0(0.0)	1(9.0)	0(0.0)	0(0.0)
7	10	0.7	2 (25.5)	0(0.0)	2(25.0)	3(30.0)	2(18.1)	3(25.0)	4(33.3)
6	10	0.6	2(25.5)	1(11.1)	4(50.0)	2(20.0)	2(18.1)	3(25.0)	3(25.0)
5	10	0.5	3(37.5)	4(44.4)	0(0.0)	2(20.0)	5(45.4)	2(16.7)	2(16.7)
4	10	0.4	1(12.5)	3(33.3)	0(0.0)	3(30.0)	1(9.0)	2(16.7)	3(25.0)
3	10	0.3	0(0.0)	1(11.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
2	10	0.2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
1	10	0.1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

n = No. of isolates, MAR index = Multiple Antibiotic Resistance index, NW = Nasarawa West Senatorial District, NS = Nasarawa South Senatorial District, NN = Nasarawa North Senatorial District

The MAR index were all 0.3 and above, suggesting that the isolates originated from an environment where antimicrobial freely available and abused.

3.5 Classification of Antimicrobial Resistance of the isolates

The distribution of *E. coli* isolated from "Suya" sold in Nasarawa State, Nigeria were grouped into different classes of antimicrobial resistance were namely; Non-Multidrug Resistance (NMDR), Multi-drug resistance (MDR), Extensive-drug resistance (XDR) and Pandrug resistance (PDR) as shown in Table 3.5

Table 3.5: Classification of Antimicrobial Resistance of *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria.

Categories of	No. (%) Resistance Isolates (n = 70)

Antibiotic Resistance	NW			NS		NN	
	Karu (n-8)	Keffi (n-9)	Nasarawa (n-8)	Lafia (n-10)	Keana (n-11)	Akwanga (n-12)	Nasarawa Eggon (n-12)
NMDR	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MDR	5 (62.5)	8 (88.8)	8(100.0)	8(80.0)	10(90.0)	12(100.0)	12(100.0)
XDR	3 (37.5)	0(0.0)	0(0.0)	2(20.0)	1(9.0)	0(0.0)	0(0.0)
PDR	0(0.0)	1(11.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

NW - Nasarawa West Senatorial District, NS= Nasarawa South Senatorial District, NN= Nasarawa North Senatorial District: NMDR = Non-Multidrug Resistance (non-resistance to a class of antimicrobial categories); MDR = Multidrug Resistance (non-resistance to ≥ 1 agent in ≥ 3 antimicrobial categories); XDR = Extensive Drug Resistance (non-resistance to ≥ 1 agent in all but ≤ 2 antimicrobial categories); PDR = Pan Drug Resistance (non-resistance to all antimicrobial listed) (Magiorakos *et al.*, 2012).

3.6 Phenotypic Characterisation of Extended Spectrum Beta-Lactamaes -producing *Escherichia coli* isolates

Overall 14(20%) of *E. coli* isolates were phenotypically confirmed by DDST as ESBL producers with the highest proportion of 5(62.5%) recorded at Karu in Nasarawa West Senatorial District followed by Nasarawa 2(22.2%) with Lafia, Keana and Akwanga having the least 1(10.0%), 1(9.1%) and 1(8.3%) recorded at Nasarawa South and North Senatorial Districts respectively(Table 3.6).

Table 3.6: Extended Spectrum Beta-Lactamase producing *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria.

Senatorial District	Locations	No. (Cefotaxime and/Ceftazidime) Resistant Isolates	No. (%) ESBL producers (n=14)
NW	Karu	8	5(62.5)
	Keffi	9	2(22.2)
	Nasarawa	8	2(25.0)
NS	Lafia	10	1(10.0)
	Akwanga	12	1(8.3)
NN	Keana	11	1(9.1)
	Nasarawa Eggon	12	2(16.7)
	Total	**70**	**14(20.0)**

ESBL: Extended Spectrum Beta-lactamase, NW = Nasarawa West, NS= Nasarawa South, NN = Nasarawa North

3.7 Confirmation of *Escherichia coli* using the 16SrRNA gene

A total of 14 *E. coli* isolates which were confirmed by PCR targeting *uidA gene* were further screened by *16SrRNA* gene by PCR and 14 isolates of the same type having identical restriction fragment length patterns were reconfirmed as *E.coli* isolates (Plate 1).

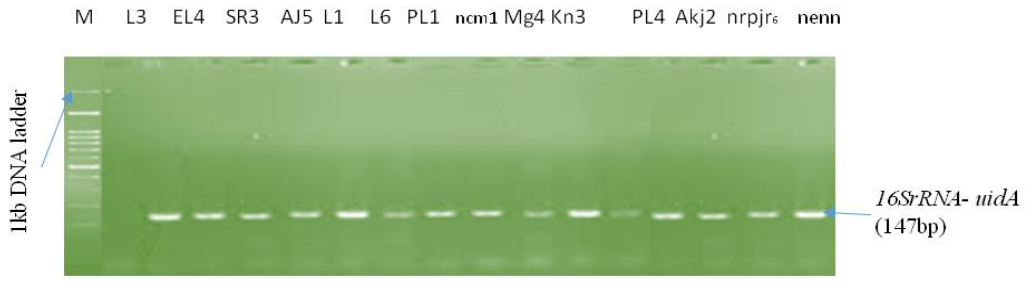

Plate 1: Agarose gel electrophoresis of the amplified 16SrRNA *gene* of bacteria isolates. Lane NC= Negative control, Lane PC= Positive control, Lane L1, EL4, SR3, AJ5, L1, L6, PL1, ncm1, Mg4, Kn3, PL4, Akj2, nrpjr6 and Lane nenn represent the expression of the *16SrRNA - uidA* gene at 147bp for *Escherichia coli*, while Lane M represents 1kb DNA molecular ladder.

KEY

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, nrpj,PL4=Keffi, Akj2, Aj5 = Akwanga, ncm1,Mg4 =Nasarawa and SR3, Kn3 = Karu.

3.8 Extended Spectrum Beta-Lactamse-producing *Escherichia coli* genes

Twenty one (21) *E. coli* isolates harbored the ESBL genes distributed as follows: *blaSHV* (14, 66.7%), *blaTEM* (3, 14.3%), *blaOXA-1* (2, 9.5%), *blaCTX-M-4* (1, 4.8%), and *blaCTX-M-9* (1, 4.8%) genes as shown in Table 3.7 and Plate 2

Table 3.7: Distribution of Extended Spectrum Beta-lactamase Genes in *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria

Amplified Genes	Amplicon sizes	No. of ESBL producers	No (%) ESBL Genes of *E. coli* (n = 21)						
			NW			NS		NN	
			Karu (n-2)	Keffi (n-4)	Nasarawa (n-2)	Lafia (n-3)	Keana (n-4)	Akwanga (n-3)	Nasarawa Eggon (n-3)
blaTEM	973bp	3	0(0.0)	2(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)
blaCTX-M-9	757bp	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)
blaSHV	615bp	14	2(100.0)	2(50.0)	2(100.0)	2(66.7)	2(50.0)	2(66.7)	2(66.7)
blaCTX-M-4	501bp	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)
blaOXA-1	494bp	2	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	1(33.3)	0(0.0)
	Total	**21**	**2(9.5)**	**4(19.0)**	**2(9.5)**	**3(14.3)**	**4(19.0)**	**3(14.3**	**3(14.3)**

ESBL = Extended Spectrum Beta-Lactamase, *bla* = beta-lactamse, TEM =Temoniera, CTX-M =Cefotaxime-Munich,, SHV = Sulphydryl Variable, bp = base pair, NW = Nasarawa West Senatorial District, NS = Nasarawa South Senatorial District, NN = Nasarawa North Senatorial District.

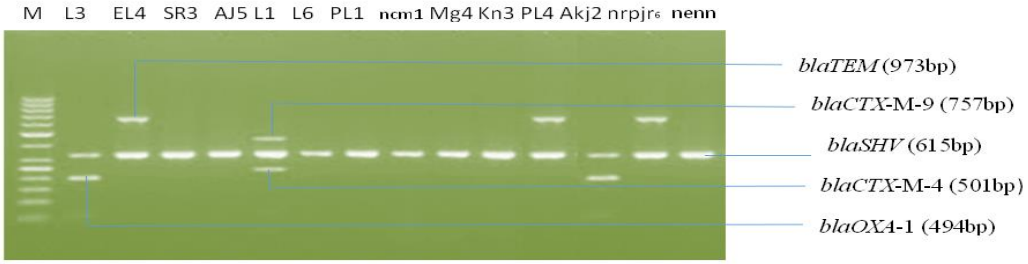

Plate 2: Agarose gel electrophoresis of the amplified ESBL genes from *Escherichia coli*. Lane L1, EL4, SR3, AJ5, L3, L6, PL1, ncm1, Mg4, Kn3, PL4, Akj2, nrpjr6 and Lane nenn represent the expression of the *blaSHV gene (615bp)*; Lane L3 and Lane Akj2 represent the expression of the *blaOXA-1 gene* (494bp), Lane L1 represents the expression of the *blaCTX*-M-4 gene (501bp) and *blaCTX*-M-9 gene (757bp) and Lane EL4, PL4 and Lane nrpjr6 represent the expression of the *blaTEM* gene at 973bp.

Key:

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, nrpj,PL4 = Keffi, Akj2, Aj5 = Akwanga, ncm1,Mg4 =Nasarawa and SR3, Kn3 = Karu.

3.9 Phylotyping

The phylogenetic grouping confirmed 24 ESBL-producing *E. coli* isolates with 1(25.0%) assigned to group A while 2(50.0%) B then 2(100.0 %) B1 and 1(33.3%) D respectively.The distribution of different phylogenic groups indicated 10 (41.7%) phylogroup B2 predominated with the highest prevalence of 2(50.0 %) detected each at Karu, Keana, Akwanga and Nasarawa Eggon of Nasarawa West, South and North Senatorial Districts and that of 1(33..3%) each in Nasarawa and Lafia This was followed by 7(29.2%) isolates which were assigned phylogroup D while 2(50.0%) in Akwanga, 1(25.0%) each in Karu, Keana and Nasarawa Eggon then 1(33.3%) each in Nasarawa and Lafia. However, no isolate was detected at Keffi nor assigned phylogroup. However, 4(16.7%) isolates were assigned phylogroup B1 of environmental origin with a prevalence of 2(100.0%) from Keffi and 1(33.3%) each from Karu and Keana then 3(12.5%) assigned phylogroup A with 1(25.0%) each at Karu, Keana and Nasarawa Eggon.

All the ESBL-producing *E. coli* isolates in this study harbored virulence gene with phylogroup A harbouring y*jaA* genes amplified at 211bp while B1 harboured *tspE4.C2* genes at 152bp then B2 *chuA* genes at 279bp *and Yja*A genes at 211 bp respectively while that of phylogroup D harboured *chu*A *genes at* 279bp (Table 3.8 and Plate 3).

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Table 3.8: Distribution of different phylogenic groups of *Escherichia coli* from "Suya" sold in Nasarawa State, Nigeria

Amplified Genes	Phylogenic groups	No. of ESBL producers	No (%) Phylogenic Groups of *E. coli* (n = 24)						
			NW			NS		NN	
			Karu (n-4)	Keffi (n-2)	Nasarawa (n-3)	Lafia (n-3)	Keana (n-4)	Akwanga (n-4)	Nasarawa Eggon (n-4)
YjaA (211bp)	A	3	1(25.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	1(25.0)
TspE4.C2 (152bp)	B1	4	0(0.0)	2(100.0)	1(33.3)	1(33.3)	0(0.0)	0(0.0)	0(0.0)
chuA (279bp and *YjaA* (211bp)	B2	10	2(50.0)	0(0.0)	1(33.3)	1(33.3)	2(50.0)	2(50.0)	2(50.0)
chuA (279bp)	D	7	1(25.0)	0(0.0)	1(33.3)	1(33.3)	1(25.0)	2(50.0)	1(25.0)
	Total	**24**	**4(16.7)**	**2(8.3)**	**3(12.5)**	**3(12.5)**	**4(16.7)**	**4(16.7)**	**4(16.7)**

bp = base pair, NW= Nasarawa West Senatorial District, NS = Nasarawa South Senatorial District, NN = Nasarawa North Senatorial District

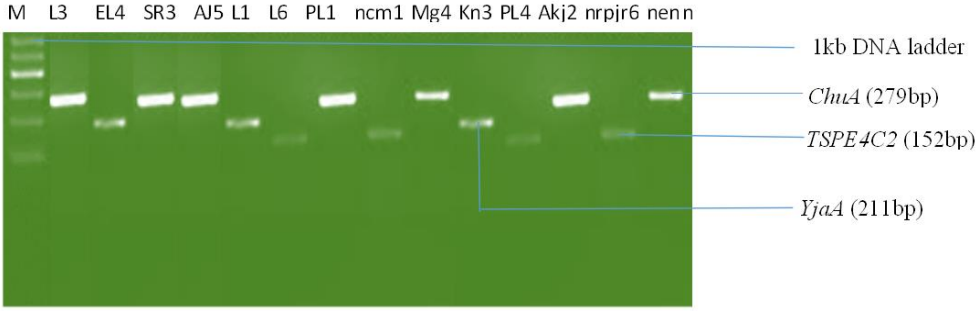

Plate 3: Agarose gel electrophoresis of the amplified phylogenetic group genes of *Escherichia coli isolates*. Lane L3, SR3, AJ5, PL1, Mg4, Akj2, and Lane nenn represent the expression of the *ChuA* gene at 279bp; Lane EL4, L1 and Kn3 represent the expression of the *YjaA* gene (211bp), Lane L6, ncm1, PL4 and Lane nrpjr6 represents the expression of the *tspE4.C2* gene at 152bp. Lane M represents 1kb DNA molecular ladder.

Key

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, nrpj, PL4=Keffi, Akj2, Aj5 = Akwanga, ncm1,Mg4 =Nasarawa and SR3, Kn3 = Karu.

4.DISCUSSION

In this study, seventy (70) ESBL *E. coli* isolates were isolated from 210 "Suya" samples collected from "Suya" spots at Karu, Keffi, Nasarawa, Akwanga, Nasarawa Eggon, Lafia and Keana in Nasarawa State, Nigeria. The *E. coli* isolation rates exhibited no significant difference at $P = 0.0931$ being higher than the significance level of 0.05 in the occurrence of *E. coli* among the study area. This translates that the *E. coli* isolation rates exhibited significant circulation in the study area as all the Senatorial Districts harbored *E. coli* irrespective of their locations. The existence of *E. coli* from the "Suya" sample in this area signals poor food handling, (Ritchie and Roser, 2019), sanitation practices and lack of education for food handlers which if not controlled might risk food borne illness.The highest occurrence rate of 12(40.0%) was recorded at Akwanga and Nasarawa Eggon in Nasarawa North Senatorial Districts. This was followed by Keana 11(36.6%) and Lafia 11(33.3%) in Nasarawa South Senatorial Districtwhile that of Keffi 9(30.0%) with least from Karu and

Nasarawa 8(26.6%), all in Nasarawa West Senatorial District. "Suya" samples from all the Senatorial Districts harbored *E. coli* in their respective locations. The high isolation of 12(40.0%)*E. col*i in this study area inferred lack of personal hygiene amongst the sellers of "Suya", dusty nature of the area where meat shops are located or "Suya" displayed on tables with no wire mesh or net protecting it from flies, hence predisposed the product to contamination.This finding in the study agreed with Falegan *et al.*, (2017) and Okonkwo *et al*., (2012) who reported the isolation of *E. coli* isolates from "Suya" implied poor handling practices and processes that poses great health risks as a predisposing factor that risk treatment options most especially in immune-compromised individuals such as people living with HIV/AIDS, pregnant women, children, diabetic patients Shisana *et al*., (2005).

The lowest isolation was detected at Karu and Nasarawa in Nasarawa West Senatorial District with occurrence rate of 26.6% each which aligned with the previous study conducted by Danladi *et al*., (2021). This low isolation in this study area suggest that most "Suya" vendors either warm their products before serving their customers or possibly most buyers are aware of the health implication of consuming over stayed "Suya". Hence, the sellers have to prepare it fresh always. Therefore, some measures as described by Adzitey, (2016) is recommended to be adapted to control and prevent bacterial foodborne infections from the consumption of "Suya" in this area.

The isolates in the study area showed high resistance to amoxicillin/clavulanic acid, streptomycin,cefoxitin, co-trimoxazole, ofloxacin and ceftazidime. The high antibiotic resistance observed could be that they are the most commonly available antibiotics used for treatment as well as growth promoters and in routine chemoprophylaxis among livestock as speculated by (Olatoye, 2010). This portends a major challenge in both human and animal medicine because these drugs are commonly used in the treatment of common human ailments. The isolates in the study area also showed less resistance to ampicillin which had 3(27.2%) in Keana, 2(22.2%) ciprofloxacin in Keffi and gentamicin 2(16.7%) in Nasarawa Eggon and Akwanga. These susceptibility levels of the isolates to these antibiotics could possibly yield good therapeutic results in treating infections of antibiotic resistant *E. coli*. All the isolates in this studyexhibited Multiple Antibiotic (MAR) index ranged from 0.3 (resistant to three antibiotics) to 0.8 (resistant to eight antibiotics). This finding showed that most of Suya sellers or consumers in the study area did not adhere to strict hygiene which could be a predisposing factor to faecal contamination (Adzitey *et al*., 2016) of the product.

Most of the isolates *E. coli* in this study were multi-drug resistant isolates with the order of occurrence in the Senatorial Districts as:

Nasarawa West Senatorial District

Karu : MDR 5(62.5%) > XDR 3(37.5%) > NMDR and PDR 0(0.0%)
Keffi: MDR 8(88.8%) > PDR1 (11.1%)>NMDR and XDR 0(0.0%)
Nasarawa: MDR 8(100.0%) > XDR, NMDR and PDR 0(0.0%)

Nasarawa South Senatorial District

Lafia: MDR 8(80.0%) > XDR 2(20.0%) > NMDR and PDR 0(0.0%)
Keana: MDR 10(90.0%) >XDR 1(9.0%)>NMDR and PDR 0(0.0%)

Nasarawa North Senatorial District

Akwanga : MDR 12(100%) > XDR, NMDR and PDR 0(0.0%)
Nasarawa Eggon: MDR 12(100%) >XDR, NMDR and PDR 0(0.0%)

This finding showed that the *E. coli* isolates might have originated from a high-risk source of contamination where several antibiotics or growth promoters were indiscriminately used which eventually superseded the drug sensitive microorganisms from an antibiotic saturated environment (Al-Kobaisi, 2007; Amoako *et al*., 2020). This is worrisome as Adzitey *et al*., (2011) and Mishra *et al*., (2013) asserted that MAR index of less than 0.4 and above is associated with nonhuman faecal contamination and therefore sensitivity patterns and treatment should be guided by laboratory investigations.

The ESBL encoding genes were found in 21 isolates with *blaSHV* detected in 14 (66.7%), *blaTEM* 3(14.3%), *blaOXA-1* (9.5%) *blaCTX-M-9* and *blaCTX-M-4* 1(4.8% isolates each. The existence of these genes in mobile

genetic elements raises concerns about their potential horizontal transfer in the food chain hence, posing a risk to public health and therefore requires closing monitoring with enhanced effort for surveillance

The phylogenetic grouping confirmed 24 ESBL-producing *E. coli* isolates with 1(25.0%) isolate assigned to group A, 2(50.0%) B, 2(100.0 %) B1 and 1(33.3%) D. The *E. coli* from the study locations in Nasarawa State belonging to different phylogroups were not dispersed randomly but associated with a specific isolation source ("Suya"). The distribution of phylogenetic grouping in the study area showed high prevalence of phylogroup B2 at Karu, Akwanga, Keana and Nasarawa Eggon at 2(50.0 %) each, followed by Lafia and Nasarawa 1{33..3%) each while none at Keffi.It was also noted that 10(41.7%) isolates from the study locations harboured the virulence genes *chuA at* 279bp *and Yja*A at 211bp which encoded for phylogroup B2. and 7(29.2%) isolates for phylogroup D with *chuA* at 279bp. This high proportion of virulence strains could be their critical silent role in the spread and dissemination of ESBL-resistance genes (Abdallah *et al*., 2015; Martínez-Vázquez *et al*., 2022).. This implies a potential risk to humans in the study population as the finding has established phylogrroups B2 and D to extraintestinal pathogenic group, commensals strains to group A and B1 while that of intestinal pathogenic strains to A, B1 and D.

4. 1 Conclusion

This study has established "Suya" as potential reservoir of ESBL *E. coli* isolates from the environment (phylogroup B1) and to a lesser extent of commensal *E. coli* (phylogroup A) and a greater extent of virulence *E. coli* of phylogroup B2 and D in Nasarawa State, Nigeria, and signals the possible occurrence of foodborne diseases in the study area if not controlled. All the isolates harbored resistance to common antibiotic with MAR indices that suggest earlier exposure of the isolates to these antibiotics. Multidrug resistant (MDR) isolates were detected in all the Senatorial Districts; and extensively drug resistant (XDR) was detected in Karu, Lafia and Keana isolates; and pan drug resistant (PDR) was detected only in Keffi isolates while none in non-MDR. Multi-Drug Resistance (MDR) strains and therefore could act as potential reservoirs of antibiotic resistance in "Suya".

Observable phenotypically high resistance of the isolates to the studied antibiotics by the conventional susceptibility profiling gave clues to health practitioners in the area on the best antibiotics of choice in treating *E. coli* infections.

Further genomics and bioinformatics analyses are recommended for classification of virulence markers which could be used for source tracking and transmission routes of contamination in *E. coli*.

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