



PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING *Escherichia coli* FROM LIVESTOCK AND HERDERS IN SOME STATES IN NORTH-EASTERN NIGERIA

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ABSTRACT

The emergence and dissemination of ESBL producing bacteria of food-animals and humans origin are responsible for numerous infections with challenging treatment and control measures which calls for considerable concern of both Medical and Veterinary practitioners all over the World. Phenotypic methods of ESBL screening are widely used for their simplicity, cost-effectiveness and suitability for routine clinical laboratories. The aim of this study was to isolate MDR *E. coli* isolates and screen them phenotypically for ESBL production using the Double Disk Synergy Test (DDST). A total of 1152 samples comprising of 384 each from cattle sheep and Herders were collected, processed, inoculated and incubated at 37 °C for 24 hours. One hundred and eighty one (181) samples yielded positive bacterial growths out of which 111 (61.3%) of them were *E. coli* isolates and 70 (%) were other members of the gram negative bacilli. Out of the 111 *E. coli* isolates, 91 (81.9%) were multidrug resistant of which 37 (36.3%) were phenotypically detected to be an ESBL producing phenotypes. This study revealed significant difference (P value < 0.05) between the *E. coli* and other members of the gram negative bacilli isolated. Calves, lambs and young herders below 10 years of age found in closed-contact with livestock as well as those living in an over-crowded and dirty environment were more prevalent to infection by ESBL producing bacteria. Therefore, proper sanitary measures be adopted and maintained in farms where calves and Lambs less than 1 year are kept. Young herders' below 10 years old be properly monitored and those with diarrhoea be promptly treated.

Keywords: Phenotypic Detection, Extended Spectrum Beta-Lactamase (ESBL), *Escherichia coli*, Livestock and Herders

INTRODUCTION

Extended Spectrum Beta-Lactamase (ESBL) is collective name of enzymes often located on the plasmids mostly of gram-negative bacteria and are capable of hydrolyzing penicillin, broad spectrum cephalosporins and monobactams. These ESBLs are transferable from strain to strain and between bacterial species such as *Escherichia coli* (*E. coli*) *Shigella*, *Proteus*, *Salmonella*, *Enterobacter*, *Klebsiella* and *Citrobacter species* which are responsible for numerous infections with challenging treatment measures (Hawser *et al.*, 2009). Detecting ESBL production is very crucial for appropriate antibiotics therapy and effective infection control. Several researchers have indicated that ESBLs are clinically significance and when detected, indicate need for choice of appropriate antibiotics (Peirano *et al.*, 2010). Phenotypic methods of ESBL detection are important because they rely on observing the bacterial growth response to specific antibiotic combinations rather than detecting the enzyme directly. Common phenotypic techniques like Double Disk Synergy Test (DDST), Combined Disk Test (CDT), E-Test ESBL strips and Disk Replacement Method (DRM) are widely used for their simplicity, cost-effectiveness and suitability for routine clinical laboratories. However, they may have some limitations in detection of other variants of co-existing resistance mechanisms (Bradford and Bush, 2001) Although *E. coli* are usually harmless microbes but some strains are the most common cause of community-acquired bacteremia and also the fifth common cause of nosocomial infections (Freidman *et al.*, 2002; Welch *et al.*, 2002; Nicolas-Chanoine *et al.*, 2008).

Antimicrobial resistance among *E. coli* infected patients is globally on the increase and this has been associated with the emergence and spread of ESBL which are also associated

with resistance to Oxy-imino cephalosporin, Ciprofloxacin and Amino glycosides (Hawser *et al.*, 2009). So far, there is paucity on documentary information on the comparative prevalence of ESBL production among domesticated food animals and their handlers (herders), zoonotic nature and antimicrobial resistance profile in the context of emergence of ESBL producing *E. coli* strains. Therefore, this study was designed to isolate the MDR *E. coli* isolates from livestock and herders in North-Eastern states of Nigeria and phenotypically screened them for ESBL production.

MATERIALS AND METHODS

Description of the Study Area

The study area is located in the North-East Sub-region of Nigeria, covering three (3) Livestock populated States of Adamawa, Bauchi and Taraba. Adamawa State lies between latitude 7° and 11° North and between Longitude 110° and 140° East, covering a landmass of about 38,741 km², and currently having twenty-one (21) Local Government Areas namely: Demsa, Fufore, Ganye, Girei, Gombi, Guyuk, Hong, Jada, Lamurde, Mayo- Belwa, Madagali, Maiha, Michika, Mubi North, Mubi, South, Numan, Shelleng, Song, Toungo, Yola-North and Yola- South with Yola as its State Capital (Adebayo, 1999). Out of which twelve (12) Local Government Areas (Demsa, Fufore, Girei, Gombi, Guyuk, Lamurde, Mayo belwa, Numan, Shelleng, Song, Yola-North and Yola-South) were sampled while remaining nine (9) were not sampled because of reasons ranging from insecurity, difficult terrain and economic constraint. The State has a tropical wet and dry seasons, with dry season last for about five (5) months (November to March) while the wet season lasting for about seven (7) months (April to October)

recording mean annual rainfall range of 700 mm to 1600 mm (Adebayo, 1999).

The State shares boundary with Taraba State in the south-west, Gombe State in its North-west and Borno state to the North. The State has an international boundary with the Republic of Cameroon along its Eastern part. Ecologically, the state is divided into guinea and Sudan savannah zones. Adamawa state has an estimated human population of 5,236,948 million people and about 80% of people of the state are engaged in large scale crops, and Livestock farming (Adebayo, 1998). The state is bordered is by seven (7) states; namely Kano and Jigawa to the north, Taraba and Plateau to the south, Gombe and Yobe to the east and Kaduna to the west. The state is known for its vast fertile grass land for Livestock rearing and crop production (Come to Nigeria Staff, 2011) (Figure 1C).

Bauchi State lies between latitude 9^o.3' and 12^o.3' North of the Equator with a total Land mass of 49,110 km² and presently having twenty (20) Local Government Areas with Bauchi city as its State Capital. These include: Alkaleri, Bauchi, Bogoro, Dambam, Darazo, Dass, Gamawa, Ganjuwa, Giade, Itas/Gadau, Jama'are, Kaltungo, Kirfi, Misau, Ningi, Shira, Tafawa-Balewa, Toro, Warji and Zaki Local Governments respectively.

Out of these, 11 Local Government Areas (Alkaleri, Bauchi, Darazo, Dass, Gamawa, Ganjuwa, Kirfi, Misau, Ningi, Tafawa-Balewa and Toro) were sampled.

Geographically, the State has two distinct vegetation zones, namely Sudan Savannah which covers the southern part of the State where grasses with less uniformly bid tall trees are found and the Sahel Savannah (semi desert zone) starting from the middle of the State to its extreme North, where predominantly short grasses with isolated stands of thorny shrubs are found. The state is known for its vast fertile grass land for cattle

rearing and crop production (Come to Nigeria Staff, 2011). The estimated human population for Bauchi state as at the year, 2020 was 7,540,663 million people. The people of state are predominantly involved in livestock production. Bauchi State has a projected livestock population of 3,612,463 million comprising of Cattle, sheep, goats, rabbit and pigs. The state is bordered is by seven (7) states; namely Kano and Jigawa to the north, Taraba and Plateau to the south, Gombe and Yobe to the east and Kaduna to the west (Figure 1A).

Taraba state lies between Latitude 6° 30' and 8°30' north of the Equator and between Longitude 9° 00' and 12° 00' east of the Greenwich Meridian with land mass of 60,291km². The State is bordered to the west by Nasarawa and Benue States, to the North-west by Plateau State, to the North by Bauchi and Gombe States, to the North-east by Adamawa State, and to the East and South by Republic of Cameroon. Taraba State lies largely within the middle of Nigeria and consists of undulating landscape dotted with a few mountainous features. These include the scenic and prominent Mambilla Plateau with an altitude of 1,800 meters (6000 feet) above sea level and has temperate climate all year round (Idris and Musa 2021).

The state lies largely within the tropic and has a vegetation of low forest in the southern part and grassland in the northern part. The major occupation of people of the State are Crop production and Livestock (Cattle, Sheep & Goat) rearing. Similarly, some farmers rear other Livestock like poultry, rabbit and pig farming in fairly large scale (Zeitlyn and Connell, 2003). According to Magnitude and trends, Taraba State projected Livestock population for 2018 was 23, 549,583 Million, comprising of 5,577,980 cattle, 3,061,666 sheep, 3,686,973M goats, 3,212,979M pigs and 8,009, 986M poultry respectively (Ayi et al., 2020) (Figure 1B).

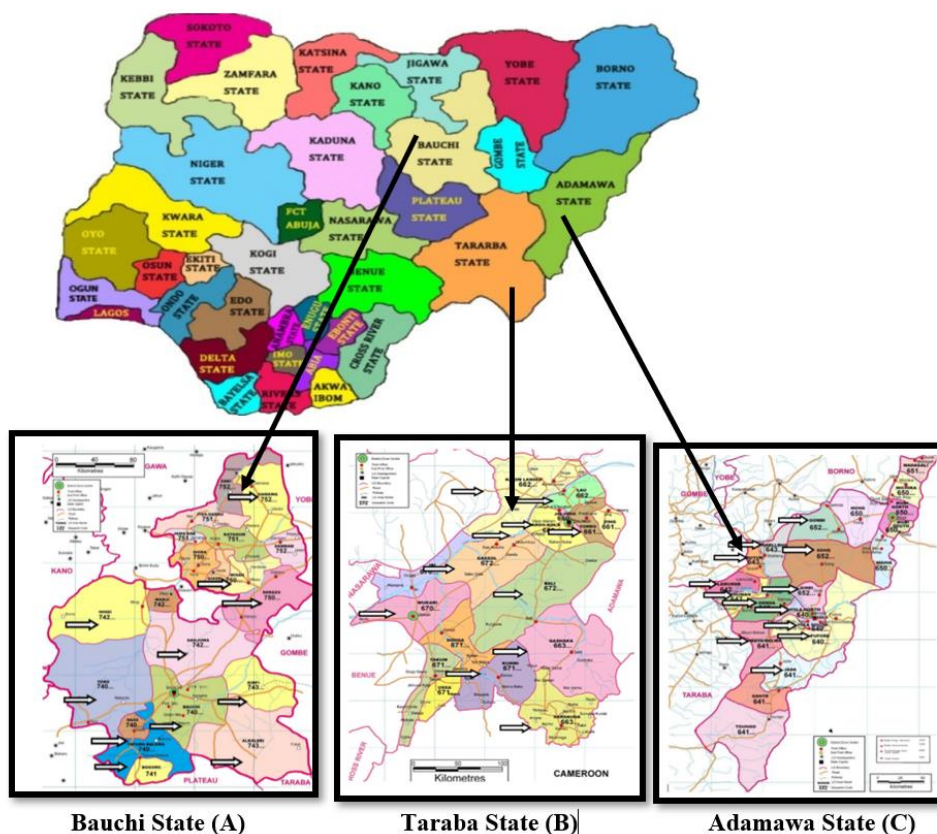


Figure 1: Combined Maps of Bauchi, Taraba and Adamawa States showing the Local Government Areas sampled (arrows) (Source: Google scholar)

Determination of Samples Size

The sample size was calculated by using the formula: $n = t^2 \times p(1-p) \div m^2$ from each sample source as described by FAO (1990). Where n = number of samples to be collected for this study, t = confidence level at 95% (standard value is 1.96), m = margin of error at 5% (standard value is 0.05). p = existing prevalence rate within the study area.

Since the prevalence rate within the study area is unknown, it was assumed to be 50% (0.5). Therefore sample size 'N' = $1.96^2 \times 0.5(1-0.5) \div 0.05^2$

$n = 3.8416 \times 0.5 \times 0.5 \div 0.0025 = 384.16$. This figure was multiplied by 3, for the 3 states

Therefore, a total of 1152 samples comprising of 384 samples from each State within the study area were collected and processed for this study.

Study Population and Sampling Procedures

A cross-sectional descriptive studies were conducted on ESBLs producing *E. coli* from white Fulani breed of cattle, balami breed of sheep which is the most predominant breed in north-eastern Nigeria and their herders themselves in Adamawa, Bauchi and Taraba States located in the North-east Sub-Region of Nigeria were sampled. The sampling involved 384 of white Fulani breed of cattle (from 0 - 11 years of age; 384 of balami breed of sheep (0 – 9 years old and 384 of the herders (0 – 70 years of age (Pehlivanoglu et al., 2016) were sampled using the random sampling method. Swab samples were directly and non-repeatedly collected from the rectum of each sampling animals as follows:

The sampling animal was properly restrained and the tail was raised with one hand and a sterile swab stick was inserted in to the rectum directly in a rotatory manner until its touches the faeces in the rectum. The swab soiled-stick was removed and labeled. All remaining samples were collected using the same procedure. While stool samples from the herders were collected in to sterile sample bottles at the point of defecation by themselves. The bottles were properly labeled. The remaining samples from the herders were collected using the same procedure. All samples were transported to ATBU postgraduate Microbiology Laboratory on ice-park for culturing procedures.

Enrichment, Inoculation and Incubation of Samples

All samples transported to the Laboratory were sterilely enriched, aseptically inoculated and incubated at 37 °C for 24hours. An enrichment procedure was performed to increase the bacteria population before inoculating the samples. The swab was soaked in 10% buffered peptone water (PW) (Lab. M. UK) and incubated at 37°C for 24 hours. A 10% suspension of stool sample in buffered peptone water (Lab. M. UK) was prepared using a vortex vibrator and incubated at 37°C for 24 hours in aerobic condition. Then a loop full of the suspension was picked and inoculated on to Eosin Methylene Blue Agar (EMB agar OXOID, U. K) by streak method and incubated at 37 °C for another 24 hours aerobically for observation of growth.

Macroscopic and Microscopic Examinations

The culture media [EMB and, MH agars (OXOID U.K)], aseptically prepared were observed for 24 hours before inoculation. After 24 hours of inoculation, cultures were grossly observed for bacterial growth and characteristic colonial appearance. Mixed cultures were sub-cultured to obtain pure cultures. The presumptive *E. coli* isolates were inoculated onto Mueller-Hinton agar slants and incubated at 37°C for 24 hours until growth was observed.

Slides prepared from pure cultures were first gram-stained and examined microscopically to determine the gram reaction of the isolates according to method developed by Gram (1884) and described by Naveena and Joy (2014). The slants of the pure cultures were preserved in refrigerator for subsequent studies.

Biochemical Identification of Bacterial Isolates

The Pure culture isolates of *E. coli* were subjected to the four biochemical tests such as Indole (I), Methyl-Red (MR), Voges-Proskauer (Vp), and Citrate utilization represented by the Acronym (IMViC) which differentiate *E. coli* from other *Enterobacteria*. Other tests like Triple Sugar Iron (TSI) and Motility tests were also conducted as described by Naveena and Joy (2014). The characteristic isolates were identified on the basis of sugar utilization, IMViC reaction patterns and production of gas, ability to ferment lactose/sucrose in triple-sugar Iron (TSI) slants for SIM Medium (sulphide, indole and Motility), Methylene Red, Voges-proskauer reaction and Citrates utilization (OXOID, UK).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed according to guidelines recommended by the Clinical Laboratory Standard Institute (CLSI, 2020) on Mueller Hinton agar plates (Oxoid). All discs for diffusion techniques were obtained from Oxoid with the following concentrations: Amikacin (25µg), Streptomycin (30µg), Amoxicillin (30µg), Ceftazidime 25µg), Cefotaxime (25µg), Ciprofloxacin (15µg), Ofloxacin (10 µg), Pefloxacin (25 µg) Bactrin (30ug) and Co-Trimoxazole (30µg).

0.5 McFarland standard suspension (inoculum) of the test isolate was made in normal saline using the McFarland vortex device and sterile swab sticks. The suspension of the inoculum was streaked uniformly as lawn on a Mueller Hinton Agar (MHA) plate. The required disks were placed at the appropriate place and incubated at 37°C for 24 hours.

Identification and Preservation of Multidrug Resistant (MDR) *E. coli* Isolates

All *E. coli* isolates found resistant to three (3) or more classes of antibiotics were sorted out as multidrug resistant (MDR) *E. coli* isolates and preserved in the refrigerator for subsequent studies.

Phenotypic Detection of ESBL Producing MDR *E. coli* Isolates

The ESBL producing *E. coli* isolates were phenotypically detected using the Double Disc Synergy Test (DDST) protocols developed by Jarlier et al., (1988) and modified by Jena et al., (2018). *E. coli* NCTC 10418 was used as ESBL-negative control while *K. pneumoniae* ATCC 700603 was used as ESBL-positive control. The test isolates were evenly spread onto Mueller Hinton Agar plates and two antimicrobial discs were placed 30mm apart (center to center) with one of these containing amoxicillin/clavulanic acid and the other containing expanded spectrum cephalosporin (ceftriaxone, cefotaxime or ceftazidime) and incubated for 24 hours. Enhancement of zone of inhibition in between the discs indicates positive result. The presence of ESBL was confirmed by demonstrating synergy between cephalosporin and clavulanic acid with reduced halo.

Statistical Analysis

Chi-square test of relation (Fishers test) was used to compare and determine the level of significance between the variables by using SPSS computer software version 20.0.

RESULTS AND DISCUSSION

Bacteria Isolated from Livestock and Herders in Relation to Sample Sources in this study

The results of bacterial isolated from Livestock and Herders in Relation to Sample Source are presented below:

Out of the 181 positive cultures recorded in this study, 102 were from Cattle, 48 were from Sheep and 31 were from Herders respectively. Out of the 102 positive cultures recorded from Cattle, 64/181 (35.3%) were *E. coli*, 12/181 (6.6%) were *Salmonella* and 26/181 (14.4%) were *Shigella*

species. Of the 48 positive cultures obtained from Sheep, 27/181 (14.9%) were *E. coli*, 4/181 (2.2%) were *Salmonella* and 17/181 (9.4%) were *Shigella* species. And of the 31 positive cultures from herders, 20/181 (11.0%) were *E. coli*, 3/181 (1.7%) were *Salmonella* and 8/181 (4.4%) were *Shigella* species respectively (Table 1). These 111 *E. coli* isolates were preserved in the refrigerator for further studies while the *Salmonella* and the *Shigella* isolates were sterilized and discarded in line with the exclusion criteria of this study.

Table 1: Distribution of Bacteria Isolates from Livestock and Herders In Relation To Sample Sources

Sample source	No. of Samples (N=1152)	No. (%) of positive cultures (n=181)	No. (%) of Bacteria isolated (n=181)		
			<i>E. coli</i> (n=111)	<i>Salmonella</i> (n=19)	<i>Shigella</i> (n=51)
Cattle	384	102 (56.4)	64(35.4)	12(6.6)	26(14.4)
Sheep	384	48 (26.5)	27(19)	4(2.2)	17(9.4)
Herders	384	31(17.1)	20(11.0)	3(1.7)	8(4.4)

Although *E. coli* are usually harmless microbes but some strains are the most common cause of community-acquired bacteremia and also the fifth common cause of nosocomial infections (Friedman *et al.*, 2002). The pathogenic serotypes of *E. coli* are defined by their somatic antigen (O) and flagella antigen (H). *E. coli* have been identified to cause Coli-septicaemia, Coli-bacillosis, Coli-mastitis and Urinary tract infection (UTI) (Nicolas-Chanoine *et al.*, 2008; (Peirano *et al.*, 2010).

In this study, an overall prevalence rate of *E. coli* isolates in bot cattle, sheep and herders was 61.3% with relative prevalence of 35.4%, 14.9% and 11.0%, Cattle, Sheep and herders respectively. The distribution of prevalence of *E. coli* isolates among the sample source showed that cattle are the most prevalent, suggesting cattle are the most important reservoirs for *E. coli* in this setting, followed by sheep and the least were the Fulani herders. Gyles *et al.* (2007), reported that cattle are well-known reservoirs of *E. coli* especially the pathogenic strains (O157:H7) which are frequently isolated from cattle faeces. Reporting that the rumen and intestine of cattle often provide favourable environment for *E. coli* colonization which explains the high isolation rate (35.4%) in the current study. Several studies positioned cattle as the major reservoirs of *E. coli* particularly with the pathogenic strains (O157:H7). Hussein *et al.* (2007), found *E. coli* prevalence in cattle ranging from 10% to 40% depending on region and sampling methods. Similarly, Chapman *et al.* (1997), reported. *E. coli* isolation rate from cattle faecal samples as consistently high, often exceeding 40% in pastoral communities.

In contrast, the overall *E. coli* isolation rate of 61.3% obtained in this study appears somewhat higher than some isolation rates reported in some studies around the World. Example, the overall *E. coli* isolation rate of 15% reported by Tadesse *et al.* (2012) from livestock in Ethiopia is so low as compared to the report of the current study which suggests difference in sampling location, methods of sampling and / or sampling from extremely healthy subjects.

Comparably, sheep harbour generally lower prevalence rate of *E. coli* than cattle. A study conducted in Nigeria by Ojo *et al.* (2010), reported prevalence rate of 15.0% which is in total agreement with the report of this study.

In contrast, Manning *et al.* (2007) reported very low prevalence rate of *E. coli* isolation (5%) in sheep in part of Europe, which could be probably due to difference in grazing behaviour, husbandry practice and antimicrobial stewardship policies of this part of the World.

The prevalence of *E. coli* isolation from the Fulani herders (11.0%), indicates zoonotic transmission potential. This suggests that close contact between animals and herders coupled with poor sanitation and congestion can facilitate *E. coli* colonization or infection in humans (Nguyen *et al.*, 2016). This study reported 11.0% of *E. coli* isolation rate from herders. However, human carriage of *E. coli* varies widely depending on hygiene, animal-human contact, and socio-economic factors. Studies in pastoral communities as conducted by Kiiru *et al.* (2013), reported *E. coli* colonization in humans up to 15% to 20% of people in close contact with animals. This appears higher than the 11.0% obtained in this study. Analysis of this result showed significant difference between the rate of isolation of *E. coli* from cattle on one hand and sheep/herders on the other hand (P-value < 0.05). This explains the common fact observed during the sampling of this study that sheep and goats were mostly found in clusters right in the settlement of the herders during the nights while cattle were at distance. This suggests that close association with livestock may pose a risk of contracting and transmission of coliform infections. Further study by phylogenetic analysis is required to concretize relationship between the Fulani herders and their livestock in terms of transmission of colibacillosis.

Antimicrobial Resistance (AMR) Profile of *E. coli* Isolates from Livestock and Herders in this Study

Out of the 111 *E. coli* isolates subjected to antimicrobial susceptibility testings in relation to sample sources (Cattle, Sheep and Herders), 102 isolates comprising of 60 from cattle, 23 from sheep and 19 from herders showed antimicrobial resistance (AMR). And of these 102 resistant isolates, 91 comprising of 51 from cattle, 23 from sheep and 17 from herders respectively were multidrug resistance (MDR) (Table 2).

Table 2: Distribution of Antimicrobial Resistance (AMR) Profile of *E. coli* Isolates from Livestock and Herders in This Study

Antibiotics (concentrations)	No. of <i>E. coli</i> isolates tested (n=111)			No. (%) of MDR- <i>E. coli</i> isolates (n=91)		
	Cattle (n=64)	Sheep (n=27)	Herders(n=20)	Cattle (n=51)	Sheep (n=23)	Herders (n=17)
Amoxicillin (30µg)	64	27	20	48 (94.1)	20 (86.9)	17 (100)
Ceftazidime (25 µg)	64	27	20	40 (78.4)	16 (69.6)	10 (58.8)
Cefotaxime (25 µg)	64	27	20	39 (76.5)	17 (73.9)	10 (58.8)
Ofloxacin (10 µg)	64	27	20	42 (82.4)	15 (65.2)	09 (52.9)
Pefloxacin (25 µg)	64	27	20	41 (80.4)	14 (60.9)	09 (52.9)
Ciprofloxacin (15 µg)	64	27	20	36 (70.5)	16 (69.6)	13 (76.5)
Streptomycin (30 µg)	64	27	20	26 (50.9)	08 (34.8)	08 (47.1)
Amikacin (25 µg)	64	27	20	26 (50.9)	07 (30.4)	06 (35.3)
Sulphonamide (30 µg)	64	27	20	42 (82.4)	14 (60.9)	09 (52.9)
Trimethoprim(30µg)	64	27	20	32 (62.7)	15 (65.2)	11 (64.7)

(CLSI, 2020).

The discovery, development and classification of antibiotics was by no doubt one of the greatest breakthrough in the field of modern medicine. The emergence of bacterial resistance to some commonly prescribed classes of antibiotics is quite alarming and their use in the treatment of infections due to gram negative bacteria is questionable and this sends a very serious warning to both Medical and veterinary practitioners all over the world (Igbal *et al.*, 2017). The United States Centres for Disease and Prevention (CDC) estimated that antibiotics resistance is responsible for more than 2 million infections and 23,000 deaths each year in U.S. (CDC, 2013). In Europe an estimate of 25,000 deaths were attributed to antibiotic-resistant infections (EMA and CDC, 2009). In this study, following the guidelines recommended by Clinical Laboratory Standard Institute (CLSI, 2020) at 37 °C for 24 hours, difference antimicrobial profiles were observed and interpreted into three (3) categories as resistant (R), intermediate- susceptible (IS) and susceptible (S) respectively. Resistant strains of *E. coli* isolates are those which can be able to grow in the presence and even on the antibiotic disc while susceptible strains, were those isolates inhibited by the drug at 37 °C, within 24 hours of incubation. However, in between the two, were intermediate-susceptible (IS) category which can become susceptible either by increasing the concentration of the drug, or incubation period or both. Using four (4) commonly prescribed classes of antibiotics (Aminoglycosides, Beta-lactams, Flouroquinolones and Sulphonamides), beta-lactam antibiotics (Amoxicillin, Ceftazidime and Cefotaxime) recorded the highest AMR rates with amoxicillin recording multidrug resistance (MDR) rate of 100% in *E. coli* isolates from herders, 94.1% from cattle and 86.9% from sheep respectively. Among the Flouroquinolones, Ofloxacin recorded moderate to higher AMR rates with MDR rates of 82.4% in cattle, 65.2% in sheep and 52.9% in herders. In contrast, Aminoglycosides, recorded the lowest AMR rates with amikacin recording the lowest MDR rates of 30.4%, 35.3% and 50.9% from sheep, herders and cattle respectively. Among the Sulphonamides (Co-Trimoxazole and Trimethoprim/ sulfamethoxazole), accounted for yet another moderate to higher AMR rates with Co-Trimoxazole recording the MDR rates of 82.5%, 60.9% and 52.9% from cattle sheep

and herders respectively aligning very closely with the MDR rates recorded by the aminoglycosides class of antibiotics.

Although, infections by ESBL producing *E. coli* are well known for conferring resistance to various classes of antibiotics however, some drugs are still exceptionally good in their antimicrobial activities. In this study, the overall lowest MDR rates of *E. coli* isolates from all the sample source, were recorded from among Aminoglycosides (amikacin and streptomycin). Amikacin recorded the overall lowest MDR rate of 30.4% from the isolates of sheep followed by the 35.3% of isolates of herders' origin respectively. This is lower than the MDR rate of 48.4% reported by Phelivanoglu *et al.* (2016). This suggests that antibiotics of the aminoglycosides class may likely be drug of choice for treatment of coliform infections in livestock and herders. This finding corroborates with the reports of Daga *et al.* (2019). Furthermore, members of sulphonamides class, Co-Trimoxazole (SXT) presented yet another high MDR rates of 70.0%, with the isolates of cattle, 60.9% with isolates of sheep and 47.4% with the *E. coli* isolates of herders respectively. Sulfamethoxazole /trimethoprim (TMP) presented an MDR rates of 53.3% with the isolates of cattle, 56.5% with the isolates of sheep and 57.9% with the isolates of herders respectively.

The prevalence of ESBL producing phenotypes among *Enterobacteriaceae* including *E. coli* is rapidly on the increase and this call for reliable Laboratory screening methods which will identify the presence or absence of these ESBL phenotypes in clinical samples (Bradford, 2001). However, various susceptibility testing methods differ in their ability to detect MDR-ESBL producing *E. coli* isolates. In the course of ESBL detection by several screening methods, some ESBL producing isolates may test falsely positive or falsely negative especially when compared to the gold standard method.

Phenotypic Detection of ESBL Producing *E. coli* Isolates Based on Sample Source in Livestock and Herders

Out of the 91 (100%) MDR *E. coli* isolates subjected to ESBL production tests using the Double Disc Synergy Test (DDST), 37 (40.7%) ESBL producers were detected. Out the 37 ESBL Producers, 21(23.1%) were cattle, 09(9.9%) were sheep and 07(7.7%) were herders respectively (Table 3).

Table 3: Distribution of ESBL-*E. Coli* Producers from Livestock and Herders Using Double Disc Synergy Test (DDST) in Relation to Sample Source

Sample source	MDR <i>E. coli</i> isolates (n=91)	No. of ESBL producing MDR <i>E. coli</i> isolates (n=37)	Percentage (%)
Cattle	51	21	23.1
Sheep	23	09	9.9
Herders	17	07	7.7

Phenotypic screening for ESBL producing bacteria is a broad based detection method which identifies all resistant factors such as plasmid mediated ESBL, AmpC-beta-lactamases, chromosomal ESBL, hypermuco-viscosity and carbapenemases. ESBL detection involves two important steps. Firstly, an indicator test with antibiotics looking for multidrug resistance (MDR) or reduced susceptibility to identify isolate's likelihood to harbouring ESBL genes. Secondly, to detect the synergy between an oxyimino-cephalosporin and clavulanic acid, distinguishing isolates with ESBL from those that are resistant due other factors (Gay et al., 2018). Multidrug resistant in this context, refers to those *E. coli* isolates that showed resistance to at least three (3) or more classes of antibiotics (Magiorakos et al., 2012). In this study, out of the 91 (100%) MDR *E. coli* isolates subjected to ESBL production tests using the Double Disc Synergy Test (DDST) 37 ESBL producers were detected presenting an overall prevalence rate of 40.7% (37/91). This is in total agreement with the report of Mohammed et al (2024) who detected 37 *E. coli* isolates as ESBL producers out the 165 isolates screened for ESBL using DDST method. Out the 37 ESBL Producers 23.1% (21/91) were from cattle, 9.9% (9/91) were from sheep and 7.7% (7/91) were from herders respectively.

In previous studies, Giriyaapur et al. (2011) reported an overall prevalence rate of 63.9% of ESBL producing phenotypes; Singal et al. (2005) reported a prevalence rate of 63.6% and Ravi et al. (2011) at 20mm inter-disc distance, detected a prevalence of 56.2% ESBL producing phenotypes among gram-negative bacilli using DDST which are all higher than the 40.7% recorded in this study. These variations might be due to difference in geographical location, hospital settings, patient demographics, sample size, sampling methods, bias sampling and/ or proper antibiotics stewardship programs of the various study areas..

In the current study, Cattle on one hand accounts for the highest prevalence rate of 23.1% as against 9.9% and 7.7% of Sheep and Herders on the other hand. This suggests that cattle are likely more vulnerable to infection by ESBL producing *E. coli* pathogens. The ESBL rate of 23.1% from cattle in the current study suggest moderate to high prevalence in livestock and is consistent with the report of Lever stein-van Hall. (2011) who reported ESBL production rates of 20% -30% in cattle in Europe.

Schmid et al. (2013) in their studies conducted in Europe and Asia reported ESBL production rates ranging from 5% - 15% in sheep. However, the ESBL production rate of 9.9% from sheep in this study fits within the expected range and is lower than that of the cattle. The herders ESBL carriage rates vary widely depending on the study population. Community ESBL carriage rates in healthy persons ranges from 3% - 15%. Sometimes it may be higher in risk regions with high antibiotic use (Woerther et al., 2013). The 7.7% ESBL detection rate in herders is within the expected range of community carriage and is lower as compared to that of the livestock. However, studies focused on individuals in close-contact with livestock (farmers / herders), may report higher carriage rates suggesting zoonotic transmission.

CONCLUSION

In conclusion, 91(81.9%) *E. coli* isolates were found to be multidrug resistant (MDR) in this study which revealed significant difference (P-value < 0.05) between the MDR *E. coli* isolates detected from cattle on one hand and sheep/herders on the other hand. Unvaccinated calves/ lambs at the first few weeks of life and young herders below 10 years of age particularly those found in closed proximity to livestock as well as those living in dirty environment where antibiotics are indiscriminately administered showed high rate of multidrug resistance. Therefore, surveillance, infection control measures, proper farm sanitation, health hygiene, and vaccination will help reduce infection by MDR *E. coli* and/or spread of MDR strains of bacteria.

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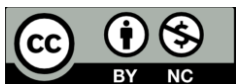
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