



Isolation and Identification of Multidrug Resistance (MDR) *Escherichia coli* Isolates from Livestock and Herders in Some States in North-Eastern Nigeria

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ABSTRACT

The emergence and horizontal transfer ESBL genes among bacteria of food-animals and human origin as well as indiscriminate use of antibiotics are responsible for multiple drug resistance with challenging treatment measures which in turn call for considerable concern of both Medical and Veterinary practitioners all-over the World. Therefore, the aim of this study was to isolate and identify the multidrug resistance (MDR) *E. coli* isolates from Livestock and Herders with view to assessing the significant relations between the former and the latter. A total of 1152 samples comprising of 384 each from cattle sheep and Herders were collected, processed, inoculated and incubated at 37⁰ C for 24 hours. One hundred and eighty-one (181 (15.7%) samples yielded positive bacterial growths of which 111 (61.3%) of them were *E. coli* isolates and of these, 91(81.9%) were detected to be multidrug resistant. This study revealed that there was 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. Calves, lambs at the first week of life and young herders below 10 years of age especially those found in closed proximity to livestock as well as those on which antibiotics were indiscriminately administered showed high rate of multidrug resistance. Therefore, proper sanitary measures be adopted and maintained in farms where calves and Lambs are kept. Young herders should be properly monitored and those with diarrhea be promptly treated using excellent antibiotics combinations. Herders with known history of prolonged hospital stay be examined for nosocomial infections by MDR *E. coli* isolates for better management.

Keywords: Multidrug resistance (MDR), Isolation, Identification, Livestock, Herders and *Escherichia coli*

INTRODUCTION

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 Aminoglycosides. Multidrug resistant (MDR) refers to those *E. coli* isolates that showed resistance to at least 3 or more classes of antibiotics (Hawser *et al.*, 2009).

Escherichia coli are Gram-negative, facultative anaerobes, non-spore forming, typically rod-shaped, about 2.0 micrometre (µm) long, 0.5 µm in diameter and cell volume of 0.6–0.7µm³ (Kubitschek, 1990). *E. coli* is widespread intestinal commensal organism found in humans and animals resulting from fecal contamination or contamination during food animal slaughter. 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* has been identified to cause Colisepticaemia, Colibacillosis, Colimastitis and Urinary tract infection (UTI) (Nicolas-Chanoine *et al.*, 2008; Peirano *et al.*, 2010). *E. coli* constitutes 80-85% of the aetiologic agents of UTI in community Hospitals of Chennai and Tamil Nadu in India (Rajan, 2012).

Colibacillosis have been described as one of the most commonly occurring and economically devastating disease of poultry and other birds worldwide which manifests as acute fatal septicaemia, pericarditis, cellulitis, Salpingitis and gastroenteritis (Lisa, 2019). Recently, Walter (2020), reported that other vertebrates like cattle, sheep, goats, pigs and even humans are at risk of septicaemic-colibacillosis especially at the first few weeks of life and with challenging treatment and control measures.

Livestock (white Fulani breed of cattle/balami breed of sheep) and herders in north-eastern States of Nigeria are particularly at risk due to close contact with livestock and also limited access to healthcare facilities. Several studies have highlighted the potential risk of multidrug resistant due to *E. coli* in the livestock and its transmission to humans through food animals or direct contact (Catawba *et al.*, 2012). The emergence of multidrug resistance *E. coli* in North-Eastern Nigeria poses a very serious threat to livestock and public as these can cause series of severe infections that can be difficult to treat with conventional antibiotics. Understanding the prevalence, mechanisms of resistance in *E. coli* isolates of livestock and herders' origin, is important for developing robust control measures.

In this study we intend to isolate and identify MDR *E. coli* strains in livestock and herders within the study area. Using the biochemical characterization and antimicrobial profiles of these isolates, will provide to some extent an

in-depth knowledge on the spread of antimicrobial resistance among livestock and their herders in this region of the country. The current research is desirable and timely since prevalence of multidrug resistance bacteria are in the increase and also have alarming impact on human health in this region. However, it may also contribute to knowledge, if MDR *E. coli* isolates from food animals and their handlers especially (Fulani herdsman) with signs of coliform infection in North-Eastern states of Nigeria is isolated and characterized to gene levels.

MATERIALS AND METHODS

The Study Area

The study area covers three (3) Livestock populated States; of Adamawa, Bauchi and Taraba in North-Eastern Nigeria. Adamawa State lies between latitude 7° and 11° North and between Longitude 110° and 140° East, covering a land mass of about 38,741 km², and currently having twenty-one (21) Local Government Areas with Yola as its State Capital (Adebayo, 1999). Bauchi State lies between latitude 9° 3' and 12° 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 (Come to Nigeria Staff, 2011). 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 Livestock rearing and crop production (Come to Nigeria Staff, 2011).

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 Mammilla Plateau with an altitude of 1,800 meters (6000 ft) 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 occupations 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).

Study Population and Sampling Procedures

Cross-sectional descriptive studies were conducted on ESBs producing *E. coli* from white Fulani breed of cattle and 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 white Fulani breed of cattle (from 0 - 11 years old; 384 balami breed of sheep (0 - 9 years old and 384 of the herders (0 - 70 years old (Pehlivanoglu et al., 2016) were sampled using the simple randomized sampling method. Swab samples were directly and non-repeatedly collected from the rectum of each sampling animals as follows:

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

Clinical Microbiological Procedures

Preparation of culture media

The culture media [EMB and, MH agars (OXOID U.K)], in this study were prepared aseptically in ATBU Postgraduate Microbiology Laboratory according to the manufacturer's instructions.

Enrichment, Inoculation and incubation of samples

All samples transported to the Laboratory were sterilely enriched by peptone water. An enrichment procedure was performed to enhance the growth of bacteria population before inoculation of the samples. The swab sample 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

After 24 hours, inoculated 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. These slants cultures were preserved in refrigerator for subsequent studies.

Gram-staining

Slides prepared from pure colonies 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).

Biochemical identification tests

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), Methyle 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), Cefotaxime 25µg), Cefotaxime (25µg), Ciprofloxacin (15µg), Ofloxacin (10 µg), Pefloxacin (25 µg) Bactrim (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.

Selection and preservation 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.

Statistical Analysis

Chi-square test of relation 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

Out of the 1152 samples collected, processed and inoculated on Eosin Methylene Blue (EMB) agar, 181 (15.7%) cultures comprising of 65(5.8%), from Adamawa, 60 (5.2%), Bauchi and 56 (4.7%), Taraba had positive bacterial growths. The results of Characteristics of Bacterial Isolated from Livestock and Herders in relation to Sample Source are presented in Table 1. 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 in this study, 35.3% (64/181) were *E. coli*, 6.6% (12/181) were *Salmonella* and 14.4% (26/181) were *Shigella* species. Of the 48 positive cultures obtained from Sheep, 14.9% (27/181) were *E. coli*, 2.2% (4/181) were *Salmonella* and 9.45% (17/181) were *Shigella* species. And of the 31 positive cultures from Fulani Herders in this study, 11.05% (20/181) were *E. coli*, 1.7% (3/181) were *Salmonella* and 4.4% (8/181) were *Shigella* species respectively. These 111 *E. coli* isolates were preserved in the refrigerator for further studies while the *Salmonella* and the *Shigella* isolates were discarded in line with the exclusion criteria of this study (Table 1)

Table 1: Distribution of bacteria Isolates from Livestock and Herders in Relation to Sample Sources

Sample source	No. of Samples Collected (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(14.9)	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, some strains are producers of ESBL and themselves are common cause of community-acquired bacteraemia 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* has been identified to cause Colisepticaemia, Colibacillosis, Colimastitis and Urinary tract infection (UTI) especially at first few weeks of life with challenging treatment and control measures (Walter, 2020); (Nicolas-Chanoine *et al.*, 2008). In study conducted by Pooja *et al.* (2017) among patients suspected of urinary tract infection (UTI) in Alka Hospital during which a total of 2209 samples cultured, only 451 (20.4%) showed significant growth. Out of the 451 positive cultures, 365 (80.9%) were all *E. coli* while 86 (19.1%) were other members of the *Enterobacteriaceae*.

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 extremely 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 shows that there was 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). However, this explains the common fact observed during the sampling 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 susceptibility profile of *E. coli* Isolates from Livestock and Herders in this Study

Out of the 111 *E. coli* isolates comprising 60 of cattle, 23 of sheep and 19 of herders' origin subjected to antimicrobial susceptibility tastings in accordance with the guidelines recommended by Clinical Laboratory Standard Institute (CLSI, 2020), the highest resistance rates of 76.6% was recorded by amoxicillin, followed by 59.5% and 58.6% of ceftazidime and cefotaxime, all being beta-Lactams. Among the fluoroquinolones; ofloxacin, pefloxacin and ciprofloxacin, recorded moderately higher MDR rates of 59.5%, 57.7%, and 59.5% respectively. From the Aminoglycosides; Amikacin and streptomycin recorded the lowest MDR rates of 33.3% and 37.8% respectively. Among the Sulphonamides (Trimethoprim / sulfamethoxazole) and Co-Trimazole accounted for yet another higher MDR rate of 52.30% and 57.7% respectively (Table 2).

In this study, following the guidelines recommended by Clinical Laboratory Standard Institute (CLSI, 2020), 111 *E. coli* isolates were subjected to antimicrobial multidrug resistance testing at 37 °C, for 24 hours using four

commonly prescribed classes of antibiotics (Aminoglycosides, Fluoroquinolones, Sulphonamides and Beta-lactams). However, difference rates of susceptibility reactions were obtained. Multidrug resistant (MDR) refers to those *E. coli* isolates that showed resistance to at least 3 or more classes of antibiotics (Magiorakos et al., 2012). The results were interpreted into three (3) categories as resistant, intermediate-susceptible (IS) and susceptible (S) respectively. Resistant strains of *E. coli* isolates were those which were able to grow in the presence and even on the antibiotic disc, while susceptible, were those inhibited by the drug at 37 °C within 24hrs of incubation with significant zones of inhibition. However, in between the two, were the intermediate-susceptible category (IS) which can be susceptible either by increasing the concentration of the drug, or incubation period or both.

The result of the antimicrobial susceptibility testing on *E. coli* isolates reveals concerned level of resistance to multiple classes of antibiotics. The highest resistance rates observed were among the Beta- Lactams (AMX, CAZ, CTX), moderately higher resistance rates were observed among the Flouroquinolones (OFL, PEF, CPX) and the Sulphonamides (TMP, SXT) suggesting a potential challenge in the treatment of infections due to grams negative bacterial.

According to study conducted by Hooper and Jacoby, (2015), the increasing prevalence of antibiotics resistance in gram- negative bacteria, including *E. coli*, is a global concern that may amount to treatment failure and increased morbidity and mortality. These resistance mechanisms can be attributed to over-use/ mis-use of antibiotics as well as horizontal transfer of resistance genes among bacterial population. Despite the high resistance rates observed in other antibiotic classes, aminoglycosides (amikacin & streptomycin), presented the lowest

resistance rates of 33.3% and 37.7% respectively, which suggests that antibiotics of this class could be a drug of choice for treatment of infections by gram-negative bacteria. The low antibiotics resistance recorded by amikacin and streptomycin in this study is quite a promising finding and can be attributed to their ability to bind to the bacteria ribosome thereby disrupting the protein synthesis. This mechanism may make it more difficult for the organism to develop resistance as compared to the other classes antibiotics, or it could be indicative of limited pressure from the use of these antibiotics in clinical settings. Bye and

large, the critical importance of prudent antibiotics uses and infection control to combat the growing trend of antibiotic resistance in gram-negative bacteria pathogens is very crucial.

However, the average MDR rate (35,5%) recorded in aminoglycosides in this study 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 management and treatment of coliform infections in livestock and herders. This finding corroborates with the reports of Daga *et al*, (2019).

Table 2: Antimicrobial susceptibility profile of *E. coli* Isolates from Livestock and Herders in this Study

Classes of antibiotics	Names of antibiotic	No. (%) resistant (n=613)	No. (%) susceptible (n= 487)
Beta-Lactams	Amoxicillin (AMX) 30µg	85(76.6%)	26 (23.4%)
	Ceftazidime (CAZ) 25µg	66(59.5%)	35 (31.5%)
	Cefotaxime (CTX) 25µg	65(58.6%)	46 (41.4%)
Flouroquinolones	Ofloxacin (OFL) 10µg	66(59.5%)	45 (40.5%)
	Pefloxacin (PEF) 25µg	64(57.7%)	47 (42.3%)
	Ciprofloxacin (CPX) 15µg	66 (59.5%)	45 (40.5%)
Aminoglycosides	Amikacin (AMK) 25µg	37(33.3%)	74 (66.7%)
	Streptomycin (STR) 25µg	42 (37.8%)	69 (62.2%)
Sulphonamides	Trimethoprim (TMP)30µg	58 (52.3%)	53 (47.7%)
	Co-Trimazole (SXT) 30µg	64 (57.7%)	47 (42.3%)

Key: CLSI 2020

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

Following subjection of 111 *E. coli* isolates comprising 64 isolates of cattle, 27 isolates of sheep and 20 isolates of herders' origin, 91 isolates comprising 51 of cattle, 23 isolates of sheep and 17 isolates of herders' origin were tested to be multi drug resistant. Out of the MDR isolates, 38 were resistant to four classes of antibiotics and 53 isolates were resistant to three classes of antibiotics. While 20 isolates were either resistant to one or two antibiotics class and as per definition of MDR, were considered as non-MDR isolates (Table 3).

Indeed, the discovery and development of antibiotics was by no doubt one of the greatest breakthroughs in the field of modern medicine. However, the emergence of bacterial resistance to antibiotics is frightening to the efficacy of many antimicrobial agents. Prescription of these antimicrobial agents are very common by both Medical and Veterinary doctors and their used is questionable (Igbal *et al.*, 2017). Flouroquinolones, aminoglycosides, beta-lactams and sulphonamides are so often prescribed for the treatment of gram-negative bacterial infections and emergence of bacterial resistance to antibiotics of these classes would be a very serious warning to both Medical and Veterinary practitioners all

over the world (Suleiman *et al.*, 2014). Antimicrobial resistances among *E. coli* infected patients are globally on the increase and these have been associated with the emergence and spread of ESBL, prolonged and indiscriminate use of antibiotics which has swung several researchers in to action.

The current study revealed that out of the 111 *E. coli* isolates tested, 91 (82.0%) exhibited multidrug resistance (MDR). Adding the MDR –*E. coli* isolates resistance to 4 and 3 classes of antibiotics together on table 3 in relation to sample source, will reveal that out of the 91 (82.0%) MDR- *E. coli* isolates, 51 (56.0%) were from cattle, 23 (25.3%) were from sheep and 17(18.7%) were from herders respectively. This overall high prevalence of MDR *E. coli* (82.0%) is consistent with other findings globally; although, the exact proportions and sources may vary depending on the geographical location, type of animal husbandry practices and pattern of antibiotics usage. Several studies all over the World have also reported high prevalence of MDR *E. coli* in cattle, supporting the findings of the current study. Wong *et al.* (2019) reported overall MDR- *E. coli* isolates rate of 75% from cattle in the United States which were resistance to atleast three (3) classes of antibiotics and this aligns closely with the 82.0% MDR rate found in this study. In another study conducted by Tadesse *et al.*,

(2019) in Ethiopia, also reported high MDR rate of 70% in cattle and 60% in sheep, which reflect again a similar trend of high MDR rate in cattle and sheep. These also suggest that the MDR *E. coli* rate is on the increase globally.

The 18.7% MDR isolates from herders suggest possible zoonosis between livestock and humans. Liu *et al.* (2020), reported MDR *E. coli* colonization rate of 20% in farm workers in China which is comparable with the 18.7% from herders in this study. However, the high ratio of 56.0%: 25.3% MDR rate from cattle to sheep is consistent with the report of Nguyen *et al.* (2016) who reported MDR – *E. coli* ratio of 60% : 30% cattle to sheep in Vietnam.

In contrast, Abraham *et al.* (2021), reported MDR *E. coli* rate of only 40% in cattle in part of Europe which may possibly due to strict/effective antimicrobial stewardship policies.

However, an estimate of 58,000 neonatal septic deaths was attributed to drug-resistance infections in India alone (Laxminarayan *et al.*, 2015). 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 is attributed to antibiotic-resistant infections (EMA and CDC, 2009).

Table 3: Distribution of Multi-drug Resistance (MDR) Profile of *E. coli* Isolates from Livestock and Herders in this Study

Sample source	No. of <i>E. coli</i> isolates tested	No. (%) of MDR <i>E. coli</i> isolates; 4 - 3 classes of antibiotics (n=91)		No. (%) of Non-MDR <i>E. coli</i> isolates; 2-1classes of antibiotics (n=20)	
		Four classes	Three classes	Two classes	One class
Cattle	64	27 (29.7)	24 (26.4)	09 (45.0)	04(20.0)
Sheep	27	05 (5.5)	18 (19.8)	00 (0.0)	03(15.0)
Herders	20	06 (6.6)	11 (12.1)	02 (10.0)	02 (10.0)
Total	111	38 (41.8)	53 (58.2)	11 (55)	09 (45.0)

CONCLUSION

In conclusion, this study revealed that of 111 *E. coli* isolates tested, 91 (82.0%) exhibited MDR of which 51(56.0%) were from cattle, 23 (25.3%) were from sheep and 17 (18.7%) were from herders respectively. This showed 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. The rate of occurrence of MDR *E. coli* isolates depends on the geographical location, type animal husbandry practices, pattern of antibiotic usage, age, and sanitary condition of the herd. 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 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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