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Original Research Article

Determination of Anti-Microbial Susceptibility of *Escherichia Coli* Isolates of Cattle Faeces and Manure against Common Antimicrobial Agents and Multiple Drug Resistance Indices

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Abstract

A research was conducted to determine the anti-microbial susceptibility of some *Escherichia coli* isolates against common anti-microbial agents and Multi Drug Resistance (MDR) indices. The *Escherichia coli* isolates (n=51) obtained from cattle faeces and manure in abattoirs, cattle farms and livestock markets of Bauchi, Darazo and Katagum local governments of Bauchi state, Nigeria were screened for anti-microbial susceptibility against 12 common anti-microbial agents. The susceptibility test was carried on all the 51 isolates to determine their antibiotic resistant profiles using the disc diffusion method developed by Kirby – Bauer and standardized by the World Health organization (WHO), commercially available antimicrobial disks impregnated with the different antibiotics were then tested. The results revealed highest resistances of the isolates to Cephazolin (86.2%), followed by Sulfamethoxazole (82.4%) and Enrofloxacin (78.4%). The research also revealed that the highest level of susceptibility of the isolates to the antimicrobial agents was found against chloramphenicol (78.4%), followed by gentamycin and Imipenem with (68.6%) and Ceftriaxone (58.8%) respectively, the results further indicated 50(98%) of the isolates resistance to 3 or more of the antibiotics. All isolates showed Multi-drug resistance pattern (MDR), some among the isolates showed resistance against up to 8 antimicrobial agents belonging to 7 different groups of the antimicrobial agents. The MDR indices of *E. coli* strains indicated resistance against the antimicrobial agents at various levels of antibiotic groups.
Keywords: Antimicrobial, susceptibility, *Escherichia coli*, Multi-drug, resistance, pattern.

INTRODUCTION

The development of antimicrobial resistance can be seen as a global problem in microbial ecology and is the best-known example of a rapid adaptation of bacteria to a new ecosystem [1]. Widespread use of these drugs led to the emergence of antibiotic resistance in many important pathogens [2]. This resistance contributes to higher rates of morbidity and, in the case of severe bacterial infections, therapeutic failure [3]. Degrees of antimicrobial susceptibility/resistance in clinical isolates are often defined in terms of the Minimum Inhibitory Concentration (MIC) of an antimicrobial compound required to prevent bacterial growth. Bacteria can be defined as being resistant to an antimicrobial compound, when its MIC is higher than its wild-type counterpart [4, 5]. This study is basically aimed to determine the anti-microbial susceptibility of the isolates obtained from cattle faeces and manure against common anti-microbial agents and their Multi Drug Resistance (MDR) indices.

The global increase in antibiotic-resistant bacteria is of major concern and thus, antibiotic use for medical and agricultural applications is a major risk factor for the increased occurrence of resistant organisms [6,8]. Besides medical use in humans, there is the troubling issue of their use in agriculture, specifically in livestock production where antibiotics have long been regularly used not only for the treatment of infections, but also as a means of getting animals to market faster through growth promotion by adding feed-based antibiotics constantly.

Antibiotics accumulate in the tissues of animals and hence, can be ingested by consumers whose own resident micro flora may become resistant [9]. Thereby serving as an important means of dissemination of resistance in humansas through the food chain [10]. Beta–lactamases target the peptidases of bacterial cell-wall biosynthetic process. Extended-spectrum beta-lactamases (ESBLs) have evolved through a series of substitutions of amino acids and provide resistance to third generation cephalosporins. ESBLs are produced by gram-negative bacteria and have been reported from many species [11].

Antibiotic resistance in pathogenic bacteria has been an increasing medical problem for decades [12]. Microbes may develop resistance to antibiotics under selective pressure, or they may acquire antibiotic resistance determinants without direct exposure to an antibiotic. Furthermore, acquired resistance determinants are spread among different species and even genera which include potential and obligate pathogens [12]. This is especially favored in settings that allow the close association of densely packed microorganisms such as the intestine of humans and animals [13]. Consequently, antimicrobial-resistant bacteria are selected for; thereby posing a serious public health threat in that antimicrobial treatment effectiveness may be reduced [14].

Certain management practices have shown evidence that antimicrobial drug residues in livestock effluent can contribute to elevated antimicrobial resistance (AMR) levels, it is important to improve our understanding of how current management systems may impact AMR transmission to the public. Pan-microbial approaches enable access to this microbial resistance ecology and provide information on how livestock production practices influence the density and composition of antimicrobial resistance genes (ARGs) (i.e., the resistome) [15]. With this insight, practical mitigation strategies can be proposed to minimize the flow of ARGs into aquatic, terrestrial and atmospheric ecosystems. However, very little is currently known about the cattle production resistome. The few published studies are descriptive in nature, utilizing samples taken from a small number of non-commercial animals [16, 17].

Materials and Methods

Antibiotic susceptibility test was carried on all the isolates to determine their antibiotic resistant profiles using the disc diffusion method developed by Kirby – Bauer [18] and standardized by the World Health organization (WHO) as modified by Sozmen *et al.*, [19]. Commercially available antimicrobial disks impregnated with Imipenem (10µg), tetracycline (30µg), Sulfamethoxazole (23.75µg), ciprofloxacin (5µg), chloramphenicol (30µg), Amoxicillin / clavulanate (10+20µg) Ticarcillinclavulanate (75+10µg), gentamycin (10µg), Erythromycin (15µg), Enrofloxacin (5µg), Ceftriaxone (30µg) and Cefazolin (30µg) were obtained from Oxoid (UK).

The antibiotic susceptibility test procedure consisted of inoculating the bacteria on tryptone soy broth and incubated at 37^{o}C for 24hrs. The turbidity of the broth was adjusted to 0.5 McFarland standard and a sterile swab stick was eluted into the overnight culture broth and excess moisture was expressed by pressing the swab against the side of the tube and the surfaces of the Mueller – Hinton agar were swabbed completely and then turned at 90^{o} and the swabbing process was repeated until the entire circumference of the plate was covered. The media were allowed to dry for about 5 mins before placing the antibiotic discs using antibiotic dispenser. Then each disc was lightly touched with a sterile inoculating loop to make sure it was in good contact with the agar surface, and then incubated upside down at 37^{o}C overnight.

The result of the incubated of the culture with the anti-microbial discs was interpreted by using a transparent plastic metric ruler across the zone of inhibition (Z I), at the widest diameter to measure from edges of the zones in millimeters. In a situation where there was no zone at all, it was recorded as 0. A chart was used to report result on a table as sensitive, resistant or intermediate [20].

Table-1: Antimicrobial susceptibility of *E. coli* isolates from cattle faeces and manure

LGA	Sample Type	No. Isolates	Antimicrobial agents											
			Imipenem (IPM) (Disc content = 10µg)			Tetracyclines (TE) (Disc content = 30µg)			Sulfamethoxazole (SXT) (Disc content = 23.75µg)			Ciprofloxazole (CIP) (Disc content = 5µg)		
			S ≥23	I 20-22	R ≤19	S ≥15	I 12-14	R ≤11	S ≥16	I 11-15	R ≤10	S ≥21(30)	I 16-20	R ≤15(20)
Bauchi	Faeces	5	5	0	0	0	0	5	0	0	5	4	0	1
	Manure	0	_	_	_	_	_	_	_	_	_	_	_	_
Darazo	Faeces	6	4	2	0	1	0	5	0	1	5	4	2	0
	Manure	0	_	_	_	_	_	_	_	_	_	_	_	_
Katagum	Faeces	36	24	5	7	19	4	13	0	8	28	14	8	14
	Manure	4	1	1	2	3	0	1	0	0	4	1	3	0
Total		**51**	_	_	_	_	_	_	_	_	_	_	_	_

Key: S =Sensitive, I=Intermediate, R=Resistance

RESULTS AND DISCUSSION

Results

The fifty-one (51) *E. coli* identified positive from faecal and manure samples were found to be 2(4.2%) from the abattoirs, 22(45.8%) were from cattle farms while the remaining 24(50.0%) were from livestock markets. The 51 isolates that were found positive for *E. coli* were subjected to antibiotic susceptibility tests against 12 commonly used antimicrobial agents (Table 1-3). The results showed highest resistances of the isolates to Cephazolin (86.2%), followed by Sulfamethoxazole (82.4%) and Enrofloxacin (78.4%). It was also observed that the highest level of susceptibility of the isolates to the antimicrobial agents was found against chloramphenicol (78.4%), followed by gentamycin and Imipenem (68.6%) and Ceftriaxone (58.8%).

Table-2: Antimicrobial susceptibility of *E. coli* isolates from cattle faeces and manure

LGA	Sample Type	No. Isolates	Antimicrobial agents											
			Chloramphenicol (C) (Disc content = 30µg)			Amoxicillin clavulanate (AMC) (Disc content = 10 +10µg+20µg)			Ticarcillinclavulanate (TIM) (Disc content = 75 +10µg)			Gentamycin (CN) (Disc content + 10µg)		
			S ≥18	I 13-17	R ≤12	S ≥18	I 14-17	R ≤13	S ≥20	I 15-19	R ≤14	S ≥15	I 13-14	R ≤12
Bauchi	Faeces	5	4	1	0	1	3	1	2	1	2	5	0	0
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Darazo	Faeces	6	4	2	0	2	0	4	4	1	1	3	1	2
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Katagum	Faeces	36	29	4	3	13	10	13	15	11	10	25	4	7
	Manure	4	3	0	1	0	2	2	2	1	1	2	1	1
Total		51	-	-	-	-	-	-	-	-	-	-	-	-

Key: S =Sensitive, I=Intermediate, R=Resistance

Table-3: Antimicrobial susceptibility of *E. coli* isolates from cattle faeces and manure

LGA	Sample Type	No. Isolates	Antimicrobial agents											
			Erythromycin (E) (Disc content = 50µg)			Enrofloxacin (ENR) (Disc content = 5µg)			Ceftriaxone (CRO) (Disc content = 30µg)			Cefazolin (KZ) (Dsc content = 30µg)		
			S ≥18	I 13-17	R ≤12	S ≥18	I 14-17	R ≤13	S ≥20	I 15-19	R ≤14	S ≥15	I 13-14	R ≤12
Bauchi	Faeces	5	0	2	3	0	1	4	4	1	0	0	0	5
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Darazo	Faeces	6	0	3	3	0	1	5	2	3	1	0	0	6
	Manure	0	-	-	-	-	-	-	-	-	-	-	-	-
Katagum	Faeces	36	0	10	26	0	9	27	22	3	11	0	**7**	29
	Manure	4	0	1	3	0	0	4	2	1	1	0	**0**	4
Total		51												

Key: S =Sensitive, I=Intermediate, R=Resistance

The antibiotic susceptibility pattern of the isolates (from faecal and manure samples) were determined against twelve (12) antibiotics and the results showed chloramphenicol 40(78.4%) to have the highest susceptibility to the isolates followed by Gentamycin and Imipedin with 35(68.6%) each. The isolates tested were found not susceptible to four (4) of the antibiotics (Enrofloxacin, Cefazolin, Erythromycin and Sulfamethoxazole). With regards to resistance Cefazoline 44(86.2%) was found to show the highest resistance followed by sulfamethoxazole and Enrofloxacin with 42(82.4%) and 40(78.4%) respectively (Table-4 and Figure-1).

Table-4: Antimicrobial susceptibility pattern of 51 *E. coli* isolates from cattle faeces and manure in Bauchi state, Nigeria

Antibiotic tested	Antimicrobial sensitivity pattern		
	No. (%) Susceptible	**No. (%) Intermediate**	**No. (%) Resistant**
Amoxicillin clavulanate	16 (31.4)	14 (27.5)	21 (41.1)
Cefazolin	0 (0.0)	7 (13.8)	44 (86.2)
Ceftriaxone	30 (58.8)	8 (15.7)	13 (25.5)
Chloramphenicol	40 (78.4)	7 (13.8)	4 (7.8)
Ciprofloxacin	23 (45.0)	13 (25.5)	21 (29.5)
Enrofloxacin	0 (0.0)	11 (21.6)	40 (78.4)
Erythromycin	0 (0.0)	16 (31.4)	35 (68.6)
Gentamicin	35 (68.6)	6 (11.8)	10 (19.6)
Imipenem	35 (68.6)	8 (15.7)	8 (15.7)
Tetracycline	23 (45.0)	4 (7.84)	24 (47.0)
Ticarcillinclavulanate	23 (45.0)	14 (27.5)	14 (27.5)
Sulfamethoxazole	0 (0.0)	9 (17.6)	42 (82.4)

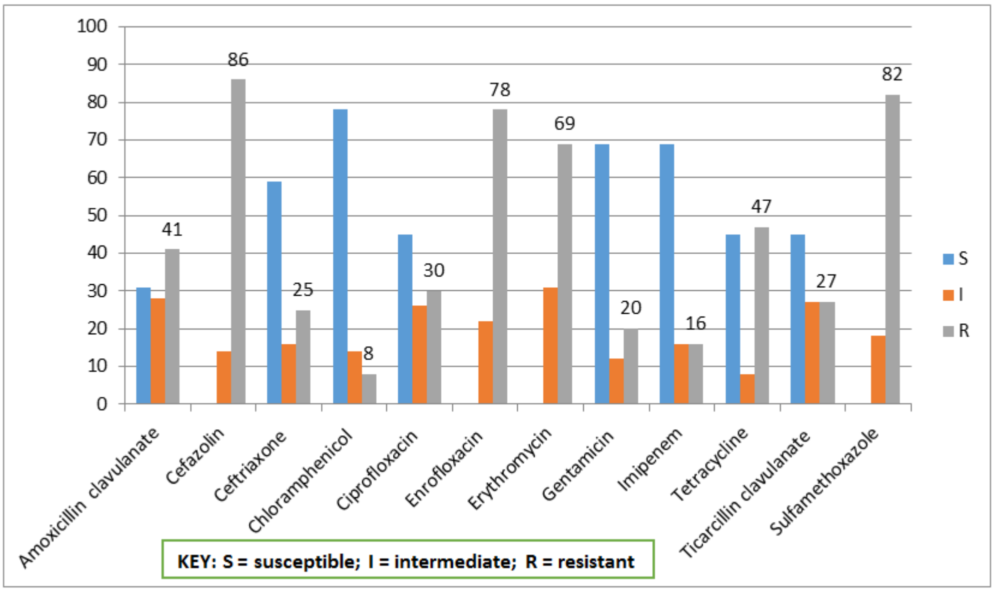

Fig-1: Percentage of Multiple Drug Resistance indices *E .coli*from cattle faeces and manure

All isolates showed Multi-drug resistance pattern (MDR). Some of the isolates showed resistance against up to 8 antimicrobial agents belonging to 7 groups of antimicrobial agents (Table 5 and 6a). Out of the 51 *E. coli* isolates tested against 12 anti-microbial agents 3 sets of isolates were found to be resistant to 4 different types of anti-microbial agents that belong to 4 groups of antibiotics. And also another 3 sets of isolates showed resistance to 5 different antimicrobial agents that belong to 4 different groups of antibiotics. It was also observed that 2 other sets of isolates tested were resistant to 4 and 6 anti-microbial agents that belong to 5 and 3 antibiotic groups respectively. While all other isolates indicated resistance to only one set of antimicrobial agents ranging from 2 – 8 that belongs to a range of 3 – 7 groups of antibiotics (Table 5 and 6).

Table-5: Multi-drug resistance pattern of *E. coli* isolates

Sample No.	Anti-microbial agents showing resistance to *E. coli* isolates	No of Antibiotics	No. of Groups
1, 27, 28	TE, SXT, ENR, KZ	4	4
02	SXT, E, ENR, KZ, AMC	5	4
03	TE, SXT, KZ	3	3
04	TE, SXT, CIP, ENR, KZ, AMC, TIM	7	6
5,6	TE, SXT, E, ENR, KZ	5	4
07	TE, SXT, ENR, CRO, KZ, TIM	6	5
08	IPM, E, CRO, KZ, AMC, TIM	6	4
09	TE, C, SXT, E, CIP, ENR, KZ, AMC	8	7
10, 33, 39	SXT, E, CIP, ENR, KZ	5	4
11	SXT, E, CRO, KZ,	4	3
12	SXT, E, KZ, TIM	4	4
13	TE, E, ENR, CRO, CN, KZ, AMC	7	4
14	E, ENR, CRO, KZ, AMC	5	3
15	IPM, SXT, ENR, CN, KZ, TIM	6	5
16, 17	TE, SXT, E, ENR, KZ, TIM	6	5
18	TE, SXT, E, ENR, KZ, AMC	6	5
19	SXT, E, CRO, KZ, AMC	5	4
20	TE, C, E, ENR, CRO, KZ, AMC	7	5
21	C, SXT, ENR, KZ, AMC	5	5
22	SXT, E, CRO, KZ, AMC	5	4
23	SXT, E, CIP, ENR, CRO, CN, KZ,	7	4
24	TE, SXT, E, ENR, CN, KZ, AMC	7	5

Table-6: Multi-drug resistance pattern of *E. coli* isolates

Sample No.	Anti-microbial agents Resistance to *E. coli* isolates	No of Antibiotics	No. of Groups
29	TE, ENR, KZ, AMC	4	4
30	SXT, E, CIP	3	3
31	TE, SXT, E, CN, KZ	5	4
32	SXT, E, ENR, CN, KZ	5	3
34	SXT, CIP, ENR, CN, KZ, TIM	6	5
35	SXT, ENR, TIM	3	3
36, 37	SXT, E, ENR, TIM	4	3
38	TE, E, CIP, ENR, KZ,	5	4
40	IPM, TE, C, SXT, ENR, CRO, KZ, TIM	8	7
41	TE, SXT, E, CIP, CRO, KZ, AMC	7	6
42	SXT, ENR, CRO, KZ	4	3
43	ENR, KZ,	2	2
44	TE, SXT, E, ENR, CRO, KZ,	6	4
45	SXT, E, CIP, CN, KZ,	5	4
46	SXT, CIP, ENR, KZ, AMC, TIM	6	5
47	IPM, TE, E, CIP, ENR, CN, AMC	7	5
48	IPM, TE, SXT, E, CIP, ENR, KZ, AMC	8	7
49	IPM, E, CIP, KZ,	4	4
50	IPM, SXT, E, ENR, KZ, AMC	6	5
51	IPM, SXT, ENR, AMC	4	4
25	TE, SXT, ENR, CN, KZ, AMC	6	5
26	TE, SXT, E, ENR, KZ	5	4

Furthermore, the results indicated that of the 51 isolates, 50(98%) were resistant to 3 or more of the antibiotics. The results further showed that 3(5.9%) of the isolates were resistant to 8(66.7%) of the antimicrobial agents used in the study, while 7(13.7%) others were resistant to 7(58.3%) different antimicrobial agents and 11(21.6%) of the isolates indicated resistance to 6(50%) antimicrobial agents. Also only 4(7.8%) of the isolates showed a resistance to less than 4(33.3%) of the antimicrobial agents (Table-7).

Table-7: Multiple drug resistance indices (MDR) of *E. coli* isolated from cattle faeces and manure in selected Local Government Areas of Bauchi State, Nigeria

No. of *E. coli* isolates tested	% of Isolates resistance to the drugs tested	MDR Index
1	1.9	0.1
4	7.8	0.2
9	17.6	0.3
16	31.4	0.4
11	21.6	0.5
7	13.7	0.6
3	5.9	0.7
51	**100**	

Discussion

Results of the antibiotic susceptibility testing for *E. coli* isolates indicated many of the isolates showing resistant to commonly used antimicrobial agents and this is an indication of higher risk of urinary tract infections (UTI) and possibly increased hemolytic uremic syndrome (HUS) in individuals especially those associated with the use of manure as stated by Christy *et al.*, [21] that pathogenic bacteria population of public health and environmental significance needs to be controlled by a combination of two or more methods depending on the feasibility and economic viability of the farm. It was also observed that antimicrobial agents were available and accessible to the public even at remote areas and being indiscriminately used, the results also indicated no control of prescription for drugs hawked and are also being openly used and that situation is cautioned by the WHO [22], document that the current practice of indiscriminate use of antibiotics is increasingly leading to resistance to medicines, persistence of infections and treatment failure.

Some of the factors observed to facilitate the above situations include patent chemist shops operated by non-qualified personnel as stated by the WHO [23] that the increasing use of antibiotics has resulted in the development of resistant microorganisms which are causing diseases in the community and hospitals. Poor environmental health due to open gutters was also seen around the study areas. The MDR patterns of *E. coli* O157:H7 for this research is similar to one obtained in a recent work conducted in Zaria by Igwe *et al.*, [24] where they evaluated phenotypic virulent characters of the organism that contributes to the expression of MDR properties of *E. coli*. Their work showed *E. coli* to be largely resistant to tetracycline, sulfonamide etc. and have also observed a similar MDR pattern obtained in this research with a significant number 51(59.3%) of the *E. coli* resistant to 4 and above antibiotics tested in their work.

The MDR pattern of *E. coli* is of concern as shown in this research work and as in a similar research carried out in Lagos by Adenipekun *et al.*, [25] which showed that the emergence of MAR of two (2) or more antimicrobials encountered in the treatment of infected patient with UTI. The studies demonstrated circulating MDR *E. coli* in Nigerian community and suggested monitoring of it as to be of paramount importance. The result also found that a similar research indicates food producing animals in Nigeria as a reservoir of MDR *E. coli* that may be transferred from animals to humans via the food chain. The MAR index of 0.2 observed in this research i.e. 98% of the *E. coli* isolates have multiple resistance to 3 or more of the antimicrobial agents used, the results clearly showed an indiscriminate use of different classes of the antimicrobials which is an indication of lack of control or monitoring of use of such antibiotics in the prevention or treatment of animals or as growth promoters in some instances. This is as indicated in a research by Alien, *et al.*, [26] who stated that the indiscriminate use of antibiotics in animal husbandry should be discouraged because of the resistant pathogens that can be transmitted to humans through consumption of foods harvouring such pathogens.

Of great importance and concern to this research is the MAR Index of 98% recorded especially when compared to MAR Index of 96.4% recorded by Umaru *et al.*, [27] in a separate research carried out in Zaria, the value shows a slight increase in the MAR index despite the variation in samples and location where the research was carried out. This situation actually calls for enforcement of control measures against the indiscriminate use of antibiotics by farmers in the treatment of their animals.

The resistance patterns of the 51 *E. coli* isolates showed all (except one) were resistant (98%) to three antibiotics or more, with highest resistance recorded on Cefazolin (86.2%), Sulfamethoxazole (82.4%), Enrofloxacin (78.4%) and Erythromycin (68.6%) this result is similar though with slightly lower MAR Index than what was obtained by Avijit *et al.*, [28] in Khulshi Bangladesh with 100% MAR index for the *E. coli* isolates to the antimicrobial agents tested. And this research also indicated an upward increase trend of antimicrobial resistance in pathogens which was as predicted by Tadesse *et al.*, [29]. Also, the antimicrobial resistance pattern showed by the isolates in this research is similar to what has been obtained by Bello *et al.*, [30] and therefore serves as an indication that adequate measure need be taken to regulate drug use in both humans and animals in order to minimize the risk of increasing antimicrobial resistance.

The high MAR and the multi-drug resistance pattern observed in this research work is alarming especially considering the fact that drugs that are not known to be used for the treatment of animals in the study area were found to show resistance. The above phenomenon may be attributed to horizontal gene transfer especially considering the fact that this same research has detected antimicrobial resistance genes in some of the isolates showing these MAR, and could be linked to what has been stated by Igwe *et al*., [31] that it is imperative to checkmate the rate at which over the counter drugs are sold and antibiotic misused in animal feeds, as it will play a key role in decreasing the emergence of resistance bacteria trains within our environment.

Moreover, the higher susceptibility indicated by some of the *E. coli* isolates to Chloramphenicol (78.4%), Gentamycin (68.6%), Imipenem (68.6%) and Ceftriaxone (58.8%) give some respite that few common antimicrobial agents among the commonly used ones in the study area have showed high level of susceptibility and this is in line with the statement by Alonso *et al*., [32] that rapid increase in the rate of antimicrobial resistance (AMR) reinforced by some opposite tendency in development of new active drugs may no longer be a health threats as recognized by World Health organization (WHO).

CONCLUSION

All the 51 isolates that were tested for antimicrobial susceptibility for this research work showed that 2(4.2%) were from the abattoirs, 22(45.8%) from the CF while 24(50.0%) from the LM. And that all the isolates tested for susceptibility showed MDR with some isolates showing resistance to up to 8 antimicrobial agents belonging to 7 groups of antimicrobial agents. The MAR index of 0.2 observed for this research work i.e. 98% of the *E. coli* isolates have multiple resistance to 3 or more of the antimicrobial agents used.

Government should enforce laws preventing the indiscriminate and unauthorized use of drugs for treatment and prevention of animal. Relevant agencies should collaborate to organize stakeholders' meetings on ways to curtail the abuse and unauthorized use of antibiotics. Clinicians should avoid or minimize unnecessary use of multiple antibiotics in the treatment of clinical disease conditions

REFERENCES

1. Carattoli, A. (2001). Importance of integrons in the diffusion of resistance. *Veterinary research*, *32*(3-4), 243-259.
2. Levy, S. B. (2002). Active efflux, a common mechanism for biocide and antibiotic resistance. *Journal of Applied Microbiology*, 92:65-71.
3. Carattoli, A. (2003). Plasmid-mediated antimicrobial resistance in Salmonella enterica. *Current issues in molecular biology*, *5*(4), 113-122.
4. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). (2000). Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. *Clinical microbiology and infection*, *6*(9), 503-508.
5. Acar, J., & Rostel, B. (2001). Antimicrobial resistance: an overview. *Revue Scientifique et Technique-Office International des Epizooties*, *20*(3), 797-810.
6. Neu, H. C. (1992). The crisis in antibiotic resistance. *Science*, *257*(5073), 1064-1073.
7. Witte, W. (1998). Medical consequences of antibiotic use in agriculture, *Science*, 279: 996-997.
8. Alhaj, N., Mariana, N. S., Raha, A. R., & Ishak, Z. (2007). Prevalence of antibiotic resistance among Escherichia coli from different sources in Malaysia. *International Journal of Poultry Science*, *6*(4), 293-297.
9. Stewart, P. S., & Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. *The lancet*, *358*(9276), 135-138.
10. Timothy, R., Keller, O. C., Pancorbo, W. C., & Barnhart, H. M. (1997). Antibiotic resistance of bacteria in biofilms. *The lancet*, *358*(9276), 135-138.
11. Barton, M. D. (1998). Does the use of antibiotics in animals affect human health?. *Australian veterinary journal*, *76*(3), 177-180.
12. Danishta, I., Ismet, M., Sonatun, D., & Jaufeerally-Fakim, Y. (2010). Antibiotic resistance of Escherichia Coli isolates from environmental and waste water samples in Mauritius. *Advances in Environmental Biology*, *4*(1), 1-9.
13. Kastner, S., Perreten, V., Bleuler, H., Hugenschmidt, G., Lacroix, C., & Meile, L. (2006). Antibiotic susceptibility patterns and resistance genes of starter cultures and probiotic bacteria used in food. *Systematic and Applied Microbiology*, *29*(2), 145-155.
14. Salyers, A. A., Gupta, A., & Wang, Y. (2004). Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends in microbiology*, *12*(9), 412-416.
15. Schroeder, C. M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D. G., ... & Meng, J. (2002). Antimicrobial resistance of Escherichia coli O157 isolated from humans, cattle, swine, and food. *Applied and Environmental Microbiology*, *68*(2), 576-581.
16. Noyes, N. R., Yang, X., Linke, L. M., Magnuson, R. J., Dettenwanger, A., Cook, S., ... & Yang, H.

(2016). Resistome diversity in cattle and the environment decreases during beef production. *Elife*, *5*, e13195.
17. Durso, L. M., Harhay, G. P., Bono, J. L., & Smith, T. P. (2011). Virulence-associated and antibiotic resistance genes of microbial populations in cattle feces analyzed using a metagenomic approach. *Journal of microbiological methods*, *84*(2), 278-282.
18. Wichmann, F., Udikovic-Kolic, N., Andrew, S., & Handelsman, J. (2014). Diverse antibiotic resistance genes in dairy cow manure. *MBio*, *5*(2), e01017-13.
19. Hautbergue, G. M., Castelli, L. M., Ferraiuolo, L., Sanchez-Martinez, A., Cooper-Knock, J., Higginbottom, A., ... & Alam, S. M. (2017). SRSF1-dependent nuclear export inhibition of C9ORF72 repeat transcripts prevents neurodegeneration and associated motor deficits. *Nature communications*, *8*, 16063.
20. Sozmen, F., Uysal, B., Oksal, B. S., Kose, E. O., & Deniz, I. G. (2011). Chemical composition and antibacterial activity of Origanum saccatum PH Davis essential oil obtained by solvent-free microwave extraction: Comparison with hydrodistillation. *Journal of AOAC International*, *94*(1), 243-250.
21. CLSI (Clinical and Laboratory Standards Institute). (2014). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. National Committee for Clinical Laboratory Standards, Wayne, Pennslyvania, Approved Standard M 100-S24, (11).
22. Manyi-Loh, C. E., Mamphweli, S. N., Meyer, E. L., Makaka, G., Simon, M., & Okoh, A. I. (2016). An overview of the control of bacterial pathogens in cattle manure. *International journal of environmental research and public health*, *13*(9), 843.
23. World Health Organization. (2014). The role of pharmacist in encouraging prudent use of antibiotics and averting antimicrobial resistance: A review of policy and experience in Europe. *WHO Regional Office for Europe Publications*.
24. Igwe, E. O., & Charlton, K. E. (2016). A systematic review on the health effects of plums (Prunus domestica and Prunus salicina). *Phytotherapy Research*, *30*(5), 701-731.
25. Adenipekan, E. D., Jackson, C. R., Oluwadun, A., Iwalokun, B. A., Fry, J. G., Barrett, J. B., Hiott, L. M., &Woody, T. A. (2016). Prevalence and Antimicrobial resistance in *E. coli* from food animals in Lagos, Nigeria. *Microbial Drug Resistance*, 30(5):256-266.
26. Alian, F., Rahimi, E., Shakerian, A., Momtaz, H., Riahi, M., & Momeni, M. (2012). Antimicrbial Resistance of staph aureus islatedfrom bovine, sheep and goat raw milk Global Veterinarian, 8(2): 111-114.
27. Umaru, G. A., Kwaga, J. K. P., Bello, M., Raji, M. A., & Maitala, Y. S. (2016). Antibiotic resistance of staphylococcus aureus isolated from fresh milk in settled Fulani herds in Kaduna state, Nigeria. *Bull Animal Health Production Africa*, 64,173-183.
28. Aviji, D. M., & Sabuj, K. (2016). Multi-drug resistance pattern of *E. coli* isolated from hospital effluent and detection of tetracycline resistance gene. *Journal of Infectious Molecular Biology*.
29. Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950–2002. *Emerging infectious diseases*, *18*(5), 741-749.
30. Bello, F. D., Lavorel, S., Lavergne, S., Albert, C. H., Boulangeat, I., Mazel, F., & Thuiller, W. (2013). Hierarchical effects of environmental filters on the functional structure of plant communities: a case study in the French Alps. *Ecography*, *36*(3), 393-402.
31. Igwe, O. U., & Okwu, D. E. (2013). GC-MS Evaluation of bioactive compounds and antibacterial activity of the oil fraction from the seeds of Brachystegia eurycoma Harms. *Asian Journal of Plant Science and Research*, *3*(2), 47-45.
32. Alonso, C. A., Zarazaga, M., Ben Sallem, R., Jouini, A., Ben Slama, K., & Torres, C. (2017). Antibiotic resistance in Escherichia coli in husbandry animals: the African perspective. *Letters in applied microbiology*, *64*(5), 318-334.