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Identification of *Escherichia coli* Isolated from Cow, Cow Workers, Farms, Shared Farm Environments in Karbala Governorate and Evaluation of its Antibiotic Resistance

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Abstract: Cow farming involves frequent contact among animals, workers and farm environments. To investigate *E. coli* that occurs on cow farms and its antibiotic susceptibility, a pilot study included samples from cow workers, cows, and the farm environments from five farms in Karbala governorate. Samples were taken from the nasals and hands of consenting workers (n=100), teats and nasals of selected cows (n=100) and shared environmental (n=200). Samples were processed bacteriologically and the isolates were tested with morphological and a number of biochemical tests for confirmation and identification. Also, diagnosis was done by Vitek- 2 system after subculture and purification. According to the standard morphological and biochemical protocols for isolation and identification that revealed an overall prevalence of 33(8.25%) of the collected 400 samples were contaminated with *E. coli*. All isolates presumptive *E. coli* given positive result for Vitek-2 system identification. *E. coli* possessed resistance to some antibiotics. In contrast, antibiotic such as Ertapenem, Imipenem, Meropenem and Nitrofurantoin were effective against *E. coli* (100%). Result indicates the presence of *E. coli* on cows, human and shared environmental, and possessed varying degrees of resistance to antibiotics.

Keywords: *E. coli*, cow farms, antibiotic resistance.

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Introduction

Escherichia coli were first isolated from the feces of a child in 1885 by the Austrian pediatrician Theodor *Escherich*(1). *It* has been frequently isolated from the farms and is ubiquitous in environment (2). Feed has been involved as a source that not only acts as a vehicle but could possibly allow for replication. Several sources have been involved with feed and fecal matter being the most common followed by water, flies and wildlife (3). *E. coli* was reported as a dominant inhabitant of the healthy human gut microbiome (4). It is mainly enteric bacteria in animals and can also survive in the environment, like dairy products and materials contaminated with faucal (5).

Among the major infectious agents, *E. coli* has been associated with milk and some of dairy products (6). *E. coli* that belong to the family Enterobacteriaceae, is Gram-negative, facultative anaerobic, rod-shaped and highly motile bacteria and a normal inhabitant of the intestines of animals

and humans (7, 8), however, its recovery from food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to wide variety of enteric and extraintestinal diseases in animals (8).

Escherichia coli are chemo-organotrophic microorganism. Approximately 95% of *E. coli* strains are indole and methyl red positive, but are Voges-Proskauer and citrate negative. It is oxidase negative, catalase positive, fermentative of glucose, D-mannitol, D-sorbitol, arabinose, maltose, reduces nitrate and are motile at 36°C. They ferment Lactose in MacConkey agar to produce lactic acid. *E. coli* is positive in beta glucuronidase identification assay due to the presence of the beta glucuronidase enzyme activity in *E. coli*(9, 10). Its isolates exhibited bright pink color with lactose fermentation on MacConkey agar plates, metallic sheen on Eosin Methylene Blue agar plate and gram-negative, pink-colored, small rod-shaped organisms arranged in single with pairs or short chains on Gram's staining (11).

The development of Antimicrobial Resistance for *E. coli* in the intestinal tract is assumed due to resource competition with other microbial communities (12). Since commensal bacteria are the reservoir of antibiotic resistance genes present in a community, and the uncontrolled use of antibiotics is associated with an increase in antibiotic resistance in pathogens (13). Around 50–60% of *E. coli* infections acquired in the community have become resistant to commonly used oral antibiotics (e.g., amoxicillin, cefixime, and ciprofloxacin), making outpatient treatment difficult (14). Some studies have illustrated multidrug-resistant pathogens in the soil and water of farm environments. The ESBL-variants detected corresponded to those previously found in animals or humans living in a farm environment (15). In Iraq, there is great concern about the use of veterinary drugs by livestock farmers who have no knowledge in treating cattle without first determining their infection status. Therefore, this study aimed to isolate, identification and evaluation of antimicrobial resistance of *Escherichia coli* from cow farms, workers and shared farm environments in Karbala Governorate-Iraq.

Materials and methods

Study area

The study was conducted in Karbala governorate, Iraq. Five locations from Karbala (Al-Ibrahimia, Al-Amriyah , Umm Al-Hol , Al-Atishi and Abu Qatna) were included in this study.

Sampling, sample types, and sample collection

A total of 400 specimens were collected during period from June 2021 to October 2021, ten cows from each farm were selected for sampling, as well as, all workers who consented and could be sampled during a field visit. Environmental sampling based on taking at least two samples for each area. At least total 50 samples of cow's milk, 50 swabs from cows' noses, 50 swabs from udder teat, 50 swabs from the noses and 50 swabs from hands of farm workers and environmental samples (50 swabs from milking tools, 50 swabs from the feeding place and 50 swabs from the cows' shelter).

Isolation of *E. coli*

All samples were transferred to the laboratory where they were processed within 24 hr of collection as previously described (16). The samples were cultured on MacConkey agar and blood agar and. Cultured samples were incubated aerobically at 37 °C for 24 hr. Colonies developed show characteristic growth; the suspected colonies were cultured for further determination. Almost all workers wore gloves during milking. Farm workers were invited to volunteer to participate in the survey and sampling.

Identification of *Escherichia coli*

Twenty-five grams of sub-samples of feed place were analyzed. Under aseptic conditions, one ml of each milk sample was drawn. For swabs 1ml of sampling buffer was squeezed out and sampled. Then transferred into sterile container and 225 ml of TSB were added supplemented with vancomysin (0.8 mg/L) or no according to Brando *et al*. (17) and incubated for 6-24 hr at 42 °C. After 24 hr enrichment, aliquots of 100 µl of the cultivated broth were streaked onto Eosine methylene Blue (EMB) agar to presumptively identify isolates as . *E. coli* have exhibited blue-black color with metallic green sheen colonies, and onto Sorbitol MacConkey agar to test for sorbitol non-fermenting bacteria (colorless colonies) and sorbitol fermenting bacteria. The purified colonies were then streaked onto nutrient broth and incubated at 37 ^{0}C for 18-24 hr for further identification. Samples that tested positive were then subject to confirmatory methods.

Biochemical characteristics and Vitek- 2 System diagnostic

The isolated strains were subjected to a number of different biochemical tests to confirm *E. coli.* Methyl red test,indole production test, Oxidase test, Voges-Proskauer test,Motility Test and Urea test were performed on all suspected isolates to confirm the *E. coli*. Presumptive positive *E. coli* isolates were then confirmed using the Vitek-2 system according to manufacturer's directions.

Antimicrobial susceptibility testing

All *E. coli* isolates were subjected to antibiotic susceptibility tests using the Vitek- 2 card (bioMérieux) according to the manufacturer's instructions.The antimicrobial used in the experiment included Ampicillin, Ampicillin/Clavulanic Acid, Piperacillin/ Tazubactam, Cefotaxime, Ceflazidime, Cefepime, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Norfloxacin, Fosfomycin, Nitrofurantoin, Trimethoprim /Sulfamethoxazole. All the results of antimicrobial susceptibility were interpreted according to CLSI guideline (18).

Statistical analysis

Data was analyzed by the Statistical Analysis System- SAS program (19). Chi-square test was used to significant compare between percentage (0.01 probability) in this study.

Results and discussion

Isolation and identification of *Escherichia coli*

In current study, a total of 400 samples were collected and processed bacteriologically and biochemical tests

were performed to detect *E. coli*. The results of the present study revealed that out of 400 samples, 33 samples were found to be positive for *E. coli*. Isolates were characterized as bright pink color on MacConkey agar plates and showed blue-greenish metallic sheen on Eosin Methylene Blue (EMB) agar plate (Figure1). Upon Gram's staining of the isolates under 1000x using light microscope by using oil immersion, pink-colored, small rod-shaped organisms arranged in single, pairs or short-chain bacilli and Gram-negative were identified. On EMB agar pure colonies of *E. coli* appear to be green metallic sheen colonies and as 2-3 mm diameter (20). These results were agreed with other researchers (21, 22, 11).

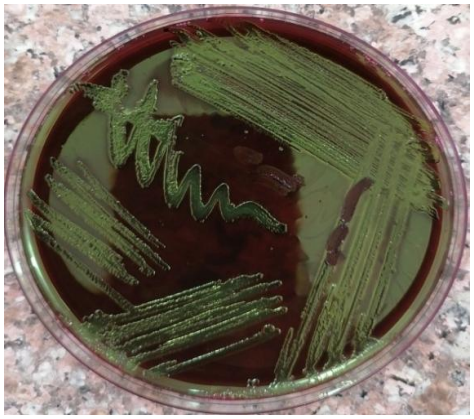

Figure (1): Colonies of presumptive *E. coli* on EMB agar.

Biochemical characterization

A total of 33 isolates were identified as *E. coli* confirmed using standard biochemical tests for *E. coli* were given positive results. *E. coli* isolates were identified by positive indole and methyl red tests, and negative for Voges-Proskauer test. These results coincide with the results of (24,19,11). Also, positive oxidase and motility, and negative for urea test (Table1) which are in agreement with the reports of (23, 20).

Table (1): Biochemical characterization of *E. coli*

Biochemial Test	Reaction
Indole Production	Positive
Methyl Red	Positive
Voges- Proskauer	Negative
Oxidase	Positive
Motility	Positive
Urease	Negative

Identification by using viteck-2 system

The colonies were confirmed by using Vitek- 2 identification Gram negative cards (bioMérieux). The results shown that all 33 isolates presumptive *E. coli* given positive result. Vitek-2 system identification method is an acceptable automated method for the rapid identification of Gram-negative bacteria (24). Ali (22) reported ability of Vitek-2 system to identify more than 163 fermentative and non-fermentative Gram-negative bacilli

that was identified previously by API 20 E.

Antibiotic sensitivity testing

It is not uncommon that drugs are different in their efficacy. Thus, 33 samples positive for *E. coli* from 400 samples were tested for antimicrobial susceptibility analyzed by Vitek-2 system (Table2). In this study, findings revealed that all *E. coli* isolates were significantly (P≤0.01) susceptible to the 16 drugs tested except for Norfloxacin, with a susceptible to Ertapenem, Imipenem, Meropenem and Nitrofurantoin (100%)) which disagreed with reports published by (25) that all *E. coli* isolates exhibited susceptibility to ciprofloxacin and sulfamethoxazole. All *E. coli* isolates showed considerable sensitivity to imipenem or meropenem 100% (Table2), which are considered the last line of defense in the treatment of infections caused by multi-resistant Gram-negative bacteria, similar results obtained by (26). The association of determinants at the human-animal-environment interface can alter microbial genomes, resulting in resistant superbugs in various niches (27).

It was found that *E. coli* possessed varying degrees of resistance to 12 antibiotics with significant resistance (P≤0.01) for Norfloxacin 17/52%). Antimicrobial resistance patterns were observed most commonly to ampicillin (30%), Trimethoprim/Sulfamethoxazole (30%) and less frequently to Piperacillin/ Tazubactam (9%). Cefotaxime (9%) and Ciprofloxacin (12%) (Table2). Which disagreed with the results of (28) who reported that the highest rate of resistance was against amoxicillin (90.6%), followed by 77.7% resistance to cefotaxime, 75.7% to cefuroxime, and 70.8% to ofloxacin. Interestingly, only 3% of *E. coli* isolates were resistant to amoxicillin-clavulanic acid, while (25) and (29) reported a significantly higher percentage 14 and 100%, respectively. Antimicrobial resistant bacteria are considered one of the major public health concerns worldwide. Mastitis is the most common cause of antimicrobial use in dairy herds, so bovine milk is considered as potential source of multidrug-resistant bacteria in the agricultural environment (30). This minimizes the effectiveness of treatments and the ability to control infectious diseases in animals and humans, facilitating the spread of bacteria resistant to antimicrobials (31, 32), mainly due to the close relationship established in dairy farms between different animal species and humans (33, 34).

Results of this study showed 2(6%), 1(3%), 3(9%) and 3(9%) were intermediate. However, the results reported by (35) and (25) were relatively similar to current study where the in *vitro* growth *E. coli* was restrained by gentamicin, ciprofloxacin.

Table (2): Antibiotic susceptibility patterns of *E. coli* isolates

Antibiotic	R^{+}	I^{++}	S^{+++}	P-value
Ampicillin	10(30%)	0(0%)	23(70%)	0.0001 **
Ampicillin/Clavulanic Acid	1(3%)	2(6%)	30(91%)	0.0001 **
Piperacillin/Tazubactam	3(9%)	0(0%)	30(91%)	0.0001 **
Cefotaxime	3(9%)	0(0%)	30(91%)	0.0001 **
Ceflazidime	8(24%)	0(0%)	25(76%)	0.0001 **
Cefepime	6(18%)	1(3%)	26(79%)	0.0001 **
Ertapenem	0(0%)	0(0%)	33(100%)	0.0001 **
Imipenem	0(0%)	0(0%)	33(100%)	0.0001 **
Meropenem	0(0%)	0(0%)	33(100%)	0.0001 **
Amikacin	5(15%)	3(9%)	25(76%)	0.0001 **
Gentamicin	5(15%)	3(9%)	25(76%)	0.0001 **
Ciprofloxacin	4(12%)	0(0%)	29(88%)	0.0001 **
Norfloxacin	17(52%)	0(0%)	16(48%)	0.0001 **
Fosfomycin	3(9%)	0(0%)	30(91%)	0.0001 **
Nitrofurantoin	0(0%)	0(0%)	33(100%)	0.0001 **
Trimethoprim /Sulfamethoxazole	10(30%)	0(0%)	23(70%)	0.0001 **
** ($P \leq 0.01$).				

#= 33 *E. coli* isolates. R^{+}= Resistant; I^{++}= Intermediate; S^{+++}= sensitive.

Multi drug resistance (MDR) is defined as resistance of an isolate to more than two antimicrobials tested. In terms of the *E. coli* multi-drug resistance in current study, it can be divided into three classes: the first is resistant to one antibiotic, the second is resistant to two antibiotics, and the third is resistant to more than two antibiotics. Similar results were obtained by (36) was 64% of *E. coli* isolates were resistant to one antimicrobial; 18% were resistant to two antimicrobials; and 18% were resistant to > 2 antimicrobials. Also are consistent with the findings of (37) who reported that multiple drug resistance was also seen in 15 (18.3%) *E. coli* and 5 (14.3%) *E. coli* O157:H7 isolates, and with the findings of (38,39) who observed MDR in 15 of the isolates, they detected eleven different MDR patterns including one isolate resistant to seven antimicrobials.

Conclusions

Overall, the study found a low prevalence rate of *E. coli* which varied between the five areas. The study revealed that relatively a number of strains are resistant to the antibiotics commonly used in the therapeutic protocol of many human and animal infections. Therefore, antimicrobial susceptibility test should be carried out at a regular basis and proper hygienic practices should be introduced at farm level. The data from this study showed that these farms can serve as reservoir of multi-drug resistant organism.

References

1. Meng, J. and Schroeder, C. M. (2007). *Escherichia coli. In* Foodborne Diseases. S. Simjee and N.J. Totowa, NJ (eds.). Humana Press, p. 1–25.
2. Sheng, H.; Shringi, S.; Baker, K.N.; Minnich, S.A.; Hovde, C.J. and Besser, T.E. (2015). Standardized *Escherichia coli* O157:H7 exposure studies in cattle provide evidence that bovine factors do not drive increased summertime colonization. Applied Environmental Microbiology., 82 (26607594): 964-971.
3. Berry, E. D., & Wells, J. E. (2010). Escherichia coli O157: H7: recent advances in research on occurrence, transmission, and control in cattle and the production environment. Advances in Food and Nutrition Research, 60, 67-117.

4. Nazareth, J.; Shaw, A.; Delate, K. and Turnbull, R. (2021). Food safety considerations in integrated organic crop–livestock systems: prevalence of *Salmonella* spp. and *E. coli* O157:H7 in organically raised cattle and organic feed. Renewable Agriculture and Food Systems, 36: 8–16.
5. Mohammed, A.N.; Abdel-Latef, G.K.; Abdel-Azeem, N.M. and El-Dakhly, K.M. (2016). Ecological study on antimicrobial-resistant zoonotic bacteria transmitted by flies in cattle farms. Parasitology Research, 115: 3889–3896.
6. Bedasa, S.; Daniel, S.; Ashebr, A. and Tesfanesh, M. (2018). Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157:H7 from food of animal origin in Bishoftu town, Central Ethiopia. International Journal of Food Contamination, 5:2.
7. Virpari, P., Nayak, J., Thaker, H.and Brahmbhatt, M. (2013). Isolation ofpathogenic *Escherichia coli* from stool samples of diarrhoeal patients with history of rawmilk consumption. Veterinary World, 6(9): 659-663.
8. Asmelash, T. (2015). Isolation, Identification, Antimicrobial Profile and Molecular Characterization of Enterohaemorrhagic *E. coli* O157: H7 Isolated from Ruminants Slaughtered at Debre Zeit Elfora Export Abattoir and Addis Ababa Abattoirs Enterprise M.S.c Thesis. Faculty of Veterinary Medicine, Addis Ababa University.
9. Balows, A.; Hausle,r W. J.; Herrmann, K. L.; Isenberg, H.D. and Shadomy ,H.J.(1991). Manual of clinical Microbiology.
10. Eppinger, M.; Mammel, M.K.; Leclerc, J.E.; Ravel, J. and Cebula, T.A (2011). Genomic anatomy of *E. coli* O157:H7 outbreaks. Proc. National. Academy Science U S A., 108: 20142- 20147.
11. Megersa, R.; Mathewos, M. and Fesseha, H. (2019). Isolation and identification of *Escherichia coli* from dairy cow raw milk in Bishoftu Town, Central Ethiopia. Chives of Veterinary and Animal Sciences, 1(1):1-7.
12. Baumgartner,M.;Bayer,F.; Pfrunder-Cardozo, K.R.;Buckling,Aand Hall,A.R.(2020). Resident microbial communities inhibit growth and antibiotic-resistance evolution of *Escherichia coli* in human gut microbiome samples. PLoS Biology.,18(4): e3000465.
13. Singh, A.K.; Das, S.; Singh, S.; Gajamer, V.R.; Pradhan, N.; Lepcha, Y.D. *etal*.(2018). Prevalence of antibiotic resistance in commensal *Escherichia coli* among the children in rural hill communities of Northeast India. PLoS One,13(6): e0199179.
14. Laxminarayan, R.; Duse, A.; Wattal, C.; Zaidi, A.K.; Wertheim, H.F.; Sumpradit, N. *et al*. (2013). Antibiotic resistance the need for global solutions. Lancet. Infectious Diseases,13 (12):1057–98.
15. Purohit, M.R.; Chandran, S.; Shah, H.; Diwan, V.; Tamhankar,A.J. and Stålsby Lundborg, C.(2017). Antibiotic resistance in an Indian rural community: A 'One-Health' observational study on commensal coliform from humans, animals, and water. International Journal Environmental Research Public Health,14: 386.
16. Soge, O.O.; No, D.; Michael, K.; Dankoff, J.; Lane, J.; Vogel, K. *et al.* (2016). Transmission of MDR MRSA between primates, their environment and personnel at a United States primate centre. J. Antimicrobial Chemotherapy.71:2798–803.
17. Brando, R. J.; Miliwebsky, E.; Bentancor, L.; Deza , N. ; Baschkier, A. ; Ramos, M. V. ,*et al*. (2008). Renal damage and death in weaned mice after oral infectionwith Shiga toxin 2-producing *Escherichia coli* strains. Clin. Exp. Immunol., 153:297–306.
18. CLSI (Clinical and Laboratory Standards Institute). (2018). Performance Standards for Antimicrobial Susceptibility Testing. M100- Ed28. Wayne, PA.
19. Statistical Analysis System (SAS).(2018). User's Guide Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
20. Hsien, C. J. (2010). Isolation and Identification of *Escherichia coli* from Raw Vegetables, in Kuching, Sarawak. Bachelorthesis. Faculty of Resource Science and Technology University, Malaysia, Sarawak.
21. Hasan, M.S.; Yousif, A.A and Alwan, M.J. (2016). Detection of virulent genes in *E. coli* O157:H7 isolated from puppies and adult dogs by polymerase chain reaction. Research Journal Veterinary Practice., 4(1): 1-6.
22. Ali, S.H. (2017). Expression of shiga toxin gene in *Escherichia coli* serotype O157: H7 and O104:H4 isolated from clinical and food samples before and after treatment with probiotics. Ph.D. Thesis. Institute of

Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

23. Adams, M.R. and Moss, M.O. (2008). Food Microbiology. The Royal Society of Chemistry, Cambridge: UK.
24. Naser, A. A. H. (2016). Special biochemical profiles of Escherichia coli strains isolated from humans and camels by the VITEK 2 automated system in Al-Ahsa, Saudi Arabia. African Journal of Microbiology Research, 10(22), 783-790.
25. Shalaby, H.Y.; Baraka, K.; Ibrahim, M.S. and Khalaf, E.M. (2019). Characterization of bacterial pathogens associated with milk microbiota in Egypt. African Journal of Microbiology Research, 13(28):580-608.
26. Fahim, K.M.; Ismael, E.; Khalefa, H.S.; Farag, H.S. and Hamza, D. A. (2019).Isolation and characterization of *E. coli* strains causing intramammary infections from dairy animals and wild birds. International Journal of Veterinary Science and Medicine, 7(1): 61-70.
27. Aslam, M.; Nattress, F.; Greer, G.; Yost, C.; Gill, C. and McMullen, L. (2003). Origin of contamination and genetic diversity of *Escherichia coli* in beef cattle. Appl. Environ. Microbiol., 69, 2794–2799.
28. Olowe, O.A.; Aboderin, B.W.; O Idris, O.; Mabayoje, V.O.; Opaleye, O. O.; Adekunle, O.C. *et al*. (2014). Genotypes and phenotypes of Shiga toxin-producing *Escherichia coli* (STEC) in Abeokuta, Southwestern Nigeria. Infection and Drug Resistance,7: 253 – 259.
29. Nobili, G.; Franconieri, I.; Basanisi, M.G.; La Bella, G.; Tozzoli, R.; Caprioli, A. *et al*. (2016). Short communication: Isolation of Shiga toxin-producing *Escherichia coli* in raw milk and mozzarella cheese in southern Italy. Journal of Dairy Science, 99(10):7877-7880.
30. Chandrasekaran, D.; Venkatesan, P.; Tirumurugaan, K. G.; Nambi, A. P.; Thirunavukkarasu, P. S.; Kumananet, K. *et al.*(2014a). Pattern of antibiotic resistant mastitis in dairy cows. Veterinary World, 7 (6):389–394.
31. Chandrasekaran, D.; Venkatesan, P.; Tirumurugaan, K. G.; Nambi , A. P. ; Thirunavukkarasu, P. S. ; Kumananet, K. *et al.* (2014b). A study on methicillin resistant *Staphylococcus aureus* mastitis in dairy cows. Journal of Applied and Natural Science, 6(2):356–361.
32. Silva, VA, J. G.; Araujo, W.J.; Leite, E.L.; Dias, L.M.; Vasconcelos, P. C.; Silva, N.M.V. *et al*. (2021). First report of a livestock- associated methicillin resistant *Staphylococcus aureus* ST126 harbouring the *mecC* variant in Brazil. Trans bound and Emergence Disease, 68(3):1019-1025.
33. Silva, N. C. C. ; Guimarães, F.F.; Manzi, M.P.; Fernandes Junior, A.; Gomez-Sanz, E.; Gomez, P. *et al*. (2014). Methicillin-resistant *Staphylococcus aureus* of lineage ST398 as cause of mastitis in cows. Letters in Applied Microbiology, 59(6): 665–669.
34. Raymundo, N. K. L.; Bersot, L-D. S. and Osaki, S.C. (2018). Consumer profile and problems associated with uninspected raw milk consumption in western Paraná. Arquivos do Instituto Biológico, 84:1–8.
35. Tadesse, H.A.; Gidey, N.B.; Workelule, K.; Hailu, H.; Gidey, S.; Bsrat A. *et al*. (2018). Antimicrobial Resistance Profile of *E. coli* Isolated from Raw Cow Milk and Fresh Fruit Juice in Mekelle, Tigray, Ethiopia. Vetrinary Medicine International, 19:8903142.
36. Al-harbi, H.; Ranjbar, S.; Moore, R.J. and Alawneh, J.I. (2021). Bacteria isolated from milk of dairy cows with and without clinical mastitis in different regions of Australia and their AMRprofiles. Front Veterinary Science, 8:743725, 17 pages.
37. Tassew, A. (2015). Isolation, Identification, Antimicrobial Profile and Molecular Characterization of Entrohaemorrhagic *E. coli* O157:H7 Isolated from Ruminants Slaughtered at Debre Zett Elfora Export Abattoir and Addis Ababa Abattoirs Enterprise.MS.c. Thesis, College of Veterinary Medicine and Agriculture of Addis, Ababa University, Ethiopia.
38. Cho, S.; Hiott, L.M.; Barrett, J.B.; McMillan, E.A.; House, S.L.; Humayoun, S.B. *et al*. (2018). Prevalence and characterization of *Escherichia coli* isolated from the Upper Oconee Watershed in Northeast Georgia. PLoS One, 13(5): e0197005, 15 pages.
39. Ali, F. A. (2018). Distribution of CTX-M gene among *Escherichia coli* strains isolated from different clinical samples in Erbil City. Iraqi Journal of Biotechnology, 17(1)