Benha Veterinary Medical Journal 45 (2023) 141-146

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Molecular exploring of multidrug resistance update of *E. coli* causing neonatal calves' diarrhea.

Aya M. Esmail[1], Amal M. Rgab[2], Amira M. Rizk[1]

[1]*Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2]*Animal Health Research Institute, Tanta branch, Egypt.*

ARTICLE INFO

Keywords

E. coli

Calves

LT gene

blaTEM gene

Received 04/09/2023
Accepted 17/11/2023
Available On-Line 31/12/2023

ABSTRACT

Cattle producers usually face considerable economic losses due to neonatal Calf diarrhea (NCD). Aiming to delineate this problem, we conducted bacteriological, serological, and molecular studies on 100 fecal swabs collected from diarrheic calves (1 day to 3 months) old from different farms in Gharbia Governorate, Egypt, from December 2021 to May 2022. The *E. coli* isolation rate was 34%, with the multi-drug resistance being a general trend in most isolates. Sero-grouping of *E. coli* resulted in the encountering of O1, O26, O44, O55, O115, O125, O127, O128 (5% for each type), O18, O119 (10% for each type) and K99 (25%). Virulence and resistance genes molecular investigation using PCR in 2 isolates of *E. coli* serotype(K99) revealed the presence of virulence genes (LT, STa) and resistance genes (*qnr*A, *aac(6)-IB-cr*, *bla*TEM, KPC, *Sul*, and *aad*B). We concluded that the higher the stress the *E. coli* face from improperly using anti-bacterial drugs, the more advanced and new mechanisms the bacteria develop to overcome this challenge. Therefore, multi-drug resistant strains of *E. coli* are continuously emerging, posing serious threats to human and animal health.

1. INTRODUCTION

Diarrhea in calves is a common devastating clinical sign of pre-weaning infection by gut microbial agents. This is caused by multiple pathogens such as *Escherichia coli*, *Clostridium perfringens*, *Salmonella enterica* species, Rotavirus, Bovine coronavirus, Cryptosporidium species and Eimeria species. Some factors may predispose to the development of such disease, such as failure to acquire passive immune protection from mothers through colostrum and other intertwined environmental factors. Of the most important factors that contribute to the incidence of calf scour are the different determinants that affect intestinal permeability, especially in the first hours and days after birth, as calves with higher diarrhea incidence were reported to have higher intestinal permeability in the first 2 hours after birth compared to calves that do not have scours (Araujo *et al.*, 2015). This was suggested due to variations in the development of calves' GIT microbiome from different maternal fluids and tissues during the parturition process (Taschuk and Griebel, 2012). In addition to the host genome, which affects the population of the commensal bacteria, it impacts the GIT microbiota establishment microbiota (Benson *et al.*, 2010).

Newborn calf death due to diarrhea in the first few days of the calves' life reached 57%, as reported by the National Animal Health Monitoring System (NAHMS) for U.S. dairy report in 2007 (Cho and Yoon, 2014). This devastating death rate, in addition to the high costs paid for the diagnosis, treatment, labor, and veterinary intervention, imposes a high economic loss on the whole economic profit of the dairy or fatting business. In Egypt, bovine newborn *E. coli* infection is the primary cause of calf death, which ranges between 27.4% and 55% of the mortality in newborn calves (Ahmed et al., 2009). The ability of any pathogenic agent to produce gut disturbance depends on its ability to colonize the GIT and destroy the host tissue.

To produce diarrhea, *E. coli* rely on its adhesion antigens that facilitate the bacteria to hang on enterocytes. The enterotoxigenic E *coli* (ETEC) was accused of being the primary cause of calves' diarrhea in the 1st four days of life due to oral exposure to fecal matter contaminated by coliforms. This results in GIT colonization with commensal flora that continues to move caudally with the ingesta. Ingesta with a high ETEC load with the virulence factors K99 fimbria, and heat-stable toxin will produce diarrhoea. The fimbrial antigen K99 enables the ETEC to attach to and colonize the intestinal wall (Foster and Smith 2009). This attachment to the intestinal wall allows the bacteria to maintain lodging and expansion to the extent that about 80% of the organisms are attached to the intestinal wall in the calves having scour compared to only about 20% being attached in the calves without scour (Hadad and Gyles 1982). After colonizing the ileum, ETEC proliferates and attains the ability to secret a heat-stable toxin that causes secretory diarrhea (Sherwood *et al.*, 1983; Constable, 2003). This heat-stable toxin (STa) binds to an intestinal villi brush

* Corresponding author: ayam54936@gmail.com

border membrane enzyme called guanylecyclas-C (GCC) (Dreyfus and Robertson 1984; Krause *et al.* 1994). This binding raises the intracellular concentration of cGMP, leading to cGMP-dependent protein kinase, which in turn phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR) protein that consequently activates and moves to the cell surface, leading to chloride secretion to the lumen (Golin *et al.,* 2005) which attract water overriding the villous absorptive ability (Argenzio 1985). Without causing structural damage to enterocytes, Enterotoxins secreted by enterotoxigenic *E-coli* activate intestinal hypersecretion and decrease intestinal absorption, therefore causing calf scour (Quinn *et al*., 2002), thus leading to dehydration, acidosis and electrolyte loss that end in death of the animals.

Escherichia coli isolates are classified into serotypes and serogroups by exploring the bacterial cell wall antigenic determinants, whether somatic (O antigens), flagellar (H antigens) or capsular (K antigens). Thus, *E. coli* that were encountered in fecal samples from diseased calves were serotyped into O119, O111, O157:H7, O111, O126 and O26, and O78 (Dean-Nystrom *et al*., 1997; Tamaki *et al.,* 2005 and Badouei *et al.,* 2010). Due to the massive uncontrolled and improper use of antibacterial drugs, most bacterial species have developed antimicrobial resistance to most of the competing medicines. This raises an emergent global concern in treating new infections and prophylaxis to decrease bacterial infections in farm animals. *E. coli* can attain resistance genes by horizontal gene transfer (HGT) through interspecies and intraspecies routes (Poirel *et al.* 2018). The HGT mechanism varies, including plasmids, transposons, and pathogenicity islands (PAIs) insertion into the recipient *E. coli* genome (Javadi *et al*. 2017). In this concern, controlling *E. coli*-induced neonatal diarrhea is still important. It continues to be a significant economic problem in cattle farming. It necessitates continuously updating the bacterial sensitivity in each locality to determine its emergent resistant genes complex to control and treat calves' diarrhea adequately. The goal of the current study was to elucidate the new characteristics of *E. coli* isolated from diarrheic neonatal calves and to update our current knowledge of antimicrobial sensitivity and the newly developed drug-resistance genes in *E. coli* isolates from diarrheic calves' fecal samples to provide veterinarians with the ability to make an efficient plan for precise controlling, managing and treatment of neonatal calves' diarrhea in the governorate of Gharbia Governorate, Egypt.

2. MATERIAL AND METHODS

2.1. Samples:

From December 2021 to May 2022, fecal swabs were taken from 100 diarrheal calves (aged one day to 3 months) from various farms in the Gharbia governorate. Without waiting, all swabs were transmitted through transport media and transported in an ice box to the lab for bacteriological analysis.

2.2. Bacteriological and biochemical examination:

Each fecal swab was inoculated in 3 mL of peptone broth (Oxid, UK) and incubated for 24 h at 37 °C according to Quinn *et al.* (2002), a loopful from broth was inoculated into MacConkey's agar (Oxid, UK) and incubated at 37 °C for 18–24 h. Lactose fermenter (pink) colonies were streaked onto eosin methylene blue agar (EMB) (Oxid, UK) and identified by cultural and morphological characters, then validated as *E. coli* using the standard biochemical tests according to Collee *et al*. (1996).

2.3. Serological identification:

By performing a slide agglutination test with conventional polyvalent and monovalent *E. coli* antisera (Denka Seiken-Co., Ltd., Tokyo, Japan), *E. coli* isolates were identified serologically according to Edward and Ewing (1972).

2.4. Antimicrobial sensitivity test:

E. coli isolates were tested for their sensitivity to 8 different antimicrobial discs (Oxoid, UK), including amoxicillin-clavulanic acid (20+10 µg), linezolid (30 µg), gentamycin (10 µg), ceftriaxone (30 µg), meropenem (10 µg), trimethoprim-sulfamethoxazole (1.25+23.75 µg), norfloxacin (10 µg) and levofloxacin(5µg) by using disc diffusion method on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI, 2021).

2.5. Molecular examination:

Two selected *E. coli* isolates (K99) were examined for detection of virulence genes (LT, STa) and resistance genes (*qnr*A, *aac(6)-IB-cr*, *bla*TEM , KPC, *sul*, *aad*B). By using QIAamp® DNA Mini Kit instructions (Catalogue no.51304). The PCR program used the primers shown in Table (1) and running conditions as detailed in Table (2). PCR products were separated by electrophoresis in 1% agarose gel. Bands were visualized by ethidium bromide (Sambrook *et al.* 1989). The run was stopped after about 30 min, and the gel was transferred to the UV cabinet. A gel documentation system photographed the gel, and the data was analyzed through computer software.

Table 1 Primers used for the detection of virulence genes and resistance genes of *E. coli* (k99) isolates.

Gene	Sequence(5´ - 3´)	Amplified product	Reference
qnrA	F- ATTTCTCACGCCAGGATTTG	516 bp	Shams *et al*., 2015
	R- GATCGGCAAAGGTTAGGTCA		
aac(6)-IB-cr	F- TTGCGATGCTCTATGAGTGGCTA	482 bp	Hu *et al.*, 2013
	R- CTCGAATGCCTGGCGTGTTT		
*bla*TEM	F- ATGAGTATTCAACATTTCCGTGT	861 bp	Azam *et al*.,2016
	R- TTACCAATGCTTAATCAGTGAGG		
KPC	F- ATG CTT AGT GCT GGT TTA GG	798 bp	Yigit *et al*., 2001
	R- GCC TTC ATC ATT TCG CTT TC		
Sul	F- TGG CCA GAA CTG ACA GGC AAA	433 bp	Tosini *et al*., 1998
	R- TTT CTC CTG AAC GTG GCT GGC		
*aad*B	F- GAGCGAAATCTGCCGCTCTGG	404 bp	Frana *et al*., 2001
	R- CTGTTACAACGGACTGGCCGC		
LT	F- AGGATTGACTGCCTTTTTG	132 bp	Nishikawa *et al.* ,2002
	R- ATTTGCTGATTTCGCTCG		
STa	F- ATTTTTMTTTCTGTATTRTCTT	190 bp	Stacy *et al.*, 1995
	R- CACCCGGTACARGCAGGATT		

Table 2 Cycling conditions of the different primers during PCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
*qnr*A	94˚C	94˚C	58˚C	72˚C	35	72˚C
	5 min.	30 sec.	40 sec.	45 sec.		10 min.
aac(6)-IB-cr	94˚C	94˚C	54˚C	72˚C	35	72˚C
	5 min.	30 sec.	30 sec.	30 sec.		7 min.
*bla*TEM	94˚C	94˚C	51˚C	72˚C	35	72˚C
	5 min.	30 sec.	30 sec.	30 sec.		7 min.
KPC	94˚C	94˚C	55˚C	72˚C	35	72˚C
	5 min.	30 sec.	40 sec.	45 sec.		10 min.
Sul	94˚C	94˚C	54˚C	72˚C	35	72˚C
	5 min.	30 sec.	40 sec.	45 sec.		10 min.
aadB	94˚C	94˚C	54˚C	72˚C	35	72˚C
	5 min.	30 sec.	40 sec.	40 sec.		10 min.
LT	94˚C	94˚C	40˚C	72˚C	30	72˚C
	5 min.	30 sec.	40 sec.	40 sec		7 min.
Sta	95˚C.	94˚C	56 ˚C	72˚C	35	72˚C
	5 min	30 sec.	40 sec.	40 sec.		7 min.

3. RESULTS

3.1. Bacteriological examination

Out of 100 fecal swabs, 34 *E. coli* isolates were detected and identified via morphological characters (Gram negative non sporulated motile bacilli), cultural characters (lactose fermenter pink colonies on MacConkey's agar and green metallic sheen colonies on EMB agar) and biochemically identification (positive result with Indole and Methyl red tests while Voges Proskaeur, Citrate, Urease and H2S tests were negative).

3.2. Serotyping of *E. coli* isolates

Seroptyping of 34 *E. coli* isolates showed that O1, O26, O44, O55, O115, O125, O127, and O128 were present in two isolates (5.88 %) for each type, 3 isolates (8.82%) for both O18, O119, and 8 isolates K99 (23.53%), and 4 isolates untypable (11.76%).

3.3. Antimicrobial sensitivity test

All *E. coli* (34) isolates show (100%) resistance to amoxicillin-clavulanic acid (20+10 µg), ceftriaxone (30 µg); linezolid (30 µg) and trimethoprim-sulphamethoxazol (1.25+23.75 µg). On the other hand, the sensitivity to meropenem was (100%), 35.3 % (12/34) to gentamycin (10 µg), 20.6% (7/34) to levofloxacin (5µg).and 5.9 % (2/34) to norfloxacin (10 µg).

3.4. Molecular identification

Detection of virulence genes: The tested two *E. coli* K99 isolates (No. 1, 2) were positive to the virulence genes (*LT* gene bands at 132bp. and *STa* gene bands at 190 bp) (figure 1, 2 respectively).

3.5.Detection of resistance genes of *E. coli* K 99:

The *E. coli* K99 isolates No. (1 & 2) testing for resistance genes (*qnr*A, *aac(6)-IB-cr*, *bla*TEM, *Sul* and *aad*B) revealed the expression of all these genes in both isolates at 516, 482, 861,433 and 404 bp. respectively (figure 3, 4, 5, 7,8). While KPC gene expression was detected in isolate K99 No.1 only (gene bands at 798 bp) and absent in isolate No. 2. (figure 6).

Figure 1 Agarose gel electrophoresis showing PCR amplification of LT gene bands at 132bp. L: marker (100pb plus ladder), Pos: +ve control*, Neg: -ve control*, Lane (1-2): No. of *E. coli* (K99) isolates (were positive for LT gene).

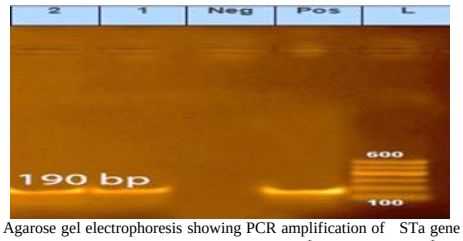

Figure 2 Agarose gel electrophoresis showing PCR amplification of STa gene bands at 190 bp. L: marker (100pb plus ladder), Neg: -ve control*, Pos: +ve control* ,Lane (1-2): No. of *E. coli* (K99) isolates (were positive for STa gene).

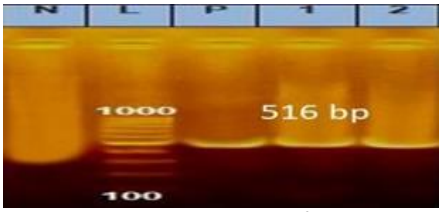

Figure 3 Agarose gel electrophoresis showing PCR amplification *qnr*A gene bands at 516 bp. L: marker (100pb plus ladder), N : -ve control*, P : +ve control* ,Lane (1-2): No. of *E. coli* (K99) isolates (were positive for *qnr*A gene).

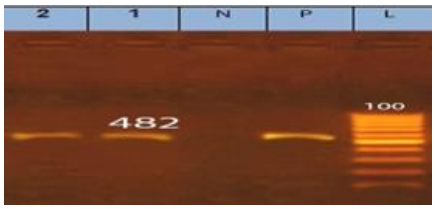

Figure 4 Agarose gel electrophoresis showing PCR amplification of aac(6)-IB-cr gene bands at 482 bp. L: marker (100pb plus ladder), N : -ve control*, P : +ve control* ,Lane (1-2): No. of *E. coli* (K99) isolates (were positive for aac(6)-IB-cr gene).

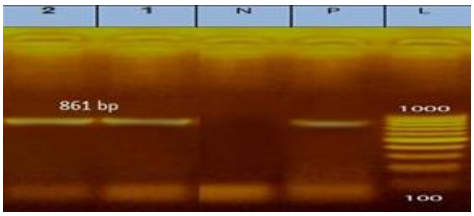

Figure 5 Agarose gel electrophoresis showing PCR amplification of *bla*TEM gene bands at 861 bp. L: marker (100pb plus ladder), N : -ve control*, P : +ve control* ,Lane (1-2): No. of *E. coli* (K99) isolates (were positive for *bla*TEM gene).

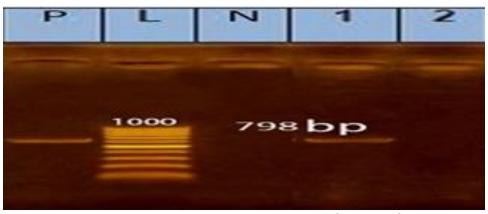

Figure 6 Agarose gel electrophoresis showing PCR amplification of KPC gene bands at 798 bp. L: marker (100pb plus ladder), N : -ve control*, P : +ve control* ,Lane (1-2): No. of *E. coli* (K99) isolates (nu. one only was positive for KPC gene).

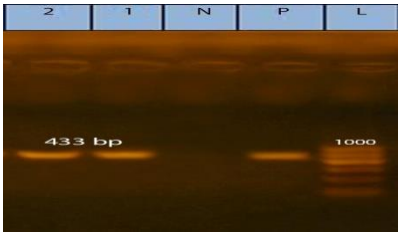

Fig (7): Agarose gel electrophoresis showing PCR amplification of *sul* gene bands at 433 bp. L: marker (100pb plus ladder), N : -ve control*, P : +ve control* ,Lane (1-2): No. of *E. coli* (K99) isolates (were positive for *sul* gene)

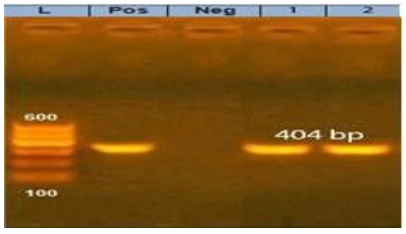

Fig (8): Agarose gel electrophoresis showing PCR amplification of *aad*B gene bands at 404 bp. L: marker (100pb plus ladder), N : -ve control*, P : +ve control* ,Lane (1-2): No. of *E. coli* (K99) isolates (were positive for *aad*B gene)

* Positive and negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference Laboratory , Animal Health Research Institute, Egypt.

4. DISCUSSION

ue to calves' diarrhea, bovine breeders suffer significant financial losses, which poses a major threat to the cattle industry (Cho and Yoon 2014). In this research, deciphering the bacterial causes of the newborn calf scours in the studied area revealed E.coli is the leading cause as its isolation rate was 34%, consistent with the previous isolation rate of E.coli of 37% from calf diarrheic samples in Ethiopia (Gebregiorgis and Tessema 2016) and a 28.8% in Egypt (Algammal et al., 2020). This current study results are also parallel with a previously reported *E. coli* being the most predominant bacterial pathogen causing newborn calves' diarrhea (Cho and Yoon 2014). However, *E. coli* isolation rate in this work is far lower than the isolation rate from calves' scour in other studies undertaken in other localities from Egypt reported an 80%, 70.7%, and75.6% isolation rate of *E. coli* (El-Seedy et al., 2016; Arefet al. 2018; Awadet al., 2020). However, the *E. coli* isolation rate in the current work is far higher than other reported isolation rates in other countries, as 13.5 % was reported in a study in Ethiopia (Darsema 2008) and 5.5% in Switzerland (Uhde et al., 2008). This discrepancy between the rates of *E. coli* isolation from different localities and countries may be attributed to the differences in management and control programs and environmental conditions. It also indicates the uncontrolled and hazardous use of antibiotics in food animal management and prophylaxis programs in Egypt. It stresses the bacteria, pushing it to develop drug resistance to overcome routine management and control measures and guarantee its presence and multiplication. It also should represent an alarming problem that necessitates national-wide strictly implemented rules to reduce antibiotics' uncontrolled and hazardous use.

The serotyping results of the presence of O1, O26, O44, O55, O115, O125, O127, and O128 (5% for each type),10% for both O18, O119, and K99 (25%) representing 85% with the presence of 15% un-serotyped isolates is consisted with a previous study (Algammal et al., 2020). This study's recognized serotypes differ from previously reported serogroups isolated and sampled from diarrheic newborn humans and animals (Uhde et al., 2008; Vaghand Jani, 2010; Enany et al., 2019). Moreover, the pattern and distribution of serotypes retrieved in the study are nearly similar to the recently reported serogroups of isolates from Egypt (Algammal et al., 2020).

Heat-labile (LT) and heat-stable (STa and STb) toxins are the mediators by which Enterotoxigenic *E. coli* (ETEC) induce its pathogenicity in humans and animals (Olaogun et al., 2016). Investigation of *E. coli* isolates in this study showed their expression of these two toxin-encoding genes, proving its biotyping as ETEC type and indicating its pathogenicity. ETEC toxin LT activates adenylate-cyclase enzyme in the affected animal's enterocytes, while STa acyivates guanylate-cyclase enzyme, leading to severe watery diarrhea. The encounter of these two toxins-encoding genes in all isolates comes in accordance with a previous study that showed the expression of (LT) in all tested *E. coli* isolates either alone or in association with the STa, pointing to their importance in ETEC-pathogenicity (Algammal et al., 2020).

The improper and hazardous use of antibacterial drugs for prophylaxis and infection treatment in food animals and poultry has led to the emergence of the globally high threat spread of antimicrobial-resistant bacterial strains (Abavisani et al., 2023). *E. coli* can attain resistance genes by horizontal gene transfer (HGT) through interspecies and intra-species routes (Poirel et al. 2018). The E. coli's HGT mechanisms include plasmids, transposons, and pathogenicity islands (PAIs) insertion into the recipient *E. coli* genome (Javadi et al. 2017).

Complicating this harmful scenario, *E. coli* can work not only as a recipient of HGT from other bacteria but also as a donor of resistance genes to other bacteria (Poirel et al., 2018), thus acting as an essential player in the widespread drug-resistance genes among pathogenic bacteria. Compared to a recent study on diarrheic *E. coli* from Egypt (Algammal et al., 2020), the current study showed a higher multi-drug resistance pattern as 100% of the E coli isolates (34) were resistant to amoxicillin-clavulanic acid (20+10 µg), ceftriaxone(30 µg); linezolid (30 µg) and trimethoprim-sulfamethoxazole (1.25+23.75 µg), 65% to gentamycin (10 µg), 80% to levofloxacin (5µg) and 95% to norfloxacin (10 µg) compared to 50% resistance to trimethoprim/sulfamethoxazole (50%) and (42.8%) resistance to gentamycin (Mousa et al., 2010). This can be attributed to increasing microbial drug resistance development or differences in antibiotic stress on *E. coli* due to differences in the management and preventive control measures in other studied localities. Recent studies have reported strains of *E. coli* with advanced resistance to most antibiotic classes (Xiao et al. 2019, Zhang et al. 2021). As *E. coli* is considered the most active and smart bacteria for developing drug-resistance genes, the more advanced the antibacterial stress it faces, the more resistant genes emerge. The problem is expected to be seriously complicated when these new genes are transmitted to other pathogenic bacteria, representing a health concern in animals and humans. An example of this scenario is the multi-drug resistance of the isolated bacteria in the current study, as all antibacterial drugs were either totally or partially ineffective whenever the only drug that could efficiently inhibit *E. coli* growth and multiplication was meropenem. This agrees with a previously reported 100% sensitivity of *E. coli* isolates to meropenem (Wu et al., 2021). Moreover, the 100% resistance of the currently studied isolates (34) to amoxicillin/clavulanic acid is far higher than the recently reported 21.5% resistant *E. coli* (Algammal et al., 2020). This multi-drug resistance ability

of *E. coli* isolates is mediated through the presence of drug resistance genes (qnrA, aac (6)-IB-cr, blaTEM, sul, and aadB) that were shown in this study to be expressed in all the examined isolates. The discrepancy between the drug-resistance pattern in this work and the previously reported one may be caused by the presence of blaTEM, sul, and aadB resistance genes at a higher rate in this work compared to a lower expression rate in the previous study (Algammal et al., 2020). This work consisted of the expression of multi-drug-resistance genes aadB, sul1, and blaTEM genes in all the tested isolates; these genes were reported to be prevalent in all the tested isolates resistant to aminoglycosides, sulfonamides, and ampicillin (Du et al., 2005).

5. CONCLUSIONS

This study throws some light on the importance of continuously updating the knowledge of the constantly inventing and developmental changes of the microbial community in each locality. Moreover, *E. coli* remains the main cause of calf diarrhea, resulting in substantial financial losses. The higher the stress the *E. coli* are facing by improperly using anti-bacterial drugs, the more advanced and new mechanisms the bacteria develop to overcome this challenge. Therefore, multi-drug resistant strains of *E. coli* are continuously emerging which pose severe threats to human and animal health. The differences between different localities in the pattern of drug resistance are related to the levels of improper antibiotic use in food animals and poultry prophylaxis and management in such areas.

6. REFERENCES

1. Abavisani, M.; Bostanghadiri, N.; Ghahramanpour, H.; Kodori, M.; Akrami, F.; Fathizadeh, H.; Hashemi, A. and Rastegari-Pouyani, M., 2023. Colistin resistance mechanisms in Gram-negative bacteria: a Focus on *Escherichia coli*. Letters in Applied Microbiology, 76, 2:ovad023. doi: 10.1093/lambio/ovad023.
2. Ahmed, A.M., Younis, E.E., Osman, S.A., Ishida, Y., El-Khodery, S.A. and Shimamoto, T., 2009. Genetic analysis of antimicrobial resistance in *Escherichia coli* isolated from diarrheic neonatal calves. Vet. Microbiol.;136 3-4.:397-402.
3. Algammal, A.M., El-Kholy, A.W.; Riad, E.M.; Mohamed, H.E.; Elhaig, M.M.; Yousef, S.A.A.; Hozzein, W.N. and Ghobashy, MOI., 2020. Genes Encoding the Virulence and the Antimicrobial Resistance in Enterotoxigenic and Shiga-Toxigenic *E. coli* Isolated from Diarrheic Calves. Toxins Basel.;12 6.:383.
4. Araujo, G.; Yunta C.; Terré,M.; Mereu .; Ipharraguerre, I. and Bach, A., 2015. Intestinal permeability and incidence of diarrhea in newborn calves. J. Dairy Sci; 98 10.:7309-7317
5. Aref, N.M.; Abdel-Raheem, A.A.; Kamaly, H.F.andHussien ,S.Z., 2018. Clinical and sero-molecular characterization of *Escherichia coli* with an emphasis on hybrid strain in healthy and diarrheic neonatal calves in Egypt. Open Vet. J., 8 4.:351-359.
6. Argenzio, R.A.,1985. Pathophysiology of neonatal calf diarrhea. Vet. Clin. North. Am. Food. Anim. Pract. ;1 3.:461–469.
7. Awad, W.S.; El-Sayed, A.A.; Mohammed, F.F.; Bakry, N.M.; Abdou, N.M.I.andKamel, M.S., 2020. Molecular characterization of pathogenic *Escherichia coli* isolated from diarrheic and in-contact cattle and buffalo calves. Trop Anim. Health Prod.; 52 6.:3173-3185.
8. Azam, M., Arif, T.J., Qazi, Mr., 2016. Bla CTX-M-152, a novel varient of CTX-M-group-25, identified in a study performed on the prevalence of multidrug resistance among natural inhabitant of river Yamuna, India. Frontiers in Microbiology 7 176.:1-13.
9. Badouei, M.A., Salehi, T.Z., Khorasgani, M.R., Tadjbakhsh, H., Brujeni,G.N. and Nadalian, M.G., 2010. Virulence gene profiles and intimin subtypes of Shiga toxin-producing *Escherichia coli* isolated from healthy and diarrheic calves. Vet. Rec.;167 22.:858-861.
10. Benson, A.K., Kelly, S.A., Legge, R., Ma, F., Low, S.J., Kim, J., Zhang, M., Oh, P.L., Nehrenberg, D., Hua, K., Kachman, S.D., Moriyama, E.N., Walter, J., Peterson, D.A. and Pomp, D., 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Natl. Acad. Sci.;107 44.:18933-18938.
11. Cho, Y.I. and Yoon, K.J., 2014. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. J. Vet. Sci.;15 1.:1-17.
12. Clinical Laboratory Standards Institute CLSI.. 2021. Performance Standards for Antimicrobial Disk Susceptibility Tests. 31st Ed. CLSI standard M100. Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing.
13. Collee, J.G., Miles, R.S. and Wan, B., 1996. Tests for the identification of bacteria. In: Collee, J.G., Fraser, A.G., Marmion, B.P. and Simmons, A., Eds., Mackie and Mac-Cartney Practical Medical Microbiology, 14th Edition, Churchill Livingstone, Edinburg, 131-150.
14. Constable, P.D., 2003. Pathophysiology of calf diarrhea: new concepts. Presented at the ACVIM Forum Proceedings. Charlotte, NC.
15. Darsema, G., 2008. Major causes of calf mortality in dairy farms and two cattle ranches in western Amhara region, northwestern Ethiopia, Ethiopian Veterinary Journal, 12, 59–68.
16. Dean-Nystrom, E.A., Bosworth, B.T. and Moon, H.W., 1997. Pathogenesis of O157:H7 *Escherichia coli* infection in neonatal calves. Adv Exp Med Biol.; 412:47-51.
17. Dreyfus, L.A. and Robertson, D.C.,1984. Solubilization and partial characterization of the intestinal receptor for *Escherichia coli* heat-stable enterotoxin. Infect Immune ;46 2:537–43.
18. Du, X.; Shen, Z.; Wu, B.; Xia, S.andShen, J., 2005.Characterization of class 1 integrons-mediated antibiotic resistance among calf pathogenic *Escherichia coli*. FEMS Microbiol. Lett., 245, 295–298.
19. Edward, P. R. and Ewing, W. H., 1972. Edward's and Ewing's identification of Enterobacteriacae,3rd Ed. Burgess, Minneapolis.
20. El-Seedy, F.R.; Abed, A.H.; Yanni, H.A.andAbd El-Rahman, S.A.A., 2016. Prevalence of Salmonella and E. coli in neonatal diarrheic calves. Beni Suef Univ. J. Basic Appl. Sci.; 5, 1.:45-51.
21. Enany, M.; Algammal, A.M.; Nasef, S.A.; Abo-Eillil, S.A.M.; Bin-Jumah, M.N.; Taha, A.E. and Allam, A., 2019. The occurrence of multidrug resistance MDR. and the prevalence of virulence genes and QACs resistance genes in *E. coli* isolated from environmental and avian sources. AMB Express, 9, 1.:192-200.
22. Foster, D.M. and Smith, G.W., 2009. Pathophysiology of diarrhea in calves. Vet Clin North Am Food Anim Pract.; 25, 1.:13-36.
23. Frana, T. S., Carlson, S. A. and Griffith, R. W., 2001. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in Salmonella enterica serotype Typhimurium phage type DT104. Applied and Environmental Microbiology 67, 445–448.
24. Gebregiorgis, A. and Tessema, T.S, 2016. Characterization of *Escherichia coli* isolated from calf diarrhea in and around Kombolcha, South Wollo, Amhara Region, Ethiopia. Trop Anim. Health Prod.; 48, 2.:273-281.
25. Golin-Bisello, F.; Bradbury, N. and Ameen, N., 2005. STa and cGMP stimulate translocation to the surface of villus enterocytes in rat jejunum and is regulated by protein kinase G. Am. J. Physiol, Cell Physiol; 289, 3.: 708–716 .
26. Hadad, J.J. and Gyles, C.L.,1982. The role of K antigens of enteropathogenic *Escherichia coli* in colonization of the small intestine of calves. Can. J. Comp. Med., 46, 1.:21–26.

27. Hu,X. ; Xu,B. ; Yang,Y. ; Liu,D. ; Yang,M. ; Wang,J. ; Shen,H. ; Zhou,X. and Ma,X., 2013. A high throughput multiplex PCR assay for simultaneous detection of seven aminoglycoside-resistance genes in Enterobacteriaceae. BMC Microbiology, 13, 1.,1-9.
28. Javadi, M., Oloomi, M. andBouzari, S., 2017. In Silico Signature Prediction Modeling in Cytolethal Distending Toxin-Producing *Escherichia coli* Strains. Genomics Inform.;15, 2.:69-80.
29. Krause, W.J.; Cullingford, G.L. and Freeman, R.H.,1994. Distribution of heat-stable enterotoxin/guanylin receptors in the intestinal tract of man and other mammals. J. Anat.;184 Pt 2:407–17.
30. Mousa, M.; Akeila, M.; Khaliel, S. and Abdellrazeq, G., 2010. Virulence factors of *Escherichia coli* isolated from diarrheic sheep and goats. J. Vet. Sci., 30, 137–147.
31. Nishikawa Yoshikazu, Zhijiang Zhou, Atsushi Hase, Jun Ogasawara, TeruyoKitase, Niichiro Abe, Hiromi Nakamura, Takayuki Wada, Eiji Ishii, KosukeHaruki and Surveillance Team, 2002. Diarrheagenic *Escherichia coli* isolated from stools of sporadic cases of diarrheal illness in Osaka City, Japan between 1997 and 2000: prevalence of enteroaggregative *E. coli* heat-stable enterotoxin 1 gene-possessing E. coli. Jpn. J. Infect. Dis.; 55, 6.:183-90.
32. Olaogun, S.; Jeremiah, O.; Jubril, A. and Adewuyi, O., 2016. Calf Diarrhea: Epidemiological Prevalence and Bacterial Load in Oyo and Ogun States, Nigeria. Alex. J. Vet. Sci.; 51:90.
33. Poirel, L.; Madec, J.Y. and Lupo, A., 2018. Antimicrobial resistance in *Escherichia coli*. Microbiol Spectrum; 6:4–6.
34. Quinn, P. J.; markey, b. K.; carter, m. E.; donnelly, w. J. and leonard, f. C., 2002. Enterobacteriaceae. In veterinary microbiology and microbial disease. Oxford, Blackwell Science. Pp 106-123.
35. Sambrook, J., Fritsch, E. F., & Maniatis, T. 1989.. Molecular cloning: a laboratory manual No. Ed. 2.. Cold Spring Harbor Laboratory Press .
36. Shams, E., Firoozeh, F., Moniri, R., & Zibaei, M. 2015. Prevalence of Plasmid-Mediated Quinolone Resistance Genes among Extended-Spectrum β-Lactamase-Producing Klebsiella pneumoniae Human Isolates in Iran. Journal of Pathogens, 2015. doi: 10.1155/2015/434391
37. Sherwood, D.; Snodgrass, D. R .and Lawson, G.H.,1983. Prevalence of enterotoxigenic *Escherichia coli* in calves in Scotland and northern England. Vet. Rec.; 113 10.:208–212.
38. Stacy-Phipps, S., Mecca, J.J. and Weiss, J.B., 1995. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during course of infection. Journal of Clinical Microbiology, 33, 1054–1059.
39. Tamaki,Y., Narimatsu, H., Miyazato, T., Nakasone, N., Higa, N., Toma, C. and Iwanaga, M., 2005.The relationship between O-antigens and pathogenic genes of diarrhea-associated *Escherichia coli*. Jpn. J. Infect. Dis., 58, 2.:65-69.
40. Taschuk, R. and Griebel, P. J., 2012. Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract. Anim. Health Res. Rev. 13:129–141.
41. Tosini F., Visca P., Luzzi I., Dionisi A.M., Pezzella C., Petrucca A. and Carattoli A., 1998. Class 1 integronborne multiple-antibiotic resistance carried by IncFI and IncL/M plasmids in Salmonella enterica serotype Typhimurium. Antimicrob Agents Ch.; 42: 3053–3058 .
42. Uhde, F.L.; Kaufmann, T.; Sager, H., Albini, S.; Zanoni, R.; Schelling, E. and Meylan, M., 2008. Prevalence of four enteropathogens in the faces of young diarrheic dairy calves in Switzerland, Veterinary Record, 163, 362–366.
43. Vagh, A. and Jani, R., 2010. Prevalence and comparative studies of some major serotype of *E. coli* from cattle and buffalo calf scour. Vet. World. 3, 10., 458- 459.
44. Wu, D., Ding, Y., Yao, K., Gao, W. and Wang, Y., 2021. Antimicrobial Resistance Analysis of Clinical *Escherichia coli* Isolates in Neonatal Ward. Front Pediatr.,9:670470.
45. Xiao, T., Yang, K., Zhou, Y., Zhang, S., Ji J., Ying, C., Shen, P. and Xiao Y., 2019. Risk factors and outcomes in non-transplant patients with extended-spectrum beta-lactamase-producing *Escherichia coli* bacteremia: a retrospective study from 2013 to 2016. Antimicrobial Resistance& Infection Control, 8, 1, 1-11.
46. Yigit, H., Queenan, A.M., Anderson, G.J., Domenech-Sanchez, A., Biddle, J.W., Steward, C.D., Alberti, S., Bush, K. and Tenover, F.C., 2001. Novel Carbapenem-Hydrolyzing Beta-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of Klebsiella pneumoniae. Antimicrobial Agents and Chemotherapy, 45, 4., 1151-1161.
47. Zhang,Y., Wang, H., Li, Y., Hou, Y. and Hao, C., 2021. Drug susceptibility and molecular epidemiology of *Escherichia coli* in bloodstream infections in Shanxi, China. Peer J., 9: e12371.