Chapter Four

Discussion

Diarrhoea in newborn farm animals, particularly calves under 30 days of age, is one of the most common disease complexes that the large-animal clinician encounters in practice. It is a significant cause of economic loss in cattle herds and continues to assume major importance as livestock production becomes more intensified. The effective treatment and control of herd epidemics of diarrhoea in calves can be frustrating and unreliable. Considerable progress has been made in the treatment of the effects of diarrhoea such as dehydration and acidosis but less so in the control of these disease complexes(Radostits *et al*., 2007).

Although the problem of diarrhoea is complex and attaining hazardous proportions, yet few fragmented studies have been carried out in Sudan (Salih, 1993; Mutasim, 1997; Kamal, 2000; Ellaithi, 2004; Mohamed, 2009; Elgaddal, 2009).

In this study which lasted for 2 years, 500 dairy farms in different localities of Khartoum State were investigated for the problem of calf diarrhoea.

According to Questionnaire survey of dairy farms, the general evaluation of the housing condition was poor for 59.6% of the farms, 79.6% of the stall surfaces were clay and the hygiene level was poor in 63% of the farms. According to Acres and Radostits (1985) finding, these factors increase the incidence of any disease, especially calf scour.

Matching with Svensson *et al*. (2003) who found that diarrhoea is one of the most common diseases reported in calves up to three months old. The present survey results proved that the main health problem in calves was calf diarrhoea (78%).

According to Radostits *et al*. (2007), several owners' practices revealed during survey, may help to decrease health problems among herds, for example the availability of the veterinary services, adoption of vaccination program and colostrums giving to the calves during first hours of birth.

In the present survey's results concerning calf diarrhoea, all owners considered that the two first weeks of calf's age are the most hazardous and the risk decreases with old ages, and this in accord with the findings of Curtis *et al*. (1988). According to owners' records, there were high mortality rates due to calf diarrhoea and in support of Svensson *et al*. (2003) who estimated that 75% of early calf mortality in dairy farms is caused by acute diarrhoea in the pre-weaning period. The analysis of the data on treatments adapted to the affected calves in the areas of the study showed different drugs with different percentages of adoption: Sulphaguanidine and neomycin (77.6%), Tetracycline (69.2%), Sulphonamides (58%), Ciprofloxacin (35.8%), Gentamycin and sulphadimidine (31.8%), fluid therapy (15%) and no treatment of calf diarrhoea and only decreased frequency of milk sucking of calves (9.4%). These treatment strategies were also recommended by Radostits *et al*. (2007) with different routes of administration of drugs. The same author also mentioned that, diarrhoeic calves are commonly treated with oral fluids and electrolytes and left with the cow. However, it is a common practice to reduce the milk intake of diarrhoeic hand-fed dairy calves for up to 24 hours or until there is clinical evidence of improvement. The withholding of milk from diarrhoeic calves has been based on the observations that lactose digestion is impaired and that 'resting' the intestines for a few days minimizes additional osmotic diarrhea caused by fermentation of undigested lactose in the large intestine. Thus it has seemed logical not to feed the animal with milk, which must be digested, but rather to provide readily absorbable substances such as oral glucose-electolyte mixture.

In this study the incidence rate of calf diarrhoea in Khartoum State was 60% and Radostits *et al*. (2007) mentioned that colibacillosis led to morbidity which may reach 75%. Also the incidence rate was greater in Bahry and East Nile localities (64.5%) than Omdurman locality (60.6%) and Khartoum and Gabal Awleia localities (52.9%). This may be attributed to high population density in Bahry and East Nile localities and also the stall surfaces in the animal farms were different. The incidence rate was higher in female calves (78.0%) than male calves (22.0%) and this leads to considerable economic loss due to losses of calving and milk production in future. Also the incidence rate was high in fist calves of heifers than calves of older cows and according to Radostits *et al*. (2007). This is due to low concentration of immunoglobulin in colostrums in first- and second-calf heifers than in third or subsequent lactations. The incidence of calf diarrhoea was higher during autumn seasons of the years, 2010 and 2011 (91.4% and 82.9% respectively) and winter seasons of the years 2010, 2011 and 2012 (71.4%, 57.1% and 47.1% respectively) than summer seasons of the years 2010 and 2011 (40.0% and 33.3% respectively) and that matches with Radostits *et al*. (2007) who mentioned that the incidence rate of calf diarrhoea will be high during winter and wet seasons. Waltner-Towes *et al.* (1986) found that diarrhea occurred with greater frequency during the winter and reached a second peak in July. Also Curtis *et al*. (1988) found that morbidity was higher in winter than in summer.

In this study the mortality rate among diarrhoeic calves was higher during first and second weeks of age (29.8% and 20.0% respectively) than the third week (19.1%). And that support the study of Curtis *et al*. (1988) who found that Calves were at highest risk for diarrhea during week one of life; the risk declined to a low level after 4 week of age. Also Waltner-Towes *et al.* (1986) found that the incidence rate of diarrhea in calves peaked at week 2 of life and

the average mortality rate in dairy caves under one month of age varied from 30 to 50%. In Sudan El Nour (1994) found that the death as the result of diarrhea ranged from the first day to 45th days of age, also he reported a mortality rate of 17 to 49% among calves in Arabian Company Farm at El Bagair area due to diarrhoea.

Two hundred and fifty two (96.9%) out of 260 *E. coli* isolates were isolated from watery yellowish diarrhoea, and 10(2.3%) were isolated from watery-mucoid diarrhoea and (0.8%) from bloody-watery diarrhoea. This high percentage of watery diarrhoea confirmed the involvement of *E. coli* as studied by Wastesson (2002), Levine (1987), Gyles (1993), Nataro and Kaper (1998) and Abubaker *et al* (2006).

Out of 300 diarrhoeic samples obtained from dairy farms in Khartoum State a total of 342 bacterial isolates were obtained. These were *Escherichia coli* (76.0%), *Escherichia fergusoni* (2.3%), *Escherichia vulneris* (0.6%), *Klebsiella pneumonia sub spp. Ozaenae* (6.4%), *Proteus mirabilis* (8.8%), and *Enterococcus faecalis* (5.8%). In some cases more than one isolate was recovered from the same sample.

It was found that the Gram-negative bacteria were the most prevalent organisms (94.2%) and the Gram-positive bacteria constituted about (5.8%). This result is similar to that reported by Ali (2002).

In this study we found that *E. coli* represented the predominant bacterial spp. among Gram-negative aerobes. The result is similar to that found by Holland *et al*. (1990), Firehammer and Myers (1988), Radostits *et al*. (2007), Aiello and Mays (1998), Debanth *et al*. (1987), Moon (1974), Smith and Halls (1967),Tzipori (1981), Perez, *et al*. (1998), Ellaithi (2004), Elgaddal (2009), Mohamed (2009), Abdel Rahman *et al*. (1995), Omeima (1993), and Perez *et al*. (1998).

Elgaddal (2009) findings *E. fergusonii*, *E. vulneris,* were also isolated. Other members of Enterobacteriaceae were isolated during this study, these were: *Klebsiella pneumonia sub spp. Ozaenae* (6.4%), *Proteus mirabilis* (8.8%).This result was similar to that found by Salih (1993), Perez and *et al*. (1998) and Quinn *et al*. (2011). *Proteus mirabilis* was thought to be involved in cases of diarrhoea (Carter, 1985; Quinn *et al*., 2011). Gram positive bacterium isolated in this study was *Enterococcus spp*. The result is similar to the findings of Elgaddal (2009).

During the present investigations, *E. coli* isolates were characterized by their rapid and satisfactory growth on different ordinary laboratory media. This is due to the ability of the microorganism to grow on minimal medium that contains a carbon compound such as glucose (which serves both as a carbon source and an energy source) and salts which supply nitrogen, Phosphorus and trace elements. Growth in rich medium (e.g blood agar) was more rapid than on minimal medium due to the presence of amino acids nucleotide precursors, vitamins and other metabolites that the organism would otherwise have to synthesize. MacConkeys lactose bile salt agar proved to be a satisfactory medium for primary isolation. These findings agree with Ausubel *et al* (2012).

In this study 210 (83.3%) of *E. coli* isolates were motile. This result agrees with Ellaithi (2004) who reported 80.0% of *E. coli* isolates were motile, Elgaddal (2009) who reported 81.0% of *E. coli* isolates were motile, Ewing (1986) who reported 77.4% of *E. coli* isolates were motile and Quinn et *al*. (2011) who reported that *E. coli* isolates from clinical cases were motile or non-motile.

In this study, 43 (17.1%) *E. coli* isolates were haemolytic. This finding agrees with Boro *et al* (1983) who reported that 52% out of 75 *E. coli* isolates were haemolytic and El gaddal (2009) who reported 18.8% of isolated *E. coli*

were hamolytic. Luis (2004) mentioned that *E. coli* is usually haemolytic. However, the most studies in haemolysin production were taken as a differentiation characteristic, while others described the production of haemolysins as a virulent factor. Quinn *et al* (2011) described the haemolytic activity of *E. coli* isolates on blood agar as a characteristic of certain strains. Kauffmann (1969) mentioned that mouse virulent strains of *E. coli* were often haemolytic and serologically more haemogenous than non-haemolytic one.

The results of biochemical reactions revealed many differences between the isolates although they fulfill the requirements for identification as *E. coli* isolates. This agrees with findings of Sojka (1971), Kauffmann (1969), Ewing (1986) and Orskov (2005). All *E. coli* isolates were positive for IMVC test, Catalase test, Nitrate reduction test, O/F test and Eijkman's test and negative for oxidase test, Gelatin hydrolysis test, Arginine test, KCN test and H2S test. Only 15 (6.0%) of the isolates were positive for Urease test.

In this study sugars fermentation results were varied. Glucose was fermented by all isolates (100%), Lactose by 230(91.1%), Mannitol by 222(88.1%), Sorbitol by 150 (59.5%), Rhamnose by 129 (51.2%), Sucrose by 96 (38.1%), Inositol by 82 (32.5%) isolates and Only 15 (3.0%) isolates fermented cellobiose.

The results of 90 *E. coli* isolates on API 20E strips showed different identification percentages ranged from 99.8% to 89.8%. This ranging was due to different results of biochemical tests included in Api rapid system identification strips. All *E. coli* isolates gave positive results with Ortho-nitro-phenyle-galactoside test and Lysine test and gave negative results with Arginine test, Sodium citrate test, Sodium thiosulphate test, Tryptophane test, Sodium pyruvate test and Kohn's gelatin test. Eighty one point one percent (81.1%) *E. coli* isolates gave positive results with Ornithine test, 93.3% with Urease test and 95.6% with Indole test.

Results of sugars fermentation tests on Api 20E rapid system were as follows:

76.7% of *E. coli* isolates fermented Glucose, 86.7% fermented Mannitol, 35.6% fermented Inositol, 56.7% fermented Sorbitol, 58.9% fermented Rhamnose, 40.0% fermented Sucrose, 52.2% fermented D-melibiose, 17.8% fermented Amygdalin and 86.7% fermented Arabinose.

Slight variations in the biochemical behavior of the microorganism may be attributed to variations in the genetic constituents of different stains resulting in different phenotypic characteristics. These genetic variations may be of chromosomal or plasmid origin. In both circumstances, transfer of genetic material between strains of the same species does occur through different mechanisms. These findings generally fulfilled the API 20E requirements for identification as *E. coli* isolates (Holmes *et al*., 1978; Willis and Cook, 1975).

Twenty *E. coli* isolates were selected randomly and inserted into VITEK2 automated identification system. The isolates scored high probability percentages and there were slight variations in the results of their biochemical tests. All isolates were positive for D-maltose, D-mannitol, D-trehalose, Succinate alkalinisation, Lysine decarboxylase, D-glucose, D-mannose, α-galactosidase, Coumarate, β-galactosidase, Fermentation of glucose, D-sorbitol and β-glucoronidase tests. All isolates were negative for Ala-phe-pro-Arylamidase, H2S production, β-Glucosidase, Glycine arylamidase, Adonitol, B-N-actyl-glucoseaminidase, Lipase, D-tagatose, α-glucosidase, GLU-Gly-Arg-Arylamidase, L-pyrrolydonyl-arylamidase, Glutamyl Arylamidase pNA,

Palatinose, L-malate assimilation, L-arabitol, Sodium citrate, β-N-acetyl-galactosaminidase, L-histidine assimilation, D-cellobiose, γ-glutamyl-transferase, β-xylosidase, Urease, Malonate and Phosphatase.

90.0% for Sucrose and O/129 resistance (comp. vibrio), 80% for Amygdalin,70.0% for L-lactate alkalinisation and Ornithine decarboxylase, 45% for Tyrosine arylamidase, 40% for Ellman and 5-keto-D-Gluconate and only 10% of *E. coli* isolates were positive for L-proline Arylamidase test. Although the results of biochemical reactions revealed many differences between the isolates, they fulfill the requirements for identification as *E. coli* isolates by VITEK2 system according to Shetty *et al*., (1998) and David (2005).

According to the present findings Vitek2 as an automated system provided very good and trustable accuracy and reproducible results as shown in repeated samples of same source. Actually its developed methods are used for identification of bacterial samples that conventional methods can't recognize properly. Use of conventional culture media instead of TSS or PVX branded culture media (Biomerieux) for preparing bacterial samples didn't influence the accuracy level in vitek2 results. Automated systems gain 24 hours time saving in diagnosis process which is a favorite advantage and sometimes a vital issue. Identification results showed good advantages of automated system against manual API 20's methods where the average confidence level (probability) of results raised about 10%.

In spite of good accuracy and short identification time, Vitek2 still

depends on conventional microbiology techniques like isolation and it needs high concentration of pure isolate suspensions to process. The preparation of pure suspension of desired bacterial sample, needs about 48 hours time before processing of sample by analyser. The second 24 hours of this long time is the major limitation of using automated systems for all of arriving samples in microbiology labs. Meanwhile at the same time (second day) conventional tests could be run by only one isolated colony of desired bacteria. The other

limitation of Vitek2 autoanalyzer is the capacity of machine which is another reason for selective sample processing. There are two compact (30 samples) and ordinary (60 samples) models of Vitek2. These limitations lead the lab to use automated system only for a selective group of the samples.

In cost comparison between API Microsystems and vitek2 automated system only the cost of differences were considered. The total cost of analyze by API and vitek2 systems are higher because there are other common costs like primary isolation or culture costs which are almost same for both of systems. The common costs are neglected to make comparison easy. In case of vitek2 there is a high fixed capital cost for the machine, but there is no fixed capital cost for API Microsystems and all needed material is consumed during daily process. API 20E strips seems even cheaper than vitek GN cards.

With success against these three limitations automated systems could be replaced of manual conventional methods in clinical microbiology lab. Economic studies estimate a good future for automated systems in microbiology lab (Funke, 2004; Ling *et al*., 2001; Ling *et al*., 2003; Otto-Karg *et al*., 2009; O'Hara and Miller, 2003; Simoons-Smit and Maclaren, 1994).

This is the first report in Sudan of using VITEK2 automated identification system for identification of *E. coli* isolates.

Isolation of *E. coli* doesn't necessarily means the presence of the disease unless, virulence factors are identified i.e. toxins and/or fimbriae (Sack, 1980; Nakazawa *et al.,* 1987; Quinn *et al*., 2011; Hirsh, 2004). In this study 110 *E. coli* isolates from different localities of Khartoum State selected randomly and tested for specific fimbrial antigens possession. The percentages of K99 (F5), K88 (F4) and 987p (F6) fimbrial antigens in Khartoum State were: 17.3%, 15.5% and 1.8% respectively. The percentages of F5 were higher (20%) in

Bahry and East Nile and Khartoum and Babal Awleia localities than Omdurman locality (12.0%). The percentage of K88 was higher in Bahry and East Nile locality (16.0%) than Omdurman and Khartoum and GabalAwleia localities (15.0%).

Two *E. coli* isolates (5.0%) in Omdurman locality, as a new finding, was found to be positive for F6. The reasonable explanation for that may be the availability of contact between pigs which rose by some people and dairy herd.

These findings are supported by the findings of Gyles *et al*., (1993); Quinn *et al*. (2001) and Hirsh (2004) who reported that the most significant fimbrial antigens of pathogenic *E. coli* strains to domestic animals are F4, F5, F6 and F41 fimbrial antigens. Morin *et al*. (1978) identified F5 in 29% of *E. coli* isolated from intestinal tract of diarrhoric calves. Tzipori (1985) reported the detection of F41 in 60%, F4 in 20% and F5 in 20% of isolated *E. coli* from diarrhoeic calves. In Sudan, Salih *et al*. (1998) detected only F41 and F4 fimbrial antigens of *E. coli* strains isolated from diarrhoeic camel calves. Elgaddal (2009) detected *E. coli* F4, F5 and F6 fimbrial antigens from diarrhoeic camel calves in Butana, Upper Atbara River and Northwest Kordofan areas. Ellaithi (2004) detected *E. coli* F4, F5 and F41 adhesion antigens in diarrhoeic calves in Khartoum North, Butri, Masoudia, Um Haraz, River Nile State and Kenana areas. These findings collectively proved the significance of fimbrial antigens.

In this study 50 randomly selected *E. coli* isolates were tested by the Suckling mouse test (SMT) for production of heat-stable (STa) enterotoxin. Forty five isolates (90%) gave positive results with SMT test. This finding agrees with Ellaithi (2004) who found that 85.7% of isolated *E. coli* produced

STa enterotoxin. The reliability of the suckling mouse was also confirmed by Dean *et al*. (1972). This study disagrees with Gyles (1971) who stated that the infant mouse test is unsatisfactory as a method for detection of STa enterotoxin.

The same Fifty *E. coli* isolates used in SMT test, were subjected to LT enterotoxins production ability test by using RPLA (Oxoid, TD 0920A) kit. Ten isolates (20%) gave positive results, i.e. agglutination was noticed as a net onto the bottom of the wells. These ten isolates were also proved to possess STa by SMT test. The percentage of positive test was higher in Bahry and East Nile locality (25.0%) than Omdurman (17.6%) and Khartoum and GabalAwleia (15.4%) localities. Same findings were reported by Salih *et al* (1998) who detected LT in 8 (27.6%) of 29 *E. coli* isolated from camel calves and also by Elgaddal (2009) who detected LT in 16.6% *E. coli* isolated from camel calves.

This is the first report in Sudan of detection of LT in *E. coli* isolated from diarrhoeic dairy calves by using RPLA (Oxoid, TD 0920A) kit. Also 10 *E. coli* isolates reported to have both STa and LT enterotoxins and according to Quinn *et al*., (2011), the possession of the two enterotoxins increases the bacterium virulence.

In this study 150 isolates out of 260 *E. coli* isolated from diarrhoeic samples were subjected to sensitivity tests using 10 antimicrobials in use for treatment of calf diarrhoea in Sudan. Ninety six point seven percent (96.7%) of the isolates were sensitive to Gentamycin, 90.7% to Ampicillin, 84.0% to Ciprofluxacin, 80.0% to Sulphamethoxazole -trimethoprim, 76.0% to Cephalothin, 74.6% to Kanamycin, 74.0% to Chloramphenicol, 70.0% to Tetracycline, 15.3% to Erythromycin and 0.0% to Procaine Penicillin.

According to Quinn *et al*. (2011) *E. coli* showed variation in their susceptibility to various chemotherapeutic agents in use for treatment of calf diarrhoea such as: Amoxicillin, Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamycin, Kanamycin, Nalidixic acid, Streptomycin, Sulphamethoxazole, and Erythromycin. According to Radostits *et al*. (2007) Gentamycin has improved stool consistency in calves with experimentally induced *E. coli* diarrhoea. Ellaithi (2004) reported that all *E. coli* isolates were sensitive to choramphenicol and Erythromycin. 97% of the isolates showed different patterns of sensitivity to other antibiotics used (Nalidixic acid, Neomycin, Tetracycline, Ampicillin, Gentamycin, Sulfamethoxazole/trimethoprim,and Streptomycin).

Mohamed (2009), reported that *E. coli* isolates were highly sensitive to Nitrofuran(98%), Colistin sulphate (98%), Gentamycin (97%) and Cotrimoxazole (82%) and resistant to the rest of antibiotics (Streptomycin, Tetracycline, Nalidixic acid and Ampicillin).

On other hand, the resistance of some isolated *E. coli* to antimicrobials used in this study was explained by Radostits *et al*. (2007) as follows: The CTX-M-14-like enzyme has been detected in the *E. coli* recovered from the faeces of diarrheic dairy calves in Wales. The enzyme is an extended-spectrum beta-lactamase (ESBL), which confers resistance to a wide range of beta-lactam (Penicillin and Cephalosporin) compounds. Organisms possessing ESBL are considered to be resistant to second-, Third-, and

fourth- generation Cephalosporines, and in vitro resistance to Amoxicillin/Calvulanate among producers is variable, reflect in the amount of beta-lactamase produced. In addition to this enzyme, the isolates produced a TEM-35 (IRT-4) beta-lactamase that conferred resistance to the Amoxicillin/ Calvulanate combination. Oxytetacycline or Chlortetracycline is also not

recommended for the treatment of bacteraemia, although Tetracyclines may have some efficacy for treating *E. coli* bacterial overgrowth of the smaller intestine. Tetracycline antimicrobials are bound to calcium, and oral bioavailability when administered with milk is 46% for Oxytetracycline and 24% for Chlortetracycline.

Conclusion

This study is concluded in the followings:

1. According to results of Questionnaire survey of dairy farms in different localities of Khartoum State, the main health problem in calves is calf diarrhoea (78%).

2. The incidence rate of calf diarrhoea in Khartoum State is 60%. It is higher in female calves (78.0%) than male calves (22.0%) and this causes economic loss due to losses of calving and milk production in future. Also it is higher in fist calves of heifers than calves of older cows. The prevalence of calf diarrhoea is higher during autumn seasons of the years, 2010 and 2011 (91.4% and 82.9% respectively) and winter seasons of the years 2010, 2011 and 2012

(71.4%, 57.1% and 47.1% respectively) than summer seasons of the years 2010 and 2011 (40.0% and 33.3% respectively).

3. The mortality rate among diarrhoeic calves is higher during first and second weeks of age (29.8% and 20.0%) than the third week (19.1%).

4. *E. coli* represents the predominant Bacterial spp. (76.0%), isolated fromdiarrhoeic calves. Other bacteria are: *Escherichia fergusoni* (2.3%), *Escherichia vulneris* (0.6%), *Klebsiella pneumonia sub spp. Ozaenae* (6.4%), *Proteus mirabilis* (8.8%), and *Enterococcus faecalis* (5.8%).

5. The results of 90 *E. coli* isolates on API 20E strips showed different identification percentages ranged from 99.8% to 89.8%.

6. This is the first report in Sudan of using VITEK 2 autoanalizer machine for identification of *E. coli* isolates. The isolates scored high probability percentages ranged from 96% to 99%.

7. The percentages of K99 (F5), K88 (F4) and 987p (F6) fimbrial antigens in Khartoum State are: 17.3%, 15.5% and 1.8% respectively**.** Two *E. coli* isolates (5.0%) in Omdurman locality, as a new finding, is found to be positive for F6.

8. *E. coli* isolates were tested by the Suckling mouse test (SMT) for production of heat-stable (STa) enterotoxin. Forty five isolates (90%) gave positive results with SMT test.

9. This is the first report in Sudan of detection of LT in *E. coli* isolated from diarrhoeic dairy calves by using RPLA**.** Ten isolates (20%) gave positive results, i.e. agglutination was noticed as a net onto the bottom of the wells.

10. Ninety six point seven percent of the isolates are sensitive to Gentamycin, 90.7% to Ampicillin, 84.0% to Ciprofluxacin, 80.0% to Sulphamethoxazole -trimethoprim, 76.0% to Cephalothin, 74.6% to

Kanamycin, 74.0% to Chloramphenicol, 70.0% to Tetracycline, 15.3% to Erythromycin and 0.0% to Procaine Penicillin.

Recommendations

1. Further studies should include a survey of more animals in different farms and an extensive study of the significance of *E. coli* in calf diarrhoea.

2. Further studies should be carried out to investigate the predisposing factors related to the incidence of neonatal calf diarrhoea and to identify different causes of calf diarrhoea.

3. Pregnant cows should be isolated within the last two weeks before calving. Moreover, feeding colostrums during the first day is strongly advised. Other management factors should not be underestimated.

4. Serotyping of *E. coli* isolates obtained from different areas should be given more attention.

5. The role of *E. coli* heat-stable (STb) enterotoxin, verotoxins (VTs) and cytotoxic necrotizing factors (CNF) in disease causation should be further investigated.

6. The application of PCR for *E. coli* isolates using ERIC primers will be helpful for study the epidemiology of neonatal calf diarrhoea caused by *E. coli.*

7. A comprehensive study of *in vitro* susceptibility of pathogenic *E. coli* to different antimicrobial drugs and the genetics of antimicrobial resistant *E. coli* isolates should be further investigated.