Bacteria Associated with Diarrhea in Lambs Goats child and Antibiotic Susceptibility in North Kordufan State-Sudan

البكتريا التي تسبب الإسهال في الحملان والجديان وحساسيتها للمضادات الحيويه في ولاية شمال كردفان - السودان

By

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DEDICATION

This work I dedicated

To

My mother and my father

My family

My brothers

My sisters

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Firstly, praise to Almighty Allah for giving me the strength and stamina to finish this work with a great touch of pleasure and gratitude.

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ABSTRACT

Lambs and goat kid's diarrhea is one of the main sign disease infecting in great lost of lambs and goat kid's as result of bacterial infections of the gastrointestinal tract. This study was carried out to identify bacterial agents associated with lamb and goat kids diarrhoea, to investigate the occurrence of Enterobacteriaceae in lamb and goat kid's diarrhoea and to determine the sensitivity of the bacterial isolating to commonly used and antibiotics (Tetracycline (30mcg), Gentamicin (10µg) Trimethoprim (5µg), Ampicillin (10µg), Ceftriaxone (30mcg), Ciprofloxacin (5mcg), Norfloxacin (10µg), Penicillin, Erythromycin (15µg), Chlormphenicol (30µg), Colistin (10µg) and Lincomycin (15µg)). Bacteriological method were used for identification of isolates and API20E strips were used for confirmation of the result. Agar diffusion method was used for Antibiotic sensitivity testing for the identified bacteria. A total 150 samples were collected from diarrhoeic lamb and goat kids of age 1 to 60 days from North Kordufan state areas during August and September 2017. The number of isolates were 91(60.7%) consisted 27(29.7%) *Escherichia coli,* 8(8.8%) *Interobacter cloacae,* 7(7.7%) *Pseudomonus aeruginosa*, 5(5.5%) *Proteus mirabills*, 5(5.5%) *Stenotrophomonas maltoplila,* 4(4.4%) *Klebsiella pnemoniae,* 3(3.3%) *Serratia macenscens,* 3(3.3%) *Salmonella Arizona,* 2(2.2%) *Yersinia pestis,* 2(2.2%) *Pseudomonas oryzihabitans,* 2(2.2%) *Serratia liquefaciens,* 1(1.1%) *Pseudomonas luteola,*1(1.1%) *Shigella spp* 1(1.1%) *Enterobacter gergoviae,*1(1.1%) *Citrobacter freundii,*1 (1.1%) *Citrobacter braakii,*1 (1.1%) *Citrobacter youngae. Escherichia coli* were found the most cause of diarrhea in lamb and goat kid's, followed by *Enterobacter cloacae.* Most isolates are highly sensitive to Gentamycine, Trimethoprim, Ceftriaxon, Norfloxacin, colistin and

Ciprofloxacin respectively, while highly resistant to Penicillin and Lincomycin.

ملخص الأطروحه

الاسهال في الحملان وصغار الماعز يعتبر واحد من اهم العلامات المرضيه التي تسبب فقدان كبير في صغار المجترات الصغيره نتيجه للاصابات البكتيريه في الجهاز الهضمي.هذه الدراسه اجريت للتعرف علي البكتريا سالبه الجرام المتعلقه بالاسهال في الحملان وصغار الماعز وتحديد حساسية البكتيريا المعزولة للمضادات الحيوية شائعة الاستخدام مثل (التتراسيكلين (30 ميكروغرام) ، الجنتاميسين (10ميكروجرام)، تريميثوبريم (5 ميكروجرام)، أمبيسلين (10ميكروجرام)، سيفترياكسون (30ميكروجرام)، سيبروفلوكساسين (5 ميكروجرام)، نورفلوكساسين (10ميكروجرام)، بنسلين، إريثروميسين (15 ميكروجرام)، كلورمفينيكول (30 ميكروجرام)، كوليستن (10 ميكروجرام)، لينكومايسين (15مايكروحرام). تم استخدام الطريقة البكتريولوجية لتحديد العزلات واستخدمت شرائح API20E لتأكيد النتيجة .تم استخدام طريقة انتشار الأجار لاختبار حساسية المضادات الحيوية للبكتيريا التي تم عزلها. تم جمع إجمالي 150 عينة من الحملان وصغار الماعز المصابه بالإسهال الذين تتراوح أعمارهم من يوم الي 60 يومًا من ولاية شمال كردفان خلال شهري أغسطس وسبتمبر 2017. وكان العدد الكلي للعزلات هو91 عزله، تم تعريف العزلات وفقاً للفحص المجهري والخصائص المزرعيه والبايوكيميائيه. وقد شملت العزلات 91(60.7%) عزله بكتيريه سالبه لصبغة جرام منها 27(27.7%) اشيريشيا كولاي، 8(8.8%) انتيروباكتر كلواكا، 7(7.7%) سدموناس اريجنوزا، 5(5.5%) بروتس ميربلس، 5(5.5%) استينوتروفومونس، 4 (4.4%) كلبسيلا نيمونيا، 3 (3.3%) سراتيا مارسنسس، 3(3.3%) سامونيلا اريزوني، 2(2.2%) يسرينيا ستاسي، 2(2.2) سدموناس اورزيهبيتنس، 2(2.2) سراتيا لكويفاسينس، 1(1.1) سدموناس ليوتيولا، 1(1.1) شايقلا، 1(1.1) انتيروباكتر جيرجوفيي، 1(1.1) ستروباكتر فريندي، 1(1.1) ستروباكتربراكي ، 1(1.1) ستروباكتر ينقي. وجد ان الاشريشيا كولاي اكثر بكتريا تسبب الاسهالات في الحملان وصغار الماعز ثم تليها انتيروباكتر كولواكا. معظم العزلات حساسة للجنتامايسين ، تريميثوبريم ، سيفترياكسون ، نورفلوكساسين، كوليستين وسيبروفلوكساسين على التوالي ، بينما كانت شديدة المقاومة للبنسلين واللينكومايسين.

Introduction

Infectious diarrhea is the most significant cause of morbidity and mortality in neonatal sheep and goat kid's throughout the world**.** Despite improvements in management practices, prevention and treatment strategies, diarrhea is still the most common problem and costly disease affecting neonatal small ruminants. Diarrhea in lambs and goats is a complex, multi-factorial disease involving the animal, environment, nutrition and infectious agents. The four major causes of diarrhea in lambs and kids during the first month of life are: *E. coli*, which is most common, rotavirus, *Cryptosporidium* spp. and *Salmonella* spp. (Schoenian, 2007). In a study at sheep experiment station in USA, it was found that diarrhea accounted for 46% of lamb mortality and the neonatal mortality among control group of kids was 1.0% compared to 3.2% in the other non control group. The study also indicated that separating the neonates from adult animals, not vaccination of dams against infectious diseases especially against pasteurellosis, colibacillosis and enterotoxaemia, and miss mothering acts as predisposing factors at the disease in the newborn which characterized by progressive dehydration and rapid death, sometimes in less than12 hours. In the sub- acute form, diarrhea may persist for several days and result in emaciation and stunted growth (Sethi, 2019).

This study is the first trial on the investigation of diarrhea among lambs and goat kid's in North Kordufan State as a breeding area of sheep and goats.

Objectives of the study

1- To identify the isolated bacteria by conventional bacteriological methods and rapid test.

2- To evaluate the antimicrobial reactions of the isolates using agar diffusion method.

3- To demonstrate the most effective antimicrobial agent for treatment of lamb and goat kid's diarrhea.

Chapter One

Literature Review

1.1. Definition of diarrhea

Diarrhea often results in fluid and electrolyte losses due to infectious diseases. As long as the ruminant neonate can compensate for losses, it will remain hemodynamically stable and continue to nurse. However, if losses exceed intake, systemic، effects will be observed on clinical examination. Fluid loss from the vascular compartment leads to hypovolemia dehydration, hypotension, and shock. Metabolic acidosis develops as a result of intestinal and fecal loss of sodium bicarbonate, increased L-lactate from hypoperfused tissues, and increased absorption of L-lactate and D-lactate produced by bacterial fermentation in the intestinal tract (Lorenz *et al*., 2005).

1.2. Pathogenesis of diarrhea

Diarrhea in neonatal ruminant is usually associated with disease of the small intestine and can be caused by either hypersecretion or malabsorption. Hypersecretory diarrhea occurs when an abnormal amount of fluid is secreted into the gut, exceeding the resorptive capacity of the mucosa. In malabsorptive diarrhea the capacity of the mucosa to absorb fluid and nutrients is impaired to the extent that it cannot keep up with the normal influx of ingested and secreted fluid (Aiello and Mays, 1998). Depending on the causative agent; intestinal malabsorption may be a result of different pathological mechanisms which are: Osmotic effect: when a substance within the lumen of the intestines raises the osmotic pressure to a level that permits excessive fluid to move into the lumen of the intestine, the fluid is not reabsorbed and therefore accumulates in the lumen creating circumstances conducive to diarrhea. Acute or chronic

inflammation and necrosis of the intestinal mucosa will result in both net increase in fluid production and inflammatory products, including lose of serum proteins and electrolytes. Examples include many of the diseases caused by bacteria, viruses, fungi, protozoa and chemicals. Enteric colibacillosis is an example of diarrheal increases resulting from intestinal hypersecretion. The villi along with their digestive and absorptive capabilities, remain intact, however, their secretion is increased beyond the absorptive capacity of intestine, resulting in diarrhea and the outcome is a varying degree of dehydration, electrolytes imbalance, acidosis, hypocalcaemia and when acidosis is severe circulatory failure, shock and death occur (Radostits *et al.*, 1994).

1.3. Predisposing factors

Neonates obtaining insufficient passive immunity (antibodies) from colostrums, this might be due to either a quantitative or qualitative deficiency, intensive husbandry practice lead to a rapid transmission of the pathogenic *E.coli* strains **,**poor hygiene often allows a build-up of pathogenic strains in the young animals environment , the normal flora of the intestine is not fully established , naive immune system of neonate ,receptors for the adhesions of *E .coli* are present for the first week of life only in calves and piglets(Quinn *et al*., 1994).

1.4. Etiology of neonatal lambs and goat kid's diarrhea

Enteropathogenic bacteria and viruses play important role in causes diarrhea in livestock worldwide (Adesiyun *et al*., 2001). Other pathogens may also have a role in enteric diseases including: *Clostridium Perfringens*, *Giardia*, *Eimeria* spp, *Campylobacter*, *Klebsiella* spp and *Proteus* (Muñoz *et al.*, 1996).

Several agents may be cause diarrhea; more of these agents are affecting the host without inducing any clinical sings (Smith and Sherman, 1994).

There are four major causes of diarrhea in lambs and kids during the first month of life; *E.coli*, which is most common, rotavirus, *Cryposporidum* spp and *Salmonella* spp (Susan, 2007). *E. coli* bearing virulence attributes different from those typical of ETEC (verotoxigenic, F1 7+ or attaching affecting *E. coli*), have been found in diarrhoeic and healthy lambs and goat kids but the pathogenic significance for these species is unknown (Beutin *et al.,* 1993;Cid *et al*.,1993;Adesiyun and Kaminjolo,1994; Drolet *et al*.,1994).

1.4.1. Aerobic bacteria associated with lambs and goat kid's diarrhea

Lamb enteritis is economically important problem because several agents may be involved in the etiology. In study conducted in Behera province from 1200 examined lambs, 650 (54.16 %) showed enteritis and was also observed that enteritis rate was higher in closed system (83.70 %) than in open system (46.80 %)and the mortality rate was 4.16 % and case fatality was 7.69 %. Bacteriological examination of fecal samples revealed that 190 (29%) of the lambs were positive for pathogenic bacteria culture. The isolated bacteria were *E. coli* pathogenic form from 65 (34.20 %) which was the most predominant bacterial isolated. Other bacteria isolated were *Salmonella* 10 (5.26 %), *Clostridia* spp. 15 (7.89 %), *Proteus* spp isolated 25 (13.10 %), *Shigella* isolated 20 (10.52 %), *Klebsiella* Spp. 15 (7.89 %) (Mohammad *et al*., 2014).

1.4.1.1. *Enterobacteriacea*

Enterobacteriaceae are Gram-negative, non-spore forming, rods, up to 3µm in length and 0.6µm in breadth. They ferment glucose and

wide range of carbohydrate, oxidase negative, catalase positive, motile or non-motile, the motile strains have peritichous flagellate, they grow well in macConkey agar. This group of micro-organisms can be divided into three categories: Major pathogens, opportunistic pathogens and non pathogens. The major animal pathogens are *E coli*, *Salmonella* spp., and *Yesinia* spp., which can cause both enteric and systemic diseases (Quinn *et al*., 2001).

1.4.1.1.1. *Escherichia coli* (*E.coli*)

E.coli is worldwide in distribution, many are part of normal flora of the intestinal tract of human and animals (carter and Wise, 2004). The bacteria are a Gram-negative, motile, facultative anaerobe, non-spore forming. Most *E. coli* strains are part of gastrointestinal tract flora, but some strains possess virulence factors that enable them to cause diarrhea in neonatal farm animals and humans (Nguyen *et al*., 2005).The strains of *E. coli* can be divided into five groups or pathotypes of diarrhoeagenic *E. coli* include: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggregative (EAEC) and enterohaemorrhagic *E. coli* (EHEC). The strains are capable of producing toxins very similar to the one produced by *Shigella dysenteriae* type1. Therefore, these bacteria are often called Shiga toxin-producing *E.coli* (STEC). Active Shiga toxins may be detected using Vero cell toxicity test (Beutin *et al.,* 2007).

1.4.1.1.2. Type of pathogenic *E.coli*

Pathogenic *E.coli* defferent from non-pathogenic *E.coli* by the presence of virulence factors in chromosome or plasmid. According to the variation in DNA and distribution of genomic location, pathogenic *E.coli* divided into eight categories (Puente and Finlay, 2001).

1.4.1.1.2.1 Enterotoxigenic *E.coli* (ETEC)

Enterotoxigenic *E.coli* is important and wide cause watery diarrhea in new-born (sucking) calves and suckling and weaned pigs (Nagy and Feket , 1999). ETEC is divided into two virulence factor according to production of diarrhea. Fibril antigens (K99 or K88) enable to attach and colonize the villi of the small intestine and to secrete enterotoxins include LT (heat labile), ST (heat stabile) toxin (Aiello and May, 1998).

1.4.1.1.2.1.1 Enterotoxins

Enterotoxins are plasmid-regulated secreted peptides acting on the intestinal epithelium, representing the following enterotoxins categories large molecular weight (88 kDa) heat-labile enterotoxins (LT); small molecular weight (11-48 amino acid containing) heat stable peptide toxins (ST). The LT toxins have good antigenicity; can be divided into two antigenically and biologically distinct but structurally similar groups: LTI and LTII (O'Brien and Holmes, 1996).

Heat-labile enterotoxins

Mechanism of action of LT and ST enterotoxins is the production of functional change in small intestine epithelial cells leading to increase secretion of H_2o and CL^- and decrease of fluid absorption and dehydration and acidosis. LT is functionally and structurally related to *vibrio toxin (*O'Brien and Holmes,1996).

Heat-stable enterotoxins

The ST(STa) is a 2-kDa peptide containing 11-18 amino acids it can Induce small intestinal fluid secretion in newborn but not in weaned pigs, STb which can induce fluid secretion in both age groups of pigs and rats (Nagy and Fekete,1999; Whipp, 1991).

1.4.1.1.2.2 Enteropathogenic *E.coli* (EPEC)

Enteropathogenic *E. coli* (EPEC) have a number of virulence attributes that distinguish them from non-pathogenic strains. Adherence to the mucosal cells of the gut and production of toxins it essential to enteropathogenisity EPEC usually the peristalsis of the small intestine by sticking to the enterocytes with the adhesive antigen K99. Enterotoxins are produced near the mucosal receptor sites, which lead to leakage of fluid into lumen, and cause diarrhea (Donnenberg and Kaper, 1992).

1.4.1.1.2.3. Enteroinvasive *E.coli* (EIEC).

EIEC adhere to the cells of the distal small intestine, invade the enterocytes and deeper layers of the intestinal mucosa, they reach lymphatic system where their multiplication exits. The death of some *E.coli* cells occurs and endotoxin is released. The virulence factors adhesions and alpha-hemolysin are important as survival factors for these invasive strains, which are responsible for colisepticaemia (Quinn *et al*., 1999).

1.4.1.1.2.4 Enteroaggregative *E.coli* (EAggEC)

These strains are associated with persistent with diarrhea in young children, and have ability to attach tissue culture cells in aggregative manner, the bacteria adhere to intestinal mucosa and cause non-bloody diarrhea without causing of inflammation, they also produce a hemolysine related to the hemolysin produced by *E.coli* strains involved urinary tract infections (Todrak, 2002).

1.4.1.1.2.5. Attaching and effecting *E.coli* (AEEC)

The AEEC colonize the intestinal mucosa, attach to target cells and kill them. These strain have been Isolated from calves and rabbits with enteric disease (Quinn *et al.,* 1999).

1.4.1.1.2.6. Entero hemorrhagic or verotoxigenic *E.coli* (EHEC)

Enterohemorrhagic *E. coli* (EHEC) cause hemorrhagic colitis and are often associated with devastating or life-threatening systemic manifestations. The most severe sequelae, the hemolytic uremic syndrome (HUS), results from Shiga toxins (Stxs) produced by the bacteria in the intestine and act systemically on sensitive cells in the kidneys, brain, and other organs (Gyles, 2007). Although most EHEC strains produce Stxs, EHEC O157:H7 are especially virulent and are responsible for the majority of HUS cases of bacterial etiology worldwide (Gyles, 2007; Serna and Boedeker, 2008).

1.4.1.1.2.8. Uropathogenic *E.coli* (UPEC)

It causes urinary tract infection, UPEC encode several adhesion factors, and both fembrial and non fembrial adhesions like type one Pilli. Many of EPEC produce hemolysin which may be involved in kidney disease in addition to cytotoxin necrotizing factors (Johnson,1991).

1.4.1.1.8.1. *Escherichia coli* O157:H7

E. coli O157:H7 is gram negative bacillus. The "O" refers to somatic antigen and the "H" refers to flagella antigen. The organism has a cell structure Similar to Gram-negative cells and posses an outer membrane with lipo-polysaccharide component that is distinct from the cytoplasmic membrane. The O157 antigen is defined by the carbohydrate composition and structure within the lip- polysaccharide. The H7 antigen

is determined by the unique polypeptide composition of the flagella. E. coli are serologically differentiated based on three major surface antigens: O(somatic),H (flagella), and K (capsule) (Figure 1). A total of 173O antigens,56H antigens,and103K antigens have been identified(Bettelheim and Thomas, 2005). Enterohaemorragic *E. coli* O157:H7 and related organisms are also referred to as Shiga toxin-producing *E. coli* (STEC) because of their ability to produce Stxs (formerly called Shiga-like toxins). EHEC O157 are present in fecal flora of the number of animals including cattle, sheep, goats, pigs, dogs, cats, chickens and gulls, with the primary source of infection for man considered to be cattle and bovine-derived meat products (Griffin and Tauxe, 1991).

1.4.1.1.3. Pathogenesis of *E.coli*

Diarrhea in neonatal ruminants is usually associated with disease of the small intestine and can be caused by hypersecretion or malabsorption. Hypersecretory diarrhea develops when an abnormal amount of fluid is secreted into the gut, exceeding the resorptive capacity of the mucosa. In malabsorptive diarrhea, the capacity of the mucosa to absorb fluid and nutrients is impaired to the extent that it cannot keep up with the normal influx of ingested and secreted fluids. This is usually the result of villous atrophy, in which the loss of mature enterocytes at the tips of the villi results both in a decrease in villous height (with a consequent decrease in the surface area for absorption) and in loss of the brush border digestive enzymes. The natural habitat of *E. coli* is the gastrointestinal tract of warm-blooded animals, and in humans, this species is the most common facultative anaerobe in the gut. Although most strains exist as harmless symbionts, there are many pathogenic *E. coli* strains that can cause a variety of diseases in animals and humans (Whittam 1996). Pathogenic *E. coli* strains differ from those that predominate in the enteric flora of

healthy individuals in that they are more likely to express virulence factors molecules directly involved in pathogenesis but ancillary to normal metabolic functions. Expression of these virulence factors disrupts the normal host physiology and elicits disease. In addition to their role in disease processes, virulence factors presumably enable the pathogens to exploit their hosts in ways unavailable to commensal strains, and thus to spread and persist in the bacterial community (Quinn *et al*., 1994).

The enterovirulent *E. coli* rank among the most common causative agents of bacterial diarrhea in several animal species as well as in humans (Nataro and Kaper, 1998).

1.4.1.1.4. *Klebsiella Pnemoniae* and *Klebsiella.oxytoca*

Klebsiella pneumoniae is a Gram-negative, non-motile, capsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. Although found in the normal flora of the mouth, skin, and intestines (Ryan *et al*., 2004). *Kleb. pneumoniae* has been demonstrated to increase crop yields in agricultural conditions (Riggs *et al*., 2001). The most common condition caused by *Klebsiella*, bacteria outside the hospital is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients have an increased tendency to develop lung abscess, cavitations, emphysema, and pleural adhesions.

1.4.1.1.5. *Proteus mirabilits* and *Proteus.vulgaris*

Natural habitat is the faeces of mammals and environment. *Proteus* spp causes urinary tract infection of dogs and horses and also associated with otitis externa in dogs and cats. On occasion, these species are

thought to be involved in diarrhea in young lambs, calves, goats and other animals (Quinn *et al*., 1999; Carter and Wise, 2004).

1.4.1.1.6. *Yersinia enterocolitica*

It is a gram-negative cocobacilli, which shows bipolar staining and motile when incubated at 22°C and non motile at 37°C. Also is aerobic and facultative anaerobic, selective media of this organism as MacConke's agar or SSA. This organism has been isolated from cattle, sheep, goats and others animals. It causes infection in animals in the form of ileitis, gastroenteritis and mesenteric inflammation (Carter and Wise, 2004).

1.4.1.1.7. *Enterobacter cloacae*

Enterobater Spp. have many features in common with *Kleb.* Spp. The organism is gram-negative motile bacteria, and can be found in intestinal tract of man and animals, soil, sewage, watery and diary product (Quinn *et al.,* 1999). Some strain may produce haemolysin. An outer protein termed Ompx may be pathogenic factor for strain of *Enterobacter.cloacae*. This protein appears to reduce production of porins, leading to decrease sensitivity to beta lactam antibiotics and might play role in host cell invasion (Greenwood *et al.,* 2002).

1.4.1.1.8. *Salmonella* spp

Salmonellae are Gram-negative rods; non-capsulated, non-sporulated and most of the species are motile by peritrichous flagella (Adesiyun *et al.,* 2001).

1.4.1.1.9. Diagnosis of *enterobacteriaceae*

Members of the *Enterobacteriaceae* are small Gram negative, non-sporing straight rods. Some genera are motile by means of peritrichous

flagella except *Tatumella*, *Shigella* and *Klebsiella* species which are non-motile. They are facultatively anaerobic and most species grow well at 37°C, although some species grow better at 25-30°C. They grow well on peptone and meat extract media. Some strains grow on D- glucose as the sole source of carbon and energy, but other strains require vitamins and or amino acids. Acid is produced during the fermentation of D-glucose and other carbohydrates (Holt *et al*., 1994).

1.4.1.1.9.1. Clinical examination:

All animals were subjected to through clinical examination including general health condition and body temperature, pulse, respiration, character of mucous membranes, auscultation of chest and abdomen and characters of the diarrhea.

Fecal samples:

Rectal swabs were taken from diarrhoeic (lambs) by means of sterile cotton swabs and transported to laboratory as soon as possible in sterile nutrient broth that incubated at 37°C for at least 4-8 hours to increasing chances of isolation (Kelly, 1984).

1.4.1.1.9.2. Bacteriological examination:

The samples (rectal swabs or tissue organs) from lambs were cultivated aerobically and anaerobically and bacterial isolates were subjected to characterization by studying their morphological, cultural, and biochemical characteristics as well as their motility according to *(*Quinn *et al.*, 2002*)*.

1.4.1.1.9.3. Serological identification of *E. coli* isolates:

The *E. coli* isolates were confirmed biochemically and subjected to serological identification by using poly-specific and mono-specific antisera, using slide agglutination test using somatic (O) antisera with heat inactivated bacteria (Ewing 1986)**.**

1.4.1.1.9.4. Conventional bacteriological method.

A-primary test

Traditional culture methods, including sample preparation, enrichment, dilution, planting, enumeration, and isolation of single colonies for further count. Selective and/or differential media are used for enumeration of specific class of bacteria and pathogens (Lau *et al*., 2003; Gracias and McKillip 2004; Kuria *et al*., 2009). Culturing and isolation of the test organisms a sterile wire loop has been used to inoculate stool samples on Macconkey agar, then incubated at 37°C for 24 hours. The pale colonies show the presence of both *Shigella* and *Salmonella*, while *E. coli* appears pinkish in color. *Shigella, Salmonella* and *E. coli* are all gram negative bacteria (Cheesbrough, 2000).

B-Secondary biochemical test: -

All isolates were subjected to the following biochemical tests: - (IMVIC) Indole, methyl red, Voges-Proskauer and Citrate. Both the methyl red and Voges-Proskauer tests were commonly used in conjunction with the indole and citrate tests to form a group of tests known as IMVIC which aid in the differentiation of *Enterobacteria* (Holt, 1994).

1.4.1.1.9.5. Rapid test

1.4.1.1.9.5.1. Api 20E strip

The API 20E kit is an identification system for *Enterobacteriaceae* and other non-fastidious Gram-negative rods, which uses 21 standardized and miniaturized biochemical tests and a database. It consists of 21 microtubes containing dehydrated substrates. These tubes are inoculated with a bac-terial suspension which reconstitutes the media. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the table provided and the identification is done using

the software provided by the manufacturer on the Internet, the apiweb. A seven-digit profile is obtained. API 20E ratings are based on three parameters, including the likelihood of a match between the unknown organism's profile and the computer profile, the relative value between the likelihood of the first and the likelihood of the second choice, and the number of tests against the first choice (Brown and Leff 1996).

1.4.1.1.9.5.2. Automated vitek 2 compact

Automation in clinical microbiology started much later than other clinical laboratories. Automation in clinical lab began in clinical chemistry with introducing chemical analyser machines based on "continuous flow analysis (CFA)". It was invented by Leonard Skeggs in 1957, and commercialized by Technicon® Corporation in 1960 decade (Galen 1990). Clinical serology and enzymes studies were joined to the automated departments. But it took more time to introduce first auto analyser in microbiology lab. Application of automated systems in clinical microbiology is different than other clinical laboratories. The main difference is the sterile working condition in microbiology and impure clinical bacterial samples which makes one-day extra operation for isolation of pure isolated samples. Automated analyse machines still are not popular in daily operation in microbiology laboratories. Where other clinical laboratories are completely replaced automated systems instead of manual methods. Sterile working condition nowadays has been replaced by higher concentrations of bacteria in feeding samples. But pure colonies preparation which is needed for identification process in microbiology still is a problem that needs conventional overnight culture plates. Thus, diagnosis process couldn't be start directly after sampling (David and Pincus, 2005).

1.4.1.1.9.6. Molecular diagnosis

Molecular diagnostics is a collection of techniques used to analyse biological markers in the genome and proteome-the individual's genetic code and how their cells express their genes as proteins-by applying molecular biology to medical testing. The technique is used to diagnose and monitor disease, detect risk, and decide which therapies will work best for individual patients, Poste (Burtis, *et al.,* 2012). By analysing the specifics of the patient and their disease, molecular diagnostics offers the prospect of personalised medicine (Hamburg and Collins 2010).

1.5. Treatments of lambs and goat kid's diarrhea

Treatment includes fluid and electrolytes replacement, alteration of the diet, antimicrobial and immunoglobulin therapy and use of anti diarrhoeal drugs and adsorbents fluid and electrolyte therapy is most important and should be instituted as soon as possible regardless of whether clinical evidence of dehydration has developed (Blood *et al.*,1990).

1.6. Antibiotic

Antibiotic used in livestock is in any antibiotics used for any purpose in the husbandry of livestock, which includes treatment when ill (therapeutic), treatment of a group of animals when at least one is diagnosed with clinical infection (Bousquet *et al.*, 2010) and used as preventative treatment (prophylaxis). Antibiotics are an important tool to treat animal as well as human disease, safeguard animal health and welfare, and support food safety (British Veterinary Association, 2019). However, when used irresponsibly, this may lead to antibiotic resistance which may impact human, animal and environmental health (Sarmah, 2014), while levels of use vary dramatically from country to country, for

example some Northern European countries use very low quantities to treat animals compared with humans, (European Medicines Agency, 2019).Worldwide an estimated 73% of antimicrobials (mainly antibiotics) are consumed by farm animals (Boeckel *et al*., 2019). Furthermore, the report also estimates that global agricultural antibiotic usage will increase by 67% from 2010 to 2030, mainly from increases in its use in developing BRIC countries (Van Boeckel *et al*.,2015).

1.6.1. Classification and mechanism of action of antibiotics

Antimicrobial agent can be divided into four groups as they affect the synthesis of protein and nucleic acid, formation of cell walls and permeability of cell membrane.

1.6.1.1. Protein synthesis inhibitors

According to inhibitors protein synthesis, they include: - -Aminogycoside e.g. gentamycine, kanamycin, tobramycine, streptomycin, tertracycline and chloramphinnicol. Macrolides, e.g erythromycin and azithromycin, and lincosamides, e.g clindamycin (Forbes *et al*., 1998; Cheesbrough,2000).Tetracycline inhibit protein synthesis by binding to the 30s ribosomal subunit so that incoming t RNA-amino acid complexes cannot bind to the ribosome, thus halting peptide chain elongation. Tetracycline has a broad spectrum of activity that includes gram-negative bacteria, gram-positive bacteria and several intracellular bacteria pathogens such as Chlamydia and Rickettsia (Forbes *et al*., 1998).Aminoglycosides inhibit bacterial protein synthesis by binding to the organism 30s ribosomal subunit. This process interrupts several steps, including initial formation of the protein synthesis complex, accurate reading of the mRNA code, and disruption of the ribosomal-mRNA complex (Forbes *et al*., 1998).Macrolides (erythromycine and

azithromycin) bind to the 50s submit of the ribosome and the binding site is a 23s r RNA (Jawetz *et al*.,1998).

1.6.1.2. Nucleic acid inhibitors

Replication of the nucleic acid of the bacterial cell is prevented directly by nalidixic acid and rifamycin and indirectly by the sulphonamides. Sulphonamides ultimately deprive the cell of nucleic acid and the presence of nalidixic acid prevents its replication (Aiello and Mays 1998).

1.6.1.3. Inhibitors of cell wall synthesis

Cell wall play essential role in the life of bacteria, which composed of peptidogycan. The cell wall of bacteria is tough and rigid and lies external to the cell membrane preventing the whole cell production from possible osmotic damage (Forbes *et al*.,1998). Several agents affect cell wall synthesis, the most important being penicillin, cloxacillin, ampicillin, amoxicillin, cephalosporine, cefuroxine, ceftazidime, vancomycin (Cheesbrough, 2000). Beta lactam antimicrobial agent are that contain the four-membrane, nitrogen-containing beta lactam ring at the core of their structure, and mode of action of these drugs that target and inhibit cell wall synthesis by binding the enzymes involved in synthesis. These beta lactam antibiotics inhibits the last step in peptidoglycan synthesis, the final cross linking between peptide side chains, mediated by bacterial carboxy peptidase and transpeptidase enzymes (Forbes *et al*., 1998).

1.6.1.4. Inhibitors of cell membrane function

Polymyxins are antibiotic used in treatment of gram negative bacteria but poor against gram-positive infection (Forbes *et al*., 1998).

They work mostly by breaking up the bacterial cell membrane. Polymyxins become firmly bound to cytoplasmic membrane and acts by damaging this structure (Thomas, 1993).

1.6. Prevention and control of lamb and goat kids' diarrhea

Because of the complex nature of diarrhea in neonates, it is unrealistic to expect total prevention and control of economic is the major objective. Incidence of the disease dependence on the balance between the levels of exposure to infectious agents and the resistance in the neonates as follows: The degree of exposure of neonates should be reduced by isolating diseased animals. Nonspecific resistance should be maximized by providing good nutrition to the dam and neonate and assuring that newborn consume ≥5% of their body weight of high-quality colostrum, preferably within 2 hrs. and certainly within 6 hrs of birth, followed by equivalent amounts at 12-hr intervals for the next 48 hr.The specific resistance of the newborn should be increased by vaccinating the dam in 6and 2weeks before parturition to stimulate antibodies to strains of ETEC these antibodies are then passed to the newborn through the colostrums (Aiello and Mays, 1998).

CHAPTER TOW

MATERIALS AND METHODS

2.1. Study areas:

The area conducted with study was North Kordufan State (Shekan, El-rahad, Om-rowaba, Bara, Jabrta-Elshekh, Omdam, Sodary). An area exceeding 700 square kilometers, coordinate 14-22N, 29-32E. North Kordufan State poses large number of farm animals as well as Sheep and Camel.

2.2. Sample collection

150 rectal swab were collect from diarrheic lambs and goat kids less than 2 month. Sterile cotton swabs were used for collection 150fecal samples then placed in sterile samples bags in ice box and transported to El-Obied Regional Veterinary Research Laboratory for bacteriological analysis.

2.3.Sterilization

Sterilization was done according to Barrow and Feltham (2003). The glass wares such as Petri dishes, test tubes, flasks and pipettes were sterilized using dry heat oven regulated at 160°C for 1 hours. The media, automatic pipette tips an distilled water were sterilized using steam autoclave at 121°C for 15 minutes (15Ib/ inch^2).

2.4. Preparation of media

All media were obtained in dehydrated form and were prepared according to the manufacturer's instructions. After taking appropriate weights, the stated distilled water was added to the media, then it was heated to dissolve completely and sterilized. Then cooled to 44 - 50°C. The media were poured aseptically (10-12 ml) into sterile plates by gentle lifting of the cover of the plate high enough to pour the medium. The

plates were then allowed to solidify within five to ten minutes and inverted (Marshall,1992)

2.5. Isolation and identification

She-camel milk samples were kept at room temperature for five to ten minutes and a loopfull was inoculated by streaking onto 10% defibrinated MacConkey agar and nutrient agar. The plates were ‹sheep blood agar incubated aerobically at 37°C for 24-48 hours for bacterial growth. The plates were examined daily by the naked eye for growth and colonial morphology and characteristics such as shape, size consistency haemolysis or pigment production. Smears were made from primary cultures, dried in the air, fixed by heating, stained by Gram's staining method and examined microscopically. Isolated bacterial colonies were purified by subcultring on nutrient agar and for suspected β-Identification of the bacterial .haemolytic streptococci on blood agar isolates was done according to the method outlined by (Barrow and Feltham ,2003).

The isolates were stored in a refrigrator and subcultured weekly on fresh sheep blood agar plates or nutrient agar and transferred to fresh medium when bacteriological studies were started.

2.5.1.Primary tests

2.5.1.1.Morphological appearance

The bacterial appearance and morphology were recorded according to Barrow and Feltham (2003).

2.5.1.2.Gram stain

Gram stain technique was done as indicated by (Harrigan and Mccance, 1976).The tested organism was picked up using sterile wire loop and put in the drop of sterile normal saline, which was previously spread, allowed to dry and then fixed ‹ put in a sterile slide. Emulsified by passing the smear three times over a flame. Crystal violet was added to the smear for one minute then washed with distilled water. Lugol's iodine was added for one minute and washed using distilled water. The slide was decolorized by ethanol for 10 seconds. The smear was then stained using

saffranin for 20-30 seconds and rinsed using distilled water. The slides were dried by filter paper and one drop of immersion oil was added to the slide for examining under the microscope. Gram positive organisms appeared purple, while Gram negative ones appeared pink.

2.5.1.3.Catalase test

On a clean microscope slide, a loopful of 3% hydrogen peroxide was placed. A colony of test culture, on nutrient agar, was picked using a wooden stick or glass rod and put in the reagent. Production of gas bubbles indicated positive result.

2.5.1.4.Oxidase test

Pieces of filter paper were soaked in 1% solution of tetramethylene phenylene diamine dihydrochloride and dried. A colony of an overnight growth, on nutrient agar, was picked with sterile bend glass rod and rubbed on the filter surface placed in a Petri dish. Development of dark violet colour within 60 seconds intecated positive test (Barrow and Feltham, 2003).

2.5.1.5.Motility test

Young broth cultures of the organism, incubated at or below the optimum growth temperature (e.g. 37 °C and 22 °C), were examined in preparations, using a high-power dry objective and "hanging drop reduced illumination. Motile organisms was indicated by their movement in different directions (Barrow and Feltham, 2003).

2.5.1.6.Sugar fermentation test

The medium was prepared by adding 1% of the required sugar to peptone water with Andred’s indicator in a Bigu vials with inverted Durham tube and inoculated with tested organism and incubated at 37 °C and examined daily. Acid production was indicated by appearance of pinkish color, while gas production was indicated by presence of empty space in the inverted Durham’s tube (Barrow and Feltham, 2003).

2.5.1.7.Oxidation Fermentation (O/F) test

Two tubes of Hugh and Leifson's medium were inoculated with the test culture. One of the tubes was covered by a layer of sterile paraffin oil to about 3cm above the surface of the medium, the other was left unsealed. Both were then incubated at 37°C and examined daily up to two weeks (Hugh and Leifson, 1953). The result read as follows:

1.Fermentative if both tubes were changed to pink color
2.Oxidative if tube without oil was changed to pink color

3.Negative result was indicated by no color changes in both tubes

2.5.2.Secondary Biochemical tests

2.5.2.1.Tube coagulase test

To 0.5 ml of 1/10 dilution of plasma in saline add 0.1 ml of an 18-24-hour broth culture of the organism was added incubated at 37 °C and and 6 h for coagulation. If negative, the tubes were left ،examined after 1 at room temperature overnight and then re-examined (Gillespie, 1943).

2.5.2.2.Methyl red (MR) reaction

Glucose Phosphate (MR) medium was inoculated by the test organism and incubated at 30 °C for 5 days. Add 2 drops of methyl red solution shaked and examined. A positive MR reaction was shown by ،were added red colour at the surface. An orange or yellow the appearance of a colour should be regarded as negative (Barrow and Feltham, 2003).

2.5.2.3.Voges-Proskaur (V.P) test

Glucose phosphate medium (M.R-V.P medium) was inoculated with the tested organisms and incubated at 37°C for 48 hours. An amount of 0.2 ml of 40% potassium hydroxide and 0.6 ml of 5% α-nephthal solution were added to one ml of culture, then shaken, placed in slope position and examined after 15 minutes and one hour, a positive reaction was indicated by bright pink or red colour (Barritt,1936).

2.5.2.4.Indole production test

Peptone Water or Nutrient Broth was inoculated with the test organism and incubated for 48 h. 0.5 ml Kovacs' reagent was added, shaked well, and examined after about 1min. A red colour in the reagent layer (Barrow and Feltham, 2003)indicates indole production

2.5.2.5.Nitrate reduction

Nitrate Broth was lightly inoculated with the test organism and incubated A .for up to 5 days. 1 ml of nitrite reagent A followed by 1 ml of reagent B were add .A deep red colour which shows the presence of nitrite and thus shows that nitrate has been reduced, indicated a positive reaction (Barrow and Feltham, 2003).

2.5.2.6.Phenylalanine deamination

Malonate-phenylalanine medium was lightly inoculated and incubated for 18-24 h and 0.1 N-HCI was added drop by drop until the medium was was added; yellow. Then 0.2ml of a 10% aqueous solution of $FeCl^3$ shaked and any colour change was observed immediately; a positive reaction was indicated by a dark green colour which quickly fades (Shaw and Clarke, 1955).

2.5.2.7.Urease activity

A slope of Christensen's Urea medium was heavily inoculated with the test organism and examined after incubation for 4 hours and daily for 5 days. Red colour indicated positive reaction (Barrow and Feltham, 2003).

2.5.2.8.Citrate test

A slope of Simmons' citrate medium was Inoculated as a single streak over the surface. Examined daily for up to 7 days for growth and colour change. Positive results were confirmed by subculture to Simmons' or Koser's Citrate medium. blue colour and streak of growth indicated citrate utilization original green colour indicated citrate not utilization (Barrow and Feltham, 2003).

2.5.2.9.Antibiotics sensitivity test

A volume of two ml of diluted culture were spread on the surface of nutrient agar. The excess fluid was discarded and the plate was allowed to dry, then Oxoid discs of ((Tetracycline (30mcg), Gentamicin(10µg) Trimethoprim(5µg), Ampicillin(10µg), Ceftriaxone(30mcg), Ciprofloxacin(5mcg), Norfloxacin(10mcg), Penicillin, Erythromycin(15µg), Chlormphenicol(30µg), Colistin(10µg), Lincomycin(15µg)).were applied to the surface of the medium by sterile forceps and incubated at 37°C for 24 hours. Zone of inhibition was determined whether the organism was sensitive, intermediate or resistant.

2.5.2.10.KCN test

1ml KCN Broth was inoculated with one loopful of an overnight broth culture or a light suspension of the organism. Cap of the bottle was screwed tight and incubated for up to 48 h and examined after 24 and 48 h for turbidity indicating growth, which constitute a positive reaction (Barrow and Feltham, 2003). Controls: positive - *Klebsiella pneumoniae*subsp. *aerogenes negative - Escherichia coli.*

2.6.Media used:

2.6.1.Nutrient broth (Oxoid,CM1)(g/L)

Contents:

Lab-lemco powder	1.0
Yeast extract (Oxoid L 20)	2.0
Peptone (Oxoid L 37)	5.0
Sodium chloride	5.0

pH 7.4 (approx).

Procedure:

Thirteen grams of the dehydrated powder were added to one litre of distilled water mixed well and distributed into bottles in 5 ml amounts and sterilized by autoclaving at 15 pressure per square inch (p.s.i) for 15 minutes. The prepared medium was kept at 4°C until used.

2.6.2.Nutrient agar (OXOID CM3) (g/L)

Contents:

Lab-lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar No 3	15

pH 7.4 (approx).

Procedure: Twenty eight grams were suspended in one litre of distilled water and brought to the boil to dissolve completely, then sterilized by autoclaving at 121°C for 15 minutes, cooled to 45-50°C and distributed into sterile Petri dishes in 15 ml portion each. The medium was kept at 4°C until used.

Peptone water (OXOID CM1049) (g/L)

Contents:

Peptone	10.0
Sodium chloride	5.0

pH 7.4 (approx).

Procedure: Fifteen grams were added to one litre of distilled water, mixed well and distributed into ten ml test tubes in three ml autoclaving at 121°C for 15 minutes. The amounts, and sterilized by prepared medium was kept at 4°C until used.

2.6.3.Blood agar base(OXOID CM0055)(g/L)

Contents:

Nutrient agar	900 ml
Sterile defibrinated blood	100 ml

Procedure:

The nutrient agar was prepared according to the manufacturer's sterilized by autoclaving at 121°C for 15 minutes, cooled to ،instructions 50°C and aseptically sterile blood was added and thoroughly mixed. Formation of air bubbles was avoided. The blood was allowed to warm to room temperature before being added to the molten agar, dispensed aseptically in 15 ml amounts in sterile Petri dishes.Each batch of the meduim was labeled by a number and date. The plates were then stored at 4°C in sealed plastic bages to prevent loss of moisture. Depending upon the agar base used, the pH was within the range of 7.2-7.6 at room temperature. The prepared medium was kept at 4°C until used.

2.6.4.MacConkey's agar (OXOID, CM7b) (g/L)

Contents:

Peptone	20
Lactose	10
Bile salts	5
Sodium chloride	5
Neutral red	0.075
Agar No 3	12

pH 7.4 (approx).

Procedure:

Fifty two grams were suspended in one litre of distilled water, boiled until dissolved completely and sterilized by autoclaving at 121°C for 15 then poured into sterile Petri dishes in portions of 15 ml and ،minutes then stored at 4°C until used.

2.6.5.Hugh and Leifson's (O/F) medium (Barrow and Feltham, 1993)

Contents:

Peptone	2.0
Sodium chloride	5.0

Agar	3.0
K2HPO4	0.3
Distilled water	1000 ml
Bromothymol blue, 0.2% eq. Sol	15 ml

Procedure:

The ingredients were dissolved by heating in water bath set at 55°C, the pH was adjusted to 7.1, then the indicator was added and the medium minutes. A volume of 10 ml of sterile glucose sterilized at 115C for ml of medium. Then the medium was solution was aseptically added to mixed and distributed aseptically in 10 ml amounts into sterile test tubes. The prepared medium was kept at 4°C until used.

2.6.6.Motility medium (Barrow and Feltham, 1993) (g/L)

Contents:

Peptone	10.0
Meat extract	3.0
Sodium chloride	5.0
Agar	4.0
Gelatin 80 Distilled water	1000ml

Procedure:

The gelatin was soaked in water for 30 minutes, then the other ingredients were added, heated to dissolve, and sterilized at 115°C for 20 minutes. The prepared medium was kept at 4°C until used.

2.6.7.MR VP medium (OXOID CM43) (g/L)

Contents:

Peptone	5.0
Dextrose	5.0
Phosphate buffer	5.0

Distilled water	1000 ml

pH 7.5 (approx)

Procedure:

Fifteen grams were added to one litre of distilled water, mixed well, then distributed into test tubes and sterilized by autoclaving at 121°C for 15 minutes. The prepared medium was kept at 4°C until used

2.6.8.Simmon's citrate agar (OXOID CM0155) (g/L)

Contents:

Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.05
Agar No 3	15

pH 7.0 (approx) .

Procedure:

Twenty three grams were suspended in one litre of distilled water, boiled to dissolve completely and sterilized by autoclaving at 121°C for 15 minutes, then the medium was distributed in ten ml portions into sterile MacCarteny bottles aseptically and allowed to set in slope position. The prepared medium was kept at 4°C until used.

2.6.9.Agar 2.0 (OXOID CM49) (g/L)

Peptone water	1000 ml

Procedure:

The ingredients were dissolved in peptone water, sterilized by autoclaving at 115°C for 10 minutes, poured into sterile MacCarteny bottles aseptically and allowed to set in slope position to solidify, then stored at 4°C until used.

2.6.10.MacConkey broth (OXOID CM5) (g/L)

Content:

Peptone	20
Lactose	10
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075

pH 7.4 (approx)

Procedure

Fourty grams was added to one litre of distilled water, mixed well, distributed into test tubes, fitted with Durham tube and autoclaved at

CHAPTER THRE

RESULTS

3.1. Isolation

Atotal of 150 fecal samples were collected from diarrheic lambs and goat kids from North Kurdofan state (Shekan, El-Rahad, Omrowaba, Bara, Jabrat El-Shekh, Om-dam, Om-kiradem and Sodari).

All samples were cultured onto biood agar and Maconky agar media and were incubated aerobically at 37°C. All fecal samples showed bacterial growth.

Tabl1: percentage of gram-negative isolates from 150 fecal samples of lambs and kids in North Kordufan state.

NO	Genus	Isolated
1	*E.coli*	27(29.7%)
2	*Klebsiella pnemoniae*	13(14.3%)
3	*Entero.cloacae*	8(8.8%)
4	*Pseudomonus. Aeruginosa*	7(7.7%)
5	*Stenotrophomonas maltoptilia*	5(5.5%)
6	*Proteus mirabilis*	5(5.5%)
7	*Enterobacter. Amnigenous*	4(4.4%)
9	*Serratia macenscens*	3(3.3%)
10	*Salmonella arizonae*	3(3.3%)

11	*Raou.ornithnolytica*	3(3.3%)
12	*Serratia liquefaciens*	2(2.2%)
13	*Yersinia pestis*	2(2.25)
14	*Pseudsomonus oryzihabitans*	2(2.2%)
15	*Citro, freundii*	1(1.1%)
16	*Citro.braakii*	1(1.1%)
17	*Citro.youngae*	1(1.1%)
18	*Shigella*	1(1.1%)
19	*Pseudomonus pneumonia*	1(1.1%)
20	*Pseudomonus luteola*	1(1.1%)
21	*Entero gergoviae*	1(1.1%)

3.1.1. *E.coli* isolaties

Twenty seven stains of *E. coli* were isolated; they were gram-negative rods, motile. On MacConkey's agar medium, convex, circular, pink and large colonies were observed. Also on EMB showed metallic sheen appears. When inoculated the bacteria in API 20E that is appear catalase positive, oxides negative, Indole-positive, methyl-red-positive, citrate-negative, urease-negative and H_2S-negative, Voges proskaur-negative. Acid and gas were produced from glucose, lactose, maltose, mannitol and xylose, According to figure1the organism was sensitive to Ciprofloxacin.

3.1.*2. Klebsiella pneumonia* isolates

Thirteen strains were isolated, they were gram-negative rod, capsulated, non spore forming in MaConky agar media, large colony appear, mucoid lactose fermentation and capsule formation. The *Klebsiella* isolates were Voges Proskaur-positive, and methyl red negative, *Klebsiella pneumonia* was indole-negative. Indicating the fermentation of both glucose and lactose, H_2S was not produced on API20E (Table4).

3.1.3. *Enterobacter cloacae*

All the strains of *Enterobacter cloacae* were isolated in this investigation were gram-negative and short rods. On nutrient agar media, colony with irregular round edges and were mucoid in nature. the isolated were citrate positive but urease and indole negative, yellow color were observed indicating the fermentation of glucose and not lactose API20E(Table4).

3.1.4. *Proteus* spp. isolates

Five strains were isolated; they were gram-negative rod, non-capsulated, non spore forming. On nutrient agar medium, colonies with distinctive smell and swarming growth were seen, urease-positive, non-lactose fermenters, and variable indol-positive, fermenting of glucose and not the lactose, on (table4).

3.1.5 .*Yersinia* spp

Two strains of *Yesinia* spp. were isolated. They were gram negative rods, non motile, catalase positive, oxides negative, lactose non fermentative, (table 4).

3.1.6. *Pseudomonus* spp.

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. It is citrate, catalase, and oxidase positive. A species of considerable medical importance. It is found in soil, water, skin flora, and most man-made environments throughout the world. If such colonization's occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal Balcht and Smith R, (1994).

3.1.7. *Salmonella arizonae*

Three strains of *salmonella* arizonae were isolated. They were gram-negative bacteria rods shaped, non spore-forming, motile, facultative anaerobes, ferment glucose and lactose; reduce nitrate into nitrite, oxidase negative and catalase positive, they usually produced H_2S, (Table 4).

3.1.8. *Citrobater* spp

Three strains were isolated (*Citro. youngae*, *Citro.freundii, Citro.braakii*, gram-negative rods, motile, non spore form cells and non-capsulated, ferment glucose, catalase positive and oixdase negative, and are genetally lactose-negative, H_2S positive, indole negative, and Voges proskaur-negative

3.1.9. *Stenotrophomonas maltoptilia*

Five strains isolates they were gram-negative rods, motile, on nutrient agar medium as round straw yellow colored colonies, ferment

glucose,maltose, and sucrose. catalase positive and oxides negative, Voges proskaur-negative (Tabl4).

3.1.10 *Shigella*

One strain was isolated gram-neegative rods, non-motile and non-spore form, catalase positive and oxidase negative, Voges proskaur-negative, urease and H_2S negative, ferment mannitol and mannose, reduction nitrate to nitrite (Table 4).

3.2. Commercial antibiotic discs which used for gram-negative bacteria isolated from diarrheic lambs and kids:-

The bacterial isolates from this study were subjected to antimicrobial susceptibility testing by disc diffusion method by using Muller-Hinten ager medium and commercial antibiotic discs (Tetracycline(30mcg), Gentamicin(10µg), Ampicillin(10µg), Ceftriaxone(30mcg), Ciprofloxacin(5mcg),Norfloxacin(10mcg),Penicillin, Trimethoprim(5µg), Erythromycin(15µg), Chlormphenicol (30µg), Colistin(10µg), Lincomycin(15µg).

Table1:

Properties of the gram-negative bacterial isolated from fecal sample

Character	E.coli	Klebsiella pneumoniae	Enterobacter cloacae	Enterobacter amnigenous	Yersinia pestis	Proteus mirabilis	citrobater youngae
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motilit	+	-	+	+	-	+	+

y							
Capsule	-	+	-	-	-	-	-
Gram stain	-	-	-	-	-	-	-
ONPG	+	+	+	+	-	-	+
ADH	-	-	+	+	-	-	+
LDC	+	+	-	-	-	-	-
ODC	+	-	+	+	-	+	-
CIT	-	+	+	+	-	+	+
H2S	-	-	-	-	-	+	+
URE	-	+	-	-	-	+	-
TDA	-	-	-	-	-	+	-
IND	+	-	-	-	-	-	-
VP	-	-	-	-	-	+	-
GEL	-	-	-	-	-	+	-
GLU	+	-	+	+	-	+	+
MAN	+	+	+	+	+	-	+
INO	-	+	-	-	-	-	-
SOR	+	+	+	-	-	-	-
RHA	+	+	+	+	-	-	+
SAC	+	+	+	+	-	-	-
MEL	+	+	+	+	-	-	-
AMY	+	+	+	+	+	-	-
ARA	+	+	+	+	-	-	+
OX	-	-	-	-	-	-	-

Chara cter	Citro bact	Shigell a	Serrattia liquefacie	Serrattia marcesc	Stenotr ophom	Pasteur ella	Pasteurell a luteola

	er braakii		ns	ens	onas maltophilia	aeruginosa	
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	-	+	+	+	-	-
Capsule	-	-	-	-	-	+	-
Gram stain	-		-	-	-	-	-
ONPG	+	-	+	+	-	-	-
ADH	+	-	+	-	-	+	+
LDC	-	-	+	+	-	-	-
ODC	+	-	+	+	-	-	-
CIT	-	-	+	+	+	+	+
H2S	+	-	-	-	-	-	-
URE	-	-	-	+	-	-	-
TDA	-	-	-	-	-	-	-
IND	+	-	-	-	-	-	-
VP	-	-	-	+	-	-	-
GEL	-	-	+	+	+	+	+
GLU	+	+	+	+	-	-	+
MAN	+	-	-	+	-	-	-
INO	-	-	+	+	-	-	-
SOR	+	-	+	+	-	-	-
RHA	+	-	-	-	-	-	-
SAC	-	-	+	+	-	-	-
MEL	+	+	+	+	-	-	+
AMY	+	-	+	+	-	-	-
ARA	+	+	+	+	-	-	+
OX	-	-	-	-	-	-	-

Character	Interobacter gergoviae	Salmonella arisonae	Pasteurella oryzihabitans	Raoultella ornithinolytica	Pasteurella Pnemoniae	Citrobacter freundii
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	-	-	-	+
Capsule	+	-	-	-	-	-

Gram stain	-	-	-	-	-	-
ONPG	+	+	-	+	-	+
ADH	-	+	-	-	-	+
LDC	+	+	-	+	-	-
ODC	+	+	-	+	-	-
CIT	+	+	+	+	-	+
H2S	-	+	-	-	-	+
URE	+	-	-	-	+	+
TDA	-	-	-	-	-	+
IND	-	-	-	+	-	+
VP	+	-	-	-	+	-
GEL	-	-	-	-	-	-
GLU	+	+	+	+	-	+
MAN	+	+	-	+	-	+
INO	-	-	-	+	-	+
SOR	+	+	-	+	-	+
RHA	+	+	-	+	-	-
SAC	+	+	-	+	-	+
MEL	+	+	+	+	-	+
AMY	+	-	-	+	-	+
ARA	+	+	-	+	-	+
OX	-	-	-	-	-	-

Table 2:
Relationship between pathogenesis of *Enterobacterieceae* and animal species.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Sheep	82	90.1	90.1	90.1
	Goat	9	9.9	9.9	100.0
	Total	91	100.0	100.0	

Table 3:
Relationship between pathogenesis *Enterobacterieceae* and animal sex.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	38	41.8	41.8	41.8
	Female	53	58.2	58.2	100.0
	Total	91	100.0	100.0	

Table4:
Relationship between pathogenesis *Enterobacterieceae* and animal age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1-30	57	62.6	62.6	62.6
	31-60	34	37.4	37.4	100.0
	Total	91	100.0	100.0	

Table 5:

Reading of AST zones which were sensitive / resistance or Iintermediate

Bacteria spp	Antibiotics sensitivity test											
	AM	E	CN	L	NOR	CL	SXT	TE	CT	CIP	CRD	P
E.coli	10	12	16	R	30	15	25	R	12	32	32	R
Klebseiallea .spp	R	10	20	R	35	R	30	25	11	25	32	R
Enterobacteria spp	13	13	25	R	35	R	25	28	12	30	25	R
Pseudomonas spp	R	10	22	R	22	R	R	22	12	26	28	R

Table 6:

Relationship between (ampicillin, gentamicin, norfloxacin, trimethoprim, ciprofloxacin, ceftriaxone) antibiotics and gram-negative bacteria isolated from lamb and goat kids diarrhea.

AM				
	Sensitive	Resistant	Intermediate	Total
E.coli	4	23	2	29
Entero.cloacae	2	5	0	7
Entero.amigenous	2	1	0	3
Kleb.pnemoniae	1	10	0	11
CN				
	Sensitive	Resistant	Intermediate	Total
E.coli	27	1	1	29
EIntero.cloacae	6	0	1	7
Entero.amigenous	3	0	0	3
Kleb.pnemoniae	9	1	1	11
NOR				
	Sensitive	Resistant	Intermediate	Total
E.coli	26	3	0	29
Entero.cloacae	7	0	0	7
Entero.amigenous	3	0	0	3
Kleb.pnemoniae	9	0	2	11
SXT				
	Sensitive	Resistant	Intermediate	Total
E.coli	27	2	0	29
Entero.cloacae	7	0	0	7
Entero.amigenous	3	0	0	3
Kleb.pnemoniae	9	1	1	11
CIP				
	Sensitive	Resistant	Intermadiate	Total
E.coli	24	3	2	29
Entero.cloacae	7	0	0	7

Entero.amigenous	3	0	0	3
Kleb.pnemoniae	11	0	0	11
CRO				
	Sensitive	Resistant	Intermediate	Total
E.coli	27	1	1	29
Entero.cloacae	7	0	0	7
Entero.amigenous	2	1	0	3
Kleb.pnemoniae	7	4	0	11

CHAPTER FOUR

DISCUSSION

Infectious diarrhea is the most significant cause of morbidity and mortality in neonatal sheep and goat kids throughout the world and economically devastating conditions encountered in the animal agriculture industry. Acute infectious diarrhea encountered in a herd is often difficult to manage because of the large number of potential enteropathogens involved, differences in individual animal immunity within the herd, population dynamics, environmental stresses, nutritional status, and difficulty in establishing an etiologic diagnosis .The etiologic diagnosis is not determined for a large percentage of cases of neonatal diarrheas. This study was aiming at identifying the bacteria associated with lambs and kid's diarrhea at the age from 1 day to 2month. One hundred and fifty were collected from diarrheic cases. Ninty one (60.7%) bacteria isolated from the swab samples were the isolated bacteria gram-negative and fifty nine (39.3%) gram-positive bacteria. The findings were in agreement with the report of Daboura (2002), who reported that gram-negative bacteria were about 80% and gram-positive bacteria about 20% in fecal samples collected from diarrheic and non diarrheic calves. In this study *E.coli* was much more prevalent with the percentage of 29.7%, *Kleb.pnemoniae* represeanted 14.3%, *Enterobacter* spp 14.3 %, *Yersinia pestis* 2.25%, *Pseudomonas* spp 12.1%, *Salmonella arizonae* 3.3%, *Proteus mirabilis* 4.49%, *Raoultella ornithinolytica* 3.37%, *Serratia* spp 5.5 %, *citrobater* spp 3.3%. These results were in line with that reported by (Ahamed *et al*.,2010) who reported *Escherichia coli* 42 (36.84%), *Salmonella spp* 18 (15.79%), *Klebsiella spp*15(13.16%), *Shigella spp* 8 (7.02%), *Proteus vulgaris* 6 (5.26%).In this study the diarrhea is more

prevalent 62.6% in lambs and kids at age between 1day and 1month, femal were more affected 58.2% and males 41.8%, lambs were more susceptible to diarrhea 90.1% than the kids. These results were in line with that reported by (Osman *et al*., 2013; Adesiyun *et al*., 2001),who detected the *E. coli* was the most frequently enteropathogen among the investigated sheep (34.7%) and goats (30.7%).

Bacteria isolated in present study were resistant to many antibiotics commonly used for treatment of bacteria diseases in animals. Members of *Enterobacteriaceae* isolated in this study showed very high resistance to Penicillin (97.7%) , Lincomycine (98.9%), Erythromycin- (68.2%) , Ampicillin(65.9%). this Result were in line that reported by Mohamed (2009) who related that the *E.coli* isolates showed resistance to ampicillin (38%) and streptomycin (31%). *E.coli* isolated in this study showed very sensitive to Ciprofloxacine (89.8%), Norfloxacine (89.8), Gentamycine (87.5%) and Trimethoprime (83%), Which agrees with Orden (2001) who found that *E.coli* strains isolated form dairy calves affected by neonatal diarrhea were very susceptible (89-95%) to Gentamycin.

Conclusions and Recommendations

Conclusions:

E. coli was the most common bacteria that caused lambs and goat kid's diarrhea.*Enterobacteriaceae* were highly sensitive for ciprofloxacine and norfloxacine.But highly resistant to penicillin and lincomycine.

Recommendations:

-Using of PCR techniques for detection of *E.coli* serotype in case of lambs and goat kids diarrhea.

-Further studies should be carry-out to investigate the predisposing factors of lambs and goat kid's diarrhea.

-Further research should carryred out to investigate the role of gram positive bacteria in lambs and goat kid's diarrhea.

-Application of sequenciy to determine the resistance genus specially for *E.coli* and *Enterobacter* spp.

References

Abate A.R., Hung, T., Sperling, R.A., Mary, P., Rotem, A., and Agresti, J.J. (2013). "DNA sequence analysis with droplet based microfluidics".13(24):4864-9.doi:10.1039/c3lc50905b. PMC 4090915. PMID 24185402.

Adesiyun, A.A., and Kaminjolo, J.S. (1994). Prevalence and epidemiology of selected enteric infections of livestock in Trinidad. Prev Vet Med; 19: 151-65.

Adesiyun, A.A., Kaminjolo, J.S., Ngeleka, M., Mutani, A., Borde, G., Kaiser, P., Rothwell, L., Galyov, E.E., Barrow, P.A., Burnside, J., and Wigley, P. (2000). Differential cytokine expression in avian cells in response to invasion by Salmonella typhimurium, Salmonella enteritidis and Salmonella gallinarum. Microbiol. 146 Pt 12: 3217-3226.

Adesiyun, A.A., Kaminjolo, J.S., Ngeleka, M., Mutani, A., Borde, G., Harewood, W., and Harper, W. (2001). A longitudinal study on enteropathogenic infections of livestock in Trinidad. *Revista daSociedade Brasileira de MedicinaTropical* 34(1): 29-35.

Ahmed,A; Egwu,G.O; Garba,H.S; and Magaji,A.A (2010). Prevalence of bacterial pathogens and serotyping of *E.coli* isolates from diarrhoeic lambs in Sokoto state, Nigeria. *Sokoto Journal of VeterinarySciences* 8(1):42-45.(ISSN 1595-093X).

Aiello, S., and Mays, A. (1998). *The Merck Veterinary Manual*. 8th Edition. Merck and Co.INC., White House Station, N.J.USA and Merial Limited

Balcht A, and Smith, R. (1994). Pseudomonas aeruginosa: Infections and Treatment. Informa Health Care. pp. 83–84. ISBN 978-0-8247-9210-7.

Ballabio A, Ranier, J.E., Chamberlain, J.S., Zollo, M., and Caskey, C.T. (1990). "Screening for steroid sulfatase (STS) gene deletions by multiplex DNA amplification" (PDF). Human Genetics. 84 (6): 571–573.doi:10.1007/BF00210812.hdl:2027.42/47626. PMID 2338343.

Barritt, M. M. (1936). The Intensification Of The Vogesproskauer Reaction By.

The Addition Of A-Naphthol. J. Path. Bact. 42,441.

Barrow and felltham, (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge University Press, Cambridge.

Barrow, G.I. And Feltham, R.K.A.,(2003).*Cowan And Steel's Manual For Identification Of Medical Bacteria* 3rd Cambridge University Press. United Kingdom

Behjati, S., and Tarpey, P.S. (2013). "What is next generation sequencing?". Education and Practice Edition. 98 (6): 236-8. doi: 10.1136/archdischild-2013-304340.PMC 3841808. PMID 23986538.

Beutin, L Geier D; Steinriick, H; Zimmermann, S; and Scheutz, F; **(1993).** Prevalence and some properties of verotoxin (shiga-like toxin)-producing Escherichia coli in seven different species of healthy domestic animals. *Journal of Clinical Microbiology. 31: 2483-8.*

Beutin, L; Steinruck, H; Krause, G; Steege, K; Haby, S; Hultsch, G; and Appel, B (2007). Comparative evaluation of the Ridascreen Verotoxin enzyme immunoassay for detection of Shiga-toxin producing strains of *Escherichia coli* (STEC) from food and other sources. *Journal of Applied Microbiology.* 102: 630-639.

Blood, D.C; Radostits, O.M; Arundel, J.H; and Gays, C.C. (1990). disease caused by *E.coli* in: *veterinary medicine,7th* edition Cathaw PLC. Chathaw; Kenjet; Great Britian. Pp. 619- 643.

Boeckel, Thomas P., Van, Glennon, Emma E., Chen, Dora, Gilbert, Marius, Robinson, Timothy P., Grenfell, Bryan T., Levin, Simon, A., Bonhoeffer, Sebastian, and Laxminarayan, Ramanan (2017). "Reducing antimicrobial use in food animals". Science. 357 (6358): 1350–1352. Bibcode: 2017Sci...357.1350V. doi:10.1126/science. aao1495. PMC 6510296. PMID 28963240. S2CID 206662316.

Boeckel, Thomas, P; Van, Pires, João; Silvester, Reshma, Zhao; Cheng, Song, Julia; Criscuolo, Nicola G; Gilbert, Marius, Bonhoeffer, Sebastian; and Laxminarayan, Ramanan (2019). "Global trends in antimicrobial resistance in animals in low- and middle-income countries" (PDF).365 (6459): eaaw1944. doi:10.1126/science. aaw1944. ISSN 0036-8075. PMID 31604207. S2CID 202699175

Bousquet-Melou; Alain, Ferran; Aude, Toutain; Pierre-Louis (2010). "Prophylaxis. Conference: 5TH International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM) At: Tel Aviv, Israel – via Research Gate.

British Veterinary Association, London (2019). "BVA policy position on the responsible use of antimicrobials in food producing animals" (PDF). Retrieved 22 March 2020.

Brown, B.J., and Leff, L.G. (1996). Comparison of fatty acid methyl ester analysis with the use of API 20E and NFT strips for identification of aquatic bacteria. *Applied and Environmental Microbiology, 62, 2183–2185.*

Burtis, C.A., Ashwood, E.R., Bruns, D.E. (2012). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Elsevier. ISBN 978-1-4557-5942-2.

Bush, Karen, Courvalin, Patrice, Dantas, Gautam, Davies, Julian, Eisenstein, Barry, Huovinen, Pentti, Jacoby, George A., Kishony, Roy, Kreiswirth, Barry N., Kutter, Elizabeth, Lerner, Stephen A.; Levy, Stuart, Lewis, Kim, Lomovskaya, Olga, Miller, Jeffrey H., Mobashery, Shahriar, Piddock, Laura J.V., Projan, Steven, Thomas, Christopher M., Tomasz, Alexander, Tulkens, Paul M., Walsh, Timothy R., Watson, James, D., Witkowski, Jan, Witte, Wolfgang, Wright, Gerry, Yeh, Pamela, Zgurskaya, and Helen, I. (2011). "Tackling antibiotic resistance". Nature Reviews Microbiology. 9(12): 894–896. doi:10.1038/nrmicro2693. PMC 4206945. PMID 22048738. S2CID 4048235.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., and Wittwer, C.T. (2009). "The MIQE guidelines: minimum information for publication of quantitative

real-time PCR experiments". *Clinical Chemistry*. 55 (4): 611–622. doi:10.1373/clinchem.2008.112797. PMID 19246619

Carter, G.R., and Wise, J.D. (2004). *Essentials of Veterinary Bacteriology and Mycology.* 6th ed. Black well publishing, lowa state press.

Chamberlain, J.S., Gibbs, R.A., Ranier, J.E., Nguyen, P.N., Caskey, C.T. (1988). "Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification". *Nucleic Acids Research.* 16 (23): 11141–11156. doi:10.1093/nar/16.23.11141. PMC 339001. PMID 3205741.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical countries*, part II. Cambirdge, England.

Chmielecki, J., and Meyerson, M. (2014). "DNA sequencing of cancer: what have we learned?". *Annual Review of Medicine*. 65 (1): 63–79. doi:10.1146/annurev-med-060712-200152. PMID 24274178.

Cid, D; Ruiz, Santa Quiteria, J.A; dela Fuente, R.F17. (1993). Fimbriae in *Escherichia coli* from lambs and kids. *Vet Rec;* 132: 251.

David, H., and Pincus (2005). *Microbial Identification Using the Biomerieux Vitek2 System*. biomerieux, MO, USA. Online published doc on net, biomerieux official website. https:// apiweb.biomerieux.com.

Doonnenbreg, M.S., and Kaper, J.B. (1992). Enteropathogenic *Escherichia coli* (Minireview*). Infection and Immunity*. 60*:*3953-3961.

Drolet, R; Fairbrother, J.M; Vaillancourt, D. (1994). Attaching and effacing Escherichia coli in a goat with diarrhoea. *Can Veterinary Journal* 35: 122-3.

European Medicines Agenc E.S.V.A.C. (2019). "Sales of veterinary antimicrobial agents in 31 European countries in 2017: Trends from 2010 to 2017" (PDF). Retrieved 22 March 2020.

Ewing, W.H. (1986). The genus Escherichia. In Edwards and Ewing's *Identification of Enterobacteriaceae*, Pp. 93-134. New York: Elsevie.

Forbes, B.A; Sahm, D.G.F; and Wessfeld, A.S. (1998*).* *Bailey and Scoot's Diagnostic Microbiology*. 10th Edition. Mosby, London.

Galen Wood Ewing and Marcel Dekker (1990). *Analytical Instrumentation Handbook*. New York ISBN-0-8247-8184-8.

Gillespie, E.B., (1943). The routine used of the coagulase test for *staphylococci*. Monthly Bulletin of the Emergency zPublic Health Laboratory Sevice, 2: 19-22.

Gracias, K.S., and McKillip, J.L. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*, 50, 883-890.

Greenwood, D., Slack, R.C.B., and Peutherer, J.F. (2002). *Medical Microbiology* 16th ed. Churchill Livinge, London. Edinburgh. NewYork. Philadelphia. Oxford. Sydney.

Griffin, P.M., and Tauxe, R.V. (1991). The epidemiology of the infectious caused by *Escherichia coli* O157:H7, other

enterohemorrhagic *E.coli,* and the associated hemolytic uremic syndrome. *Epidemiology Review* 13:60-68.

Gyles, C.L. (2007). Shiga toxin-producing Escherichia coli: an overview. *J Anim Sci*.;85: E45–E62. [PubMed] [Google Scholar].

Hamburg, M.A., Collins, F.S. (2010). "The path to personalized medicine". *The New England Journal of Medicine*. 363 (4): 301–4. doi:10.1056/NEJMp1006304. PMID 20551152.

Harrigan, W. F. And Mccance, M. E. (1976). *Laboratory Methods In Food And Dairy Microbiology*. Academic Press, London.

Hayden, M.J., Nguyen, T.M., Waterman, A., Chalmers, K.J. (2008). "Multiplex-ready PCR: a new method for multiplexed SSR and SNP genotyping". *BMC Genomics*. 9: 80. doi:10.1186/1471-2164-9-80. PMC 2275739. PMID 18282271.

Hildesheim, A., Schiffman, M.H., Gravitt, P.E., Glass, A.G., Greer, C.E., Zhang, T., Scott, D.R., Rush, B.B., Lawler, P., and Sherman, M.E. (1994). Persistence of type-specific human papillomavirus infection among cytologically normal women. J. Infect. Dis.169:235-240.

Holt, J.G; Krieg, N.R; Sneath, P.H.A; Staley, J.T. and Williams, S.T. (1994). *Bergey's Manual of Determinative Bacteriology*. Baltimore, Williams and Wilkins; pp. 175-222.

Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. J. Bact.66, 24.

Husnjak, K; Grce, M; Magdic, L; and Pavelic K. (2000). Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical cell specimens. *J. Virol. Methods88*:125-134.

Johnson, J.R. (1991). Virulence factors in *E.coli* urinary tract infection. *Clin. Microbial.Rev.,*4:80-128.

Kuria, J.K.N; Wahome, R.G; Jobalamin, M; and Kariuki, S.M. (2009). Profile of bacteria and fungi on money coins. *East African Medical Journal*, 86 (4), 151- 155.

Lau, S.K.P; Woo, P.C.Y; Hui, W.T; Li, M.W. S; Teng, J.L.L; Que, T. L; Yuen, K.Y. (2003). Use of cefoperazone MacConkey agar for selective isolation of *Laribacter hongkongensis*. *Journal of Clinical Microbiology*, 41 (10), 4839- 4841.

Lorenz, I., Gentile, A., and Klee, W. (2005). Investigations of D-lactate metabolism and the clinical signs of D-lactataemia in calves. *Vet Rec*.156 (13): 412-5.

Marshall, R.T. (1992). Standard Methods For Examination For Dairy Products. 16th Ed. American Public Health Association (Apha), Washington, Dc. Usa.

Mohammad,1. Nasr; Nabil, M; Bakeer, Alaa, A; Omar Hassan, A; and Hammouda1, (2014). Animal Medicine Department, Faculty of Veterinary Medicine, Damanhur University1 Animal Health Research Institute, Egypt. *Alexandria Journal of Veterinary Sciences*, 43: 8-16. www.alexjvs.com2047, -ISSN 1110 DOI: 10.5455/ajvs.163829.

Mohamed, S. (2009). *Escherichia coliAssociated With Neonatal Calf Diarrhoea In Khartoum North, Sudan* (M.Sc.,UOFK)

Mohanty, A. (2010). Physiochemical and antimicrobial study of polyherbal. Pharmacie globale. 4 (4) 1-3.

Muñoz, M.1; Alvarez, M; Lanza, I; Cármenes, P. (1996). Role of enteric pathogens in the etiology of neonatal diarrhea in lambs and goat kids in Spain. 117: 203-11.doi10.1017.

Nagy, B., and Fekete, P.Zs. (1999). Enterotoxigenic Esherichia coli (ETEC) Iin farm animals. *Vet.Res.,*30:441-443.

Nagy, B., and Fekete, P.Zs. (1999). Enterotoxigenic *Esherichia coli* (ETEC) Iin farm animals. *Vet.Res*. 30: 259 – 284.

Natro, J.P., and Kaper, J.B. (1998). *Diarrheogenic Esherichia coli. Clinical Microbiology*. 11: 142 – 201.

Nguyen, D; LeVan, P; Le Huy, C; Gia, K.N; and Weintraub, A. (2005). Detection and characterization of diarrhea genic Escherichia coli from young childrenin Hanoi, Vietnam. *Journal of Clinical Microbiology*. 43: 755-760.

O' Brien, A.D. and Holmes, R.K. (1996). Protein toxins of *Echerichia coli* and *salmonella*. In: *Cellular and Molecular Biology*. Neidhart, F.C., Curtiss, R., Ingraham, J.L., Lin, E.C.C., Low, KB., Magasanik, B., Reznikoff, W.S., Riley, M., Schaechter, M. and H.E.(Eds.). ASM press, Washington, DC, pp. 2877- 2802.

Olsvik, O; Wahlberg, J; Petterson, B; Uhlén, M; Popovic, T; Wachsmuth, I.K; and Fields, P.I. (1993). "Use of automated sequencing of polymerase chain reaction-generated amplicons to

identify three types of cholera toxin subunit B in Vibrio cholerae O1 strains". *Journal of Clinical Microbiology*. 31 (1): 22-25. doi:10.1128/JCM.31.1.22-25.1993. PMC 262614. PMID 7678018.

Orden, J. A; Jose, A; Cid, D; Diez, R; Martinez, S. And Ricardo de la Fuent'e. (2001). Quinolone resistance in potentially pathogenic and non-pathogenic *Escherichia coli* strains isolated from healthy ruminants.*J. Antimicrob. Chemothe*., 48 (3): 421-424.

Osman, K.M; Mustafa, A.M; El hariri, M; and Abdel Hamed, G.S. (2013). Thedistribution of Escherichia coli serovars,virulence genes, gene association andcombinations and virulence genes encoding serotypes in pathogenic *E.Coli* recovered from diarrhoeic calves, sheep and goat. *Transboundary and Emerging Diseases* 60(1): 69-78.

Pekin, D; Skhiri, Y; Baret, J.C; Le Corre, D; Mazutis, L; and Salem, C.B. (2011). "Quantitative and sensitive detection of rare mutations using droplet-based microfluidics". 11 (13): 2156-66. doi:10.1039/c1lc20128j. PMID 21594292.

Perchetti, G.A; Nalla, A.K; Huang, M.L; Jerome, K.R; and Greninger, A.L. (2020). "Multiplexing primer/probe sets for detection of SARS-CoV-2 by qRT-PCR". *Journal of Clinical Virology*. 129: 104499. doi: 10.1016/j.jcv.2020.104499. PMID 32535397.

Pettersson, E., Lundeberg, J., and Ahmadian, A. (2009). "Generations of sequencing technologies".93 (2): 105–11. doi: 10.1016/j.ygeno.2008.10.003. PMID 18992322.

Puente, J.L., and finaly, B.B. (2001). Pathogenic *E.coli* In: *Principle of Bacteria Pathogen*. Academic press pp.387-429.

Quinn, P.J; Carter, M.E; Markey, B; and Carter, G.R. (1994). *Clinical Veterinary Microbiology*, WOLFE publishing, UK. pp: 30-330.

Quinn, P.J; Carter, M.E; Markey, B; and Carter, B.R. (1999). *Clinical Veterinary Microbiogy*. page 220.

Quinn, p.j; Markey, B.K; Carter, M.E; Donnelly, w.J.c; and leonard, F.C. (2001). *Veterinary Microbiology and Microbial Disease.* Dublin.

Radostits, O.M; Blood, D.C; Hinchcliff, K.W; and Gays, C.C. (1994). *Veterinary medicine*, 8th edition (LEBS) Bailliere Tindall, London.

Riggs, P.J; Chelius, M.K; Iniguez, A.L; Kaeppler, S.M; and Triplett, E.W. (2001). "Enhanced maize productivity by inoculation with diazotrophic bacteria". *Australian Journal of Plant Physiology*. (8): 829–836. doi:10.1071/PP01045.

Ryan, K.J., Ray, C.G., (2004). *Sherris Medical Microbiology (4th* ed.). McGraw Hill. ISBN 978-0-8385-8529-0.

Serna, A.T., Boedeker, E.C. (2008). Pathogenesis and treatment of Shiga toxin-producing *Escherichia coli* infections. *Curr Opin Gastroenterol*.;24:38–47. [PubMed] [Google Scholar].

Shaw, C. & Clarke, P. H. (1955). Biochemical classification of Proteus and Providence cultures. J. gen. Microbiol. 13, 155.

Smith, and Sherman, (1994). *Alexandria Journal of Veterinary Sciences* 2014, 43: 8-16ISSN 1110 -2047, www.alexjvs

Schoenian, S. (2007). Diarrhea (Scours) in Small Ruminants, Maryland Cooperative Extension, University of Maryland**.**

Thomas, C.G.A. (1993). *Medical Microbiology*. 6th ed. Baillira Tindal, London, Cambirdge.

Todrak, K. (2002). T odor's Online *Textbook of Bacteriology*. University of Wiscon in Madison, Department of Bacteriology. http://www.textbook of Bacteriology .net

Van Boeckel; Thomas P; Brower, Charles, Gilbert, Marius, Grenfell, Bryan T; Levin, Simon ; Robinson, Timothy P; illant, Aude, Laxminarayan, Ramanan (2015). "Global trends in antimicrobial use in food animals". 112 (18): 5649–5654. Bibcode:2015 PNAS.112.5649V. doi:10.1073/pnas.1503141112. PMC 4426470. PMID 25792457. S2CID 3861749.

Whipp, S.C. (1991). Intestinal responses to enterotoxigenic *Escherichia coli* heat-stable toxin b in non-porcine species. *American Journal of Veterinary Research*. 47:615-617.

Whittam, T.S. (1996). Genetic variation and evolutionary processes in natural populations of *Escherichia coli*. In *Escherichia coli* and *Salmonella*: cellular and molecular biology. F.C. Neidhardt, editor. ASM Press. Washington, DC, USA. 2708 **-** 2720.

Appendices

AP**Table (1)**

I 20E reading table

Test	**Positive**	**Negative**	**Test**	**Positive**	**Negative**
ONPG	Yellow	Colourless	**INO**	Yellow	blue/blue-green
ADH	red/orange	Yellow	**SOR**	Yellow	blue/blue-green
LDC	Orange	Yellow	**RHA**	Yellow	blue/blue-green
ODC	red-orange	Yellow	**SAC**	Yellow	blue/blue-green
CIT	blue-green/green	pale green/yellow	**MEL**	Yellow	blue/blue-green
H_2S	black deposit	colourless/grayish	**AMY**	Yellow	blue/blue-green
URE	red/orange	Yellow	**ARA**	Yellow	blue/blue-green
TDA	dark brown	Yellow	**OX**	Violet	Colourless
IND	pink/red	pale green/yellow	**NO3**	Red	Yellow
VP	pink/red	Colourless	**NO3/Zn**	Yellow	Red
GEL	diffused black	restricted black	**MOB**	Motile	Non motile
GLU	Yellow	blue/blue-green	**MAC**	Present	Absence
MAN	Yellow	blue/blue-green	**OF**	Yellow	Green

Table 2

Zone size interpretation chart: (Quinn *et al*, 1999)

Antimicrobial	Resistant	Intermediate	Susceptible
Tetracycline(30mcg)	≤11	12- 14	≥15
Gentamicin(10µg)	≤12	13- 14	≥15
Trimethoprim(5µg)	≤10	11- 15	≥16
Ampicillin(10µg)	≤13	14- 16	≥17
Ceftriaxone(30mcg)	≤19	20- 22	≥23
Ciprofloxacin(5mcg)	≤15	16-20	≥21
Norfloxacin(10mcg)	≤12	13- 16	17
Penicillin(10 units)	28	-	29
Erythromycin(15mcg)	≤13	14- 22	≥23
Chlormphenicol (30mcg)	≤12	13- 17	≥18
Colistin(10mcg)	≤10	-	≥11
Lincomycin(15mcg)	-	-	-

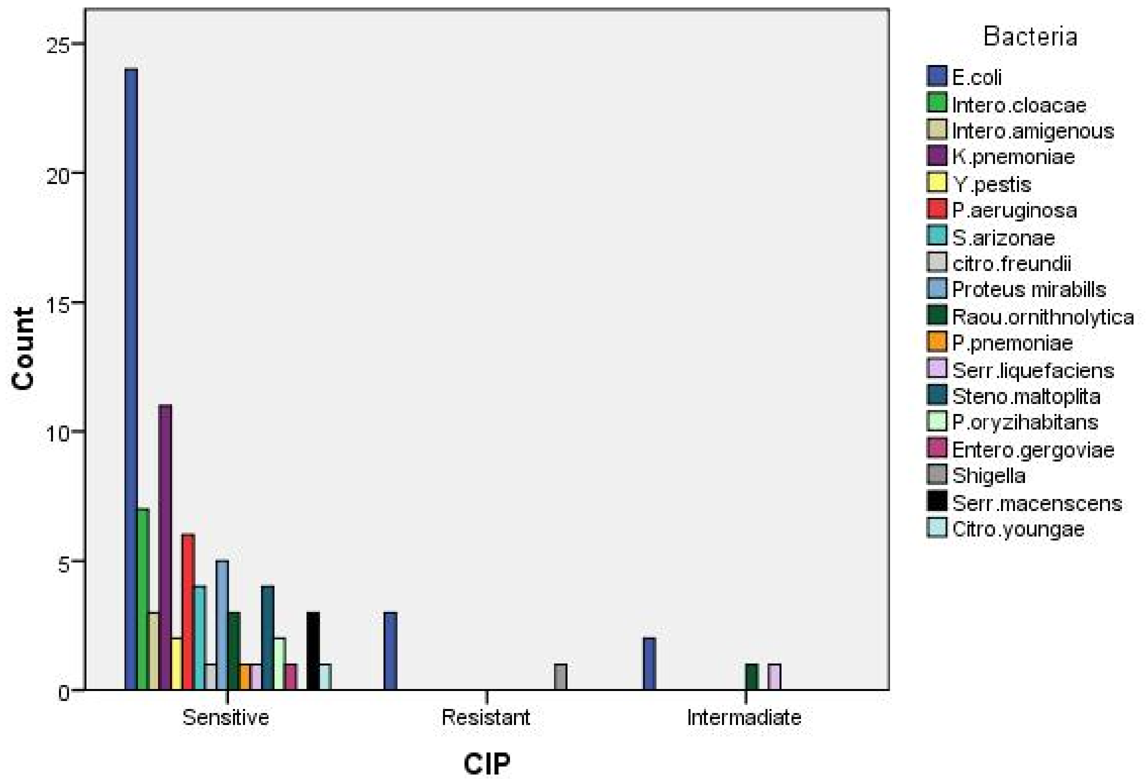

Fig1: Sensitivity of *Enterobacterioceae* to Ciprofloxacin:-

These figures determine the *E.coli* and *Klebsilla-pnemoniae* were highly sensitive to Ciprofloxacin.

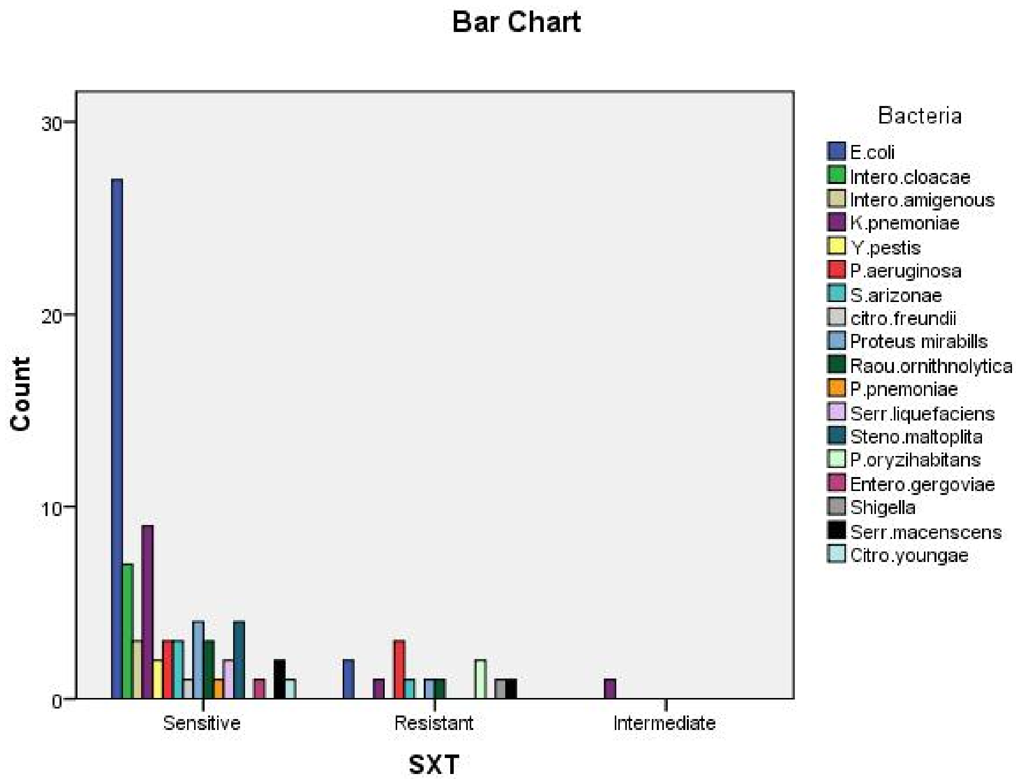

Fig 2: Sensitivity of *E.coli* to Trimethoprim:-

These figures determine the *E.coli* and *Klebsilla-pnemoniae* were highly sensitive to Trimethoprim.

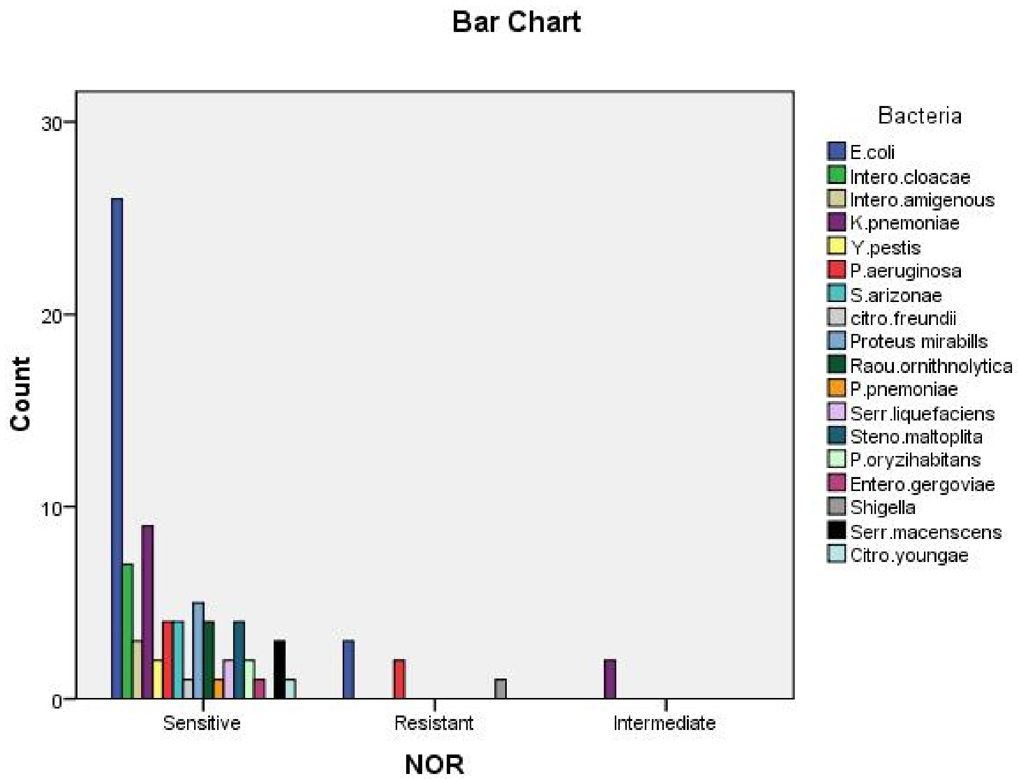

Fig3: Sensitivity of *E.coli* to Norfloxacin

These figures determine the *E.coli* and *Klebsilla-pnemoniae* were highly sensitive to Norfloxacin**.**

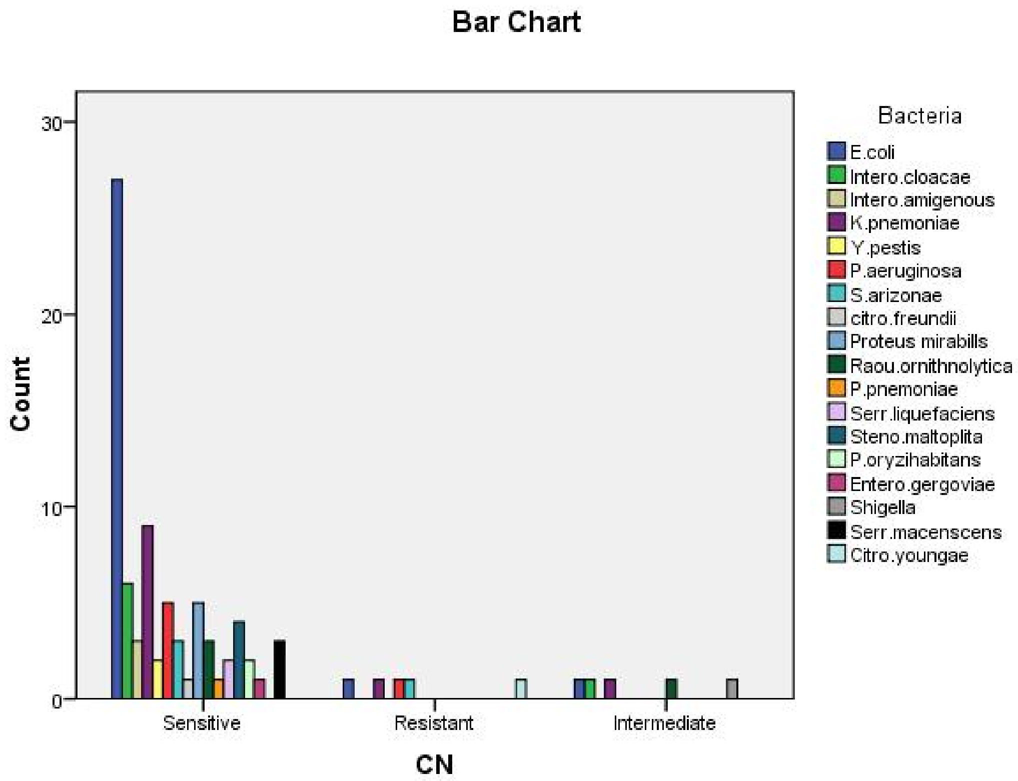

Fig 4: Sensitivity of *E.coli* to Gentamicin:-

These figures determine the *E.coli* and *Klebsilla-pnemoniae* were highly sensitive to Gentamicin.

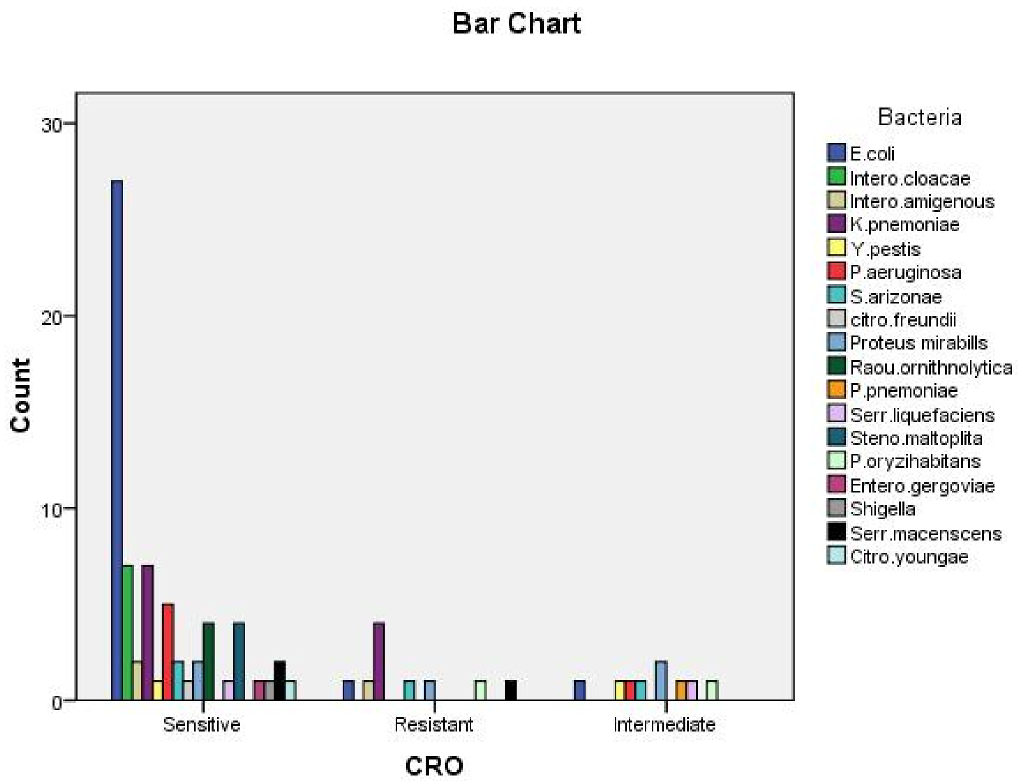

Fig 5: Sensitivity of *E.coli* to Cifitriaxon

This figure determine the *E.coli* was highly sensitive to Cifitriaxon.

Figure (6): Reaction of *E.coli* to biochemical test inAPI20E strips

This figure determine the *E.coli* was converted VP from colorless to pink and GEL from restricted black to diffuse black and fermentative of sugars from blue green to yellow color.

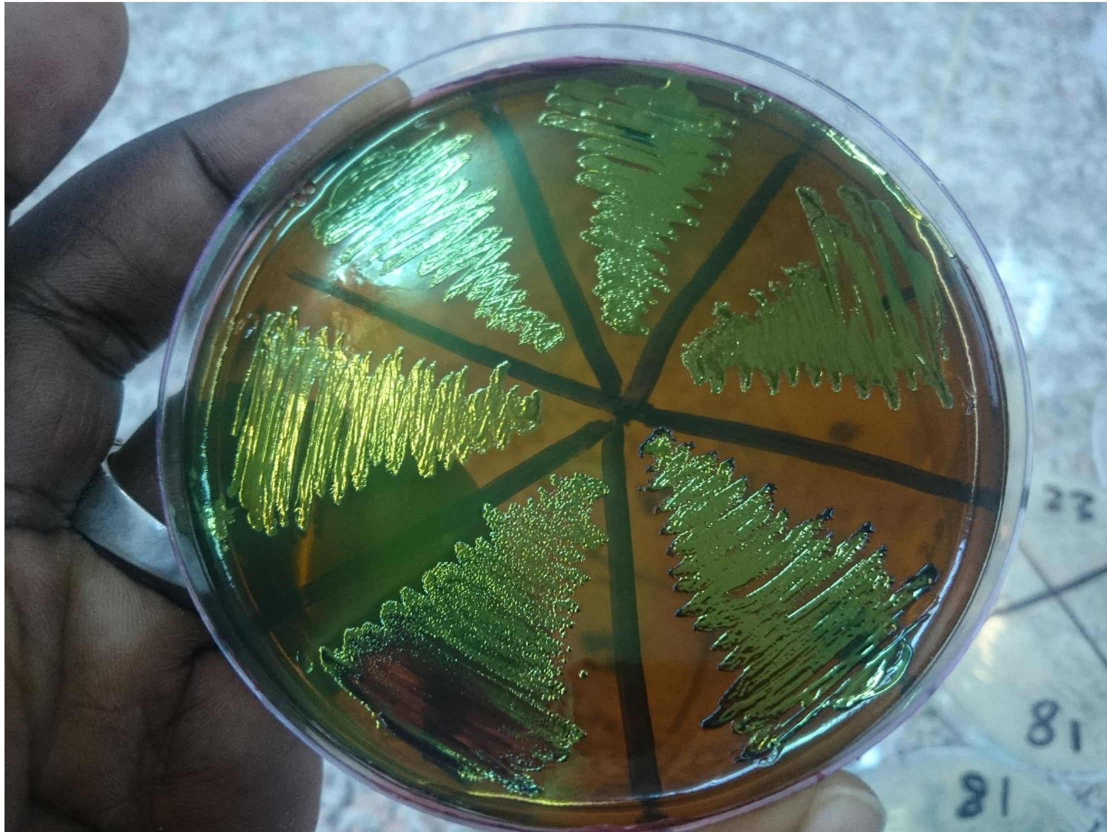

Figure (7): Appearance of *E.coli* in EMB medium (metalic sheen)

Figure (8): Samples collection from diarrheaitic lamb.