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Isolation and diagnosis of *E. coli* isolated from diarrheic sample and test the effect of some antibiotic sensitivity

A search

Submitted to the council of the college of veterinary medicine, University of Al-Qadissiya in partial fulfillment of the requirements for The Degree of Bachelors of Science in Veterinary Medicine

By

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بسم الله الرحمن الرحيم

وقل اعملوا فسيرى الله عملكم ورسوله والمؤمنون وسترادون الى عالم الغيب والشهادة فينبئكم بما كنتم تعملون

صدق الله العظيم

الشورى الآية (١٠٥)

Supervisor's Certificate

I certify that this search titled **(Isolation and diagnosis of *E. coli* isolated from diarrheic samples and test the effect of some antibiotic sensitivity)** was prepared under my supervision at the College of Veterinary Medicine of Al-Qadissiya university, as partial fulfillment of requirements of Bachelor in sciences in veterinary medicine in the view of a viable recommendations, I forward this search for debate by the examining committee.

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In view of the available recommendation, I forward this thesis for debate by the examining committee .

Certificate of Department

We head of Dept. of Int. & prev. Med. Certify that (**Mustafa Abdul Sahib Kazim**) is adequate for the debate of Graduation Project of Bachelor Degree in Science in Veterinary Medicine.

Instructor

Dr . Muthanna Hadi Hussain

Head of Dept. of Int. & prev. Med.

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Thank god lord of the worlds may Allah bless Muhammad and aspects of my thanks and appreciation to my professors valued all of what have made me of valuable guidance opinions sound as Iam pleased to draw my thanks and gratitude to Ass. Lecturer **Khetam Qaid**

Dedication

To the function of patience and optimism and hope

To each of the following in the presence of God and his Messenger , my mother dear

To the big heart my dear father

To those who have demonstrated to me what is the most beautiful of my brother's life

To the people who paved our way of science and knowledge

All our teachers Distinguished

To the taste of the most beautiful moments with my friends

I guide this research

Mustafa

Abstract

Background : Cattle diarrhea is a major economic concern in cattle industry all around the world. This study was carried out in order to investigate distribution of *E. coli* infection between bovine and sheep in housing livestock of veterinary medicine college, and antibiotic resistance properties of Escherichia coli isolated from diarrheic cases.

Results : Totally, out 71.87 % of 32 diarrheic fecal samples collected from housing livestock were positive for *E. coli* , 58.3% of 12 cows were positive and 80% of 20 diarrheic stool of sheep were positive . All isolates had the high resistance to rafmpcine (), norphloxacin () and amikacin () antibiotics.

Conclusions : Our findings should raise awareness about antibiotic resistance in diarrheic bovine and sheep. Clinicians should exercise caution when prescribing antibiotics.

1.1 Introduction

Cattle diarrhea is one of the most economic and pervasive concern in veterinary industry all around the world. Infectious agents are the most commonly detected causes of cows and sheep diarrhea **(1-3).** Several studies have been addressed the high distribution of Escherichia coli (*E. coli*) strains in infectious calf diarrhea **(3-6).** Escherichia coli is a gram-negative, rod-shaped, flagellated, non-sporulating and facultative anaerobic bacterium of the family enterobacteriaceae that classically classified in to six different groups of *Escherichia coli* associated with diarrhoea in humans and animals including entero-pathogenic *E. coli* (EPEC), entero-aggregative *E. coli* (EAEC), entero toxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), diffusely-adherent E. coli (DAEC) and shiga-toxin producing *E. coli* (STEC) **(7).** ETEC is the most common group associated with the neonatal dia rhoea in animals causing significant morbidity and mortality **(8),** necrotoxigenic (NTEC), enteroinvasive (EIEC), enteropathogenic (EPEC) and attaching and effacing *E. coli* (AEEC) pathotypes **(9).** EPEC strains are defined as eae harbouring diarrhoeagenic *E. coli* that possess the ability to form attaching-effacing (A/E) lesions on intestinal cells **(10,11)**. Diseases caused by *E. coli* often require antimicrobial therapy; however, antibiotic-resistant strains of this bacterium cause longer and more severe illnesses than their antibiotic-susceptible counterparts. Several studies have shown that antibiotic resistance in *E. coli* has increased over time **(12,13,14)**.

Aims of study

*to detect E.coli infection between housing living stock animals

*to detect the effect of Amcacin , Rifampcin ,Norpholoxacin

2.1 An overview of *E. coli*

The fecal coliform group includes rod-shaped, Gram-negative, non-spore forming organisms that live in the gastrointestinal tract of humans and other warm blooded animals, ferment lactose at 44.5 C^0 within 48 hour. A member of the fecal coliform group is Escherichia coli **(15)**. The name Escherichia comes from the name of the paediatrician Escherich, who in 1895 first isolated and characterized this bacterium as "bacterium coli commune" ('enteric bacteria') **(16)**.

E. coli is defined as a Gram-negative, non-spore forming, rod-shaped (1.1-1.5 µm x 2.0-6.0 µm) micro-organism that is often motile by means of flagella or may be non-motile, and which can grow with or without oxygen **(17)**. It is catalase positive, oxidase negative, fementative (glucose, lactose, D-mannitol, arabinose and maltose), reduces nitrate and is β-galactosidase positive. Approximately 95 % of *E. coli* strains are indole and methyl red positive **(17)**. It is characterized by lactose fermentation with gas production and indole production from tryptophan when incubated for 48 ± 2 h at 44°C to separate *E. coli* from other organisms of the fecal coliform group **(18)**. Its involvement in several cases of food poisoning has suggested that *E. coli* should be used as an indicator for sanitary quality **(19)**. The limits of temperature for growth of *E. coli* are 7-46°C and the optimum growth temperature is approximately 37°C but *E. coli* can survive for weeks at -20°C to 4°C. *E. coli* generally grows within the pH range of 4.4–9.0, at an aw of at least 0.95 **(20)**.

2.2 *Escherichia coli* Virulence Factors

Escherichia coli has many virulence factors that enable bacterial colonization, swivel and disease production. The more pathogenic members of *E. coli* isolate have similar virulence factors in all mammals. These virulence factors include adhesins (pili), senun resistance, antiphagocytic capsules and sidrrophores (aerobactin, enterobactin) **(21)**. Hemolysin production is not characteristic of poultry isolates **(22)**. Fimbriae (pili) are protein adhesions that mediate attachment of *E. coli* in a tissue and species specific way **(23)**.

2.3 Transmission of *E. coli*

The transmission of this pathogen is by the fecal–oral route. Cattle and small ruminants are the major natural reservoir of these foodborne pathogens, they can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources and mainly transmitted to humans by the consumption of contaminated food and water, or by contact with animals **(24, 25).**

2. Material and methods

2.1. Sampling

Fecal samples were collected every 4 weeks for a period of 5 months, the rectal swabs were collected with sterile cotton bud by gentle touch from 12 cows and 20 sheep in veterinary medicine college animals housing, along the period of September 2016 to February 2017. All samples were transferred in an ice box to the laboratory with minimal delay for bacteriological examination.

2.2. Bacteriological examination

One gram of each fecal sample was diluted in 3 mL of sterile saline. Samples were cultured and identified according to Quinn et al. **(26)**. For isolation of E. coli strains, a loop full from the diluted specimens was inoculated into MacConkey's agar and incubated at 37 °C for 18–24 hours. Lactose fermenter (pink) colonies were subculture and streaked in Eosin - Methyline Blue agar was performed from the suspected plates containing E. coli obtain a pure culture and confirmed as E. coli using the standard biochemical tests according to Collee et al. **(26)**.

2.3 Antibiotic susceptibility testing

All *E. coli* (isolates) recovered from diarrheic cows and sheep were tested for their antimicrobial susceptibility to 3 different antimicrobial discs including **AK**/(amikacin)(30 µg), **NOR**/(norphloxacin) (10 µg), and **RA**/(rifampicin)(5 µg). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) **(27)**. The antibiotic susceptibility was based on the induced inhibition zones according to the guidelines of the CLSI **(27)**.

4.1. Bacterial isolation

Out of 32 collected fecal samples from diarrheic animals, including 12 (58.3%) bovine bacterial isolates, 20 (80%) from sheep were positive isolate **(Table1)**.

Table **(4-1)** Prevalence of *E. coli* in diarrheic animals.

Animal species	No. of samples	No. of positive samples to E. coli		Positivity rate %
		Culture examination	biochemical examination	
Bovine	12	7		58.3
sheep	20	16		80
Total	**32**	**23**		**71.87**

% : was calculated according to the number of the collected samples.

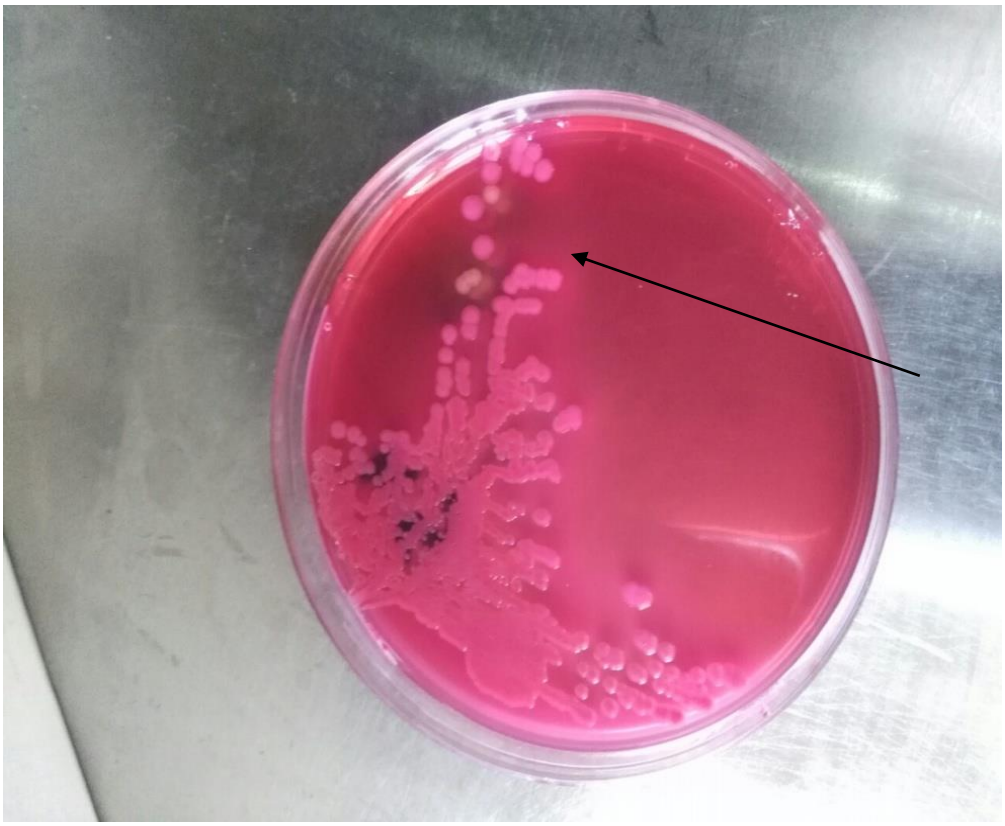

Figure (4-1) bright pink color smooth transparent colonies

Figure (4-2) greenish black colony with metallic sheen

Figure (4-3) Bio chemical test to confirm *E. coli* isolate forming the red ring in indol

Table **(4-2)** Results of cultural, morphological characteristics of the isolated *E. coli*

Sources Of Isolate	Colony Characteristics		Staining Character
	MC. Agar	EMB Agar	
Bovine Stool	Bright Pink Color Smooth Transparent	Raised Colonies/Greenish Black Colony With Metallic Sheen	Pink short rod, Gram negative bacilli
Sheep Stool	=		=

Legends : **EMB=**Eosine-methylene blue **MC** = MacConkey

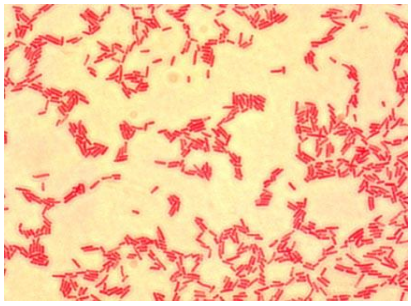

Figure **(4-4)** *E. coli* is a Gram-negative, facultative anaerobic, nonsporulating bacterium, typically rod-shaped, and are about 2.0 µm long and 0.25–1.0 µm in diameter

Table **(4-3)** Antibiotic resistance profile of *E. coli* isolated from diarrheic bovine and sheep (Disk diffusion method)

Antimicrobial agent	Code Disk	Potency	Inhibition zone size
Amikacin	AK	30 ug	22mm
Rafmpicin	AP	5 ug	15mm
Norphloxacin	NOR	10 ug	17mm

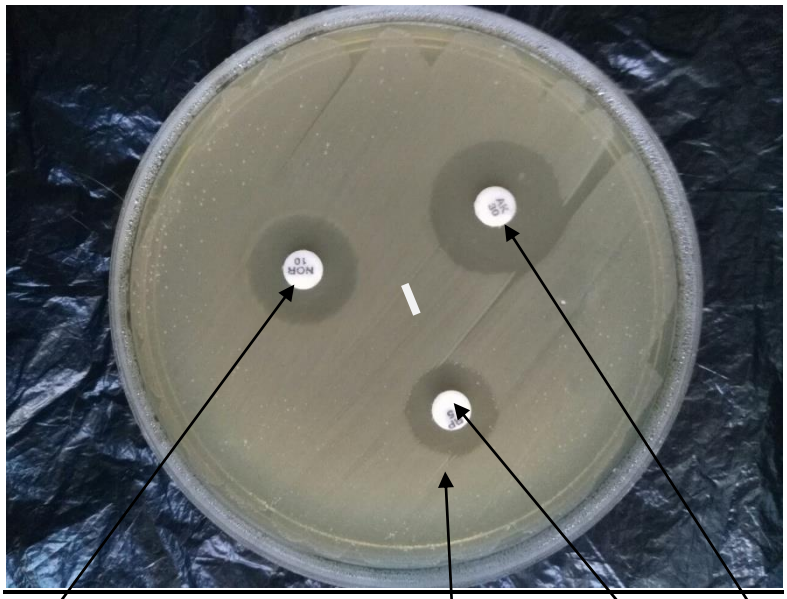

Figure **(4-5)**. Antimicrobial susceptibility test of *E. coli* on Muller Hinton agar by disc diffusion method

5. Discussion

Diarrhea remains as one of the most important problems faced by livestock, causing great economic losses. cattle are at greatest risk of diarrhea within the first month of life and the incidence of diarrhea decreases with age **(29)**.

Identification of the possible causative agent in outbreaks of diarrhea is important to allow targeted preventative measures, such as vaccination, and identification of possible risk factors or sources of infection **(30).** *E. coli* are the most common identified pathogens **(32,33,34)**. Their prevalence's vary by geographical location of the farms, farm management practices, and herd size **(35).**

In the present study, the prevalences of *E. coli* in cattle of veterinary medicine college housing livestock were 71.87% **(Table 1-4)**. The negative bacterial isolation in some fecal samples did not mean absence of microbial infection but may attribute to infection with bacteria requiring specific or enriched culture media and can't grow on the used culture media **(36)** or **(37)** elucidated that bacteria were not detectable due to the number of colony forming units are below the detection limit of the assay. Additionally, the presence of antibiotic residues may explain falsely negative bacteriological results because the withdrawal time is not regarded in our herds.

The cultural characterization of all positive bovine and sheep *E. coli* revealed greenish black colony with metallic sheen in Eosine methylene blue agar, bright pink color smooth transparent colony in MacConkey agar, table **(4-2)**, figure**(4-1)(4-2)(4-3)** which were similar to the findings of other authors **(38)**. In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative short rod arranged in single or paired table **(4-2)**.

The prevalence of *E. coli table* ***(4-2)*** in the present study between bovine 58.3% was nearly to what reported by **(39)**. The highly prevalence's (80%) of *E. coli* in sheep diarrheic samples reflect the importance role of *E. coli* as causative agent of diarrhea, also emphasize the role of sheep as major reservoir for pathogenic *E. coli* **(40)**. As a light of species and geographical locations and hygienic measures, and these factors significantly influence the prevalence of *E. coli* between housing livestock animals **(41)**.

The sensitivity to the antimicrobial agent of *E. coli* results table **(4-5)** showed that *E. coli* more sensitive to Amikacin than Norphloxacin whereas intermediate resistant to Refampicin. The results agreed with **(42)**.

6.2: Recommendations

- In particular *E. coli* was found to be widely distributed among diarrheic cattle in housing livestock. These observations raise the need to pay attention to these circulating pathogen for better control of diarrheic cases and supplied medications immediately.

- Further research is needed to determine which aspects can reduce the transmission of these infections and detection the another causes of diarrhia in stable.

References

1. **Schroeder, M.E.; Bounpheng, M.A.; Rodgers, S.; Baker, R.J.; Black, W.; Naikare, H.; Velayudhan, B.; Sneed, L.; Szonyi, B.; Clavijo, A.(2012)**. Development and performance evaluation of calf diarrhea pathogen nucleic acid purification and detection workflow. *J Vet Diagn Invest*, 24(5):945–953.
2. **Sans, P. and De Fontguyon, G.(2009).**Veal calf industry economics. *Rev Med Vet-Toulouse* 2009, 160(8–9):420–424.
3. **Nguyen, T.D.; Vo, T.T.; Vu-Khac, H.(2011).** Virulence factors in Escherichia coli isolated from calves with diarrhea in Vietnam. *J Vet Sci*, 12(2):159–164.
4. **Wani, S.A.; Hussain, I.; Nabi, A.; Fayaz, I. ; Nishikawa, Y**. **(2007).**Variants of eae and stx genes of atypical enteropathogenic *Escherichia coli* and non-O157 Shiga toxin-producing Escherichia coli from calves. *Lett Appl Microbiol*, 45(6):610–615.
5. **Arya, G. Roy, A. Choudhary, V.; Yadav; M.M.; Joshi, C.G.(2008).** Serogroups, atypical biochemical characters, colicinogeny and antibiotic resistance pattern of Shiga toxin-producing Escherichia coli isolated from diarrhoeic calves in Gujarat, India. *Zoonoses Public Health*, 55(2):89–98.
6. **Islam, M.A.; Heuvelink, A.E.; de Boer E, Sturm, P.D.; Beumer, R.R.; Zwietering, M. H.; Faruque, A. S.; Haque, R.; Sack, D. A.(2007).** Shiga toxin-producing Escherichia coli isolated from patients with diarrhoea in Bangladesh. *J Med Microbiol*, 56(Pt 3):380–385.
7. **Croxen, M.A.; Law, R.J.; Scholz, R.; Keeney, K.M.; Wlodarska, M.; and Finlay, B.B. (2013).** Recent advances in understanding enteric pathogenic Escherichia coli. *Clin. Microbiol. Rev.*, 26: 82–880.
8. **Kolenda, R.; Burdukiewicz, M. and Schierack, P. (2015).** A systematic review and meta-analysis of the epidemiology of pathogenic Escherichia coli of calves and the role of calves as reservoirs for human pathogenic *E. coli* . *Front. Cell. Infect. Microbiol.*5: 23.
9. **Kaper, J.B.; Nataro, J. P.; Mobley, H.L.(2004).** Pathogenic Escherichia coli. *Nat Rev Microbiol*, 2(2):123–140.
10. **Moxley, R.A.; Smith, D.R.(2010).** Attaching-effacing Escherichia coli infections in cattle. *Vet Clin North Am Food Anim Pract*, 26(1):29–56.
11. **Janke, B. H; Francis, D. H.; Collins, J.E.; Libal, M. C. (1989).** Attaching and effacing Escherichia coli infections in calves, pigs, lambs, and dogs. *J Vet Diagn Invest*, 1(1):6–11.
12. **Solomakos, N.; Govaris, A.; Angelidis, A. S.; Pournaras, S.(2009).** Occurrence, virulence genes and antibiotic resistance of Escherichia coli O157

isolated from raw bovine, caprine and ovine milk in Greece. *Food Microbiol*, 26(8):865–871.

13. **Cortés, P.G. Blanco, J. and Llagostera, M. (2010).** Isolation and characterization of potentially pathogenic antimicrobial-resistant Escherichia coli strains from chicken and pig farms in Spain. *Appl Environ Microbiol*, 76(9):2799–2805.
14. **Tadesse, D. A.; Zhao, S. Tong, E.; Ayers, S.; Singh, A. (2012).** Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950–2002. *Emerg Infect Dis*, 18(5):742–749.
15. **Oram, B. (2005).** Why fecal coliform testing is important–Escherichia coli? Wilkes university. Center for environmental quality environmental engineering and earth sciences (http://www.water-research.net/Watershed/ecoli.htm, accessed on 19 August, 2007).
16. **Todar, K. (2005).** Ken Todar's Microbial World. University of Wisconsin–Madison. In (ed.): Bacteriology at UW-Madison. Lectures and readings from introductory microbiology courses at the University of Wilconsin-Madison (http://www.bact.wisc.edu/themicrobialworld/Escherichia coli.html, accessed on August 17, 2007).
17. **Fratamico, P.M., Smith, J.L. (2006).** Escherichia coli infections. In: Riemann, H.P., Cliver, D.O. (3rd): Food-borne infections and intoxications. Florida: Elsevier Inc. Academic Press. pp. 205-208.
18. **ISO 7251. 2005** Standard protocol (3rd): Microbiology of food and animal feeding stuff – Horizontal method for the detection and enumeration of presumptive Escherichia coli - Most probable number technique. Switzerland: International Organization for Standardization.
19. **FAO (Food and Agriculture Organization of the United Nations) (2006).**Livestock report 2006 (A0255/E). Rome: FAO (http://www.fao.org/docrep/009/a0255e/a0255e00.HTM, accessed on 16 August, 2007).
20. **Bell, C., Kyriakides, A. (1998).** Escherichia coli: A practical approach to the organism and its control in foods. 1st ed. London, United King: Blackie Academic.
21. **ISO 7251: 2005** Standard protocol (3rd): Microbiology of food and animal feeding stuff – Horizontal method for the detection and enumeration of

presumptive Escherichia coli - Most probable number technique. Switzerland: International Organization for Standardization.

22. **FAO (Food and Agriculture Organization of the United Nations) (2006).** Livestock report 2006 (A0255/E). Rome: FAO (http://www.fao.org/docrep/009/a0255e/a0255e00.HTM, accessed on 16 August, 2007).

23. **Bell, C.; Kyriakides, A. (1998).** Escherichia coli: A practical approach to the organism and its control in foods. 1st ed. London, United King: Blackie Academic.

24. **Gross, W. G. (1994).** Diseases due to Escherichia coli in poultry. In: Escherichia coli in domestic animals and humans. C. L. Gyles, ed. CAB International, Wallingford, United Kingdom. pp. 237-259.

25. **Gyles, C. L. (1993).** Escherichia di. In: Pathogenesis of bactenal infections in animals, 2nd ed. C. L. Gyles, and C. O. Thoen, eds. Iowa State University Press, Ames, Iowa. pp. 164-187.

26. **Smyth, C. J.; M. Marron, and Smith, S. G. J.(1994).** Fimbriae of Escherichia coii. In: Escherichia coli in domestic animais and humans. C. L. Gyles, ed. CAB International, Wallingford, United Kingdom. pp. 399-435.

27. **Jeshveen, S. S; Chai, L. C.; Pui, C. F.; Son, R.(2012).** Optimization of multiplex PCR conditions for rapid detection of Escherichia coli O157:H7 virulence genes. International Food Research Journal 19 (2): 461-466.

28. **Ellingson, Jay, L. E.; Koziczkowski, J.; Anderson, J. L.(2005).** Rapid PCR detection of enterohemorrhagic Escherichia coli (EHEC) in bovine food products and feces.

29. **Quinn et al,.(2002).** Veterinary microbiology and microbial disease Blackwell, pp. 113–116.

30. **Collee et al,.(1996)**. Simmons Practical medical microbiology (14th ed) (1996).

31. **Clinical and Laboratory Standards Institute(CLSI).(2012).** Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial disk susceptibility tests (Approved Standard – 11th ed), 32. M02-A11.

32. **Garcia et al, .(2000).** Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain Comp. *Immunol Microbiol Infect Dis*, 23, pp. 175–183
33. **Izzo et al, .(2011).** House Prevalence of major enteric pathogens in Australian dairy calves with diarrhea *Aust Vet J*, 89 (5).pp. 167–173**.**
34. **Achá et al, .(2004).** Studies on calf diarrhoea in Mozambique: prevalence of bacterial pathogens Acta Vet Scand, 45 (2004), pp. 27–36**.**
35. **Cho, Y.; Yoon, K. J.(2014).** An overview of calf diarrhea-infectious etiology, diagnosis, and *intervention J Vet Sci*, 15 (1). pp. 1–17
36. **Cho et al, (2010)**. Development of a panel of multiplex real-time polymerase chain reaction assays for simultaneous detection of major agents causing calf diarrhea in feces. *J Vet Diagn Invest*, 22, pp. 509–517
37. **C.R. Fricker, Fricker, (1987)** .The isolation of salmonellas and campylobacters. *J Appl Bacteriol*, 63, pp. 99–116
38. **Hassan.(2014)**. Some studies on bacteriological causes of enteritis in calves *J Vet Adv*, 4 (5), pp. 503–507
39. **Moussa et al,. (2010).** Rapid detection of *Salmonella* species in new-borne calves by polymerase chain reaction *Int J Gen Mol Biol*, pp. 62–66
40. **Saba A. A. (2015).** Detection of hemolysin virulence factor gene of Enterohemorrhagic Escherichia coli (EHEC) isolated from feces of infected sheep by using Polymerase Chain Reaction Technique**.** *AL-Qadisiya Journal of Vet. Med. Sci*. 14 : 1.
41. **Younis *et al*,.(2009).** Molecular screening and risk factors of enterotoxigenic *Escherichia coli* and *Salmonella* spp. in diarrheic neonatal calves in Egypt *Res Vet Sci*, 87. pp. 373–379
42. **Walk et al, .(2007)**. Influence of antibiotic selection on genetic composition of *Escherichia coli* populations from conventional and organic dairy farms Appl Environ Microbiol 73. pp. 5982–5989