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BACTERIOLOGICAL AND MOLECULAR STUDY OF ANTIBIOTICS EFFECT ON GROWTH *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTION AND DIARRHEAL IN CALVES

Fatima Farhan Abbas [1], Hameedah Hamzah Ajeel [2]
1,2 Department of Microbiology, Collage of Veterinary Medicine , AL-Qasim Green University, Babylon , Iraq.
Correspondence to: Hameedah Hamzah Ajeel.
E-mail: hameedahhamza@vet.uoqasim.edu.iq.

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Abstract:

The current study included testing the effect of antibiotic effect on E. Coli bacteria isolated from cases of urinary tract infection and diarrhea in calves . 49 samples from veterinary clinics in Al-Qasim and Al-Hashimiya during the period from March to July 2022. The results showed 10 isolates of E. coli diagnosed in calves. 4 isolates (16.67%) from 24 samples UTI , while 6 isolates (24%) from 25 samples diarrhea infections in calves. The isolates were re-diagnosed according to culture tests, Gram stain and biochemical test.It was growth on the MaCconkey agar and it gave a pink color, as well as on the grom agar, it gave a green color, but on the EMBagar it gave a green metallic sheen. Antibiotic susceptibility testing was performed on isolates of Escherichia coli by kirby-bure method. The results showed that E. coli was 100% resistant to ciprofloxacin and 90% to levofloxacin, while the rest of the antibiotics were less resistant. Also, the efflux pump genes responsible for E. Coli's AcrA were detected using polymerase chain reaction (PCR) and all E. Coli isolates showed contain those genes. Therefore, this study was aimed to assess the in vitro antibiotic activity against E. coli as well as to shed spot light upon its efficacy in reducing E. coli infection in calves.

Keywords: *E. coli,* antibiotic sensitive*, PCR ,*UTI, diarrheal

INTRODUCTION:

Neonatal calf diarrhea (NCD) is a syndrome that affects calves younger than one month of age and is caused by a mix of infectious and non-infectious factors (Bashir et.al,..,2020). The four common pathogenic sources of calf diarrhea worldwide are enterotoxigenic Escherichia coli (ETEC), rotaviruses, coronaviruses, and Cryptosporidium spp. Cryptosporidium spp. and rotaviruses are the two most usually detected culprits in diarrhoeic feces samples (Reichel et.al,.2018). Intestinal pathogenic *E. coli* (DEC) and extra-intestinal pathogenic *E. coli* (ExPEC) are two types of *E. coli* pathogenic strains that trigger urinary tract infections (UTI) (Kaper et.al,.2004). UTI is still the most frequent infection in the world, leading to various disorders including pyelonephritis and cystitis; nevertheless, there have been numerous advancements in diagnostic and prevention strategies (Johnson & Nolan 2009). The E. coli studies paved the way for the establishment of bacterial community genetics (Leimbach,et.al.2013).They discovered considerable recombination in the subspecies as well as a strong phylogenetic structure with eight major phylogroups, four of which (A, B1, B2, and D) comprise the bulk of strains and four others (C, E, F, and G) are more rare (Vila et.al., 2016 ; Touchon,et.al., 2020). Antibiotics are drugs that are used to treat or prevent bacterial infections. Almost all antibiotic classes are depend on the antimicrobials structure found normally in environmental microbes, with synthetic versions of these natural structures accounting for the majority of antibiotics currently in use (Qiao ,et.al., 2018).

MATERIAL AND METHODS:-

1. Samples Collection

A total of 10 *E. coli* isolated were collected from differ clinical specimen in Babylon province during the period from March to July 2022. These specimens were collected from 49 sample obtained from animal suffered from different infection by taking swabs from diarrheal and UTI infection. 49 calves sample from diarrheal and UTI infection were collected veterinary clinic and the sick animal showed fever, Abdominal fecal consistency, sign of dehydration and weak.

2. Isolation and identification of E. coli isolates:

Classical identification of E. coli depends mainly on its growth on MacConkey`s agar plates, streaking on Eosin Methylene Blue (EMB)medium, Grom agar and biochemical examination of its typical colonies by IMVC and TSI (Quinn et.al,. 2002).

3. Antimicrobial susceptibility testing.

The susceptibility of E. coli isolates were determined by the standard disk diffusion method (Ortez .,2005) against eight antimicrobial agents including Amikacin (30µg), Aztreonam (30µg), amoxicillin+clavulanic acid (30µg), Tobramycin (10µg), Ceftriaxone (30µg), Fasfomycin (200µg), ciprofloxacin (5µg), and Levofloxacin (5µg), Trimethoprim (5µg), Tetracycline (5µg).

4. PCR amplification:-

The target DNA was amplified using PCR with specified primer pairs. It consisted of three processes that were performed for a certain number of cycles in order to obtain a PCR result (amplicon) that could then be detected after agarose gel electrophoresis. shown in Table (1).

Table (1) The thermal cycling conditions**:-**

Genes	Initial denaturation		Denaturation		Annealing		Extension		No. of cycles	Final extension		Cooling	
	Temp	time	Temp	time	Temp	time	Temp	time		Temp	time	Temp	time
AcrA	95°C	5min.	95°C	35sec	58°C	30sec	72°C	40sec	35	72°C	5m	4°C	4m.

5. Statistical Analysis:

Statistical analysis of the results by using computer program (SPSS), Version 23 one-way analysis of variance (ANOVA), the difference was considered significant at ($P \leq 0.05$) .

RESULTS :-

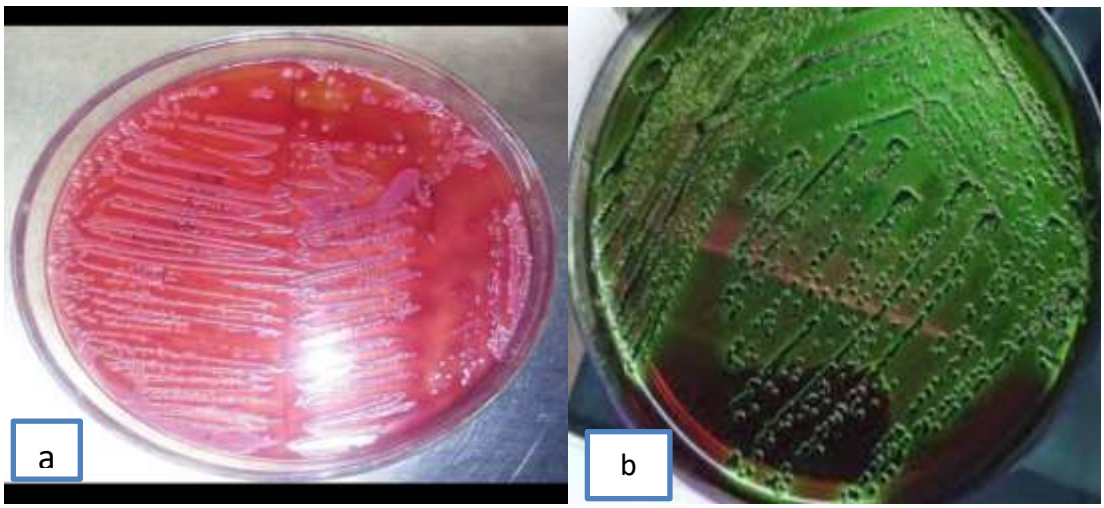
1. *E. coli* isolation by culture and biochemical tests

10 out of 49 samples (20.41%) were isolated, where. 4 (16.67%) from 24 represented UTI , while 6 (24%) from 25 represented diarrhea infections in animals .show in table 2.

Table (2) Number and Percentage of positive *E. coli* isolated from animal.

Specimen Animals	Number	Positive result	Negative result	Percentage
UTI	24	4	20	16.67%
Diarrheal	25	6	19	24%
Total	49	10	39	20.41%

And Characterization of bacterial isolates of *E. coli* on macConkey agar appear Rose pink lactose Fermenter colonies, while Grom agar appear white to grayish colony and Eosin-Methylene Blue (EMB) Agar appear circular colonies green metallic sheen.as show in fig.1



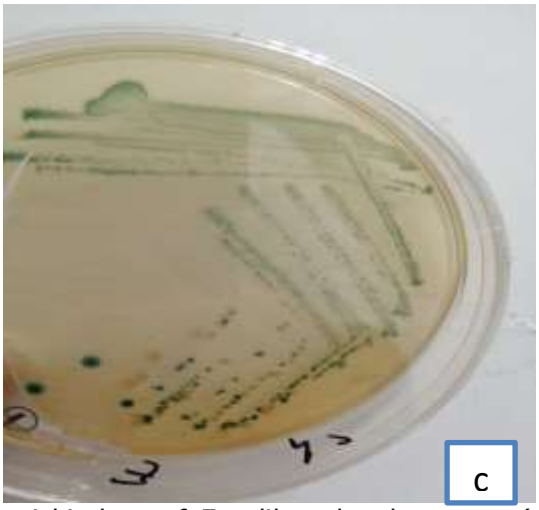


Figure (1): Characterization of bacterial isolates of *E. coli* by cultural property. (a. macConkey agar, b. EMB and c. Grom agar).

2. Antibiotic susceptibility of *E. coli*

Antibiotic sensitivity of *E. coli* isolated from diarrheal and UTI pathological samples revealed high sensitivity rates of E. coli 70% to aztreonam, amoxicillin/clavulanate and trimethoprim. While the fosfomycin and tetracycline preserved 50% sensitivity rate for each one. On the other hand, low sensitivity rates were observed for levofloxacin, amikacin, ceftriaxone, and tobramycin in a percentage 10%, 20%, 30% and 40% respectively. While ciprofloxacin showed the highest resistance by E. coli with 100%. Table (3).

Table (3) Antibiotic susceptibility profile of *E.coli* by disc diffusion Method (DD)

Type of antibiotic	Sensitivity Rate No. and %	Intermediate Rate No. and %	Resistance Rate No. and %
Amikacin (30mg)	2 (20)	1(10)	7 (70)
Aztreonam (30mg)	7 (70)	0(0)	3 (30)
Tobramycin (10mg)	4 (40)	0(0)	6 (60)
Ceftriaxone (30mg)	3 (30)	0(0)	7 (70)
Amoxicillin clavulanate (20\10mg)	7 (70)	0(0)	3 (30)
Levofloxacin (5mg)	1 (10)	6(60)	3 (30)
Trimethoprim (5mg)	7 (70)	0(0)	3 (30)
Ciprofloxacin (5mg)	0 (0)	0(0)	10 (100)
Fosfomycin (200mg)	5 (50)	0(0)	5 (50)
Tetracycline (5mg) (TET)	5 (50)	5 (50)	0(0)
X^2	69.79		
P value	0*		

*** Highly significant difference at P<0.01.**

3. Molecular Detection of Efflux Pumps (AcrA)Gene in *E.coli* by Using Polmerase Chain Reaction (PCR)

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All E.coli isolates were screened by PCR for present of efflux pump gene AcrA, and the results revealed that all isolates of E.coli 10(100%) were positive for AcrA gene with amplified size 480 pb as show in figure (2)

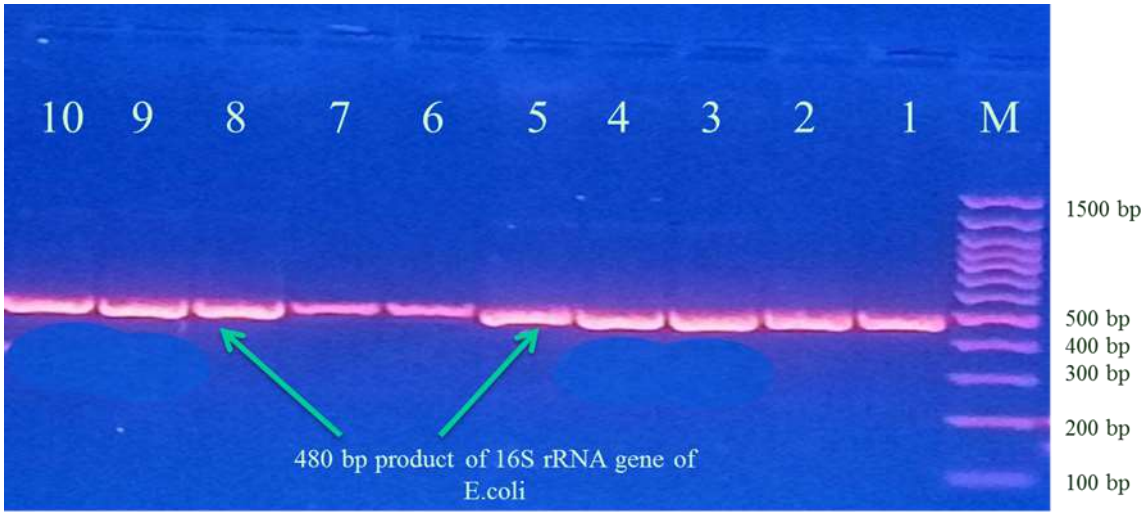

Figure (2) Electrophoresis of ethidium bromide stained agarose gel of the 480 bp fragment of the *E. coli* based *16S rRNA* gene detected from Lanes 1–10 are the positive samples. M: the 1500-100 bp molecular marker

DISCUSSION:-

In this study , found 10 (20.41%) E.coli isolates, where 6 (24%) from diarrhea infections in calves. these findings were in agreement the results recorded 28% of diarrheal specimens from calves infected with E. coli (Mailk, et.al., 2013; Ansari et.al., 2014). However, Naizir (2007) found a substantially greater frequency (60%). Additionally, 16.67% of calves specimens recorded with E. coli strain infection. A common cause of urinary tract infections (UTIs) in mammals is Escherichia coli (Foxman, 2014; Moreno et.al., 2018). E. coli isolated from distinct clinical specimens in the current investigation shown varied patterns of antibiotic sensitivity. The outcomes revealed that the E. coli was sensitive to Amoxicillin/Clavulanic Acid (AMC), Aztreonam and trimethoprim in (70%), these sensitivity results disagreement with Hussein, et.al.,(2019) From the other hand this results were in agreement with LaPlante & Sakoulas (2009) who recorded that Both E. coli strains were susceptible to aztreonam. There is a varying in a percentage of resistance of *E. coli* isolates for aminoglycosides antibiotics used in this study; When revealed 60% and 80 % of resistance to tobramycin and amikacin respectively, and these results were in agreement with other antimicrobial-susceptibility studies that found varying degrees of non-susceptibility to tobramycin and other aminoglycosides; while disagreement with the same studies when reported E. coli isolates consistently susceptible to amikacin(Plattner, et.al.,2020). Aminoglycosides (amikacin and tobramycin) had a resistance rate of 7 (70%) and 6 (60%) respectively. One of the reasons *E. coli* is resistant to aminoglycosides is that the bacterium owns the efflux pump systems as well as the Aminoglycoside Modifying Enzymes (AMEs)(Paltansing et.al., 2015; Zaman, et.al., 2017). According to the results of the current investigation, quinolones levofloxacin and ciprofloxacin had resistance rates of 100% and 30%, respectively. These results agreed with studies that found 10 E. coli isolates to be fluoroquinolone resistant (Dehbanipour et.al., 2019). This resistance is frequently caused by mutations in the gyrA and parC genes(Morgan et.al., 2009). Additionally, efflux pump genes may contribute to the resistance to fluoroquinolones (FQ) (Dehbanipour et.al., 2019). The cause of resistance to fluoroquinolones and other antibiotics is efflux pump over-expression(Poole 2005). Our findings demonstrate a relationship between the multidrug-resistant phenotype (cephalosporins and fluoroquinolones) in E and the overexpression of the efflux pump, which is mediated by AcrA. clinical isolates of E. coli Previous research(Mortimer & Piddok, 1993; Sato et.al.,2013) shown that a decrease in AcrA expression increases cephalosporin and fluoroquinolone MICs, and it was discovered that OmpF is controlled by MarA, which also regulates AcrAB expression (Jellen & Kern 2001). These data also suggest that AcrAB-TolC may affect clinical isolates' sensitivity to cephalosporins.

CONCLUSIONS

strains isolated from calves treated with antimicrobials. Improved hygiene in the calving pen could significantly reduce the risk of bacterial contamination. Further research into resistance pathogens in manure is required to determine the potential health risk.

DISCLOSURE OF INTERESTS

According to the authors, there are no conflicts of interest.

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REFERENCE :-

1. Bashir, S., Kossarev, A., Martin, V. C., & Paeshuyse, J. (2020). Deciphering the Role of Bovine Viral Diarrhea Virus Non-Structural NS4B Protein in Viral Pathogenesis. Veterinary sciences, 7(4), 169.
2. Reichel, M. P., Lanyon, S. R., & Hill, F. I. (2018). Perspectives on current challenges and opportunities for bovine viral diarrhoea virus eradication in Australia and New Zealand. Pathogens, 7(1), 14.
3. Kaper, J.B., Nataro, J.P., & Mobley, H.L. (2004). Pathogenic Escherichia coli. Nat Rev Microbiol. ,2(2):123–40. doi: 10.1038/nrmicro818.
4. Johnson, T.J., & Nolan, L.K. Pathogenomics of the virulence plasmids of Escherichia coli. (2009). Microbiol Mol Biol Rev, 73(4):750–74. doi: 10.1128/MMBR.00015-09.
5. Leimbach, A., Hacker, J., & Dobrindt, U. (2013). E. coli as an all-rounder: the thin line between commensalism and pathogenicity. Between pathogenicity and commensalism, 3-32.
6. Vila, J., Sáez-López, E., Johnson, J. R., Römling, U., Dobrindt, U., Cantón, R., & Soto, S. M. (2016). Escherichia coli: an old friend with new tidings. FEMS microbiology reviews, 40(4), 437-463.
7. Touchon, M., Perrin, A., De Sousa, J. A. M., Vangchhia, B., Burn, S., O'Brien, C. L., & Rocha, E. P. (2020). Phylogenetic background and habitat drive the genetic diversification of Escherichia coli. PLoS genetics, 16(6), e1008866.
8. Qiao, M., Ying, G. G., Singer, A. C., & Zhu, Y. G. (2018). Review of antibiotic resistance in China and its environment. Environment international, 110, 160-172.
9. Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J.C., Leonard, F.C., 2002. Veterinary Microbiology and Microbial Diseases. Blackwell Scientific Publications, Oxford, London.
10. Ortez, H.J., 2005. Test Methods, Disk diffusion testing. In: Manual of antimicrobial susceptibility testing, Coyle, B.M. American Society for Microbiology, Washington DC.
11. Mailk, S., Kumar, A., Verma, A. K., Gupta, M. K., Sharma, S. D., Sharma, A. K., & Rahal, A. (2013). Incidence and drug resistance pattern of References 113 collibacillosis in cattle and buffalo calves in Western Uttar Pradesh in India. J. Anim.Health Prod, 1(1), 15-19.
12. Ansari, A. R. M. I. H., Rahman, M. M., Islam, M. Z., Das, B. C., Habib, A., Belal, S. M. S. H., & Islam, K. (2014). Prevalence and antimicrobial resistance profile of Escherichia coli and Salmonella isolated from diarrheic calves. Journal of Animal Health and Production, 2(1), 12-15.
13. Nazir, K. & Hussain, N. (2007). Plasmid profiles and antibiogram pattern of Escherichia coli isolates of calves feces and diarrhegenic stool of infants. Journal of the Bangladesh Society for Agricultural Science and Technology, 4, 149-152.
14. Foxman, B. (2014). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect. Dis. Clin. North Am. 28, 1–13. doi: 10.1016/j.idc.2013.09.003 .
15. Moreno, L. Z., Matajira, C. E. C., Poor, A. P., Mesquita, R. E., Gomes, V. T. M., Silva, A. P. S., et al. (2018). Identification through MALDI-TOF mass spectrometry and antimicrobial susceptibility profiling of bacterial pathogens isolated from sow urinary tract infection. Vet. Q. 38, 1–8.
16. Hussein, E. A. M., Mohammad, A. A. H., Harraz, F. A., & Ahsan, M. F. (2019). Biologically synthesized silver nanoparticles for enhancing tetracycline activity against staphylococcus aureus and klebsiella pneumoniae. Brazilian Archives of Biology and Technology, 62.
17. LaPlante, K. L., & Sakoulas, G. (2009). Evaluating Aztreonam and Ceftazidime Pharmacodynamics with Escherichia coli in Combination with Daptomycin, Linezolid, or Vancomycin in an In Vitro Pharmacodynamic Model. Antimicrobial Agents and Chemotherapy, 53(10), 4549–4555. doi:10.1128/aac.00180-09.
18. Plattner, M., Gysin, M., Haldimann, K., Becker, K., & Hobbie, S. N. (2020). Epidemiologic, phenotypic, and structural characterization ofaminoglycoside-resistance gene Aac (3)-IV. International journal of molecular sciences, 21(17), 6133.
19. Paltansing, S., Kraakman, M., van Boxtel, R., Kors, I., Wessels, E., Goessens, W., Bernards, A. (2015). Increased expression levels of chromosomal AmpC β-lactamase in clinical Escherichia coli isolates andtheir effect on susceptibility to extended-spectrum cephalosporins. Microbial Drug Resistance, 21(1), 7-16.
20. Zaman, S. B., Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., & Hossain, N. (2017). A review on antibiotic resistance: alarm bells are ringing. Cureus, 9(6).
21. Dehbanipour, R., Khanahmad, H., Sedighi, M., Bialvaei, A. Z., & Faghri, J. (2019). High prevalence of fluoroquinolone-resistant Escherichia coli strains isolated from urine clinical samples. Journal of preventive medicine and hygiene, 60(1), E25.
22. Morgan-Linnell, S. K., Becnel Boyd, L., Steffen, D., & Zechiedrich, L. (2009). Mechanisms accounting for fluoroquinolone resistance in Escherichia coli clinical isolates. Antimicrobial agents and chemotherapy, 53(1), 235-241.
23. Poole, K. (2005). Efflux-mediated antimicrobial resistance. Journal of Antimicrobial Chemotherapy, 56(1), 20-51.
24. Mortimer, P. G., & Piddok, L. J. (1993). The accumulation of five antibacterial agentsin porin-deficient mutants of Escherichia coli. Journal of Antimicrobial Chemotherapy, 32(2), 195-213.

25. Sato, T., Yokota, S. I., Okubo, T., Ishihara, K., Ueno, H., Muramatsu, Y., Tamura, Y. (2013). Contribution of the AcrAB-TolC efflux pump to high-level fluoroquinolone resistance in Escherichia coli isolated from dogs and humans. Journal of Veterinary Medical Science, 75(4), 407-414.
26. Jellen-Ritter, A. S., & Kern, W. V. (2001). Enhanced expression of the multidrug efflux pumps AcrAB and AcrEF associated with insertion element transposition in Escherichia coli mutants selected with afluoroquinolone. Antimicrobial agents and chemotherapy, 45(5), 1467- 1472.