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Antibiogram of *Escherichia coli* isolates obtained from cases of neonatal calf diarrhoea in Kerala[#]

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Abstract

A study was conducted to determine the antibiogram of Escherichia coli isolated from cases of neonatal calf diarrhoea in Thrissur and Wayanad districts of Kerala. A total of 101 isolates were tested for susceptibility against 12 antibiotics. The antibiotics to which the isolates were most susceptible were Streptomycin (49.51%) and Chloramphenicol (47%). The isolates showed varying degrees of susceptibility to the other antibiotics tested such as Enrofloxacin (8.92%), Ciprofloxacin (15.85%), Gentamicin (23.77%), Tetracycline (20.8%), Amoxicillin/sulbactam (8.92%), Co-trimoxazole (20.8%), and Nitrofuratonin (25.75%). All the isolates were resistant to Cefpodoxime, Penicillin G and Cefotaxime/Clavulanic acid. Many of the isolates were found to be multi-drug resistant which has serious implications for the control of these infections through antibiotic therapy.

Keywords: *Antibiogram, Escherichia coli, neonatal calf diarrhoea, multi-drug resistance*

Farm animals are affected by different types of diseases during their neonatal period. The United States National Health Monitoring System estimated about 10.8 per cent mortality

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rate in pre-weaning dairy calves and Razzaque *et al*. (2001) reported a crude mortality rate of 44 per cent in neonatal calves of Kuwait. Neonatal calf diarrhoea (NCD) is the leading cause of mortality and morbidity inflicting heavy economic losses to cattle farmers worldwide. In Sweden, enteritis was the predominant cause of death of calves aged less than one month (Svensson *et al.*, 2006). In Norway, calf diarrhoea accounted for 40 per cent of all calf diseases (Gullisen, 2009). Studies carried out by Wu *et al*. (2010) noted that diarrhoea was responsible for more than 50 per cent of deaths in neonatal calves. Diarrhoea in neonatal calves has multiple aetiologies that make this condition a complex one. Infectious and non-infectious agents are involved in the causation of NCD, and non-infectious agents usually act as the predisposing factors for infection. Infectious agents usually responsible for NCD are *Escherichia coli*, salmonella, rotavirus, coronavirus and *Cryptosporidium* spp. Of these agents, enterotoxigenic *E. coli* (ETEC) acts as one of the major pathogens causing around 20 to 30 per cent of cases of calf diarrhoea that leads to bacteriaemia (Constable, 2009; Abubaker *et al*., 2015).

Calf diarrhoea is the leading cause of mortality in pre-weaned calves and veterinarians and farmers depend on antimicrobials for treatment (USDA-APHIS, 2008). Also, the use of antimicrobials as a preventive measure against calf diarrhoea has been proven to be beneficial in decreasing morbidity and mortality (Berge *et al*., 2009). Indiscriminate use of antibiotics may worsen the condition and lead to more diarrhoeic cases in the herd, reduction in food consumption and weight-gaining capacity of the calf. Moreover, it may lead to the emergence of antimicrobial-resistant *E. coli* in cattle that are capable of colonizing humans and causing infections with limited therapeutic options (Hammerum and Heuer, 2009). The present study was carried out to study the antibiogram of *E. coli* isolates obtained from cases of NCD in Thrissur and Wayanad districts of Kerala.

Materials and methods

Sample Collection

A total of 120 freshly voided diarrhoeic faecal samples, or rectal swabs were collected from farms in Thrissur and Wayanad districts of Kerala during the period from October 2019 to September 2022. These samples were kept in sterile containers and transported on ice to the Department of Veterinary Microbiology, College of Veterinary and Animal Science, Mannuthy (in Thrissur district) or Pookode (in Wayanad district) depending on the site of sample collection. All the samples were inoculated on Brain Heart Infusion Agar (BHIA) and incubated at 37°C for 24 hours. The colonies suggestive of *E. coli* were selected and stained by Gram's method and further inoculated on MacConkey (MAC) agar to test the lactose utilisation. The lactose fermenting pink colonies obtained on MAC agar were inoculated on Eosin Methylene Blue (EMB) agar. The colonies with green metallic sheen on EMB agar were confirmed as *E. coli* by standard biochemical tests such as indole, methyl red, Voges-Proskauer and citrate (IMViC) tests, urease, triple sugar iron (TSI) agar, sugar fermentation and Ejikman's tests, as described by Quinn *et al*. (1994).

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was carried out by the Kirby-Bauer disc diffusion method as described by Bauer *et al*. (1966). Briefly, the colonies of *E. coli* were first inoculated in five millilitres of Brain Heart Infusion Broth (BHIB) and incubated for 2–4 hours at 37°C till light to moderate turbidity developed. From this, the cultures were inoculated on Mueller-Hinton agar plates using sterile swabs. On these agar plates, 12 antibiotic discs were placed aseptically at an appropriate distance with the help of sterile forceps and incubated aerobically at 37°C for 24 hours. The antibiotic discs used for the test were Amoxicillin/ Sulbactam (AMS 30/15), Cefpodoxime (CPD 10 mcg), Cefotaxime/Clavulanic acid (CEC 30/10 mcg), Chloramphenicol (C 30 mcg), Ciprofloxacin (CIP 30 mcg), Cotrimoxazole (COT 25 mcg), Enrofloxacin (EX 10 mcg), Gentamicin (GEN 30 mcg), Nitrofuratonin (NIT 300 mcg), Penicillin G (P10 mcg), Streptomycin (S10 mcg), and Tetracycline (TE 30 mcg). By measuring the diameter of the inhibition zone, the results were recorded and categorized as resistant (R) or sensitive (S).

Results and discussion

Of the 120 samples tested, 101 (84.17 per cent) were found to be positive for *E. coli*. The antibiotics to which the isolates showed the most susceptibility were Streptomycin (49.51 per cent) and Chloramphenicol (47.53 per cent). The isolates showed varying degrees of susceptibility to Enrofloxacin (8.92 per cent), Ciprofloxacin (15.85 per cent), Gentamicin (23.77 per cent), Tetracycline (20.8 per cent), Amoxicillin/sulbactam (8.92 per cent), Cotrimoxazole (20.8 per cent), and Nitrofuratonin (25.75 per cent). All the isolates were resistant to Cefpodoxime, Penicillin G, and Cefotaxime/Clavulanic acid (Table 1)

Neonatal calf diarrhoea is an important and widespread disease condition that leads to economic losses for cattle farmers. As per the studies of Windeyer *et al*. (2014), 23 per cent of the calves had undergone treatment for NCD at least once in their life, while 8.7 per cent of calves had taken the treatment more than once. The condition can be caused by infectious agents such as bacteria, viruses and protozoa, by non-infectious factors, or by a combination of both. According to Izzo *et al*. (2011), *E. coli* was the predominant aetiological agent responsible for NCD. It causes significant mortality in young calves. The bacterium may also cause diarrhoea in weak, malnourished, debilitated and immunosuppressed calves (Malik *et al*., 2013). Death occurs as a result of dehydration and electrolyte loss due to diarrhoea. In the present study, *E. coli* could be isolated from 101 of the 120 (84.17 per cent) diarrhoeic faecal samples. Varying prevalence rates of *E. coli*-induced NCD have been reported. In a study conducted by Srivani *et al*. (2017), a high prevalence (60 per cent) of *E. coli* - associated diarrhoea was observed in the West Godavari district of Andhra Pradesh while a lower prevalence (36.6 per cent) was observed in Ranga Reddy district. They also reported that NCD was a major problem faced by farmers around the world and calves below 30 days are more susceptible to this condition. Prevalence rates of 53.37 per cent, 30 to 50 per cent, and 23.72 per cent have been reported by Navade *et al*. (2000), Malik *et al*. (2012) and Gupta *et al*. (2006) respectively. Poor managemental practices such as overcrowding and malnutrition may predispose calves to NCD and influence its spread in farms.

Antimicrobials are routinely used for disease prevention and growth promotion in farm animals. However, Constable (2004) reported that oral administration of penicillin, chloramphenicol, and neomycin increased the incidence of diarrhoea in healthy calves, caused malabsorption, or reduced growth rate. In the United States, agriculture could be responsible for as much as 70 per cent of antimicrobial drug consumption and therefore,

Table 1. Antibiogram of *E.coli* isolates obtained from cases of neonatal calf diarrhoea

No.	Antibiotics	Percentage of isolates	
		Sensitive	Resistant
1	Ciprofloxacin (CIP 30 mcg)	15.85	85.15
2	Cefpodoxime (CPD 10 mcg)	-	100
3	Cotrimoxazole (COT 25 mcg)	20.8	80.2
4	Chloramphenicol (C 30 mcg)	47.53	53.47
5	Tetracycline (TE 30 mcg)	20.8	80.2
6	Streptomycin (S 10 mcg)	49.51	51.49
7	Gentamicin (GEN 30 mcg)	23.77	77.23
8	Enrofloxacin (Ex 10 mcg)	8.92	92.08
9	Amoxicillin/Sulbactam (AMS 30/15 mcg	8.92	92.08
10	Nitrofuratonin (NIT 300 mcg)	25.75	72.25
11	Penicillin G (P 10 mcg)	-	100
12	Cefotaxime/Clavulanic acid (CEC 30/10 mcg)	-	100

agricultural animals are considered an important reservoir and arena for emerging antimicrobial drug resistance (van den Bogaard and Stobberingh, 2000). Antibiotic residues build up and resistant bacterial strains emerge as a result of antibiotic residue accumulation, continuous indiscriminate use, and inadequate use in animals.

Antimicrobial therapy is an important tool for reducing the incidence of diarrhoea and for the treatment of the condition in calves. On the other hand, widespread and indiscriminate use of antimicrobial agents leads to multi-drug resistant pathogenic bacteria in calves, making the treatment using these agents ineffective. Increased use of antimicrobials reduces the ability of normal enteric flora to resist colonization of the pathogens (Wagner *et al*., 2008). Because of these concerns, FAO/WHO/OIE (2007) warned that the use of antimicrobials in food animal production has the potential to increase antimicrobial resistance in human pathogens and that alternative measures to reduce widespread antimicrobial use were needed. Meganck *et al*. (2014) opined that routine use of antibiotics in diarrhoeic calves cannot be recommended due to increased levels of antibiotic resistance. The resistant pattern exhibited by the bacteria in different localities may vary depending on the antibiotics commonly used for treatment in that area. Because routine laboratory isolation and sensitivity testing are not feasible and expensive, frequent testing at the herd level will be beneficial in developing the best possible therapy approach (Tarekegn and Molla, 2017).

Berge *et al*. (2009) reported that the subtherapeutic use of tetracycline increased feed efficiency. The use of tetracycline along with milk as a growth promoter has been reported by several researchers. Also, a high percentage of resistance to tetracycline, ranging from 63.21 to 98.09 per cent has been reported (Constable, 2009; Pereira *et al*., 2011; Shahrani, *et al*., 2014; Srivani *et al*., 2017). Manickam and Ponnusamy (2017) also reported that 74 per cent of isolates were resistant to tetracycline which is near to the resistance rate (80.2 per cent) observed in the present study.

Numerous investigations have revealed the effectiveness of ciprofloxacin in the treatment of NCD. Chowdhury and Das (2003) reported that *E. coli* isolates were found to be very susceptible to ciprofloxacin. Kumar *et al*. (2010) reported that 84.61 per cent and 96.15 per cent of isolates were sensitive to cotrimoxazole and ciprofloxacin respectively. In contrast, in the present study, only 15.85 per cent and 20.8 per cent of the isolates were sensitive to ciprofloxacin and cotrimoxazole, respectively.

Pereira *et al*. (2011) and Shahrani *et al*. (2014) reported that 98.2 per cent and 70 per cent of *E. coli* isolates respectively were sensitive to streptomycin. However, a lower sensitivity percentage of 49.51 was obtained in the present study. In the case of chloramphenicol, a sensitivity of 47.53 per cent was obtained in the present study. This is in contrast with that reported by Rehman (2014) and Srivani *et al.* (2017) who obtained a greater sensitivity of 86.6 and 96.33 per cent respectively, while low (15.38 per cent) sensitivity was reported by Kumar *et al*. (2010). In a study conducted in Western Uttar Pradesh, Malik *et al*. (2013) reported that 51.21 per cent of *E. coli* isolates obtained were sensitive to gentamicin and that all the isolates were resistant to antibiotics such as cotrimoxazole, tetracycline, and penicillin. In the present study also, all the *E. coli* isolates obtained were resistant to Penicillin. However, the sensitivity to gentamicin was 23.77 per cent which was lower than that reported by Malik *et al*. (2013) and Srivani *et al*. (2017) (96.33 per cent). According to Kadam *et al*. (2018), *E. coli* isolates exhibited less resistance to nitrofurantoin. In contrast to their findings, 75.25 per cent of isolates were resistant to nitrofurantoin in the present study.

These results of the study indicated that many of the *E. coli* isolates obtained from NCD are multidrug resistant. Even though the use of antibiotics for treating diarrhoea may be unavoidable, their usage can be controlled for the continued effectiveness of these drugs for animals and people. Strategies to reduce antimicrobial use and to maintain calf health and welfare are needed and veterinarians should play an important role in creating this awareness among the farmers.

Whenever possible, antibiotic susceptibility testing must be performed to select the most appropriate antimicrobial. If it is impractical at least a periodic evaluation may be carried out. Therapeutic antimicrobial usage may be limited to diarrhoea cases with fever, inappetence, or depression, or when there is blood or mucosal shreds in faeces. Urgent measures to check the generation of antimicrobial resistance in livestock should be taken as this problem if left unchecked will indirectly affect human health al so.

Conclusion

A study was carried out to determine the susceptibility of *E. coli* isolates obtained from cases of NCD to commonly used antibiotics. An alarming rate of resistance was observed against the majority of the antimicrobials tested. All the isolates were resistant to three among the 12 antimicrobials tested. This study gives a snapshot of the scenario of antimicrobial resistance prevalent in bacteria associated with NCD and highlights the need to address the issue urgently.

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References

Abubaker, A., Ayis, E.I., Ali, A., Elgaddal, Y. and Almofti, A. 2015. Isolation, identification and enterotoxin detection of *Escherichia coli* isolated from calf diarrhoea and their virulence characteristics. *J. Appl. Ind. Sci.* **3**(4): 141-149.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**: 493-496.

Berge, A.C., Moore, D.A., Besser, T.E. and Sischo, W.M. 2009. Targeting therapy to minimize antimicrobial use in pre-weaned calves: Effects on health, growth, and treatment costs. *J. Dairy Sci.* **92**: 4707–4714.

Chowdhury, M. and Das, R. 2003. Incidence of drug resistance in *E. coli* strain in West Bengal. *Indian Vet. J*. **80**(1): 81-82.

Constable, P.D. 2004. Antimicrobial use in the treatment of calf diarrhoea. *J. Vet. Intern. Med.* **18**: 8-17.

Constable, P.D. 2009. Treatment of calf diarrhoea: Antimicrobial and ancillary treatments. *Vet. Clin. Food Anim.* **25**: 101-120.

FAO/WHO/OIE (Food and Agriculture Organization/World Health Organisation/ Office International des Epizooties) 2007. Report of the Joint FAO/WHO/ OIE expert meeting on critically important antimicrobials. Rome, Italy. November 26–30, 2007. http://www.who.int/foodborne_disease/resources/Reportpercent20jointpercent20CIApercent20Meeting.pdf. Accessed June 1, 2008.

Gulliksen, S.M., Lie, K.I., Loken, T. and Osteras, O. 2009. Enteropathogens and risk factors for diarrhoea in Norwegian dairy calves. *J. Dairy Sci*. **92**: 5057-5066.

Gupta, D.K., Shukla, S.K., Rajora, V.S. and Nag, L.K. 2006. Prevalence of *E. coli* in the neonatal diarrhoeic calves. *Indian J. Vet. Med*. **26**(1): 36-37.

Hammerum, A.M., and O.E. Heuer. 2009. Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. *Clin. Infect. Dis*. **48**: 916-921.

Izzo, M.M., Kirkland, P.D., Mohler, V.L., Perkins, N.R., Gunna, A.A. and Housea, J.K. 2011. Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. *Aust. Vet. J*. **89**: 167-173.

Kadam, A.S., Tembhurne, P.A. and Ingle, V.C. 2018. Bacterial profiling from the bovine calves's diarrhoea and its antibiotic sensitivity pattern around Chandrapur

district in Maharashtra. 2018. *Int. J. Sci. Envir. Technol*. **7**(6): 2019-2025.

Kumar, B., Shekhar, P. and Kumar, N. 2010. A clinical study on neonatal calf diarrhoea. *Intas Polivet* **11**(II): 233-235.

Malik, S., Kumar, A., Verma, A.K., Gupta, M.K., Sharma, S.D., Sharma, A.K.and Rahal, A. 2013. Incidence and drug resistance pattern of colibacillosis in cattle and buffalo calves in western Utter Pradesh in India. *J. Anim. Hlth. Prod.* **1**(2): 15-19.

Malik, S., Verma, A.K., Kumar, A., Gupta, M.K. and Sharma, S.D. 2012. Incidence of calf diarrhoea in cattle and buffalo calves in Uttar Pradesh, India. *Asian J. Anim. Vet. Adv.* **7**: 1049-1054.

Manickam, R. and Ponnusamy, P. 2017. Bacterial species isolated from diarrhoeic calves and their antibiotic sensitivity pattern. *Int. J. Sci. Envir. Technol.* **6** (4): 2202-2211.

Meganck, V., Hoflack, G. and Opsomer, G. Advances in prevention and therapy of neonatal dairy calf diarrhoea. 2014. A systematical review with emphasis on colostrum management and fluid therapy. *Acta. Vet. Scand.* **56:** 75.

Navade, R.B., Bhalerao, D.P. and Jagdish. 2000. Neonatal calf diarrhoea - Serotyping of *E. coli* isolates. *Indian Vet. J*. **77** (9): 815-816.

Pereira, R.V.V., Santos, T.M.A., Bicalho, M.L., Caixeta, L.S., Machado, V.S. and Bicalho, R.C. 2011. Antimicrobial resistance and prevalence of virulence factor genes in faecal *Escherichia coli* of Holstein calves fed milk with and without antimicrobials. *J. Dairy Sci*. **94**: 4556-4565.

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. 1994. *Clinical Veterinary Microbiology*, Wolfe, 648p.

Razzaque, M.A., Scharp, D., Al-Mutawa, T., Al-Muhanna, M., Abbas, S., Shalabi, A., El-Sawy, E., Al-Awadhi, A., Mulla, F. and El-Sanousi, A. 2001. Field and laboratory investigation of calf mortality in Kuwait and its economic impact on dairy production. Kuwait Institute for Scientific Research, Report No. KISR6004, Kuwait.

Rehman, M.U., Rashid, M., Sheikh, J.A., Wani, S.A. and Farooq, S. 2014. Multi-drug resistance among Shiga toxin-producing *Escherichia coli* isolated from bovines and their handlers in Jammu region, India. *Vet. Wld*. **6**(9): 655-658.

Shahrani, M., Dehkordi, F.S. and Momtaz, H. 2014. Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. *Biol. Res*. **47**: 28.

Srivani, M., Reddy, Y.N., Subramanyam, K.V., Reddy, M.R. and Srinivasa Rao T. 2017. Prevalence and antimicrobial resistance pattern of Shiga toxigenic *Escherichia coli* in diarrheic buffalo calves. *Vet. Wld*. **10**(7): 774-778.

Svensson, C., Hultgren, J. and Oltenacu, P.A. 2006. Morbidity in 3–7-month-old dairy calves in south-western Sweden, and risk factors for diarrhoea and respiratory disease. *Prev. Vet. Med*. **74**: 162–179.

Tarekegn, Y. and Molla, F.W. 2017. The Prevalence of *E. coli* from diarrheic calves and their antibiotic sensitivity test in selected dairy farms of Debre Zeit, Ethiopia. *Adv. Biotech. Micro.* **6**(1): 12-22.

USDA-APHIS (United States Department of Agriculture-Animal Plant Health Inspection Service) 2008. Antimicrobial use on US dairy operations, 2002 and 2007. N534.1008. USDA, Fort Collins, CO.

Van den Bogaard, A.E. and Stobberingh, E.E. 2000. Epidemiology of resistance to antibiotics. links between animals and humans. *Int. J. Antimicrob. Agents*. **14**: 327-335.

Wagner, R.D., Johnson, S.J. and Cerniglia, C.E. 2008. *In vitro* model of colonization resistance by the enteric microbiota: effects of antimicrobial agents used in food-producing animals. *Antimicrob. Agents Chemother*. **52**: 1230-1237.

Windeyer, M.C., Leslie, K.E., Godden, S.M., Hodgins, D.C., Lissemore, K.D and LeBlanc, S.J. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Prev. Vet. Med*. **113**: 231-240.

Wu, G., Mafura, M., Carter, B., Lynch, K., Anjum, M. F., Woodward, M.J. and Pritchard, G.C. 2010. Genes associated with *Escherichia coli* isolates from calves with diarrhoea and/or septicaemia. *Vet. Rec.* **166**: 691-692. ■