Antibiogram analysis of *Escherichia coli* isolates recovered from dog faeces

Abstract

Antimicrobial resistance is a global problem affecting animals and humans. Improper use of antibiotics by humans, inefficient prevention and control of infections, poor hygiene and sanitation, are causes for the emergence and distribution of antibiotic resistant bacteria. The role of pets is quite significant as disseminators of antimicrobial-resistant bacteria. This study was planned to analyze the antibiotic susceptibility profile of *Escherichia coli* isolated from dog faeces. The dog faecal samples (n=5) collected from Veterinary Clinical Complex, College of Veterinary and Animal Science, Navania were subjected to bacteriological examination for the isolation and identification of *Escherichia coli*. The isolates were confirmed by PCR to detect the species specific *usp*A gene. Antimicrobial susceptibility testing was done to determine the antibiotic susceptibility pattern of the isolates. In the present study, it was revealed that out of 5 dog faecal samples analyzed, *E coli* was isolated from 4 samples. The PCR analysis revealed a specific amplification of 884 bp product of the *usp*A gene among the isolates. Further on analyzing the antibiogram, it was found that chloramphenicol, oxytetracycline, azithromycin, ampicillin, co-trimoxazole and ceftriaxone were effective against *E coli.* While, the isolates were resistant to amoxicillin and trimethoprim. Therefore, it was concluded that the screening of cultures by antimicrobial susceptibility testing will not only help in deciding the right antibiotic for treatment but also identify the shifting trends of antimicrobial resistance.

Keywords: Antibiogram, dog, *Escherichia coli,* faeces, PCR

Introduction

Dogs are a reservoir of the *Escherichia coli* strains that may be the reason for various extraintestinal infections in humans. Dogs can spread pathogens through their faeces and may pollute the water sources or the vegetation. Moreover, the occurrence of dog faeces in the soil is a significant public health problem due to the existence of various microorganisms that can be transmitted to humans (Penakalapati *et al.,* 2017). Children can get the infection while playing in areas which are contaminated by the dog faeces which may lead to severe gastrointestinal disorders. *E. coli* is both a commensal and a major pathogen having public

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health significance. It is also considered as an indicator of the antimicrobial resistance burden in a large variety of environment (Valat *et al.,* 2020).

Antimicrobial resistance is a global problem affecting animals and humans. Improper use of antibiotics by humans, inefficient prevention and control of infections, poor hygiene and sanitation, are causes for the emergence and distribution of antibiotic resistant bacteria (Bryce *et al.,* 2016 and Bhoomika *et al.,* 2016). The role of pets is quite significant as disseminators of antimicrobial-resistant bacteria. The close contact between pets and humans has created conditions for interspecies transmission of multidrug-resistant (MDR) bacteria. Therefore, the present study focussed on assessing the antibiogram of *E. coli* isolated from the faecal samples of dogs which were presented to the Veterinary Clinical Complex (VCC), College of Veterinary and Animal Science (CVAS), Navania.

Materials and Methods

The dog faecal samples (n=5) collected from VCC, CVAS Navania were subjected to bacteriological examination for the isolation and identification of *E. coli*. Isolation of *E. coli* from the faecal samples was done as per the standard method (Quinn *et al.* 2011). One loopful of sample was aseptically added to 9 ml of MacConkey broth for enrichment in a test tube. The test tube was incubated at 37°C for 24hrs, followed by plating of a loopful of inoculum on MacConkey agar and incubated at 37°C for 24 hrs. After 24 hrs incubation, pink coloured (lactose fermenter) colonies were picked up and streaked on Eosin methylene blue agar (EMB). After incubation at 37°C for 24-48 hrs, the colonies showing green metallic sheen on EMB agar were selected for further confirmation.

Molecular confirmation of the *E coli* isolates

Isolation of DNA from pure culture was undertaken using by Nucleo-pore gDNA fungal/bacterial mini kit by following the manufacturer's instructions. The PCR procedure to detect the species specific *usp*A gene was standardized with certain modifications. The reaction mixture was optimized to contain 12.5µl Green Taq PCR master mix, 10 nmol (0.5µl) of each forward and reverse primer, 10.5µl nuclease free water and 1µl of DNA template. The reaction was performed in the thermal cycler with pre-heated lid (lid temp. =105°C). The cycling conditions for *usp*A gene comprised of denaturation (94°C; 1 min), annealing (55°C; 1 min) and extension (72°C; 2 mins) steps repeated for 30 cycles. PCR

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protocol for the detection of species specific, *uspA* gene was standardized having specific amplification product of 884 bp (Osek, 2001).

Antimicrobial susceptibility test

All the *E. coli* isolates were subjected to antimicrobial susceptibility test as described by Bauer *et al*., 1966. Antimicrobial susceptibility testing was done by agar disc diffusion methodand the results were interpreted as per the standard guidelines (CLSI, 2020). Pure colony was inoculated in Luria Bertani broth and incubated at 37^{0}C for 24 hrs. Then, the swab culture was smeared on Mueller Hinton agar plate and allowed to dry for 3-5 min. The antibiotic discs were placed on the surface of the inoculated agar plate. Each disc was pressed down individually to ensure complete contact with the agar surface. A total of 12 antibiotic discs comprising of amoxyclav, ampicillin, ceftriaxone, chloramphenicol, co-trimoxazole, trimethoprim, norfloxacin, erythromycin, azithromycin, gentamicin, streptomycin and oxytetracycline from the different classes of antibiotics were used for each sample. The agar plates were placed in inverted position in an incubator maintained at 37^{0}C for 24 hrs. After incubation, the diameter of the zone of inhibition were measured and compared with the zone size interpretation chart provided by the supplier so as to determine the susceptibility pattern of the isolates for the respective antibiotics.

Results and Discussion

Out of 5 dog faecal samples analyzed, *E coli* was isolated from 4 faecal samples. The *E coli* isolates were confirmed on the basis of cultural and molecular characterization. The isolates which produced pink coloured colonies on MacConkey agar were selected and were further cultured on EMB agar **(Figure 1)**. Out of 5 dog faecal samples, 4 isolates revealed green metallic sheen which were picked up and characterized by Gram's staining. On performing the Gram's staining, the isolates were morphologically identified as Gram negative bacilli arranged singly or in pairs.

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Fig. 1: Morphological characterization of green metallic sheen of *E. coli* culture on eosin methylene blue agar after 24 hrs incubation.

Molecular confirmation of *E. coli* isolates by targeting *usp*A gene

The *E. coli* was confirmed by PCR by targeting the *uspA* gene (universal stress protein) following Chen and Griffiths(1998) methodology. In the present study, electrophoresis analysis revealed a specific amplification of 884 bp product of the *usp*A gene **(Fig. 2)** in test isolates.

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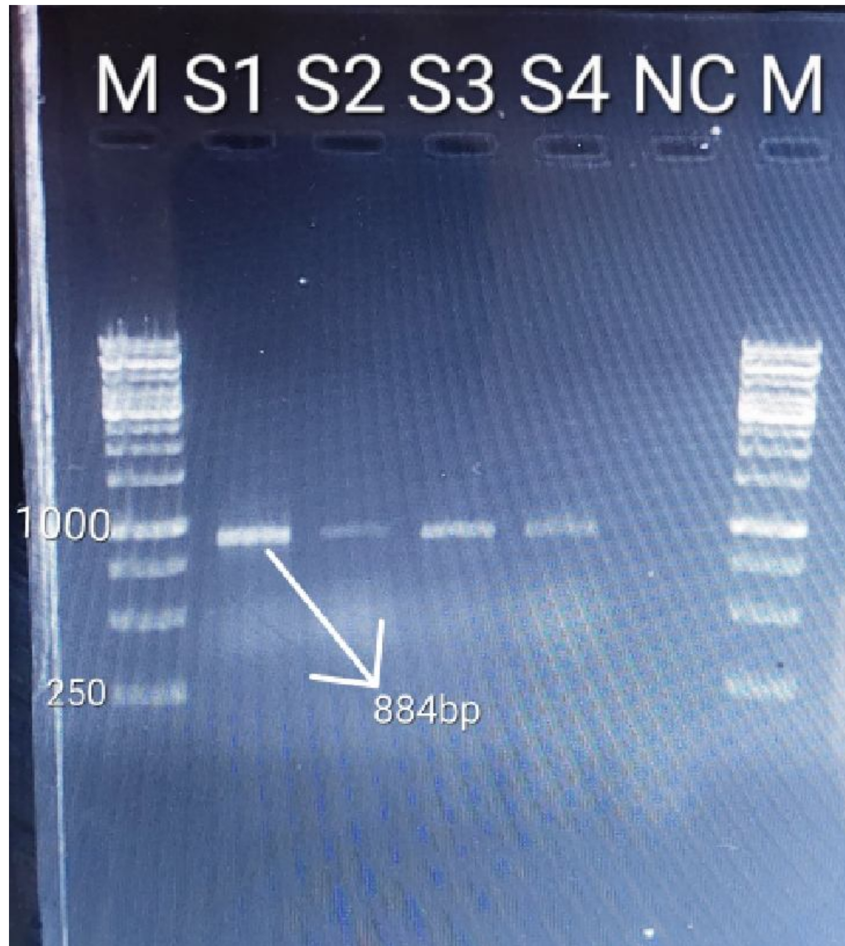

Fig. 2 Agarose gel showing PCR amplified product (884 bp) for *usp*A gene.
M=1kb DNA marker, positive samples (S1, S2, S3, S4; NC= negative control)

Antimicrobial susceptibility test of *E. coli* isolates

Out of the 4 isolates recovered from the dog faecal samples, the most effective antibiotics were chloramphenicol (100%), oxytetracycline (100%), azithromycin (75%), ampicillin (75%), co-trimoxazole (75%) and ceftriaxone (50%). In the present study, the isolates were found to be highly resistant to amoxicillin (75%) and trimethoprim (75%) **(Table 1)**. Singh *et al.* 2018 and Parussolo *et al*. 2019 have reported 100% susceptibility of *E. coli* towards chloramphenicol. Similarly, Vasquez-Garcia *et al.* 2017 have reported 100% of *E. coli* isolates as sensitive towards ampicillin. Ortega-Paredes *et al.* 2019 reported similar findings which showed high resistance towards trimethoprim. Contrasting findings were revealed by Marchetti *et al.* 2021 who reported resistance towards oxytetracycline.

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Table 1: Antimicrobial susceptibility pattern of the *E. coli* isolates recovered from faeces samples

Name of antibiotics	Antibiotic Sensitivity Pattern		
	Sensitive	**Intermediate**	**Resistant**
Gentamicin	2(50%)	2(50%)	0
Norfloxacin	2(50%)	2(50%)	0
Co-Trimoxazole	3(75%)	1(25%)	0
Trimethoprim	0	1(25%)	3(75%)
Erythromycin	1(25%)	1(25%)	2(50%)
Azithromycin	3(75%)	1(25%)	0
Ceftriaxone	2 (50%)	0	2 (50%)
Chloramphenicol	4(100%)	0	0
Ampicillin	3(75%)	1(25%)	0
Amoxycillin-clavulanic acid	1(25%)	0	3(75%)
Oxytetracycline	4(100%)	0	0
Streptomycin	1(25%)	2(50%)	1(25%)

Conclusion

In the present study, chloramphenicol, oxytetracycline, azithromycin, ampicillin, co-trimoxazole and ceftriaxone were found to be effective against *E coli*, while, the isolates were resistant to amoxicillin and trimethoprim. An antibiotic sensitivity test helps us in selecting the right antibiotic will be most effective in treating a bacterial infection. Moreover, these antibiotics must also be used judiciously following proper dose regimen. If these antibiotics are not used prudently, then it may also lead to the development of antimicrobial resistance (Franklin, 1999). This menace of antimicrobial resistance is a global problem affecting both animal and human health. Therefore, the screening of cultures by antimicrobial susceptibility testing will not only help in deciding the right antibiotic for treatment but also identify the shifting trends of antimicrobial resistance. Further studies are required to assess the role of dog faeces contamination in environment as a pathway for dissemination of antimicrobial resistance.

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References

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Truck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**(4): 493-496.

Bhoomika S.S., Patyal A. and Gade N.E. (2016). Occurrence and characteristics of extended-spectrum β-lactamases producing Escherichia coli in foods of animal origin and human clinical samples in Chhattisgarh, India. *Vet World,* **9**(9): 996.

Bryce A., Hay A.D., Lane I.F., Thornton H.V., Wootton M. and Costelloe C. (2016). Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis. *BMJ*. **352**: i939.

Chen, J. and Griffiths, M. W. (1998). PCR differentiation of *Escherichia coli* from other Gram-negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. *Letters in Applied Microbiology,* **27**(6): 369-371.

CLSI. (2020). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Franklin, A. (1999). Current status of antibiotic resistance in animal production. *Acta Veterinaria Scandinavica. Supplementum*, **92**: 23-28.

Marchetti, L., Buldain, D., Gortari Castillo, L., Buchamer, A., Chirino-Trejo, M., & Mestorino, N. (2021). Pet and stray dogs as reservoirs of antimicrobial-resistant Escherichia coli. *International Journal of Microbiology*, Article ID 6664557, 8 pages https://doi.org/10.1155/2021/6664557.

Ortega-Paredes, D., Haro, M., Leoro-Garzón, P., Barba, P., Loaiza, K., Mora, F, Fors, M., Vinueza-Burgos, C. & Fernández-Moreira, E. (2019). Multidrug-resistant Escherichia coli isolated from canine faeces in a public park in Quito, Ecuador. *Journal of Global Antimicrobial Resistance*, **18**, 263-268.

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Osek, J. (2001). Multiplex polymerase chain reaction assay for identification of enterotoxigenic Escherichia coli strains. *Journal of Veterinary Diagnostic Investigation,* **13**(4): 308-311.

Parussolo, L., Sfaciotte, R. A. P., Dalmina, K. A., Melo, F. D., Costa, U. M. and Ferraz, S. M. (2019). Detection of virulence genes and antimicrobial resistance profiles of *Escherichia coli* isolates from raw milk and artisanal cheese in Southern Brazil. *Semina: Ciencias Agrarias*, **40**(1): 163-178.

Penakalapati, G., Swarthout, J., Delahoy, M. J., McAliley, L., Wodnik, B., Levy, K., and Freeman, M. C. (2017). Exposure to animal feces and human health: a systematic review and proposed research priorities. *Environmental Science & Technology*, **51**(20), 11537-11552.

Quinn, P. J., Markey, B. K., Leonard, F. C., Hartigan, P., Fanning, S. and Fitzpatrick, E. (2011). Veterinary Microbiology and Microbial Disease, 2nd edition, Wiley-Blackwell.

Singh, K., Chandra, M., Kaur, G., Narang, D. and Gupta, D. K. (2018). Prevalence and antibiotic resistance pattern among the mastitis causing microorganisms. *Open Journal of Veterinary Medicine*, **8**(4): 54-64.

Valat, C., Drapeau, A., Beurlet, S., Bachy, V., Boulouis, H. J., Pin, R., Cazeau, G, Madec, J. & Haenni, M. (2020). Pathogenic *Escherichia coli* in dogs reveals the predominance of ST372 and the human-associated ST73 extra-intestinal lineages. *Frontiers in Microbiology*, **11**, 580.

Vasquez-Garcia, A., Silva, T. D. S., Almeida-Queiroz, S. R. D., Godoy, S. H., Fernandes, A. M., Sousa, R. L. and Franzolin, R. (2017). Species identification and antimicrobial susceptibility profile of bacteria causing subclinical mastitis in buffalo. *Pesquisa Veterinaria Brasileira*, **37**: 447-452.

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