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. Children can get the infection while playing in areas which are contaminated by the dog faeces which may lead to severe gastrointestinal disorders. This can pose a serious public health hazard. 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. In antibiogram analysis, 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.

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

Introduction

Dogs are a reservoir of the *E. coli* strains that may be the reason for various extraintestinal infections in humans. They 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 micro-organisms 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.

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 VCC, 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 method described by Quinn *et al.,* 2011. One loopful of sample was 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 hours. After 24 hours, pink coloured (lactose fermenter) colonies were picked up and streaked on Eosin methylene blue agar (EMB). Inoculated petri plates were incubated at 37°C for 24-48hrs in the bacteriological incubator. The colonies showing green metallic sheen 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 supplied along with the kit. The PCR procedure to detect the species specific *usp*A gene was standardized with certain modifications. Followed by preliminary trials, 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 is mentioned in **(Table 1)**. PCR protocol for the detection of species specific, *uspA* gene was standardized having specific amplification product of 884 bp (Osek, 2001).

Table 1: Steps and conditions of thermal cycling for detection of *usp*A gene by PCR

Primers	Cycling conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
		Repeated for 30 cycles			72ºC

*usp*A	94ºC 5 minutes	94ºC 1 minute	55ºC 1 minute	72ºC 2 minutes	5 minutes

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 method. A single isolated colony of the test culture was inoculated in Luria Bertani broth & incubated at 37^0C 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 placed on two agar plates each containing 6 antibiotic discs. The agar plates were placed in inverted position in an incubator maintained at 37^0C for 24hrs. 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. The antibiotic discs are mentioned in **Table 2**.

Table 2:Antibiotic discs used for antimicrobial susceptibility test of the *E. coli* isolates

S. No.	Class	S. No.	Antibiotic	Symbol	Disc Content	Interpretative criteria (mm)		
						R	I	S
I.	Penicillin	1.	Amoxicillin/ clavulanic acid	**AMC**	30 mcg	13	14-17	18
		2.	Ampicillin	**AMP**	10 mcg	13	14-16	17
B	Cephalosporins	3.	Ceftriaxone	**CTR**	30 mcg	19	20-22	23
C	Amphenicol	4.	Chloramphenicol	**C**	30 mcg	12	13-17	18
D	Sulphonamides	5.	Co-Trimoxazole	**COT**	25 mcg	10	11-15	16
		6.	Trimethoprim	**TR**	5 mcg	14	15-17	18
E	Quinolones	7.	Norfloxacin	**NX**	10 mcg	12	13-16	17
F	Macrolides	8.	Erythromycin	**E**	15 mcg	13	14-22	23

		9.	Azithromycin	**AZM**	15 mcg	13	14-17	18
G	Aminoglycosides	10.	Gentamicin	**GEN**	10 mcg	12	13-14	15
		11.	Streptomycin	**S**	10 mcg	11	12-14	15
H	Tetracycline	12.	Oxytetracycline	**O**	30 mcg	14	15-18	19

R = Resistant, I = Intermediate and S = Sensitive

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 and biochemical tests. On performing the Gram's staining, the isolates were morphologically identified as Gram negative bacilli arranged singly or in pairs.

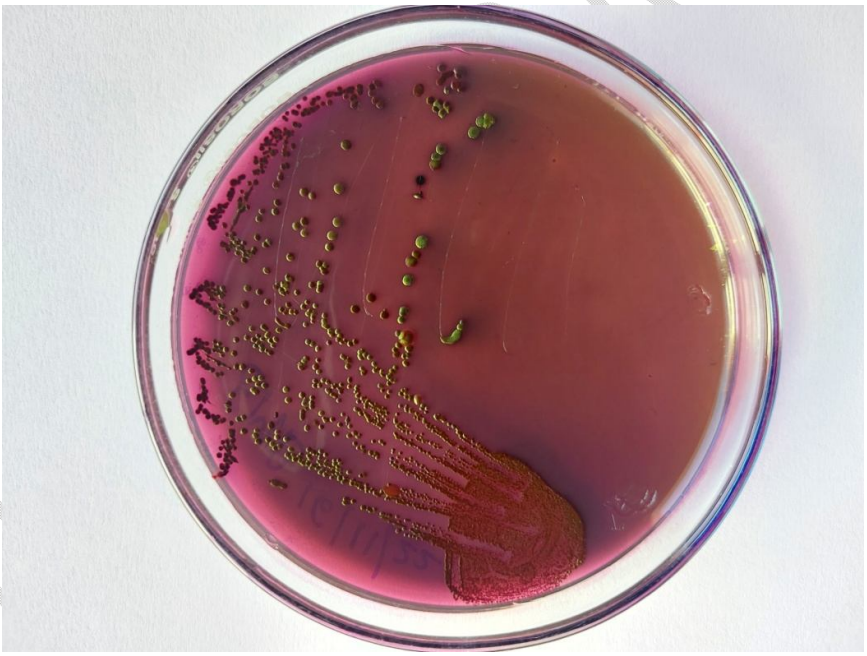

Fig. 1: Growth of the test culture on eosin methylene blue agar (EMB)

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

The *E. coli* have also been confirmed by PCR, targeting the *uspA* gene (universal stress protein) (Chen and Griffiths, 1998). PCR protocol for the detection of species specific,

uspA gene was standardized. In the present study, electrophoresis analysis revealed a specific amplification of 884 bp product of the *usp*A gene **(Fig. 2)** in test isolates.

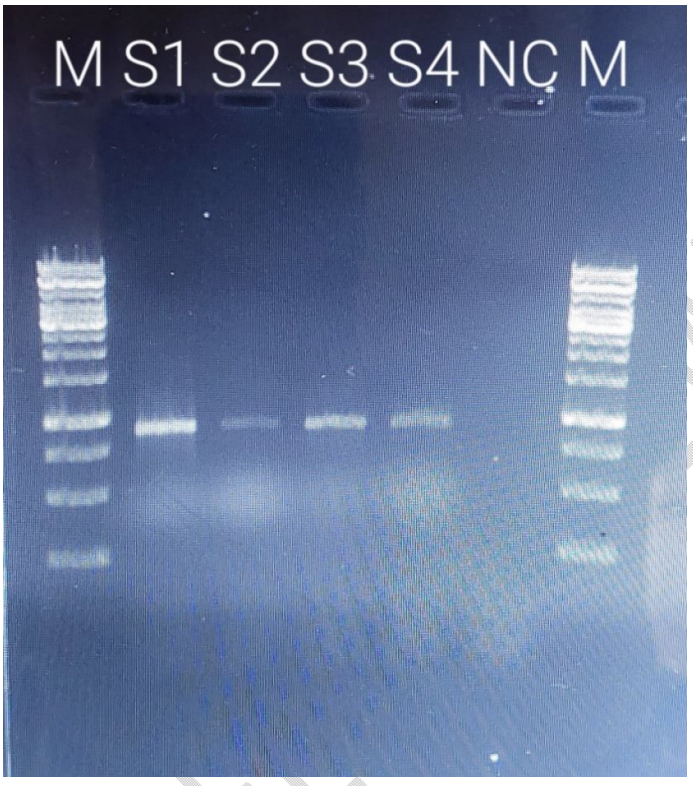

Fig. 2 Agarose gel showing PCR amplified product (884 bp) for *usp*A gene. M=1kb DNA ladder, positive samples (L1=M, L2=S1, L3=S2, L4=S3, L5=S4, L6=NC, L7=M; NC= negative control)

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 3)**. 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.

Table 3: 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%)

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 facilitates to find out which antibiotic will be most effective in treating a bacterial infection. While, 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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