Haryana Vet. (June, 2019) **58(1),** 66-69

Research Article

ISOLATION OF UROPATHOGENIC *ESCHERICHIA COLI* FROM DOGS AND MOLECULAR DETECTION OF CHLORAMPHENICOL RESISTANCE GENES

MUHAMMAD MUSTAPHA[1]*, PARVEEN GOEL[1], VINAY KUMAR[2], DIVYA AGNIHOTRI[3], TARUN KUMAR[3] and SUSHILA MAAN[2]

[1]Department of Veterinary Medicine, [2]Department of Animal Biotechnology, [3]Veterinary Clinical Complex
Collage of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, India

Received: 31.05.2018; Accepted: 20.12.2018

ABSTRACT

This study was conducted to isolate pathogenic *E. coli* from the urine of dogs suffering from urinary tract infections and to determine their antimicrobial resistance profile. A total of 103 urine samples were collected and cultured using Blood agar, MacConkey's lactose agar and Eosine methylene blue agar. Out of the 103 samples, 25 were positive for uropathogenic *E. coli*. The positive UPEC isolates were subjected to disk diffusion and conventional PCR method. The UPEC isolates were found sensitive to chloramphenicol (84%), meropenem (64%), gentamicin (44%) and amikacin (40%) while all the isolates showed resistance against ampicillin (25%), ciprofloxacin (25%) and tetracycline (25%). The PCR assay detected cmlA gene (16%) in 4 isolates. None of the isolates were positive for floR genes. This data confirm large number of UPEC strains with high level of resistance against several classes of antibiotic with respect to the prevalence of chloramphenicol resistance genes.

Key words: AST, Chloramphenicol resistance genes, Dog, PCR, UPEC

Pathogenic urinary tract infections (UTIs) occur in about 14% of dogs throughout their life. Prevalence of UTIs in dogs had a range between 5% and 30% all around the world (Chang *et al.*, 2015; Rzewuska *et al.*, 2015). UTIs can be classified as simple uncomplicated or complex complicated infections which may spread to dangerous pathogenic diseases such as pyelonephritis, cystitis, and urethritis (Rzewuska *et al.*, 2015).

Uropathogenic *Escherichia coli* (UPEC) strains are the most significant causative agent of UTIs in both humans and dogs (Chang *et al.*, 2015; Rzewuska *et al.*, 2015). It is a Gram negative, nonsporulating, flagellated, rod shaped, and facultative anaerobic bacterium which belong to Enterobacteriaceae family (Ranjbar *et al.*, 2017). Total prevalence of UTIs caused by the UPEC strains is about 30-70% (Chang *et al.*, 2015; Rzewuska *et al.*, 2015). UTIs caused by UPEC strains often require antibiotic therapy (Chang *et al.*, 2015; Ranjbar *et al.*, 2017). Nowadays, occurrence of antibiotic resistance is common and emerging issue in small animal medicine especially in dogs. UPEC strains isolated from the cases of UTIs in dogs show high prevalence of resistance (85–100%) against commonly used antimicrobial agents (Chang *et al.*, 2015; Rzewuska *et al.*, 2015).

Molecular investigations have found certain antibiotic resistance genes including the genes that encode resistance against chloramphenicol (cmlA and floR), β lactams (blaSHV, CITM), quinolones (qnr), tetracycline (tetA and tetB), trimethoprim (dfrA1), gentamicin (aac(3) IV), sulfonamide (sul1), and streptomycin (aadA1), which is the most significant reason for occurrence of antibiotic resistance in UPEC strains (Rzewuska *et al.*, 2015; Ranjbar *et al.*, 2017). Therefore, keeping in view of high degree of antibiotic resistance, the aim of the present study was to isolate and identify pathogenic *E. coli* from urine of dogs suffering from UTI and determining their chloramphenicol associated resistance genes by conventional Polymerase Chain Reaction (PCR) assay.

*Corresponding author: tanimuzimbos@gmail.com

MATERIALS AND METHODS

Sample collection: From January 2017 to February 2018, a total of 103 urine samples were collected aseptically through cystocentesis from non-medicated adult dogs of both sexes and of different breeds with a presumptive diagnosis of UTIs in small animal section of Veterinary Clinical Complex (VCC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana, India.

Bacteriological Examination: The fresh urine samples collected aseptically were inoculated and streaked onto 5% sheep blood agar (BA) (HiMedia, Mumbai, India) and MacConkey's lactose agar (MLA) (HiMedia, Mumbai, India) plates separately. The plates were incubated aerobically at 37° C for 24-48 hours till adequate growth was observed. Suspected colonies were streaked onto Eosine Methylene Blue agar (EMB), (HiMedia, Mumbai, India) and the plates were incubated aerobically at 37º C for 24 hours. The appearance of blue green colonies with a metallic luster on EMB was presumptively considered as of *E. coli*.

Biochemical Examination: Gram staining of the collected samples was performed to identify *E. coli* by their Gram reaction. Samples with rod shaped arrangements were subjected to biochemical tests (Indole, Methyl Red, Voges Proskauer, Citrate, Glucuronidase, Nitrate

reduction, ONPG, Lysine utilization, Lactose, Glucose, Sucrose and Sorbitol) using commercially available KB010 Hi *E. Coli* TM Identification Kit (HiMedia Mumbai, India) following the manufacturer's instructions.

Antimicrobial Susceptibility Testing: The *in-vitro* drug sensitivity testing of *E. coli* isolates was determined according to the method of Bauer-Kirby (Bauer *et al.*, 1966) by using commercially prepared disc (Himedia, India) with known concentration of antibiotics. The following antibiotics were used: Ampicillin (AMP) 10 mcg, Amoxyclav (AMC) 30 mcg, Amikacin (AK) 30 mcg, Ciproflaxacin (CIP) 10 mcg, Ceftizoxime (CZX) 30 cmg, Chloramphenicol (C) 30 mcg, Streptomycin (S) 25 mcg, Gentamicin (GEN), 30 mcg, Meropenem (MRP) 10 mcg and Tetracycline (TE) 30 mcg (HiMedia, Mumbai, India).

Extraction of genomic DNA: DNA of *E. coli* from all the positive isolates was extracted using commercially available Pure Link TM Genomic DNA mini kit (Invitrogen, USA) following the manufacturer's instructions. The extracted DNA was kept at -20° C until further used.

Detection of chloramphenicol resistance gene: The presence of chloramphenicol resistance genes in *E. coli* DNA extracts was determined by conventional PCR. Primers sequences, target genes, products size and references are given in table 1. The conventional PCR was performed in veriti thermo cycler (ABI, USA) in 25 volume reaction containing 6µl of template DNA, 1µl of each of the primers (10pmoles concentration),12.5µl Phusion PCR Master-mix (2X) (High Fidelity, USA), 1µl DMSO and 2.5µl of nuclease free water. Amplification procedure consisted of initial denaturation at 98°C for 30 sec, followed by 35 cycles of denaturation at 98°C for 10 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 min. The PCR products were analyzed on 1.5% agarose gel electrophoresis and visualized under UV trans illuminator GEL DOC™ (BIO RAD, India) and documented by photography for further analyses.

RESULTS AND DISCUSSION

Out of the 103 urine samples examined, 25 (24.3%) were found positive for *E. coli.* In a similar study, Chang *et al.* (2015) reported 114 (56.7%) of 201 dogs positive for *E. coli*. Moyaert *et al.* (2016) isolated E. coli in 204 (46.7%) of 437 urine samples from dogs with symptoms of UTIs. Liu *et al.* (2017) further reported a detection of *E. coli* growth in 106 (60.9%) of 174 dogs examined. The percentage of prevalence of UPEC in our study was found lower compared to these studies. This may be attributed to the use of large number of samples in their studies. These results also indicate that urinary tract infections caused by bacteria are mostly diagnosed using urinalysis, clinical findings and culture. Therefore, historically, the isolation and identification of bacteria in urine aseptically obtained through cystocentesis from the bladder and processed by routine urine culture methods has defined UTI. It has been widely accepted that negative bacterial culture signified bladder sterility, while this method continues to be the gold standard for the diagnosis of clinically relevant UTI in dogs (Price *et al.*, 2016).

Table 1
Primers for conventional PCR assays

Target genes	Primer sequence	Product size (bp)	References
cmlA	F:CCGCCACGGTGTTGTTGTTATC R: CACCTTGCCTGCCCATCATTAG	698	Keyes *et al.*, 2000
floR	F: TATCTCCCTGTCGTTCCAG R: AGAACTCGCCGATCAATG	399	Keyes *et al.*, 2000

The result of antimicrobial susceptibility test are presented in table 2. The current studies established a very high level of resistance to ampicillin, ciprofloxacin and tetracycline. This, therefore, shows that the widespread use of ampicillin and other β lactams antibiotics could be associated with the selection of antibiotics' resistance mechanisms in pathogenic and nonpathogenic *E. coli* isolates (Sunde and Surum, 1999). Resistance to β lactam antimicrobial agents in *E. coli* is primarily mediated by β lactamases, which hydrolyze the β-lactam ring and thus inactivate the antibiotics (Livermore, 1995). Resistance to ampicillin observed in this study was higher than the previously reported studies from companion animals (Lei *et al.*, 2010), although the results are in consistence with the findings of Liu *et al.* (2017).

Fluoroquinolone/Quinolones exhibit their antimicrobial effects by inhibition of DNA gyrase and

Table 2
Antibiotic Susceptibility Pattern of *E. coli* isolated from dogs (n = 25)

Antibiotics	Resistant No. (%)	Intermediate No. (%)	Susceptible No. (%)
Amikacin	9 (36.0)	6 (24.0)	10 (40.0)
Amoxyclav	12 (48.0)	3 (12.0)	10 (40.0)
Ampicillin	25 (100)	0 (0.0)	0 (0.0)
Ceftizoxime	19 (76.0)	0 (0.0)	6 (24.0)
Chloramphenicol	4 (16.0)	0 (0.0)	21 (84.0)
Ciprofloxacin	25 (100)	0 (0.0)	0 (0.0)
Gentamicin	9 (36.0)	5 (20.0)	11(44.0)
Meropenem	6 (24.0)	3 (12.0)	16 (64.0)
Streptomycin	13 (52.0)	3 (12.0)	9 (36.0)
Tetracycline	25 (100.0)	0 (0.0)	0 (0.0)

topoisomerase IV by alterations in drug target enzymes. However, the presence of mutations in the quinolone resistance- determining region (QRDR) of the DNA gyrase enzyme is the primary cause of high-level fluoroquinolone resistance in gram-negative bacteria such as *E. coli* (Ruiz, 2003). The observed high level of resistance against ciprofloxacin is probably explained by cross-resistance with other quinolones members such as enrofloxacin, nalidixic acid and norfloxacin (Literak *et al.*, 2013). This observation is similar to that found in previous studies (Liu *et al.*, 2017) while it was greater than the fluoroquinolone resistance in companion animals reported earlier by Thungrat *et al.* (2015). However, other studies have documented a higher level of ciprofloxacin resistances in *E. coli* of canine origin (Krishnan *et al.*, 2012). Furthermore, increased fluoroquinolone resistance in companion animals, may limit the treatment of uropathogenic *E. coli* infections and in turn can limit treatment options in human, who may acquire infection from their pet (Liu *et al.*, 2017).

Tetracycline is a broad-spectrum antibiotic drug that inhibits bacterial protein synthesis by preventing aminoacyl-tRNA from binding to the bacterial ribosome (Roberts, 1996). Resistance to the antibiotic is conferred by one or more of the forty currently described tetracycline resistant genes, which encode one of the three mechanism of resistance (An efflux pump, a method of ribosomal protection or direct enzymatic inactivation of the drug) (Chang *et al.*, 2015).

The relatively high levels of tetracycline resistance have been documented in other studies and probably reflect the long history of use of this antibiotic for both therapeutic and prophylactic agents (Tadesse *et al.*, 2012). However, the result of the current study tallied with the finding recorded by Liu *et al.* (2017) who isolated high level of tetracycline resistant *E. coli* in animals.

The high level of antibiotic resistance among the *E. coli* isolates recorded in this study is attributed to Veterinary Clinical Complex (VCC) hospital. This is because, it is a tertiary referral hospital that receives large number of referred dogs with complicated conditions and recurrent cases from the primary health care centers and the private practitioners, where the dogs have already been exposed to different classes of antibiotics without any prior laboratory assessment. Furthermore, the occurrence of drug-resistant *E. coli* in dogs represents a potential threat to public health. The role of livestock as a source of pathogen transmission to people has been well documented, predominantly through food-born exposure but also via direct contact (Cummings *et al.*, 2012). Although, in Hisar, dogs generally share the entire home environment with their owners and they are considered as family members. Thus, direct contact with dogs is frequent among the human population and may serve to be an important route of *E. coli* transmission to humans. In fact, an increasing body of evidence indicates that transfer of resistance bacteria or mobile resistance determinants can occur between dogs and humans through direct contact (Stockholm *et al.*, 2012).

The *E. coli* isolates were highly susceptible against chloramphenicol, meropenem, gentamicin and amikacin. This confirmed that chloramphenicol has a broad-spectrum activity against gram positive and negative organisms, while it also confers protection against anaerobic infection. Susceptibility to meropenem is very interesting, since this is one of the new and few drugs that can be used to attack infection due to extended β-lactamase producing Enterobacteriaceae (ESBLs) (Pitout and Laupland, 2008). However, meropenem effectiveness especially against *E. coli* isolated strains may be as a result of its infrequent use in clinical veterinary medicine due to its high cost and less availability for abuses. In addition, gentamicin and amikacin also showed high activity against uropathogenic *E. coli*, which may be as a result of complexity of the aminoglycoside and possibly the route of administration (Onanuga *et al.*, 2005).

The PCR analysis of the chloramphenicol resistance genes revealed the genes responsible for the resistance. The amplification of cmlA genes gave a positive PCR product only in 4 (16.0%) *E. coli* isolates and no amplification was detected for floR in all the 25 isolates as shown in fig. 1. This result also matches with AST results as shown in table 2.

In the present study, resistance to chloramphenicol was very low, with only 4 isolates resistant to the chloramphenicol. Although, active efflux pumps i.e. floR and cmlA played an important role in intrinsic and acquired chloramphenicol resistance (Chang *et al.*, 2015). However, over expression of efflux pumps affecting chloramphenicol has become increasingly common in uropathogenic *E. coli* (Blickwede and Schwarz, 2004). The floR gene was not detected in all the isolates including the 4 isolates that were found to be resistant. The only gene detected by PCR was a cmlA gene, which is responsible for the resistance in these isolates. This tallied with the findings recorded by Yousefi and Torkam (2017) who demonstrated the presence of cmlA gene in a study of uropathogenic *E. coli* in urine samples of Iranian dogs.

In conclusion, the present study identified a large number of the UPEC strains with high levels of resistance

against several classes of antibiotics with respect to the prevalence of chloramphenicol resistance genes. Monitoring antibiotic prescription and resistance patterns in a small animal medicine may serve as an early indicator of changes in the antibiotic susceptibility of clinical isolates. Using culture-based identification, disk diffusion, and PCR-based amplification of antibiotic resistance genes provide valuable data to veterinarians for the management of persistent or recurrent UTI in dogs. Prescription of chloramphenicol, meropenem, gentamicin and amikacin antibiotics can be more effective for treatment of UTIs in dogs here in Hisar.

ACKNOWLEDGEMENT

The authors wish to acknowledge the assistance of Indian Council for Cultural Relations (Ministry of External Affairs, Government of India).

REFERENCES

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

Blickwede, M. and Schwarz, S. (2004). Molecular analysis of florfenicol resistant *Escherichia coli* isolates from pigs. *J. Antimicrob. Chemother.* **53**: 58-64.

Chang, S.K., Lo, D.Y.,Wei, H.W. and Kuo, H.C. (2015). Antimicrobial resistance of *Escherichia coli* isolates from canine urinary tract infections. *J. Vet. Med. Sci.* **77(1)**: 59-65.

Cummings, K.J., Warnick, L.D., Davis, M.A., Eckmann, K., Gröhn, Y.T., Hoelzer, K., MacDonald, K., Root, T.P., Siler, J.D., McGuire, S.M., Wiedmann, M., Wright, E.M., Zansky, S.M. and Besser, T.E. (2012). Farm animal contact as risk factor for transmission of bovine associated Salmonella subtypes. *Emerg. Infect. Dis.* **18**: 1929-1936.

Keyes, K., Hudson, C., Maurer, J.J., Thayer, S.,White, D.G. and Lee, D.M. (2000). Detection of florfenicol resistance gene in *E. coli* isolated from sick chickens. *Antimicrobial. Agent. Chemother.* **44(2)**: 421-424.

Krishnan, S., Balasubramania, D., Raju, B.A. and Lakshmi, B.S. (2012). Use of naturally occurring codon bias for identifying topoisomerase mutations in ciprofloxacin resistant *Escherichia coli* using PCR and future prospects with other bacterial genera: A pilot study. *Adv. Biol. Chem.* **2**: 366-371.

Lei, T., Tian, W., He, L., Huang, X.H., Sun, Y.X., Deng, Y.T., Sun, Y., Lv, D.H., Wu, C.M., Huang, L.Z., Shen, J.Z. and Liu, J.H. (2010). Antimicrobial resistance in *Escherichia coli* isolates from food animals, animal food products and companion animals in China. *Vet. Microbiol.* **146**: 85-89.

Literak, I., Reitschmied, T., Bujnakova, D., Dolejka, M., Cizek, A., Bardon, J., Pokludova, L., Alexa, P., Halova, D. and Jamborova, I. (2013). Broilers as a source of quinolone-resistant and extraintestinal pathogenic *Escherichia coli* in the Czech Republic. *Micob. Drug. Resist.* **19**: 57-63.

Liu, X., Liu, H., Li, Y. and Hao, C. (2017). Association between virulence profile and fluoroquinolone resistance in *Escherichia coli* isolated from dogs and cats in China. *Infect. Dev. Ctries.* **11(4)**: 306-313.

Livermore, D.M. (1995). β Lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev.* **8**: 557-584.

Moyaert, H., Morrissey, I., de Jong, A., El Garch, F., Klein, U., Ludwig, C., Thiry, J. and Youala, M. (2016). Antimicrobial susceptibility monitoring of bacterial pathogens isolated from urinary tract infections in dogs and cats across Europe: ComPath Results. *Microbial. Drug Resist.* **23(3)**:391-403.

Onanuga, A., Oyi, A.R. and Onaolapa, A.J. (2005). Prevalence and susceptibility pattern of Methicillin resistant *Staphylococcus aureus* isolates amongst healthy women in Zaria, Nigeria. *Afr. J. Biotech.* **4(11)**: 1321-1324.

Pitout, J.D and Laupland, K.B. (2008). Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet. Infect. Dis.* **8(3)**: 159-166.

Price, T.K., Dune, T., Hilt, E.E., Thomas White, K.J., Kliethermes, S., Brincat, C., Brubaker, L. Wolfe, A.J., Mueller, E.R. and Schreckenberger, P.C. (2016). The clinical urine culture: Enhanced techniques improve detection of clinically relevant microorganisms. *J. Clin. Microbiol.* **54(5)**: 1216-1222.

Ranjbar, R., M. Masoudimanesh, M., Dehkordi, F.S., Jonaidi Jafari, N. and E. Rahimi, E. (2017). Shiga (Vero) toxin producing *Escherichia coli* isolated from the hospital foods; virulence factors, oserogroups and antimicrobial resistance properties. Antimicrob. *Resist. Infect. Ctrol.* **6(1)**: 4.

Roberts, M.C. (1996). Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility and distribution. *FEMS Microbiol. Rev.* **19**: 1-24.

Ruiz, J. (2003). Mechanism of resistance to quinolones: Target alterations, decreased accumulation and DNA gyrase protection. *J. Antimicrob. Chemother.* **51**: 1109-1117.

Rzewuska, M., Czopowicz, M., KizerwetterSwidia, M., Chrobak, D., Blaszczak, B. and Binek, M. (2015). Multidrug resistance in *Escherichia coli* strains isolated from infections in dogs and cats in Poland (2007-2013). *Sci. World. J.* **10 (1155)**: 1-8.

Stokholm, J., Schjorring, S., Pedersen, L., Bischoff, A., Følsgaard, N., Carson, C.G., Chawes, B., Bønnelykke, K., Mølgaard, A., Krogfelt, K.A. and Bisgaard, H. (2012). Living with cat and dog increases vaginal colonization with *E. coli* in pregnant women. *PLoS One* **7(9)**: 1-6.

Sunde, M. and Sorum, H. (1999). Characterization of integrons in *Escherichia coli* of the normal intestinal flora of swine. *Microb. Drg. Resist.* **5**: 279-287.

Tadess, D.A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M.J. and McDermott, P.F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States 1950-2002. *Emerg. Infect. Dis.* **18**: 741-749.

Thungrat, K., Price, S.B., Carpenter, D.M. and Boothe, D.M. (2015). Antimicrobial susceptibility patterns of clinical *Escherichia coli* isolates from dogs and cats in the United State: January 2008 through January 2013. *Vet. Microbiol.* **179**: 287-295.

Yousefi, A. and Torkan, S. (2017). Uropathogenic *Escherichia coli* in the Urine Samples of Iranian Dogs: Antimicrobial Resistance Pattern and Distribution of Antibiotic Resistance Genes. *Biomed. Res. Internl.* Article ID 4180490:10.