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Phylogrouping, Virulence Profile and Antibiogram of *Escherichia coli* Strains Associated with Canine Pyometra

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ABSTRACT

Background: Multi-drug resistant bacterial strains with biofilm-forming ability are one of the significant reasons contributing to the failure of medical management and recurrence of canine pyometra. The predominant bacterial agent implicated in canine pyometra is *Escherichia coli,* the different strains of which can be grouped into distinct phylogroups with the same ecological niches, characteristics and tendency to cause disease. The identification of a phylogroup of an unknown strain can facilitate the control, prevention and treatment of infections.

Methods: The isolates of *E.coli* obtained from 25 clinical cases of pyometra were identified based on the cultural, morphological and biochemical characteristics. The antibiogram of the isolates were done employing Kirby-Bauer disk diffusion method. Biofilm forming ability of theisolates were detected by tissue culture plate method and the presence of virulence genes among them were detected using a multiplex PCR. The phylogrouping of *E. coli* was done employing the novel quadruplex PCR method.

Result: In the antibiogram, most isolates were found sensitive to Amoxycillin-clavulanate (90%), Gentamicin (80%),Tetracycline (50%) and Amikacin (50%), while 100% resistance were shown towards ceftazidime-clavulanate, enrofloxacin,ceftriaxone-tazobactam, ciprofloxacin and cefotaxime-clavulanate. Among the *E. coli* isolates, strong and moderate biofilm producers showed resistance to a wide range of antibiotics compared to non-biofilm producers. Around 80% of the isolates belonged to the phylogroup B2 and all of these had the presence of *fim*H, *sfa*, and *csg*A genes. Among the isolates belonged to the phylogroup B2, 87.5% of the isolates had *pap* gene while none of them possessed *afa* gene.

Key words: Antimicrobial resistance, Biofilm, *Escherichia coli*, Phylogroup pyometra, Virulence gene.

INTRODUCTION

Pyometra is a chronic inflammatory condition of the uterus in bitches characterized by the accumulation of pus in the uterus and mostly associated with bacterial infection. Disturbances of the hormonal milieu during dioestrum concomitant with bacterial infections lead to the onset of pyometra. About 25% of bitches eventually suffer from pyometra before they reach ten years of age (Hagman, 2012). The majority of pyometra infections in bitches were reviewed to be caused by *Escherichia coli* (Hagman, 2018). Other bacterial organisms like *Klebsiella*spp., *Streptococcus* spp.,*Staphylococcus* spp. and *Pseudomonas* spp. have also been implicated in the disease (Hagman, 2018). Though the treatment of choice for pyometra is surgical ovariohysterectomy (Gogoi *et al.,* 2022), medical management of pyometra is resorted when the breeding value of the bitch is to be maintained or when the bitch is not suitable for an immediate surgery and has to be stabilized clinically (Hagman, 2018). A lack of response to antibiotics or recurrence of the condition is a documented limitation in the medical management of pyometra.

One of the significant reasons for bacterial antibiotic resistance, in general,is the biofilm-forming ability, which has also been recorded in pyometra affecting dogs (Amrutha *et al.,* 2021). *Escherichia coli* isolates possess adhesins such as *pap, sfa* and *afa,* associated with extraintestinal infections (Naziri *et al.,* 2021) and a protein called *csg*A which plays a crucial role in the formation of extracellular amyloid polymers in a biofilm (Hammar *et al.,* 1996).

Phylogenetic analysis has shown that *E. coli* strains could be grouped into distinct phylogroups such as A, B1, B2, C, D, E, F and different clades (Clermont *et al.,* 2000).

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The strains are grouped based on the presence or absence of a set of genes (*arp*A, *chu*A, *yja*A and TspE4. C2), employing a novel quadruplex PCR (Clermont *et al.,* 2013). Research conducted in different countries has documented the correlation between specific phylogroups, antibiotic resistance and biofilm-associated genes in *E. coli* (Ghanbarpour *et al.,* 2012; Olowe *et al.,* 2019). Thus, identifying the phylogroup of an unknown strain can predict its antibiotic resistance. This study was aimed at assessing the correlation between the antibiogram profile, biofilm-forming potential, presence of virulence genes and phylogroups of *E. coli* isolates associated with canine pyometra.

MATERIALS AND METHODS

Isolation and Identification

The research was conducted during the year 2022-23 at the department of Veterinary microbiology, College of veterinary and animal sciences, Thrissur, Kerala.The study was conducted upon bitches presented at the University Veterinary Hospitals, Mannuthy, and Kokkalai, Thrissur under the Kerala Veterinary and Animal Sciences University. Guarded anterior vaginal swabs were collected from a total of 25 bitches affected with open-cervix pyometra.

The collected samples were plated onto Brain Heart Infusion Agar (BHIA), MacConkey agar (MCA) and Eosin Methylene Blue (EMB) agar plates. Plates were incubated at 37°C for 24 h and the *E. coli* isolates were identifiet by biochemical identification tests (Quinn *et al.,* 1994).

Antimicrobial susceptibility testing

Escherichia coli isolates were tested for antimicrobial susceptibility using 20 antibiotic discs: Ceftazidime (30 µg), Ceftazidime-clavulanic acid (30/10 µg), Cefotaxime (30 µg), Cefotaxime-clavulanic acid (30 µg), Amoxycillin-clavulanic acid (30 µg), Ceftriaxone (30/10 µg), Ceftriaxone-tazobactam (30/10 µg), Ceftriaxone-sulbactam (10 µg),Ciprofloxacin (5 µg), Enrofloxacin (30 µg), Tetracycline (30 µg), Cefuroxime (30 µg), Cefepime (30 µg), Cefoxitin (30 µg), Amikacin (30 µg), Cefpodoxime (10 µg), Gentamicin (10 µg), Ertapenem (10 µg), Imipenem (10 µg) and Meropenem (10 µg), using the Kirby-Bauer disk diffusion method and categorized by the Clinical and Laboratory Standards Institute Criteria (2018).

Calculation of multiple antibiotic resistance index

Multiple antibiotic resistance index (MAR) was calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics to which the organism is exposed. MAR index values greater than 0.2 indicated a high-risk source of contamination where antibiotics are often used.

Detection of biofilm formation

The biofilm-forming capacity of *E. coli* isolates was determined using a microtitre plate assay as described by Christensen *et al.* (1985).

Dna extraction

Escherichia coli DNA was extracted using the HiPurA® Bacterial Genomic DNA Purification kit.

Virulence genes detection

The four adhesion genes, *pap, sfa, afa* and *fimH* were detected in all isolates using multiplex PCR. The amplification was carried out at an annealing temperature of 65°C and under the conditions described by Tewawong *et al.* (2020). The presence of the *csg*A was assessed using PCR as described by Zeighami *et al.* (2019) and referred the primer sequences and sizes of PCR products they used for detecting all the five genes (Tewawong *et al*., 2020; Zeighami *et al.,* 2019).

Phylogrouping of *escherichia coli* isolates

The phylogrouping of *E. coli* was done employing the novel quadruplex PCR under the thermal cycling conditions as described by Clermont *et al.* (2013) and referred the primer sequences and sizes of PCR products they used.

RESULTS AND DISCUSSION

A total of 10 *E. coli*, six *Klebsiella* spp., five *Streptococcus* spp. and five *Staphylococcus* spp. were isolated from 25 samples collected from dogs with pyometra.

According to the results of antibiotic susceptibility test, among the 10 *E. coli* isolates, nine were found to be sensitive to Amoxicillin-clavulanic acid followed by Gentamicin (8), Ertapenem (7), Meropenem (6), Tetracycline (5), Amikacin(5), Cefoxitin (3), Ceftriaxone (3), Cefpodoxime (2) and Ceftazidime (2). Additionally, all of the isolates were resistant towards Ceftazidime-Clavunalic acid, Enrofloxacin, Ceftriaxone-sulbactum,Ceftriaxone-Tazobactam, Ciprofloxacin and Cefotaxime-clavunalic acid, while 9 isolates were resistant to Cefuroxime. Intermediate sensitivity was exhibited towards the antibiotics such as Imipenem (5), Cefepime (5) and Cefotaxime (3). The dendrogram showing the antibiotic resistance pattern among the *E. coli* isolates obtained are depicted in (Fig 1). The MAR index of the *E. coli* isolates to the different antibiotics used are depicted in Table 1.

Microtitre plate assay revealed that one *E. coli* isolate as a strong biofilm producer, five as moderate producers, and four as non-biofilm producers. The strong and moderate biofilm-producing *E. coli* exhibited higher antibiotic resistance than non-biofilm producers.

All *E. coli* isolats carried at least one virulence gene. The most prevalent genes were *fim*H and *csg*A (pre sent in all 10 isolates) followed by *sfa* (pre sent in 9 isolates) and *pap* (pre sent in 8 isolates) (Fig 2 a,b). While *afa* was not detected in any of the isolates.

Phylogenetic analysis demonstrated that *E. coli* strains isolated from canine pyometra tend to cluster mainly in phylogroup B2: 8 (80%) followed by group A: 1 (10%) and group E: 1 (10 %) (Fig 3 a and b). The isolates included in all the three phylogroups A, B2 and E had shown

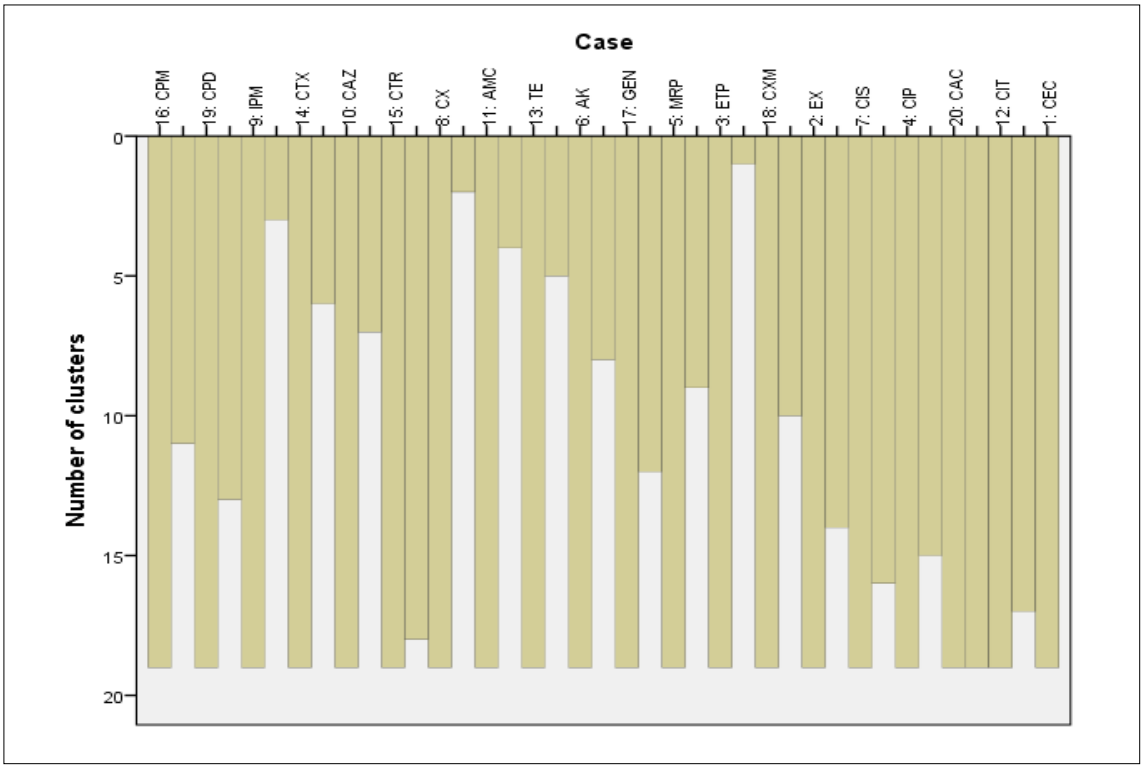


Fig 1: Dendrogram showing the antibiotic resistance pattern among the *E. coli* isolates.

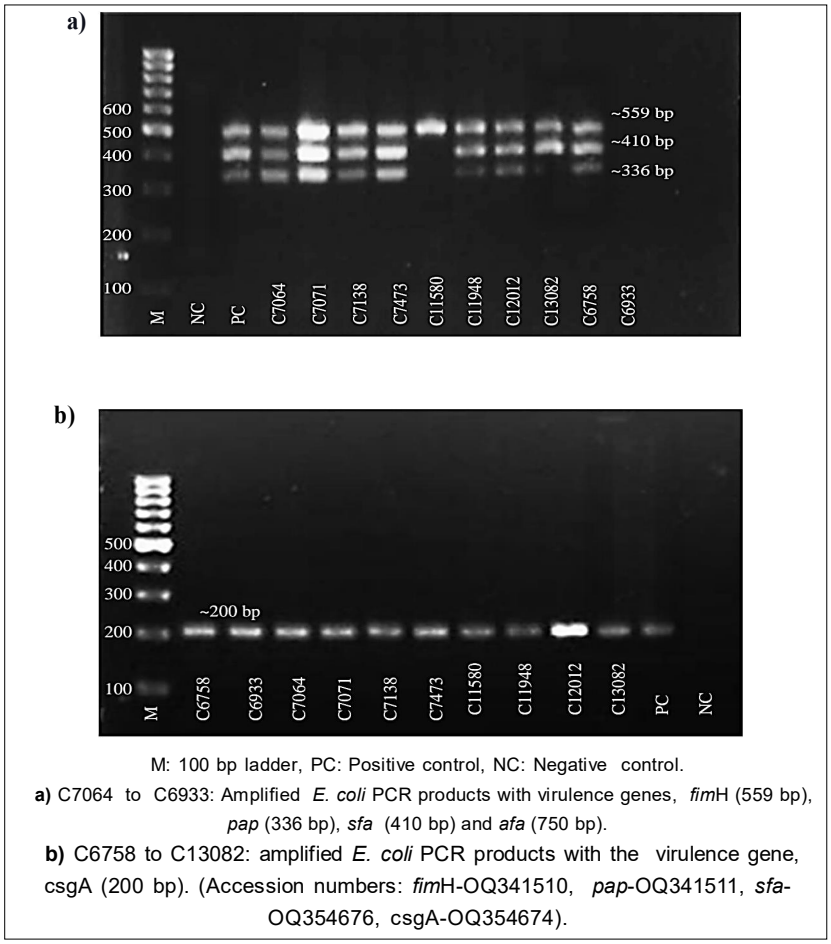
Table 1: Multiple antibiotic resistance index of the *Escherichia coli* isolates to the different antibiotics tested.

Isolates	MAR index
C 6758	0.9
C 6933	0.75
C 7064	0.5
C 7071	0.5
C 7138	0.85
C 7473	0.45
C 11580	0.55
C 11948	0.45
C 12012	0.6
C 6758	0.45

MDR. In this study, significant association was observed between virulence genes and *E.coli* phylogroup B2. Majority of the *E. coli* isolates belonged to phylogroup B2 (80%), among them 100% have shown the presence of *fim*H, *sfa*, and *csg*A, 87.5% showed the presence of *pap* and none showed *afa* (0%). The strong biofilm producer (10%) and moderate biofilm producers(40%) were included in phylogroup B2 and have shown a high virulence profile and resistance to a wide range of antibiotics.

There has been a significant increase in the number of pets, mainly dogs and cats, in the last few years, especially after the COVID pandemic. Among the various diseases affecting dogs, pyometra is a medical emergency, which affects most of the middle to old aged, intact bitches. Though the most resorted treatment option for pyometra is OHE, medical management is opted in case of valuable breeding bitches or when an immediate surgery is not tenable. One of the major causes of failure of medical treatment in pyometra-affected dogs is the presence of MDR bacterial species. The predominant organism associated with canine pyometra is *E. coli* and it plays a crucial role in the increasing extraintestinal infections in hospitals. These strains are seen to exhibit several virulence properties as well as a high rate of antibiotic resistance, which is of major concern in the management of infections.

The predominant organism isolated in the present study was *E. coli*. Other studies have recorded similar results for *E. coli* prevalence (Coggan *et al.,* 2008; Robaj *et al.,* 2016). Regarding antimicrobial resistance exhibited by the isolates, all 10 *E. coli* isolates were found to be multi-drug resistant. Most of the isolates were found to be sensitive to Amoxicillin-clavulanic acid followed by Gentamicin, Ertapenem, Meropenem, Tetracycline. All the 10 isolates were resistant towards Ceftazidime-Clavunalic acid, Enrofloxacin,Ceftriaxone-Tazobactam,Ciprofloxacin and Cefotaxime-clavulanic acid. Few of the *E. coli* isolates had intermediate sensitivity towards the antibiotics such as Imipenem (50%), Cefepime (50%) and Cefotaxime (30%).Similar observations were als reported previously (Coggan *et al.,* 2008). Contradictory results have also been observed in the sensitive pattern of *E. coli* isolates (Robaj



M: 100 bp ladder, PC: Positive control, NC: Negative control.

a) C7064 to C6933: Amplified *E. coli* PCR products with virulence genes, *fim*H (559 bp), *pap* (336 bp), *sfa* (410 bp) and *afa* (750 bp).

b) C6758 to C13082: amplified *E. coli* PCR products with the virulence gene, csgA (200 bp). (Accession numbers: *fim*H-OQ341510, *pap*-OQ341511, *sfa*-OQ354676, csgA-OQ354674).

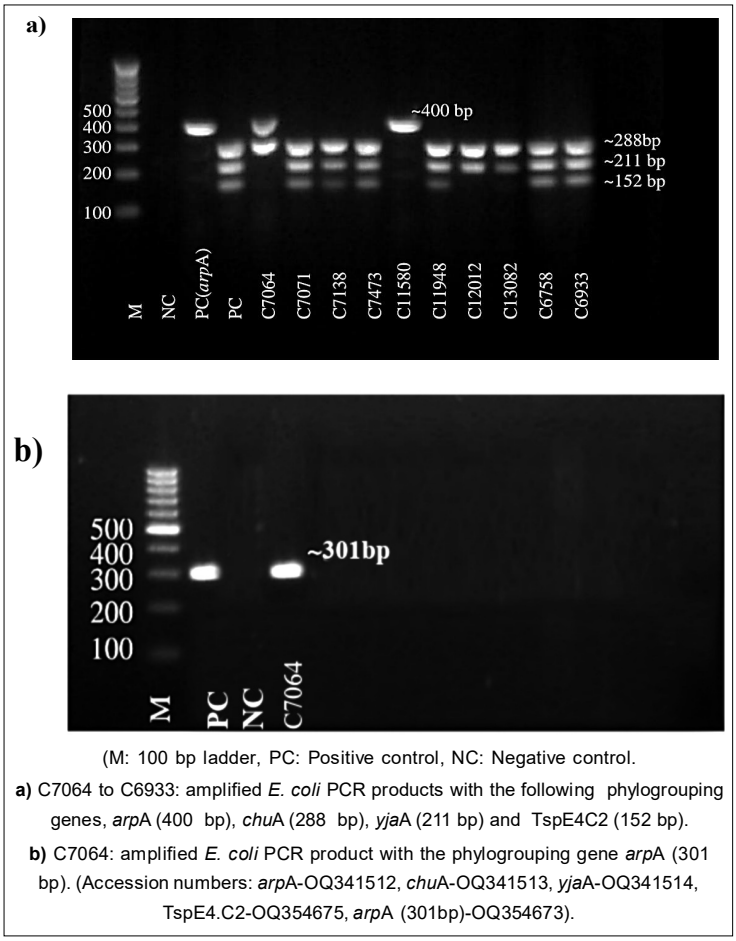
Fig 2: Representative 1.5% agarose gel electrophoresis of *Escherichia coli* virulence genes isolated from canine pyometra.

et al., 2016) and the resistance of isolates to Tetracycline and Amoxicillin-clavulanic acid (Ghanbarpour and Akhtardanesh, 2012).

The presence of plasmids with one or more resistance genes, each encoding a single antibiotic resistance phenotype is commonly associated with multiple antibiotic resistance (MAR) in bacteria. The high prevalence of multidrug resistance indicates a serious need for broad-based, local antimicrobial resistance surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens. In the pre sent study, all of the isolates showed a MAR index greater than 0.2, which implies a high-risk source of contamination, where antibiotics are often used (Osundiya *et al.,* 2013).

In our study, in the *in-vitro* detection of biofilm formation by tissue culture plate method, one *E. coli* isolate was found to be a strong biofilm producer (10%), five (50%) as moderate producers and four (40%) as non-biofilm producers. A high percentage of biofilm formation by *E. coli* isolates from pyometra was also reported previously (Fiamengo *et al.,* 2020). Several studies have revealed the relationship between antibiotic resistance and the biofilm potential of different organisms associated with pyometra and UTI. In the pre sent study, the strong and moderate biofilm-producing *E. coli* exhibited higher antibiotic resistance than non-biofilm producers. The finding indicated that uterine colonization by strong biofilm-forming *E. coli* increased the risk of pyometra. Previous study has indicated that the *E. coli* strains capable of forming biofilm were showing high antibiotic resistance (Rocha *et al.,* 2021). On the other hand, Fernandes *et al.* (2022) could not find a significant relation between the biofilm formation capacity of the clinical and commensal *E. coli* isolates and the susceptibility profile of each antimicrobial tested.

All of the isolates possessed *fim*H and *csg*A genes followed by *sfa* (90%), *pap* (80%) and *afa* was not detected in any of the isolates. These results corroborate with several investigations, which reported that among various *E. coli* isolates, the predominant gene exhibited was *fim*H (Tewawong *et al*., 2020). Many studies reported a high percentage of these virulence genes (Babacan *et al.,* 2021; Naziri *et al.,* 2021) among the *E. coli* isolates from pyometra. Contradictory results have been observed in several previous investigations done by Coggan *et al*. (2008) and Tewawong *et al*. (2020) and another study reported the presence of *afa* gene in 3.3% of *E. coli* isolates



(M: 100 bp ladder, PC: Positive control, NC: Negative control.

a) C7064 to C6933: amplified *E. coli* PCR products with the following phylogrouping genes, *arp*A (400 bp), *chu*A (288 bp), *yja*A (211 bp) and TspE4C2 (152 bp).

b) C7064: amplified *E. coli* PCR product with the phylogrouping gene *arp*A (301 bp). (Accession numbers: *arp*A-OQ341512, *chu*A-OQ341513, *yja*A-OQ341514, TspE4.C2-OQ354675, *arp*A (301bp)-OQ354673).

Fig 3: Representative 2% agarose gel electrophoresis of *Escherichia coli* genes used to classify *Escherichia coli* into different phylogroup.

from pyometra (Melo *et al.,* 2022). In a study on UPEC isolates, found *afa* gene was detected to be as 17% and the least prevalent virulence gene was *sfa* (9%) (Naziri *et al.,* 2021).

In the present study, the phylogenetic analysis indicated that the majority of uterine *E. coli* isolates were included in group B2 8 (80%) followed by group A 1 (10%) and group E 1 (10%). Studies from many different geographical areas of the world reported that the *E. coli* strains isolated from canine pyometra tend to cluster mainly in phylogroup B2 (Xavier *et al.,* 2022), but some researchers documented phylogroup B1 as the dominant phylogroup (Basu *et al.,* 2013; Olowe *et al.,* 2019). Phylogroup A and D were reported as the dominant group from other areas (Ghanbarpour and Akhtardanesh, 2012).

The isolates included in all the three phylogroups A, B2 and E had shown MDR. Similar findings were reported by Iranpour *et al.* (2015) in a study on 140 *E. coli* isolates from UTI and among them, 82.14% were MDR. In their study, 39.3% of the isolates belonged to group B2 and among them, 50% had shown antibiotic resistance. This suggests that uterine colonization by MDR strains of *E. coli* could be a risk factor that resulted in the recurrence of pyometra in dogs. Results of the present study indicated that 80% of *E. coli* isolates belonged to phylogroup B2. Among the *E. coli* isolates obtained, 100% had shown the presence of *fim*H,*sfa* and *csg*A, 87.5% showed the presence of *pap* and none showed *afa* (0%). The prevalence of virulence genes in phylogroup B2 coincided with the previous findings (Xavier *et al.,* 2022), but in some other documentation, the virulence genes were higher in groups A and D (Ghanbarpour *et al.,* 2012). The strong (10%) and moderate biofilm producers (40%) recorded in the current research were included in phylogroup B2 and had shown a high virulence profile and resistance to a wide range of antibiotics. Previous study had revealed that the biofilm-forming *E.coli* strains were mainly from phylogroup B2 (Tewawong *et al*., 2020).

CONCLUSION

To conclude, the present study ascertained that the majority of the *E. coli* isolates from dogs with pyometra belonged to phylogroup B2 and they possessed a high proportion of virulence gene profile and antibiotic resistance properties. The isolates were also potent biofilm producers. Evaluating the correlation between the antibiogram, biofilm-forming potential, virulence profile and phylogroups of *E.coli* isolates

should facilitate the control, prevention and treatment programs. Elaborate studies in this regard are needed and are the need of the hour.

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Author contributions

Archana T. S: Research work was done by Archana T.S. Surya Sankar, Hiron M. Harshan: Involved in the design of the concept of work, methodology of the work, research guidance and writing of the manuscript. Binu K. Mani: Technical and financial support to the research work. Vidya P, Amrutha Anand, Bincy K. Abraham: Technical help in the completion of the research work.

Conflict of interest

All authors declare that they have no conflicts of interest.

REFERENCES

Amrutha, M.S., Sankar, S., Mani, B.K., Harshan, H.M., Vidya and Paulson, S.T. (2022). Detection of biofilm formation by *Escherichia coli* and *Staphylococcus aureus* associated with canine pyometra. Indian Journal of Animal Research. 58(4): 648-653. doi: 10.18805/IJAR.B-4923.

Babacan, O. and Izgur, M. (2021). Detection of virulence factors of *Escherichia coli* strains isolated from urogenital system infections in dogs and cats. Veteriner Hekimler Dernegi Dergisi. 92: 132-142.

Basu, S., Mukherjee, S.K., Hazra, A. and Mukherjee, M. (2013). Molecular characterization of uropathogenic *Escherichia coli:* Nalidixic acid and ciprofloxacin resistance, virulent factors and phylogenetic background. J. Clin. Diagnostic Res. 7: 2727-2731.

Christensen, G.D., Simpson, W.A., Younger, J.J., Baddour, L.M., Barrett, F.F., Melton, D.M. and Beachey, E.H. (1985). Adherence of coagulase negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. 22: 996-1006.

Clermont, O., Bonacorsi, S. and Bingen, E. (2000). Rapid and simple determination of the *Escherichia coli* phylogenetic Group. Appl. Environ. Microbiol. pp 4555-4558.

Clermont, O., Christenson, J.K., Denamur, E. and Gordon, D.M. (2013). The Clermont *Escherichia coli* phylotyping method revisited: Improvement of specificity and detection of new phylogroups. Environ. Microbiol. Rep. 5: 58-65.

Clinical and Laboratory Standards Institute. (2018). Performance standards for antimicrobial susceptibility testing; 28th international supplement M100-S21. Wayne, PA: CLSI.

Coggan, J.A., Melville, P.A., Oliveira, C.M., Faustino, M., Moreno, A.M. and Benites, N.R. (2008). Microbiological and histopathological aspects of canine pyometra. Brazilian J. Microbiol. 39: 477-483.

Fernandes, V., Cunha, E., Nunes, T., Silva, E., Tavares, L., Mateus, L. and Oliveira, M. (2022). Antimicrobial resistance of clinical and commensal *Escherichia coli* canine isolates: profile characterization and comparison of antimicrobial susceptibility results according to different guidelines. Vet. Sci. 9: 284.

Fiamengo, T.E., Runcan, E.E., Premanandan, C., Blawut, B. and Silva, M.A.C. (2020). Evaluation of biofilm production by *Escherichia coli* isolated from clinical cases of canine pyometra. Topics Companion Anim. Med . 1: 688-5642.

Ghanbarpour, R. and Akhtardanesh, B. (2012). Genotype andantibiotic resistance profile of *Escherichia coli* strains involved in canine pyometra. Comp. Clin. Path. 21: 737-744.

Gogoi, J., Leela, V., Suganya, G., Shafiuzama, M., Vairamuthu, S. and Rajathi, S. (2022). Detailed pathophysiological investigation of canine pyometra and the curative role of ovariohysterectomy. Indian Journal of Animal Research. 1-6.

Hagman, R. (2012). Clinical and molecular characteristics of pyometra in female dogs. Reprod. Domestic Anim. 47: 323- 325.

Hagman, R.(2018). Pyometra in small animals. Vet. Clin. Small Anim. 48: 639-661.

Hammar, M, Bian, Z, Normark, S. (1996).Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli.* Proc. Natl. Acad. Sci. 93: 6562-6566.

Iranpour, D., Hassanpour, M., Ansari, H.,Tajbakhsh, S., Khamisipour, G. and Najafi, A. (2015). Phylogenetic groups of *Escherichia coli* strains from patients with urinary tract infection in Iran based on the new Clermont phylotyping method. BioMed Res. Int. 1-7.

Melo, R.T., Oliveira, R.P., Silva, B.F., Monteiro, G.P., Saut, J.P.E., Costa, L.R.M., Dias, S.D.C. and Rossi, D.A. (2022). Phylogeny and virulence factors of *Escherichia coli* isolated from dogs with pyometra. Vet. Sci. 9: 158.

Naziri, Z., Kilegolan, J.A., Moezzi, M.S. and Derakhshandeh., A. (2021). Biofilm formation by uropathogenic *Escherichia coli:* A complicating factor for treatment and recurrence of urinary tract infections. J. Hosp. Infect.117: 9-16.

Olowe, O.A., Adefioye, O.J., Ajayeoba, T.A., Schiebel, J., Weinreich, J., Ali, A., Burdukiewicz, A., Rodiger, S. and Schierack, P. (2019). Phylogenetic grouping and biofilm formation of multidrug resistant *Escherichia coli* isolates from humans, animals and food products in South-West Nigeria. Scientific Afr. 6: 1-11.

Osundiya, O.O., Oladele, R.O. and Oduyebo, O.O. (2013). Multiple antibiotic resistance (MAR) indices of *pseudomonas* and *klebsiella* species isolates in Lagos university teaching hospital. Afr. J. Cln. Exper.Microbiol. 14: 164-168.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (1994). Clin. Vet. Microbiol. (2nd Ed.). Mosby Wolf, Spain. 648p.

Robaj, A., Sylejmani, D. and Hamidi, A. (2016). Occurrence and antimicrobial susceptibility of bacterial agents of canine pyometra. Indian Journal of Animal Research. 52(3): 397-400. doi: 10.18805/ijar.v0iOF.6830.

Rocha, R.A., Ribeiro, W.M., Almeida, J.A., Santos, A.L.,Fernandes, M.R., Barbosa, M.S., Moraes Filho, A.V., Carneiro, L.C.

and Silva, C.A. (2021). Detection of resistance genes in pyometra isolated bacteria in bitches. Brazilian J. Vet. Res. Anim. Sci. 58: 1678-4456.

Tewawong, N., Kowaboot , S., Pimainog, Y., Watanagul, N., Thongmee, T. and Poovorawan Y. (2020). Distribution of phylogenetic groups, adhesin genes, biofilm formation and antimicrobial resistance of uropathogenic *Escherichia coli* isolated from hospitalized patients in Thailand. Peer J. 8.

Xavier, R.G.C., da Silva, P.H.S., Trindade, H.D., Carvalho, G.M., Nicolino, R.R., Freitas, P.M.C. and Silva, R.O.S. (2022). Characterization of *Escherichia coli* in dogs with pyometra and the influence of diet on the intestinal colonization of extraintestinal pathogenic *E. coli* (ExPEC). Vet. Sci. 9: 245.

Zeighami, H., Valadkhani, F., Shapouri, R., Samadi, E. and Haghi, F. (2019). Virulence characteristics of multidrug resistant biofilm forming *Acinetobacter baumannii* isolated from intensive care unit patients. BMC Infect. Dis.19: 629.