SHORT COMMUNICATION
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.1.275-278

Assessment of quinolone resistance in bacteria isolated from corneal ulcers in dogs

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Citation: Ashtamy,M.G., Mani,B.K., Mini,M., Ambily,R. and Anoop,S. 2023. Assessment of quinolone resistance in bacteria isolated from corneal ulcers in dogs. *J. Vet. Anim. Sci.* **54**(1):275-278
DOI: https://doi.org/10.51966/jvas.2023.54.1.275-278

Received: 05.11.2022 *Accepted: 21.12.2022* *Published: 31.03.2023*

Abstract

Corneal ulcers or ulcerative keratitis is the loss of continuity of corneal epithelium leading to exposure of corneal stroma. It is a highly painful condition in dogs. The common aetiological agents responsible for this condition are bacteria and for treatment, antibiotics are widely used. Indiscriminate and prolonged use of antibiotics can cause antimicrobial resistance (AMR) in bacteria. The present communication deals with the isolation of bacteria from corneal ulcers in dogs and the assessment of quinolone resistance. A total of 15 corneal swab samples were collected from dogs suffering from corneal ulcers, presented to the Department of Veterinary Surgery and Radiology, Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Mannuthy. They were inoculated on to brain heart infusion agar as well as blood agar and incubated at 37 °C for 24 h for the isolation of aerobic bacteria. Eighteen isolates obtained were then subjected to antibiotic susceptibility test by disc diffusion method using the quinolone group of antibiotics, viz. ciprofloxacin, enrofloxacin, ofloxacin and moxifloxacin. Majority of the isolates exhibited varying resistance to these antibiotics. Polymerase chain reaction was performed to amplify gyrA gene, which is reported to be one of the reasons responsible for quinolone resistance with respect to its mutation. Seven out of the 18 isolates amplified gyrA gene. The amplicon obtained corresponding to a representative sample, which was found resistant by disc diffusion method was sequenced and a single point mutation was detected.

Keywords: *Quinolones, antimicrobial resistance, bacterial isolates, corneal ulcers*

Antimicrobial resistance (AMR) is an emerging public health concern, which is mainly due to the indiscriminate use of antibiotics over a long period of time. Since dogs are the best companion animal, they are considered to be potential carriers of microbes with AMR genes to human beings

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(Guardabassi *et al.*, 2004). Corneal ulcers or ulcerative keratitis is one of the most common and painful eye affections in dogs. It is defined as the loss of continuity of corneal epithelium leading to exposure of corneal stroma. The predominant bacteria associated with corneal ulcers are Gram positive, *viz. Staphylococcus* spp. (Murali *et al.*, 2017), *Streptococcus* spp., *Corynebacterium* spp. and *Bacillus* spp. while, the Gram negative organisms included *Pseudomonas* spp., *Klebsiella* spp., *E. coli* and *Neisseria* spp. (Moore and Naisse, 1999). Hence, isolation of bacteria from corneal ulcers in dogs and determination of antibiotic susceptibility pattern is of great importance.

Corneal swab samples collected were inoculated onto brain heart infusion agar and blood agar and incubated at 37 °C for 24 h. Eighteen isolates could be obtained with twelve as pure cultures and three as mixed, each with two types of colonies. On Gram's staining, seven were Gram positive cocci, five Gram positive bacilli, five Gram negative bacilli and one was identified as Gram negative diplococci. Based on colony morphology, Gram's staining and biochemical tests described by Barrow and Feltham (1993), the isolates were identified as *Staphylococcus* spp. (five), *Corynebacterium* spp. (five), *Streptococcus* spp. (two), *Pseudomonas* spp. (two), *Klebsiella* spp. (two), *Neisseria* spp. (one) and *E. coli* (one). Similar results were recorded by Hewitt *et al.* (2020).

Antibiotic susceptibility testing of the isolates was carried out by disc diffusion method in Mueller-Hinton agar, using the quinolone group of antibiotics, *viz*. ciprofloxacin, moxifloxacin, enrofloxacin and ofloxacin. Antimicrobial susceptibilities for all isolates were determined according to Clinical and Laboratory Standards Institute guidelines

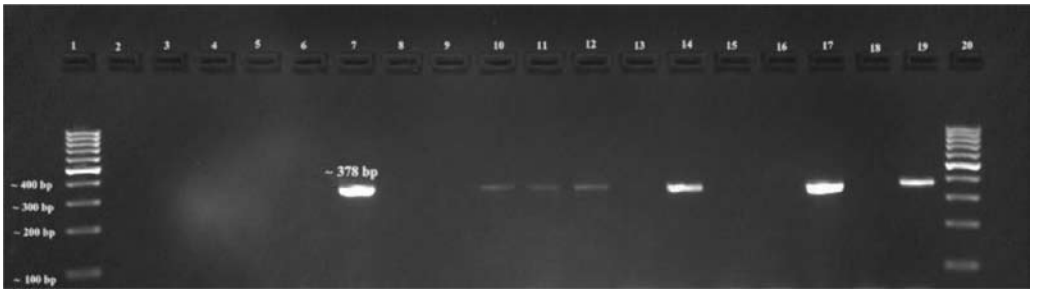

Fig. 1. Agarose gel electrophoresis depicting amplicons specific to *gyrA* gene
Lane 1, 20 – 100 bp ladder
Lane 2 to 19 – samples (S1-S6; S7C, S7N, S8-S11; S12C, S12S, S13, S14, S15C, S15S)

Fig. 2 Sequence comparison of *gyrA* gene of isolate with reference strain

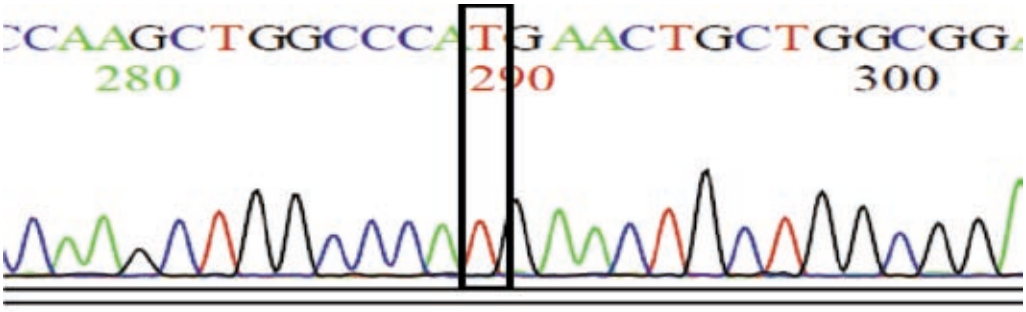

Fig. 3 Chromatogram of the sequenced isolate

(CLSI, 2020). It revealed that majority of them (88.89 per cent) were resistant to the quinolone group of antibiotics, *viz*. ciprofloxacin, moxifloxacin, enrofloxacin and ofloxacin. Only one isolate each of *Corynebacterium* spp. and *Staphylococcus* spp. were found to be sensitive to quinolones. Hindley *et al.* (2015) reported a similar finding in which isolates from canine ulcerative keratitis were found to be resistant to quinolone group of antibiotics.

Genomic DNA was extracted from these cultures and polymerase chain reaction (PCR) was performed to amplify the antibiotic resistance gene, *gyrA* in these isolates. The primer sequences used for the amplification of the gene were as follows (5'-3'): *gyrA* Forward primer, AGTCCTATCTCGACTACGCGAT; *gyrA* Reverse primer, AGTCGACGGTTTCCTTTTCCAG (Lin *et al.,* 2012). After performing the PCR, products were electrophoresed in 2 per cent agarose gel by submarine agarose gel electrophoresis and visualized by gel documentation system (Bio-Rad, USA). Only seven isolates were found to amplify this gene (Fig.1), which showed amplicon of size 378 bp, while the rest of the isolates failed to amplify this gene. Osman *et al.* (2016) and Rasool *et al.* (2020) also reported similar finding in which all the isolates in their study did not amplify the *gyrA* gene. Since the mutation in *gyrA* is the commonly reported reason for quinolone resistance, *gyrA* specific amplicon of representative isolate of *Pseudomonas* spp. in the present study, was selected for sequencing. The sequence was analysed with that of the reference strain, *Pseudomonas aeruginosa* PAO1 (Nouri *et al.*, 2016). A single point mutation in nucleotide sequence was detected in the sample sequenced (Fig. 2 and 3) but it did not alter the amino acid pattern. Hence, the mutation detected was silent. The mutation reported by Lin *et al.* (2012) was substitution of Thr at position 83 with Ile in *gyrA*. However, the association of mutation recorded in present study with AMR need further investigation. A comprehensive study is required to unravel the effect of mutations in specific gene or presence of other AMR genes to understand the resistance mechanism against quinolones.

Summary

The present study revealed that the most commonly isolated bacteria from ulcerative keratitis in dogs were Gram positive with *Staphylococcus* spp. and 88.89 per cent of the isolates were found to be resistant to quinolone group of antibiotics. The amplicon of representative isolate sequenced, possessed a silent mutation. Hence, further studies are warranted for better understanding of the quinolone resistance mechanism.

Acknowledgement

The authors are thankful to the authorities of Kerala Veterinary and Animal Sciences University for providing all facilities for the completion of this work.

Conflict of interest

The authors declare that they have no conflict of interest.

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