The Haryana Veterinarian (March, 2023) **62(SI),** 81-85 ISSN 0033-4359 (P) **Research Article**

COMPARATIVE THERAPEUTIC EFFICACY OF AMOXICILLIN-CLAVULANIC ACID AND MARBOFLOXACIN IN CLINICAL CANINE PYODERMA CASES

VICHAR NEMA*, K.K. MISHRA, KANCHAN, K. WALWADKAR, NEERAJ SHRIVASTAVA[1] and ANURADHA NEMA[2]
Department of Veterinary Medicine, [1]Department of Veterinary Microbiology,
[2]Department of Veterinary Surgery and Radiology,
College of Veterinary Science and Animal Husbandry, Rewa (MP)

Received: 29.10.2021; Accepted: 12.11.2022

ABSTRACT

Pyoderma refers to pyogenic bacterial infection of skin, caused by *Staphylococcus* spp. especially, *S. pseudintermedius*. Under the study comparative therapeutic efficacy of marbofloxacin and amoxicillin-clavulanic acid drugs was evaluated in clinical cases of pyoderma in dogs. Out of 196 dogs presented with dermatological affections, 33 dogs (16.84%) were diagnosed with staphylococcal pyoderma. Therapeutic evaluation was made on the basis of comparison of clinical dermatological scores on a scale of 1 to 5 i.e., pruritus score, presence and distribution of lesions score and coat condition score. Further, on the basis of gross dermatological score; efficacy of therapeutic drugs was assessed. Comparative trial on 12 dogs, revealed that both therapeutic regimens under the study brought significant changes in pruritus, resolution of lesions and improvement in coat condition. However, significantly higher efficacy ($p<0.05$) was observed in marbofloxacin treated group than those treated with amoxicillin-clavulanic acid.

Keywords: Amoxicillin-Clavulanic acid, Marbofloxacin, Pruritus score, Pyoderma, *Staphylococcus* spp., Therapeutic evaluation

How to cite: Nema, V., Mishra, K.K., Kanchan, Walwadkar, K., Shrivastava, N. and Nema, A. (2023). Comparative therapeutic efficacy of amoxicillin-clavulanic acid and marbofloxacin in clinical canine pyoderma cases. *The Haryana Veterinarian* **62(SI)**: 81-85.

Pyoderma refers to pyogenic bacterial infection of the skin caused by *Staphylococcal* spp. Canine pyoderma is one of the commonest reasons for prescription of antimicrobials in dogs (Summers *et al.*, 2014). Majority of (90%) canine pyodermas are caused by *Staphylococcus intermedius* Group (SIG) which includes *Staphylococcus intermedius*, *Staphylococcus pseudintermedius* and *Staphylococcus delphini* (Sasaki *et al.*, 2007). Among *Staphylococcus intermedius* group (SIG), vast majority of skin infections are associated with *Staphylococcus pseudintermedius*, a Gram-positive, β-haemolytic, coagulase -producing bacteria (Devriese *et al.*, 2005). Presently, due to improper and inadequate treatment protocols practiced in variety of skin infections, anti-microbial resistant strains are coming up with adamant skin problems (Hillier *et al.*, 2014).

MATERIALS AND METHODS

This study was carried out to compare clinico-therapeutic efficacies of amoxicillin- clavulanic acid and marbofloxacin in clinical cases of pyoderma in dogs. Cases of canine pyoderma were screened among clinical cases reported with plethora of skin problems at Veterinary Clinical Complex, Rewa (MP) from Jan-June, 21. Presumptive diagnosis of staphylococcal pyoderma was made on the basis of clinical presentation and indirect impression smear examination. Multiple skin swabs were collected as per the guidelines given by Hillier *et al.* (2014) from various skin lesions and immediately transferred to microbiology laboratory for further isolation and characterization of causative organism. Isolation of *Staphylococcus* spp. from skin swab was done as described by Gomez-Sanz *et al.* (2013). Swabs were pre-enriched into a tube containing 30 ml of Tryptone-Soy Broth (TSB) supplemented with 6.5% NaCl and incubated at 35°C for 24 hours. One loopful (10 µl) of this pre-enrichment culture was streaked onto Mannitol Salt Agar (MSA) and Baird-Parkar Agar (BPA) plates for isolation of *Staphylococcus* spp. Further, plates were incubated at 35° C for 24-48 hours. Further identification of causative agent was based on characteristic morphology (size and color) of colonies obtained i.e., pearly white and jet-black growth on the MSA and BPA plates, respectively. Colonies were checked for purity by Gram's reaction, morphology and arrangement. Pyoderma infections may vary in severity based on depth of infection, location on the body, extent of body surface involved, dog's general health status and any underlying skin disease. Characteristic lesions of superficial pyoderma consist of papules, pustules and/or epidermal collarettes, while deep skin infection generally presents with furuncles, draining tracts and/or haemorrhage from erythematous and oedematous skin (Fig. 1).

Pathogenicity of the isolates were assessed by tube coagulase reaction and only isolates with positive coagulase reaction were further characterized by Voges-Proskauer (VP) reaction, Beta-galactosidase test (ONPG test), Maltose utilization test and haemolysis pattern

*Corresponding author: vichar.nema@gmail.com

(Fig. 2). After confirmation of staphylococcal pyoderma, 12 dogs were randomly distributed into two therapeutic groups i.e., amoxicillin-clavulanic acid group (T1) and marbofloxacin group (T2). In therapeutic group (T1) Amoxicillin- Clavulanate was prescribed @ 12.5 mg/kg TID, PO and in group (T2) Marbofloxacin was prescribed @ 2 mg/kg OD, PO for 21 days. To evaluate therapeutic efficacy of drugs dermatological clinical score and gross score (sum of pruritus, presence/distribution of lesions and coat condition) were assessed on day 3, 7, 14 and 21 during the course of treatment. In the study, all animals included in the therapeutic trial were provided supportive treatment i.e., omega-6 and omega-3 fatty acid supplement (5:1), Zinc, Vitamin A and E @ 5-10 ml OD, PO for three weeks and Hydroxyzine hydrochloride @ 0.5 mg/kg OD, PO for one week. Medicated shampoo containing chlorhexidine and miconazole was applied liberally on wet body, thoroughly rubbed with a contact time of 10 minutes before rinsing and then towel dried. Medicated spray containing chlorhexidine gluconate (2% w/w) and miconazolenitrate (2% w/w) was used topically twice a day.

On the first day of visit, owners were asked to score their dog's pruritus in terms of behaviour i.e., scratching, rubbing, chewing or licking on a scale of 1 to 5:1-Absent, 2-Minimal (briefly, occasionally during the day), 3-Mild (frequently during the day), 4-Moderate (more than half of the day) or 5-Severe (day and night), and to state any changes in the behaviour on subsequent visits. Assessment of the presence and distribution of skin lesions such as erythema, papules/pustules, collarettes, crust/scale and ulcers were scored as 1 (absent), 2 (one or two lesions present), 3 (involving less than 5 per cent of the skin), 4 (involving 5 to 50 per cent of the skin) and 5 (involving more than 50 per cent of the skin). Coat condition was subjectively assessed on the basis of lustre, suppleness and odour as 1- Very good, 2-Good, 3-Fair, 4-Poor or 5-Very poor. Finally, on the basis of gross dermatological score (sum of pruritus, presence/distribution of lesions and coat condition), overall response to treatment was assessed on a scale of 1 to 15: Score upto 5-Excellent therapeutic response, 5-10-Moderateandscoreabove10-Mildtherapeuticresponse.

To test the significance of ordinal scores, non-parametric Freidman test was applied and pair wise comparison was conducted by Wilcoxon signed rank test using SPSS statistics software version 20.

RESULTS AND DISCUSSION

Study was carried out at Veterinary Clinical Complex, Rewa from Jan-June, 21. During the study 956 dogs were screened and found 196 cases reported with various skin problems. Out of 196 cases, 33 dogs were confirmed with staphylococcal pyoderma. Among these 33, twelve dogs were randomly distributed into two groups with six animals each. The dogs (07 males and 05 females) were aged between 08 months to 09 years and represented six breeds. All dogs included in the study expressed signs of pruritus in the areas of pyoderma lesions. Eight dogs (66.67%) were suffering from recurring skin disease for more than six months before including in the study and previous responses to systemic antibiotic therapy (fair to good) was reported in the clinical history. All the 12 dogs completed the therapeutic trial for study period of 21 days by virtue of good owner compliance. No adverse reactions were observed in any of the therapeutic groups. Skin lesions of pyoderma including erythematous papules, pustules, epidermal collarettes, scale, crust, multi-focal alopecia, ulcers and furuncle with oedema were found in dogs with highest distribution of lesions on the dorsum followed by lateral aspects, ventral abdomen and hind limb of the body.

Clinical Score: Based on the gross dermatological score all animals (n=6) treated with marbofloxacin showed marked improvement in the skin condition. However, in amoxicillin-clavulanic acid group, no-change was observed in one case and another dog showed worse response to treatment.

Pruritus Score: In marbofloxacin group (T2) pruritus score on day 3, 7, 14 and 21 were 2.83±0.17, 1.67±0.21, 1.17±0.17, 1.00±0.00, respectively. This group expressed significant reduction in pruritus score on day 3, 7 and 14as compared to pre-treatment score on day 0 (4.17±0.31). In amoxicillin-clavulanic acid group (T1) on day 3, 7, 14 and 21 pruritus score were 3.83±0.31, 3.00±0.26, 2.33±0.21 and 2.17±0.48, respectively. There was significant reduction in score was observed on day 7 and 14 which were higher as compared to marbofloxacin treated group. On day 21, no significant difference was observed as compared to day 14 and score was higher than marbofloxacin treatment group (Table 1). Similar findings were reported by Carlotti *et al.* (1995) who found marbofloxacin much more effective (96%) than amoxycillin-clavulanic acid (74.5%), particularly for the superficial skin infections. Paradis *et al.* (2001) also reported marbofloxacin as an excellent therapy (94.4%) in pyoderma cases of dogs. Beigh *et al.* (2013) noticed faster and excellent response with antibiotic and zinc combination in pyoderma cases and recommended that although animals respond well to the antibiotic, but to hasten recovery, zinc can be considered as supportive therapy in treatment regimen.

Present study revealed significant effect of both treatments in control of pruritus, however marbofloxacin

Table 1

Mean±SE of pruritus, presence/distribution of lesions, coat condition and Gross Dermatological Score during the treatment period

Group	Pruritus Score				
	0	3	7	14	21
T1	4.00±0.26^{a1}	3.83±0.31^{a1}	3.00±0.26^{b1}	2.33±0.21^{c1}	2.17±0.48^{c1}
T2	4.17±0.31^{a1}	2.83±0.17^{b2}	1.67±0.21^{c2}	1.17±0.17^{cd2}	1.00±0.00^{d2}
	Presence/distribution of lesions clinical Score				
T1	4.00±0.37^{a1}	4.00±0.37^{a1}	3.00±0.36^{b1}	2.83±0.40^{b1}	2.50±0.67^{b1}
T2	4.33±0.21^{a1}	3.33±0.21^{b2}	2.00±0.26^{c2}	1.00±0.00^{d3}	1.00±0.00^{d3}
	Coat Condition Score				
T1	4.17±0.31^{a1}	3.83±0.17^{a1}	3.17±0.17^{b1}	2.67±0.33^{b1}	2.17±0.48^{b1}
T2	4.33±0.21^{a1}	3.33±0.21^{b1}	2.83±0.17^{b1}	2.00±0.26^{c2}	1.33±0.21^{d2}
	Gross Dermatological Score				
T1	12.17±0.79^{a}	11.67±0.67^{a1}	9.17±0.60^{b1}	7.83±0.91^{b1}	6.83±1.62^{b1}
T2	12.83±0.60^{a}	9.50±0.50^{b2}	6.50±0.50^{c2}	4.17±0.31^{d2}	3.33±0.21^{d2}

Values having different alphabetical superscript in row and different numerical superscript in column differ significantly (P<0.05)

had a faster and effective result as compared to amoxycillin - clavulanic acid, though for control of acute pruritus anti-histamine drug and essential fatty acids were given in both treatment groups as ancillary therapy. Essential fatty acids (linoleic acid and linolenic acid) are most important for homeostasis of the skin of dogs owing to anti-inflammatory properties due to competitive inhibition of arachidonic acid metabolism which lead to a reduction of inflammatory leukotriene and prostaglandin synthesis.

Presence and distribution of lesions Score: Variety of lesions was found in cases of superficial pyoderma including scales, crust, erythema, alopecia, papules, erosions and epidermal collarettes. Similarly, furuncle, oedema and draining tracts were common findings in deep pyoderma. There was no significant difference in score value between groups on day 0 (pre-treatment). In marbofloxacin group (T2) mean ± S.E. scores on day 3, 7, 14 and 21 were 3.33 ±0.211, 2.00±0.26, 1.00±0.00, 1.00±0.00, respectively. There was significant reduction of score on day 3, 7 and 14, with complete resolution of lesions on day 14. However, in amoxycillin-clavulanic acid group (T1) significant improvement was observed from day 7 onwards, and few lesions remained till day 21 (Table 1).

Coat condition Score: Coat condition score revealed that treatment protocols had significant effect on coat condition over the days. In marbofloxacin group (T2), coat condition score on day 3,7 14 and 21 were 3.33±0.21, 2.83 ±0.17, 2.00±0.26, 1.33±0.21, respectively, which showed improvement by day 3 in marbofloxacin group (T2), which was due to resolution of lesions and pruritus. Further, there was significant improvement observed on day 14 and 21. In amoxycillin-clavulanic acid group (T1) significant improvement in coat condition was first observed on day 7 which further improved non-significantly till day 21. Coat condition score on day 21 was still significantly higher in amoxycillin-clavulanic acid group (T1) as compared to marbofloxacin group (T2) (Table 1). The above results are in close agreement with Paradis *et al.* (2001), who reported excellent improvement in coat condition with use of marbofloxacin for 21-28 days along with coat-conditioning shampoos.

Response to treatment on basis of gross dermatological score:

Response of treatment was assessed on the basis of sum of scores of pruritus, presence and distribution of lesions and coat condition. Overall clinical assessment was done on scale of 1-15. On day 0, gross dermatological scores were not significantly different, showing that there was no marked bias in scores of dogs between treatment groups. In marbofloxacin group (T2), GDS on day 3, 7, 14 and 21 were 9.50±0.50, 6.50±0.50, 4.17±0.31 and 3.33±0.21, respectively. There was serially significant reduction in gross score from day 3 to 14 which signifies progressive improvement in skin condition. On day 21, there was non-significant difference in score as compared to day 14, although it was in decreasing pattern. In amoxicillin-clavulanic acid group (T1) GDS on day 3, 7, 14 and 21 were 11.67±0.67, 9.17±0.60, 7.83±0.91 and 6.83±1.62, respectively. There was significant ($p<0.05$) reduction in score was observed on day 7 that further decreased non-significantly till day 21. Score depicted higher values at various time intervals as compared to T2 (Table 1). It depicts better therapeutic

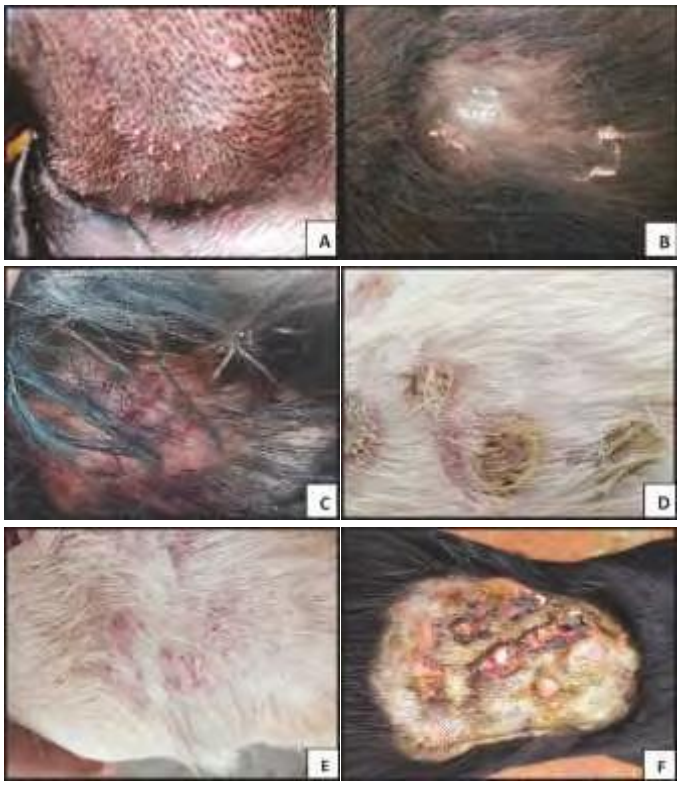

Fig. 1. Various types of skin lesions in staphylococcal pyoderma (A) Erythem -atous pustules, (B) Epidermal collarette, (C) Excoriation- erosions and uleers, (D) Large ruptured pustular moist lesions, (E) Erythematous papules and crusts with focal alopecia, (F) Ulcerative erosions with crusts, (G) Furuncles with oedema

Fig. 3. Progressive resolution of skin lesions in a dog treated with Amoxicillin-Clavulanic acid (Group T2)

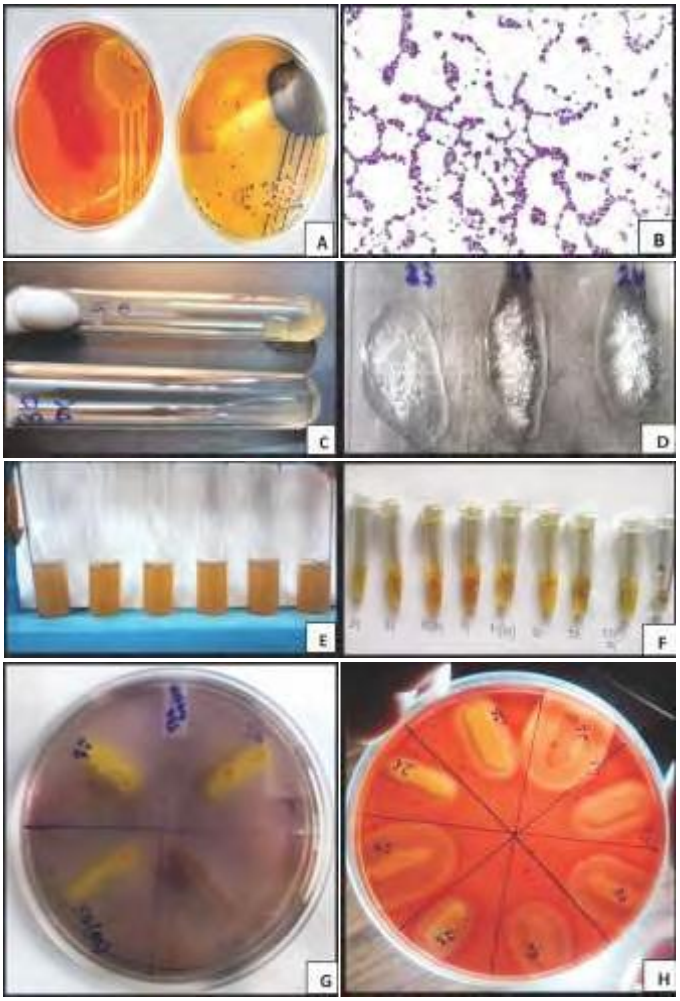

Fig. 2. Phenotypic tests used for identification of clinically significant *Staphylococcus* spp.; (A) Growth on MSA and BPA plates, (B) Gram's reaction, (C) Tube coagulase test, (D) Catalase test, (E) Voges proskaurer, (F) ONPG test, (G) Maltose fermentation *H. Haemolysis* pattern of bovine blood agar

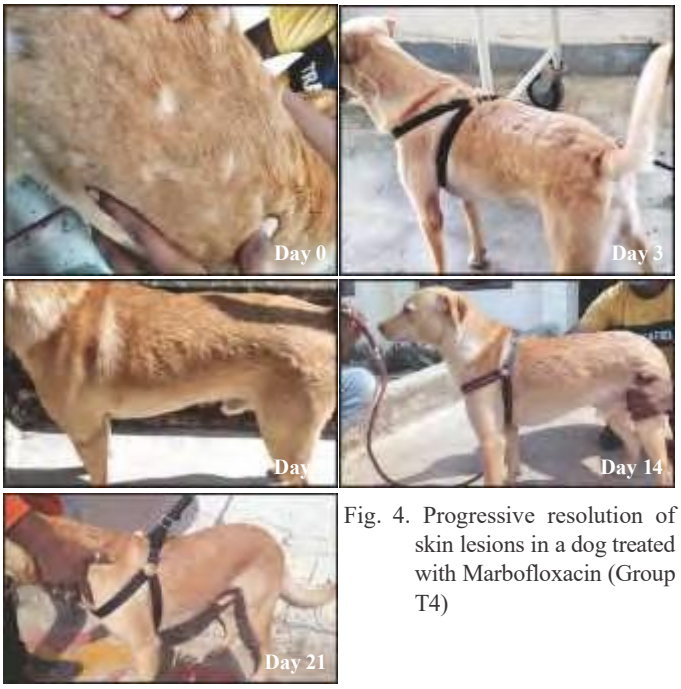

Fig. 4. Progressive resolution of skin lesions in a dog treated with Marbofloxacin (Group T4)

effect of marbofloxacin over amoxicillin-clavulanic acid.

Results based on the gross score revealed the excellent therapeutic efficacy of marbofloxacin (3.33±0.21) and moderate therapeutic efficacy of amoxicillin - clavulanic acid (6.83±1.62) on day 21 of therapeutic trial. The differences between scores in both the groups measured on day 0 and 21 were significant, indicating that these could be attributed to the treatment differences. Thus, over the days (day 0 to day 21) both treatment protocols were found to be effective in management of pyoderma cases in dogs (Figs. 3 and 4).

In general, antimicrobial efficacy of any drug depends on several pharmacodynamic and pharmacokinetic factors. Marbofloxacin, a third-generation bactericidal fluoroquinolone has been developed exclusively for animals. It acts by inhibiting bacterial DNA-gyrase enzyme, responsible for supercoiling of DNA strands of bacterial chromosomes. Disruption of above mechanism leads to degradation of DNA and lysis of bacterial cell. It has been reported to be more suitable for treatment of infectious skin diseases, because of broad spectrum activity that mainly acts on the main pathogens (*Staphylococcus* spp. and *Pseudomonas* spp.) involved in veterinary dermatology (Lloyd *et al.*, 1997). Marbofloxacin has excellent oral bioavailability (~100 %) in dogs at recommended dosage of 2 mg/kg BW (Sorgel and Kinzig, 1993). Single daily administration has been found to be sufficient to achieve minimal inhibitory concentration (MIC) for *Staphylococcus* spp. (0.23 µg/ml) (Spreng *et al.*, 1995).

Amoxicillin is broad spectrum bactericidal β-lactam penicillin and acts by impairing development of bacterial cell walls by inhibiting transpeptidase enzymes responsible for formation of cross-links between peptidoglycan strands. Transpeptidase enzymes are associated with a group of proteins called penicillin-binding proteins (PBPs). Clavulanic acid irreversibly binds and inhibits many bacterial beta-lactamase enzymes. Combination of amoxicillin with clavulanate produces a notable synergistic effect because it protects amoxicillin from hydrolysis by bacterial â- lactamase enzyme. A very short elimination half-life, i.e., 90 minutes demand frequent dosing at every 8 hours (Pachauri, 1999).

REFERENCES

Beco, L., Guaguere, E., Lorente-Mendez, C., Noli, C., Nuttall, T. and Vroom, M. (2013). Suggested guidelines for using systemic antimicrobials in bacterial skin infections: part 1-diagnosis based on clinical presentation, cytology and culture. *Vet. Rec.* **172(3)**: 72-78.

Beigh, S.A., Soodan, J.S., Tantary, H. and Tikoo, A. (2013). Comparative evaluation of antibacterial alone and antibacterial along with zinc in management of pyoderma in canines. *Intas. Polivet.* **14(2)**: 388-390.

Carlotti, D.N., Guaguere, E., Pin, D., Jasmin, P., Thomas, E. and Gulral, V. (1999). Therapy of difficult cases of canine pyoderma with marbofloxacin: a report of 39 dogs. *J. Small Ani. Pract.* **40**: 265-270.

Carlotti, D.N., Jasmin, P., Guaguere, E. and Thomas, E. (1995). Use of marbofloxacin in the treatment of canine pyoderma. *Comp. Ani. Med. Surg. Pract.* **30**: 281-293.

Devriese, L.A., Vancanneyt, M., Baele, M., Vaneechoutte, M., De-Graef, E., Snauwaert, C. and Dawyndt, P. (2005). *Staphylococcus pseudintermedius*: A coagulase-positive species from animals. *Int. J. Syst. Evol. Microbiol.* **55**: 1569-1573.

Gomez-Sanz, E., Torres, C., Ceballos, S., Lozano, C. and Zarazaga, M. (2013). Clonal dynamics of nasal *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in dog-owning household members. *Plos One.* **8(7)**: e69337.

Hillier, A., Lloyd, D.H., Scott, J., Blondeau, J.M., Boothe, D., Breitschwerdt, E., Guardabassi, L., Papich, M.G., Turnidge, J.D. and Sykes, J.E. (2014). Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis. *Vet. Dermat.* **25**: 163-e43.

Lloyd, D.H., Carlotti, D.N., Koch, H.J. and Van Den, B.A.H. (1997). Treatment of canine pyoderma with co-amoxyclav: Acomparison of two dose rates. *Vet. Rec.* **141**: 439-441.

Pachauri, S.P. (1999). Canine Preventive Medicine, (1st Edn.), Nilay Publications, Pantnagar, p. 76.

Paradis, M., Abbey, L., Baker, B., Coyne, M., Hannigan, M., Joffe, D., Pukay, B., Trettien, A., Waisglass, S. and Wellington, J. (2001). Evaluation of the clinical efficacy of marbofloxacin (Zeniquin®) tablets for the treatment of canine pyoderma: An open clinical trial. *Vet. Dermat.* **12**: 163-169.

Sasaki, T., Kikuchi, K., Tanaka, Y., Takahashi, N., Kamata, S. and Hiramatsu, K. (2007). Reclassification of phenotypically identified *Staphylococcus intermedius* strains. *J. Clin. Microbiol.* **45**: 2770-2778.

Scott, D.W., Miller, W.H. and Griffin, C.E. (2001). Small Animal Dermatology, (6th Edn.), W.B. Saunders, Philadelphia, pp. 207-273.

Sorgel, F. and Kinzig, M. (1993). Pharmacokinetics of gyrase inhibitors, Part 1: Basic chemistry and gastrointestinal disposition. *Am. J. Med.* **94**: 59-72.

Spreng, M., Deleforg, J.E., Oisrameb, B. and Drugeoh, N. (1995). Antibacterial activity of marbofloxacin: A new fluoroquinolone for veterinary use against canine and feline isolates. *J. Vet. Pharmacol. Therap.* **18**: 284-289.

Summers, J.F., Hendricks, A. and Brodbelt, D.C. (2014). Prescribing practices of primary-care veterinary practitioners in dogs diagnosed with bacterial pyoderma. *Biomed. Vet. Res.* **10**: 240.