Original Research Article

Antibiotic susceptibility pattern of bacterial pathogens isolated from canine superficial pyoderma

ABSTRACT

Out of 205 dogs affected with skin infection, 32 dogs were found positive for superficial pyoderma infections which were subjected for bacterial culture and isolation. A total of 43 bacterial isolates were recovered from the skin of 32 dogs affected with superficial pyoderma. Among the 43 bacterial isolates, *S. intermedius, S. aureus, S. epidermidis, E. coli, Klebsiella spp.* and *Pseudomonas spp.* were identified with the prevalence of 75, 18.75, 6.25, 18.75, 9.38 and 6.25 %, respectively. All the isolates were sensitive to cephalexin (100%) followed by amoxicillin clavulanic acid (95.34%), enrofloxacin (86.04 %), ceftriaxone (41.86 %), gentamicin (30.2%) and tetracycline (9.30%).

Key Words: Pyoderma, dog, staphylococcus, antibiogram

Introduction

Superficial pyoderma is one of the multi-etiological bacterial skin disease of dogs which is more common in dogs due to certain characteristics of dog's skin like thin stratum corneum with less lipid material and unprotected hair follicles that are at increased risk for bacterial invasion and subsequent colonization and overgrowth. This may lead to a higher incidence of primary inflammatory disease that affects the first-line defenses (Pinchbeck, 2010). The common lesions of this disease include follicular papules, which may or may not be crusted, erythema, alopecia, epidermal collarettes and hyperpigmentation. *Staphylococcus intermedius* is coagulase

positive microorganism which is most predominantly isolated from an infected canine. However, other causative agents like *Proteus spp, Escherichia coli, Pseudomonas spp, Actinomyces spp, Fusobacterium spp, Actinobacillus spp,* and *Mycobacterium spp* may also cause pyoderma (Debouer, 1995, Scott *et al*., 1995 and Paradis *et al*., 2001).

Staphylococcus intermedius has long been considered as the most common cause of pyoderma.. This is no longer the case, as the main canine pathogen is now known to be *Staphylococcus pseudintermedius.* This is not a new organism, but simply a new name for the organism that has always been the cause of these infections (Thomas, 2010). Recurrent superficial pyoderma is much more common than recurrent deep pyoderma. Idiopathic recurrent pyoderma is mostly due to persistent underlying skin disease, bacterial hypersensitivity, immunodeficiency, resistant strains of *Staphylococcus intermedius* and non-staphylococcal pyoderma. Currently, the diagnosis of canine pyoderma is based on history, clinical examination and complementary examinations like skin scrapping, bacterial culture and antibiotic sensitivity. Bacterial culture and sensitivity tests are only occasionally used to confirm the diagnosis and to choose an appropriate antibiotic in non-responsive cases. The nonjudicious use of antibiotics causes the resistance in animals. Keeping in view, the facts of skin affection and resistance of bacterial pathogen towards antibiotics, this study was planned to determine the antibiotic susceptibility pattern of bacterial pathogen isolated from cases of superficial pyoderma in dogs.

Materials and methods

Swab samples containing skin exudate were used for preparing smears and microscopically examining the presence of bacteria. Swabs collected from dogs affected with superficial pyoderma were inoculated into nutrient broth and incubated at 37^0C for 24 hours and then a loopful of broth culture was streaked on nutrient agar plates for primary isolation of

bacteria. Based on morphology and Gram's staining properties, cultures were inoculated into specific / selective media like Mannitol salt agar, Eosine Methylene Blue agar, MacConkey agar and Blood agar.

Primary identification of bacteria was done based on Gram's staining, colony characteristics, type of haemolysis and pure cultures were identified up to genus level as described by Holt *et al*. (1994) and by biochemical as described by

Antibiotic susceptibility test

The bacterial isolates were subjected to antibiotic susceptibility test as described by Bauer *et al*. (1966). Antimicrobial susceptibility testing was done by agar disc diffusion method. Firstly, a 0.5 McFarland turbidity standard was prepared. Further, 4-5 isolates colonies were picked up and transferred into a tube containing 5 ml tryptone soy broth and vortexed thoroughly. The bacterial suspension was then compared to the 0.5 McFarland turbidity standards (Quinn *et al*., 1994). The turbidity was adjusted by adding more amount of sterile broth or adding more bacterial culture. A sterile cotton swab was dipped into the adjusted suspension and rotated several times and finally pressed firmly on the inside wall of the tube in order to remove the excess inoculum from the swab. The swab culture was smeared on to Mueller Hinton agar plate and allowed to dry for 3-5 minutes. The predetermined antimicrobial 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. To conduct antibiotic sensitivity test, 6 antibiotic discs were selected viz., Cephalexin (CN 30mcg), Amoxicillin with Clavulanic acid (AMC10mcg), Enrofloxacin (EX 10mcg), Ceftriaxone (CTR 30 mcg), Gentamicin (GEN 30mcg) and Tetracycline (TE 30 mcg). All the 6 antibiotic discs were placed on one agar plate. The plates were placed in an inverted position in an incubator maintained at 37°C for 24 hours.

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 sensitivity pattern of the isolates for the respective antibiotics.

Results and Discussion

The present study was conducted on 205 dogs of different of sex, age and breeds which were affected with dermatological affections for a period of one year in which 32 dogs were found to be affected with superficial pyoderma. All the 32 cases were subjected for isolation and identification of bacterial pathogens and determination of their antibiotic susceptibility patterns.

Isolation and identification of bacteria

Detailed cultural examination was carried out in 32 superficial pyoderma samples from which 43 bacterial isolates were obtained and identified. All the 32 samples were found to be positive for *Staphylococcus spp.* (100%), out of which 24 isolates were *S. intermedius* (75%), six isolates were identified as *S. aureus* (18.75%) and two isolates were identified as *S. epidermidis* (6.25%). *Staphylococcus spp.* is the major bacterium reported to be cause of bacterial skin infection in dogs (Sindha *et al*., 2015; Ankita and Gandge, 2018). Eleven samples revealed mixed infection and among mixed infections, staphylococci along with *E. coli* were observed in 6 samples (18.75%), staphylococci with *Klebsiella spp.* in 3 samples (9.38%) and staphylococci along with *Pseudomonas spp.* were observed in 2 dogs (6.25%) (Table 1).

Table 1: Various bacterial isolates associated with superficial pyoderma in dogs

S.No.	Type of bacteria	Number of samples	Percentage (32)
1	***Staphylococcus* species**	**32**	**100**
a	*S. intermedius*	24	75

b	*S. aureus*	6	18.75
c	*S. epidermidis*	2	6.25
2	**Mixed bacterial infection**	**11**	**34.38**
a	*Staphylococcus + E. coli*	6	18.75
b	*Staphylococcus + Klebsiella*	3	9.38
c	*Staphylococcus + Pseudomonas*	2	6.25

The antibiotic susceptibility pattern of bacterial isolates from superficial pyoderma in dogs is presented in Table 2. Antibiotic susceptibility test was conducted on test isolates. It was revealed that all 24 isolates of *S. intermedius* analyzed in the present investigation were sensitive (100%) to cephalexin followed by 95.83% (23/24) of the isolates which were sensitive to amoxicillin with clavulanic acid while 87.50% (21/24) isolates of *S. intermedius* were sensitive to enrofloxacin. Likewise, 33.33% (8/24) and 12.50% (3/24) isolates were sensitive to ceftriaxone and gentamicin, respectively. Further it was revealed that 50%, 87.50% and 95.83% of isolates of *S. intermedius* were resistant to ceftriaxone, gentamicin and tetracycline, respectively.

Among the 6 isolates of *S. aureus*, all the isolates were 100% (6/6) sensitive to cephalexin and amoxicillin-clavulanic acid followed by 83.33% (5/6) isolates which were sensitive to enrofloxacin, 33.33% (2/6) sensitive to ceftriaxone and 16.67% (1/6) isolates were sensitive to gentamicin. Out of 6 *S. aureus* isolates, 5 (83.33%) were resistant to gentamicin, followed by 50.00% (3/6) to ceftriaxone and 100% (6/6) to tetracycline.

All the isolates of *S. epidermidis* (n=2) were sensitive to cephalexin, amoxicillin-clavulanic acid and enrofloxacin. Out of 2 isolates of *S. epidermidis* 50% (1/2) isolates were sensitive to gentamicin. The isolates of *S. epidermidis* 100% (2/2) were resistant to ceftriaxone and tetracycline.

Antibiotic sensitivity pattern of *E. coli* revealed that all the isolates were 100% (6/6) sensitive to cephalexin, enrofloxacin and ceftriaxone. Whereas, 5 (83.33%) isolates of *E. coli* were sensitive to amoxicillin-clavulanic acid followed by 66.67% (4/6) sensitive to gentamicin and 50% (3/3) sensitive to tetracycline. Among the 6 isolates of *E. coli*, 33.33% (2/6) isolates were resistant to gentamicin.

All the *Klebsiella spp.* isolates were sensitive to cephalexin and amoxicillin-clavulanic acid whereas 66.67% (2/3) isolates were found sensitive to enrofloxacin, ceftriaxone and gentamicin each. Among the 3 isolates of *Klebsiella spp*, 33.33% (1/3) were sensitive to tetracycline. One isolate (33.33%) was found resistant for ceftriaxone and gentamicin both.

All the isolates of *Pseudomonas* were found to be sensitive to cephalexin and amoxicillin-clavulanic acid and gentamicin, while 50% (1/2) isolates were sensitive to enrofloxacin. All the *Pseudomonas* isolates were resistant to ceftriaxone and tetracycline and 50 % (1/2) were resistant to enrofloxacin.

In the present study, the most effective antibiotic was found to be cephalexin which was in agreement with the findings of Jaham (2003), Blanco & Wolberg (2004), Sprucek *et al.* (2007) and Toma *et al.* (2008). Amoxicillin-clavulanic acid was also found effective to treat cases of canine superficial pyoderma in dogs. Similar findings were reported by Beco *et al.*, 2013 and Bajawa, 2016. Also, enrofloxacin was found to be effective against canine superficial pyoderma which was in accordance with the findings reported by Paradis, 1990.

Table 2: Antibiotic susceptibility pattern of bacterial isolates from superficial pyoderma in dogs

S.No.	**Antibiotic**	**Response of antibiotic**	*S. intermedius* **(24)**	*S. aureus* **(6)**	*S. epidermidis* **(2)**	*E. coli* **(6)**	*Klebsiella* **(3)**	*Pseudomonas* **(2)**

1.	Cephalexin	Sensitive	24 (100%)	6 (100%)	2 (100%)	6 (100%)	3 (100%)	2 (100%)
		Intermediate	-	-	-	-	-	
		Resistant	-	-	-	-	-	-
2.	Amoxicillin-clavulanic acid	Sensitive	23 (95.83%)	6 (100%)	2 (100)	5 (83.33%)	3 (100%)	2 (100%)
		Intermediate	1 (4.17%)	-	-	1 (16.66%)	-	
		Resistant	-	-	-	-	-	-
3.	Enrofloxacin	Sensitive	21 (87.5%)	5 (83.33%)	2 (100)	6 (100%)	2 (66.67%)	1 (50%)
		Intermediate	3 (12.5%)	1 (16.67)	-		1 (33.33%)	
		Resistant	-	-	-		-	1 (50%)
4.	Ceftriaxone	Sensitive	8 (33.33%)	2 (33.33%)	-	6 (100%)	2 (66.67%)	-
		Intermediate	4 (16.67%)	1 (16.66%)	-	-	-	-
		Resistant	12 (50%)	3 (50%)	2 (100%)	-	1 (33.33%)	2 (100%)
5.	Gentamicin	Sensitive	3 (12.5%)	1 (16.67%)	1 (50%)	4 (66.67%)	2 (66.67%)	2 (100%)
		Intermediate	-	-				-
		Resistant	21 (87.5%)	5 (83.33%)	1 (50%)	2 (33.33%)	1 (33.33%)	-
6.	Tetracycline	Sensitive	-	-	-	3 (50%)	1 (33.33%)	
		Intermediate	1 (4.17%)	-	-	3 (50%)	2 (66.67%)	
		Resistant	23 (95.83%)	6(100%)	2 (100)	-		2 (100%)

Conclusion

In the present study, it was concluded that *Staphylococcus spp*. was a predominant organism found in superficial pyoderma affected dogs. All the isolates of *Staphylococci* and majority of Gram negative bacteria were sensitive to cephalexin, amoxicillin-clavulanic acid and enrofloxacin.

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