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Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus* species in Canine Skin Infection

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A B S T R A C T

Bacterial skin infections in dogs are a very common clinical problem and almost every dermatological disease of the dog can have bacterial pyoderma as a component. This study investigated the incidence of *Staphylococcus spp*. and proportion of antimicrobial resistant patterns of Staphylococcus isolates from skin scraping samples of dog. 186 samples were collected from private pet clinic. Proceed for isolation of *Staphylococcus* species and verified for susceptibility to gentamicin, chloramphenicol, vancomycin, ceftriaxone, cephotaxime, methicillin, oxacillin and penicillin-G by agar disc diffusion method. Out of 186, 125 cases were positive for bacterial skin infection i.e., 67.2%. Among 125*Staphylococcus* recovered, 100(80%) isolates of *Staphylococcus* Intermedius, 20(16%) isolates of *Staphylococcus aureus* and 5(4%) isolates of *Staphylococcus epidermidis* were characterized by use of conventional biochemical tests and sugar fermentation test. The incidence rate of age group 1-3 years (67.18%) showed greater susceptibility to dermatological conditions. Male dogs showed greater involvement in bacterial dermatitis. *S. intermedius* were sensitive to gentamicin 98(98%) followed by chloramphenicol 70(70%) and vancomycin 66(66%), intermediate to cephotaxime 46(46%) and ceftriaxone 20(20%). *S. aureus* were sensitive to ceftriaxone 12(60%) followed by gentamicin 9(40.9%) and vancomycin 8(36.36%), intermediate to chloramphenicol 5(22.7%) and cephotaxime 6(27.27%). *S. epidermidis* were sensitive to chloramphenicol 4(80%) and vancomycin 3(60%), intermediate to ceftriaxone 1(20%) and gentamicin 1(20%), resistant to cephotaxime 3(60%) were seen in some cases. In common, gentamicin and vancomycin were the most active antimicrobial agent. All the isolates of The *Staphylococcus* isolates were found to be resistant methicillin, oxacillin and penicillin- G. Hence, multiple drug resistant were obtained in *Staphylococcus* spp. The increasing frequency of multidrug resistance complicates the selection of antimicrobial therapy.

Introduction

Dogs are the members of order Carnivore, a group of mammals with origin in the tertiary era, about 55 million years ago (Pachauri *et al.*, 1999). The strong bonding between dog owners and pet dogs leads to concern about health and well-being of their pets and therefore the affected dogs are often brought to veterinary clinics for diagnosis and

treatment of infectious skin diseases, amongst which the cases of skin infections are very common. Skin disorders are among the most common health problems in dogs. In small animal clinics, dermatological disorders constitute a majority of cases and are estimated to range between 12% and 75% as the chief or concurrent owner complaint. (Sindha *et al.,* 2015) There are so many agents that cause dermatitis including bacteria, fungi, yeasts and also other factors such as age, season and autoimmune hormonal and inadequate or unbalanced nutrition (Sharma *et al.,* 2008). The condition of dog's skin and coat is an important indicator of its general health (Manjul *et al.,* 2012). Skin disorders of dogs vary from acute, self-limiting problems to chronic or long-lasting problems requiring life-long treatment. They also need to be differentiated on the basis of being of primary or secondary (due to scratching, itch) in nature, making diagnosis is complicated. The diagnosis can be made on the basis of history, physical examination, skin scrapping examination, cultural isolation and conventional identification method by (Shalaby *et al.,* 2016).

Bacterial skin infections in dogs are a very common clinical problem and almost every dermatological disease of the dog can have bacterial pyoderma as a component (Srivastava *et al.,* 2011). Bacterial Pyodermas are a common cause of skin disease in the dog (Ihrke *et al.,* 2006) and are caused by infectious *Staphylococcus intermedius*, *Staphylococcus aureus* and *S. epidermidis* and also common secondary complication of a range of canine skin diseases, including allergic, endocrine, seborrhoeic and follicular disorders (Leib and Monoroe, 1997and Morris, 2010). The diagnosis of superficial bacterial pyoderma is based on the presence of variable gross cutaneous lesions (papules, pustules, epidermal collarets, erythema, crusting, and hyperpigmentation), positive findings on direct microscopic examination of glass slide impression smears revealing bacteria. Hot Spots or Acute Moist Dermatitis is an acutely inflamed and infected area of skin irritation created and made worse by a dog due to licking and biting itself. A hot spot can manifest and spread rapidly in a matter of hours as secondary *Staphylococcus* infection causes the top layers of the skin to break down and as pus becomes trapped in the hair.

Treatment of superficial bacterial pyoderma frequently involves the use of systemic and/or topical antimicrobial agents. The causative agents, *S. pseudintermedius*, *Staphylococcus aureus* and *S. intermedius* have historically been considered slow to acquire resistance to antimicrobial groups, with high levels of susceptibility to common, empirically chosen antimicrobials such as first-generation cephalosporin and potentiated penicillins. Methicillin-resistant *Staphylococcus* species are considered resistant to all other β-lactams, including cephalosporin and amoxycillin–clavulanate, irrespective of susceptibility test results. (Bannoehr *et al.,* 2009).

In view of this, the present research was planned for isolation and identification of bacteria and to study *in vitro* antimicrobial susceptibility pattern in order to select the effective therapeutic agent for treatment of canine skin infections.

Materials and Methods

The present study was carried out at Department of Veterinary Microbiology, Bombay Veterinary College, Parel, Mumbai during the period from January 2017 to December 2017. This study had been approved by the institutional ethics committee for veterinary clinical research. A written informed consent was obtained from the patients' owners before collecting the samples for diagnosis. The skin scraping samples of

dogs were obtained from Pet Clinics, Mumbai and Veterinary College hospitals. The specimens were preferred from dogs with symptoms and sign suggestive of clinical skin infections viz discoloration, thickening, pruritus, alopecia, red patches, crusted skin, musky odour, dry flaky skin around the ear, eyes, abdominal and limbs regions.

The suspected skin lesions of the dogs were cleaned to remove contaminants, scrapings were taken with a sterile scalpel blade and collected in a clean paper, labelled the details (Fig.1). The collected specimens were transported to the laboratory and processed as early as possible. The skin scraping samples were directly inoculated on the sterile plates of nutrient agar (Hi Media Lab. Pvt. Ltd.) and incubated at 37˚C for 24 hrs and plates were observed for appearance of typical colonies of *Staphylococcus* spp. and the smears of colonies stained with Gram's staining. The isolates showing Gram positive cocci in clusters, suggestive for *Staphylococcus* spp. were subjected to conventional bacteriological procedures for confirmation at species level.The isolates were further streaked on Mannitol salt agar (MSA) medium and Baird parker agar (BPA) and incubated at 37˚C for 24-48 hrs. The colonies showing typical characters suggestive of *Staphylococcus* spp. were further confirmed by various biochemical and sugar fermentation test viz catalase, oxidase, MR, VP, urease test, citrate test, Trehalose and maltose (Ravens *et al.*, 2014).

Antibiotic sensitivity test of *Staphylococcus* spp. were subjected by disc diffusion method as described by (Bauer *et al.*, 1966) using following Vancomycin (30mcg), Penicillin-G (10unit), Chloramphenicol (30mcg), Ceftriaxone (30mcg), Cephotaxime (30mcg), Gentamicin (10mcg), Methicillin (10mcg), Oxacillin (1mcg) antibiotic discs from Hi Media.

Results and Discussion

A total of 186 skin scraping samples were processed during investigation from suspected skin infection cases of dogs. Although number of bacteria are involved in skin infections, *Staphylococcus* spp. is the major bacteria reported to be cause of bacterial skin infection in dogs (Sindha *et al.*, 2015). Therefore, investigation of specimens was performed pertaining to *Staphylococcus* species.

In the present investigation, a total of 186 skin scraping samples were processed to find out the bacterial etiology, especially *Staphylococcus* spp. by using different media.

Similar specimens were used for attempting isolation of *Staphylococcus* spp. by Singh *et al.*, (2012), Ravens *et al.*, (2014) and Sindha *et al.*, (2015), who also achieved isolation of different species of *Staphylococcus* from the skin scraping samples successfully.

Identification of major species of Staphylococcus genus was carried out by conventional methods viz, cultural characteristics, morphology, biochemical tests, sugar fermentation tests etc (from Fig.2 to Fig.6).Talan *et al.*, (1989), Zubeira*et al.*, (2007), Rubin *et al.*, (2011) and Griffeth *et al.*, (2008) identified different *Staphylococcus* spp. by similar conventional methods.

Out of 186 skin scraping samples processed during investigation, bacterial etiology was found in 125 (67.2%) cases, this incidence rate is corroborated with Griffeth *et al.*, (2008) who reported 74% of prevalence of *Staphylococcus* spp, The incidence rate of 67.20% of present study laid between the incidence rate observed by Sindha *et al.*, (2015) and Chitra*et al.*, (2016) who reported 80% and 59% incidence rate respectively. Other workers viz. Vijaya kumar *et al.*, (2011) and Singh *et al.*, (2012) reported lower rate of

bacterial etiology i.e. 13.71% and 25% respectively. Whereas, higher prevalence of *Staphylococcus* spp. i.e. 94% is reported by Sousa *et al.,* (2014). Among 125 *Staphylococcus* spp. recovered, the incidence of *Staphylococcus intermedius, Staphylococcus aureus, Staphylococcus epidermidis* was found in100 (80%), 20(16%) and 5(4%) cases of canine skin infections respectively. The species wise results of present study correlated with Sousa *et al.,* (2014) who identified *S. intermedius* approximately 84%, and *S. aureus* whereas less common cause of bacterial infection approximately 1%. Griffeth *et al.,* (2008) isolated *Staphylococci* from 74% cases of dogs, which included *S. aureus* (16%*), S. intermedius* (92%).Thus, the result of present evaluated with result of above the authors quoted. The incidence of *Staphylococcus* spp. in age group of 1-2 yrs was 72% (fig.7) similar observation of age wise prevalence rate is reported by Vijayakumar*et al.,* (2011) while Singh *et al.*, (2012) studied the highest incidence of *Staphylococcus spp* in the dogs of 1-3 years age group (44.54%) and Singh *et al.,* (2012) revealed that the dogs of less than one year of age (41.66 %) were most susceptible followed by dogs of one to two years of age (27.78 %).

Table.1 Gender wise incidence of bacterial skin infections

Gender	Total nos. of dogs tested	Number of affected	Percentage
Male	124	101	80.8%
Female	62	24	19.2%
Total	**186**	**125**	**100%**

Table.2 Summary of antibiogram patterns of *Staphylococcus* spp isolates

Sr. No	Antibiotics used	*S. aureus*			*S.intermedius*			*S.epidermidis*		
		S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
1.	Gentamicin	9	11	0	98	2	0	1	4	0
2.	Vancomycin	8	12	0	66	16	18	3	2	0
3.	Chloramphenicol	5	15	0	70	8	12	4	1	0
4.	Ceftriaxone	12	8	0	20	78	2	1	4	0
5.	Cephotaxime	6	10	4	46	54	0	1	1	3
6.	Methicillin	0	0	20	0	0	100	0	0	5
7.	Oxacillin	0	0	20	0	0	100	0	0	5
8.	Penicillin-G	0	0	20	0	0	100	0	0	5

Fig.1 Collection of skin scraping sample from dog

Fig.2 *S. aureus* showing Mannitol fermentation on MSA

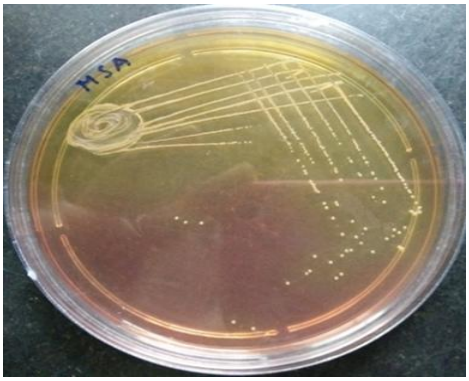


Fig.3 *Staphylococcus* spp showing grams positive cocci in clusters

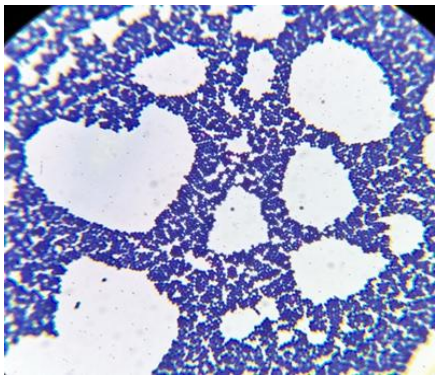


Fig.4 *S. intermedius* showing MR positive and VP negative

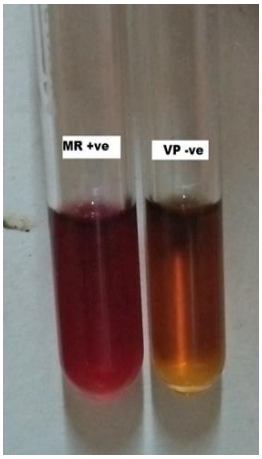


Fig.5 *S. intermedius* showing trehalose positive and maltose negative

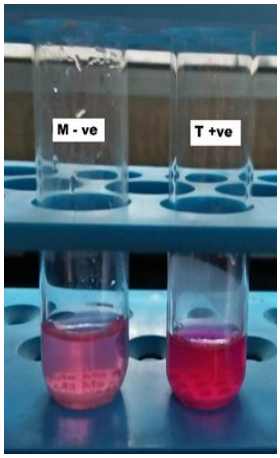
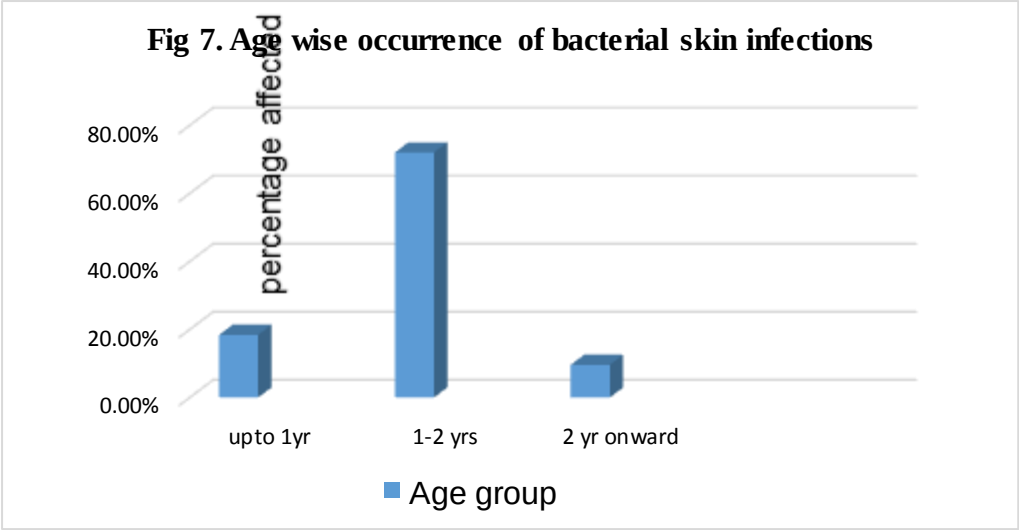


Fig.6 *S. aureus* showing citrate positive test



In present investigation, involvement of male dogs (80.8%) was observed more than female dogs (19.2%), this finding was in agreement with Patil*et al.,* (1999) and Singh *et al.,* (2012) who reported similar observations whereas, Kubesy*et al.,* (2017) reported contrast finding of higher prevalence rate in female than male (Table 1). All 125 *Staphylococcus* isolates recovered from skin scraping sample of dogs were tested for susceptible to eight suitable antibiotics by disc diffusion method (Table 2). *S. intermedius* (100) isolates showed highest sensitivity to gentamicin 98 (98%) followed by chloramphenicol 70(70%) and vancomycin 66(66%), intermediate to cephotaxime 46(46%) and ceftriaxone 20(20%), similar results obtained by Bloom *et al.,* (2014).

The result of showed multiple drug resistant Kelany *et al.,* (2011) correlated with who revealed (50%) *S. intermedius* were resistant to oxacillin contrast findings was observed by Petersen *et al.,* (2002) who observed 95% of *S. intermedius* isolates were susceptible to oxacillin.

S. aureus (20) isolates displayed highest sensitivity to ceftriaxone 12(60%) followed by gentamicin 9(40.9%) and vancomycin 8(36.36%), intermediate to chloramphenicol 5(22.7%), cephotaxime 6(27.27%) and resistant to penicillin G and oxacillin, results are supported by Lilenbaum *et al.,* (1998) who observed gentamicin as most effective antimicrobial agent and resistance to penicillin G and oxacillin.

Whereas, *S. epidermidis* (5) isolates of were highly sensitive to chloramphenicol 4(80%) followed by vancomycin 3 (60%), intermediate to ceftriaxone 1(20%) and gentamicin 1(20%), resistant to cephotaxime 3 (60%). The overall results of antibiotic susceptibility test of *Staphylococcus* spp. indicated maximum resistant to methicillin (100%), penicillin G (100%) and oxacillin (100%). The least resistant was observed to cephotaxime (3%).

The similar result of resistant pattern to penicillin group drugs was observed by Lilenbaum*et al.,* (1998) while, methicillin resistant was observed by Kania*et al.,* (2004) and oxacillin resistant was observed by Rebekah *et al.,* (2005) and Raven *et al.,* (2014).

In conclusion, canine skin infections are widely prevalent all over world and have become a major problem causing economic losses and zoonotic infections.

The current epidemiologic trends in the prevalence and knowledge of the exact causative agents of skin infections of dog may play an important key role in prevention and control of infections and modification of current treatments.

The present study emphasizes the need for bacterial isolation with species identification and antimicrobial susceptibility test in order to choose the appropriate antimicrobial agent for treatment of canine skin infections.

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