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Prevalence of Bacterial and Fungal Pathogens of Canine Dermatitis and Otitis and their Antimicrobial/Antifungal Susceptibility and Resistance Pattern in Chittoor District, Andhra Pradesh

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A B S T R A C T

Canine dermatitis and otitis cases are widely prevalent all over the world. The present study was aimed to find out the etiological agents for canine dermatitis and otitis cases and their antimicrobial/antifungal susceptibility for treatment purposes. A total of 275 skin scrapings and 189 ear swabs from canine dermatitis and otitis cases were collected and processed during the period from August, 2015 to March, 2020 at State Level Diagnostic Laboratory, SVVU, Tirupati. Out of 275 skin scrapings, parasitic -15 (5.45%), bacterial-63 (22.91%), fungal-159 (57.80%) and mixed infections -38 (13.82%) and out of 189 ear swabs from otitis cases, bacterial-33(17.50%), fungal-159 (57.81%) and mixed infections -19 (10.10%) were reported. Upon processing of skin scrapings and ear swabs, Malassezia pachydermatis was found to be major etiological agent with percent prevalence of 57.81 & 51.85 respectively. Among bacterial, Staphylococcus species found to be predominant followed by Pseudomonas species. Invitro antimicrobial sensitivity of staphylococcal isolates from dermatitis and otitis cases showed high sensitivity to Enrofloxacin and Ciprofloxacin, but resistance to Gentamycin, Ampicillin, Cefatriaxome and Pencillin. *Pseudomonas* isolates showed high sensitivity to Gentamycin and resistance to Ampicillin, Cefatriaxome, Enrofloxacin, towards canine dermatitis. Whereas isolates from Canine otitis cases showed high sensitivity to Gentamycin and Enrofloxacin but resistance to Ciprofloxacin and Streptomycin. Malassezia pachydermatis isolates showed high sensitivity to Ketoconozole and Itraconozole and resistance to Nystatin towards canine dermatitis and otitis cases.

Introduction

Skin disorders are one of the most commonly encountered health problems in pet animals. Dogs are susceptible to various skin disorders and it may be parasitic, fungal and bacterial skin infections or allergies of various origins. These conditions become worse in hot and humid climate therefore becoming difficult to resolve. This not only deteriorates the cosmetic value of the animal but also pose a risk to its general health and utility.

In small animal clinics, dermatological disorders constitute a major part and are estimated to range between 12% and 75% (Sindha *et al.,* 2015). A part from bacteria, fungi and yeasts, other factors such as age, season, hormonal, autoimmune and in-adequate or unbalanced nutrition playing a role (Sumathi *et al.,* 2009). The condition of dog skin and coat is an important indicator of its general health (Mauldin *et al.*, 2002). The skin disorders of the dogs vary from acute, self limiting problems to chronic or long – lasting problems requiring life-long treatment. They may be differentiated as primary or secondary (due to scratching and itching) in nature. The diagnosis can be made on the basis of history, physical examination, examination of skin scrapings, cultural and conventional identification methods (Shalaby *et al.,* 2016)

Otitis externa (OE) which is the inflammation of external auditory canal is the most common ear disease of canine and fenine (Guillot *et al.,* 2003; Rougier *et al.,* 2005). Although otitis externa is not a life threatening disease. It can be frustrating for both the patient and owner. The prevalence of otitis externa is estimated between 5 to 20% (Rougier *et al.*, 2005). The etiology of canine otitis externa is complicated and involves many aspects that can be classified as predisposing, primary and perpetuating. Bacteria, yeast and progressive pathologic changes are considered as perpetuating factors and they are responsible for aggravation of the process and therefore avoid spontaneous resolutions (Rosser *et al.*, 2004; Lodh *et al.*, 2014). Common organisms isolated from dogs with otitis externa include Staphylococcal species, Pseudomonas species, Streptococcus, *E.coli*, Proteus, Klebsiella species and Pasturella species and *Malassezia pachydermatis* (Keskin *et al.*, 1999).

The lipid independent species of *Malasezzia pachydermatis* is a saprophyte of normal canine skin and may act as an opportunistic agent. Although *M. pachydermatis* is a saprophyte of normal canine skin (Bond *et al.*, 1995; Lyskova *et al.,* 2007). It has been regarded as a secondary pathogen on the skin of dogs affected by seborrhoeic dermatitis and ceruminous otitis externa Manjul (2012) in which the yeast is often highly pro inflammatory. Yeast infections are becoming more prevalent. The use of antifungal agents for prophylaxis and treatment of fungal infections may result in the emergence of drug-resistant fungal pathogens.

The present study help in identifying the etiological agents of canine dermatitis and otitis and *in vitro* antimicrobial and antifungal sensitivity tests against the known etiological agents and help in taking quick decision in selecting antimicrobials for effective treatment of canine dermatitis and otitis cases.

Materials and Methods

A total of 275 skin scrapings from dermatitis cases and 189 ear swabs from otitis cases were collected from canines presented to the Clinics, College of Veterinary Science, Tirupati and Veterinary hospitals of Chittoor District during the period from August, 2015 to 2020, March. The skin scrapings were initially screened against parasitic infections. Later all the samples were subjected for both bacterial as well as fungal isolation studies.

Isolation

Inoculation into nutrient broth and Sabroud’s broth:-

Skin scrapings and ear swabs after processing were inoculated into nutrient broth and Sabroud’s broth in duplicates and keep one at room temperature and another at 37^0C for 24hrs for bacterial studies and 48 to 72 hrs for fungal studies.

b) Selective media

The samples from nutrient broth are streaked onto the nutrient agar, blood agar and Mannitol salt agar media for the isolation of pathogenic bacteria and keep at 37^0C for 48hrs. Similarly, the samples from sabouraud's broth are streaked on to the modified sabourauds dextrose agar medium and keep at one at room temperature and another at 37^0C for 4 to 7 days for isolation.

c) Bio-chemical tests

Bio-chemical tests viz. catalase test, coagulase test, oxidase test, Indole production test, Citrate test etc were performed as per the protocols mentioned in the text book of clinical Veterinary Microbiology by Markey et al (2013).

d) Direct microscopic examination

Pure colonies from nutrient agar, blood agar, Mannitol salt agar and Sabouraud's dextrose agar media stained with Grams and Methylene blue staining and observe for the characteristic morphology of the organisms under microscope.

e) Antibiogram

All the recovered isolates, both bacterial and fungal are subjected for *in vitro* antibiotic and antifungal sensitivity test according to the method of Kirby Bauer of disc diffusion method (1966). The sensitivity and resistance patterns were recorded with the zone of inhibition and compared with zone size interpretative chart furnished by the manufacturer of antibiotics (Hi-media).

Results and Discussion

A total of 275 skin scrapings from canine dermatitis cases and 189 ear swabs from canine otitis cases were subjected for processing and screened against parasitic, bacterial and fungal infections. Out of 275 skin scrapings, parasitic -27(9.82%), bacterial-63 (22.91%), fungal-159 (57.80%) and mixed -26 (9.45%) respectively were found to be positive (Table 1). Similarly, out of 189 ear swabs from canine otitis cases, bacterial-81(42.86%), fungal-98 (51.85%) and mixed infections -10 (5.29%) were recorded (Table 2).

On cultural isolation, out of 63 bacterial isolates from skin scrapings of canine dermatitis cases, 48 (76.19%) were of staphylococcal species found to be dominant pathogen followed by Pseudomonas species -15 (23.81%). Up on processing, out of 189 ear swabs,98 fungal isolates (51.85%) were found to be *Malasezzia pachydermatis* from canine otitis cases, whereas among 81 bacterial isolates from canine otitis cases, revealed the presence of Staphylococcal species -64 (71.01%) and 17 (20.99%) -Pseudomonas species respectively on cultural isolation (Table 3).

The samples which streaked on blood agar media showed hemolytic colonies indicating pathogenicity of the isolates. On mannitol salt agar, Staphylococcal species produced golden yellow with round glistening colonies on Mannitol salt agar (fig.1) Pseudomonas isolates produced large, flat, irregular clear zone of hemolytic colonies on blood agar media (fig.2), on Macconkey's agar media large pale colonies and bluish green pigmented colonies on nutrient agar media (fig.3 and 4).

The samples which streaked on saboraud's dextrose agar media showed small smooth shiny colonies round to convex shape with yellowish white color after 7 days of incubation suggestive of Malassezia.

Pure colonies from mannitol salt agar and blood agar on grams staining revealed characteristic bunch of grapes of staphylococcal species (fig.5). Colonies from blood agar and nutrient agar and macconkey's agar revealed medium sized slender gram negative rods on grams staining indicative of Pseudomonas species (fig.6). All the isolates were catalase, oxidase and urease positive further confirming pseudomonas species biochemically.

Colonies from Saboraud's dextrose agar media upon staining with methylene blue revealed characteristic footprint or bottle shaped appearance suggestive of *Malassezia pachydermatis* (Fig. 7). All the isolates were found to be positive for urease test and which was one of the important confirmatory bio-chemical test for *Malassezia pachydermatis*. *In vitro* antibiotic sensitivity and resistance pattern of Staphylococcal isolates from skin scrapings (63) revealed sensitivity to Enrofloxacin-44 (69.84%; 30.16%) followed by Ciprofloxacin-38 (60.32%; 39.68%), Amikacin-26 (41.27%; 58.73%), Pencillin-24 (38.10%; 61.90%) and Gentamycin-21 (33.33%; 66.67%) whereas for Staphylococcal isolates from 64 otitis ear swabs showed sensitivity and resistance to Enrofloxacin-42 (65.63%; 34.37%) followed by Ciprofloxacin-35 (54.69%; 45.31%), Streptomycin-29 (45.31%; 54.69%), Pencillin-24 (37.50%; 62.50%), Ampicillin-21 (32.81%; 71.89%), Amikacin-18 (28.13%; 71.89%), and Ceftrixozome-15 (23.44%; 76.56%) respectively (Table 4 and Fig. 8).

Table.1 Details of samples screened from canine dermatitis cases

S.No.	Year	Total milk samples screened/testes	Details of positivity				Total
			parasitic	bacteria	fungal	Mixed	
1.	2015-16	45	4	15	23	3	45
2.	2016-17	55	8	9	34	4	55
3.	2017-18	58	5	11	36	8	50
4.	2018-19	65	6	13	39	5	63
5.	2019-20	52	4	15	27	6	52
Grand total		275	27	63	159	26	275
% Positivity			**9.82**	**22.91**	**57.82**	**9.45**	**0.14**

Table.2 Details of samples screened from canine otitis cases

Year	Total samples screened	Details of Positivity		
		Bacterial	**Fungal**	**Mixed**
2015-16	24	9	13	2
2016-17	42	18	21	3
2017-18	39	20	17	2
2018-19	40	15	23	2
2019-20	44	19	24	1
	189	81	98	10
Percent positivity		(42.86)	(51.85)	(5.29)

Table.3 Details of bacteria isolated from canine dermatitis and otitis cases

S.No	Name of the organism isolated	Canine dermatitis	Canine Otitis
1.	*Staphylococcus*	48 (76.19%)	64 (79.01%)
2.	*Pseudomonas*	15 (23.81)	17 (20.19%)
	Total	**63**	**81**

Table.4 Details of antimicrobial susceptibility and resistance patterns for Staphylococcus species from canine dermatitis and otitis cases

S. No	Type of antibiotic used	No. of isolates	Canine dermatitis (N=48)		No. of isolates	Canine otitis (N=64)	
			Sensitivity	Resistance		Sensitivity	Resistance
1.	Enrofloxacin	44	69.84	30.16	42	65.63	34.37
2.	ciprofloxacin	38	60.32	39.68	35	54.69	45.33
3.	streptomycin	0	0	100	29	45.31	54.69
4.	Amikacin	26	41.27	58.73	18	28.13	71.89
5.	Pencillin-G	24	38.10	61.90	24	37.5	62.50
6.	Gentamycin	21	33.33	66.67	64	-	100.0
7.	Ampicillin	0	0	100	21	32.81	71.89
8.	Ceftriaxome	0	0	100	15	23.44	76.56

Table.5 Details of antimicrobial susceptibility and resistance pattern for *Pseudomonas* species isolates from canine dermatitis and otitis cases

S. No	Type of antibiotic used	No. of Isolates	Canine dermatitis (N=48)		No. of isolates	Canine otitis (N=64)	
			Sensitivity	Resistance		Sensitivity	Resistance
1.	Enrofloxacin	4	26.67	73.33	9	69.24	30.76
2.	ciprofloxacin	5	33.33	66.67	64	0	100
3.	streptomycin	7	46.67	53.33	3	17.65	82.35
4.	Amikacin	9	60.0	40.0	5	29.41	70.59
5.	Gentamycin	11	73.33	26.67	13	76.48	23.52
6.	Ampicillin	0	0	100	6	35.29	64.71
7.	Ceftriaxome	0	0	100	7	41.18	58.82

Table.6 Details of antifungal sensitivity of *Malassezia pachydermatis* from canine dermatitis and otitis cases

S.No	Antibiotic used in the study	No. of Isolates	Canine dermatitis		No. of Isolates	Otitis cases	
			Sensitivity	Resistance		sensitivity	Resistance
1.	Ketoconozole	138	86.80	13.20	64	65.31	34.69
2.	Itraconazole	122	76.73	23.27	77	78.57	21.43
3.	Fluconozole	96	63.88	36.62	48	48.98	51.02
4.	Nystatin	73	45.91	54.09	29	29.60	70.40

Fig.1 Golden yellow color colonies of *S. aureus* on Mannitol salt agar

Fig.2 *P.aeruginosa* on Blood Agar

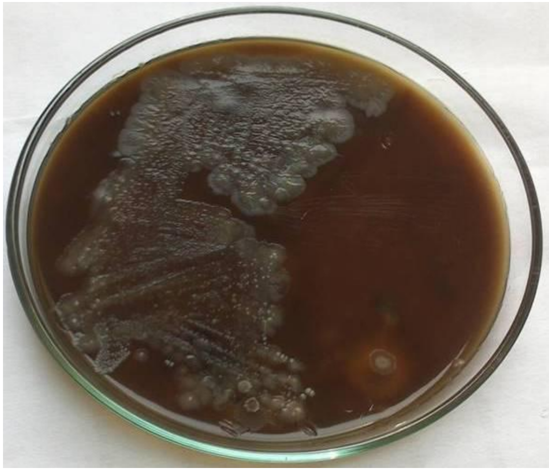


Fig.3 *P. aeruginosa* on Macconkey's agar

Fig.4 Bluish green pigmented colonies on Nutrient agar

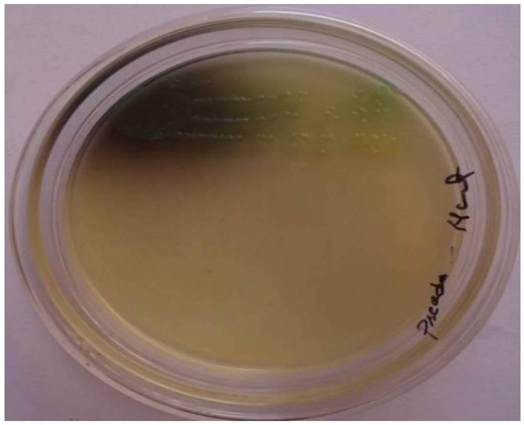


Fig.5 Gram stained smear of *Staphylococcus aureus*

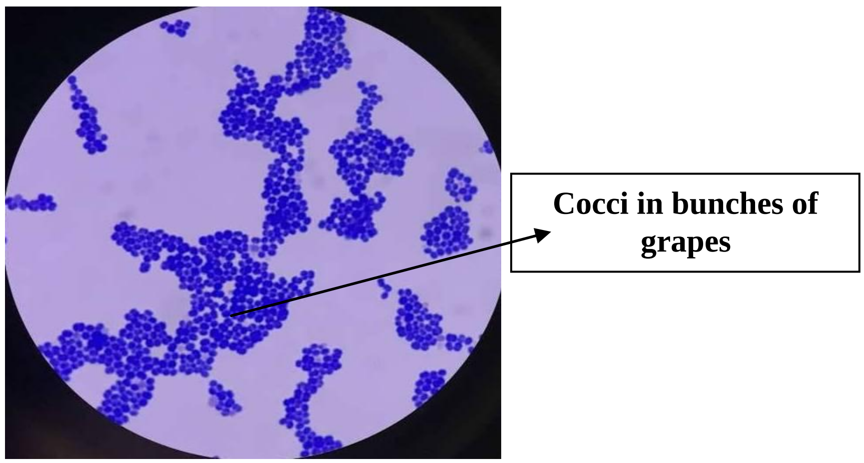


Fig.6 Gram staining smear of *Pseudomonas aeruginosa*

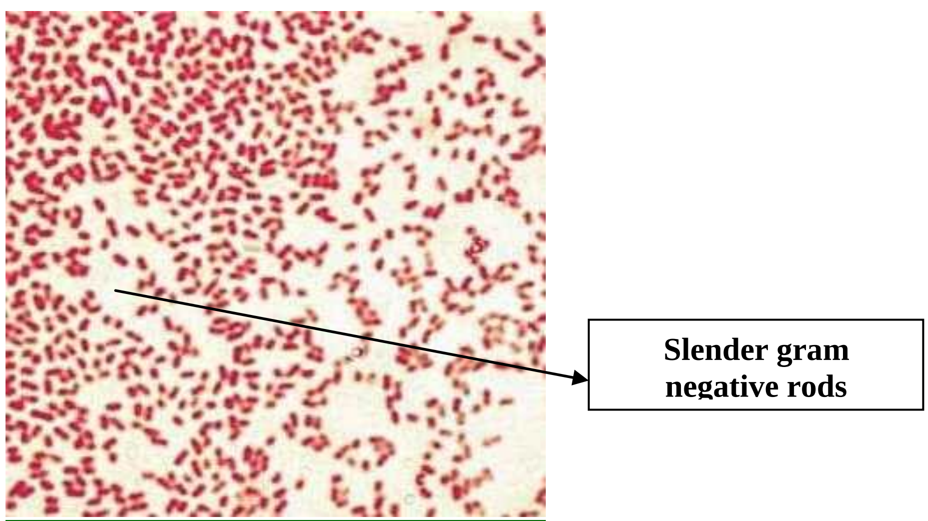


Fig.7 Methylene blue staining of malassezia in cultural smear

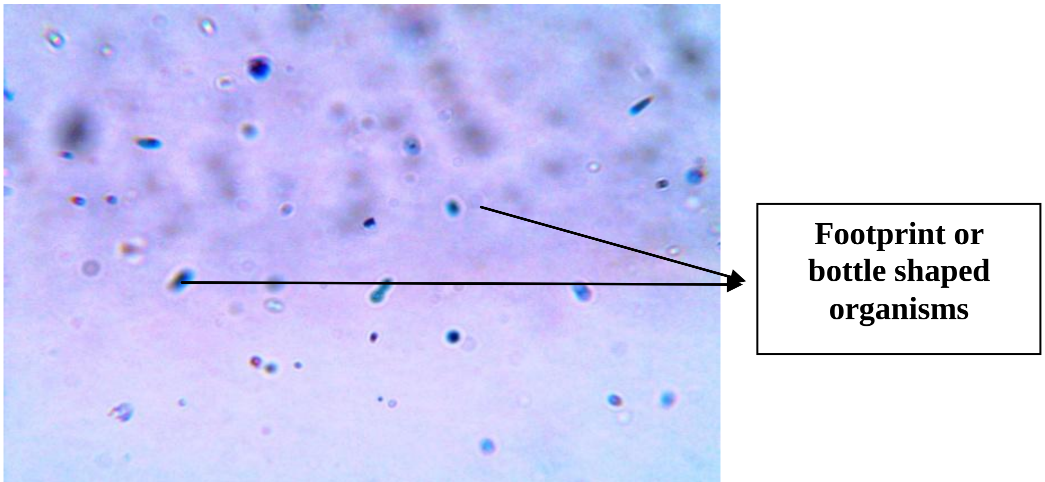


Fig.8 Details of antimicrobial susceptibility and resistance patterns for Staphylococcus species from canine dermatitis and otitis cases

Fig.9 Details of antimicrobial susceptibility and resistance patterns for *Pseudomonas* species from canine dermatitis and otitis cases

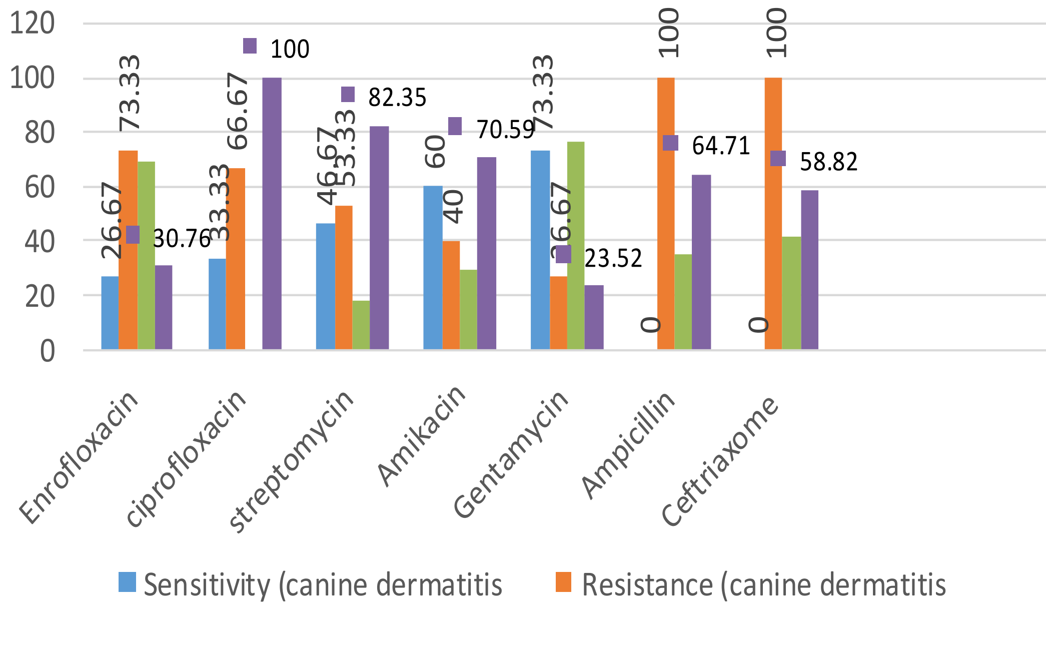
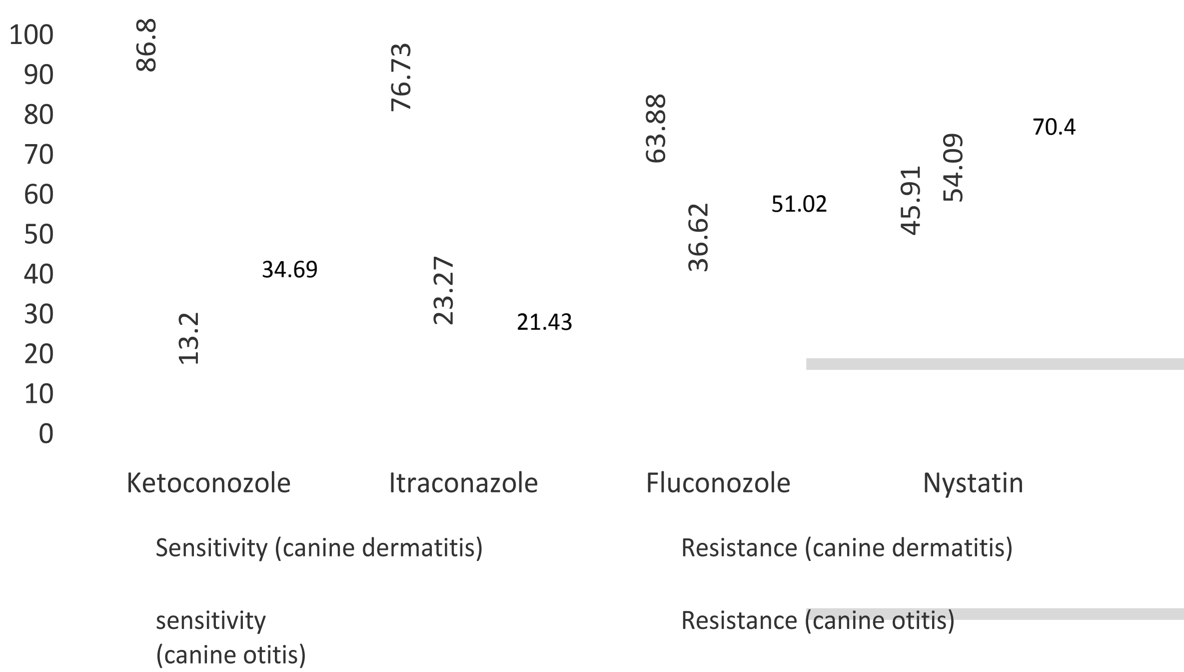


Fig.10 Details of antifungal sensitivity of *Malassezia pachydermatis* from canine dermatitis and otitis cases



Similarly, Pseudomonas isolates (15) from skin scrapings of canine dermatitis cases revealed sensitivity and resistance to Gentamycin-11 (73.33%; 26.67%) followed by Amikacin-9 (60.0%; 40.0%), Streptomycin-7 (46.67%; 53.33%), Ciprofloxacin-5 (33.33%; 66.67%) and Enrofloxacin -4 (26.67%; 73.33%) and isolates from canine otitis ear swabs showed sensitivity and resistance to Gentamycin -13 (76.48%; 23.52%) followed by Enrofloxacin-9 (69.24%; 30.76%), Ceftrixome-7(41.18%; 58.82%), Ampicillin-6 (35.29%; 64.71%), Amikacin-5 (29.41%; 70.59%) and Streptomycin-3 (17.65%; 82.35%) respectively. All Malassezia isolates (fungi) from skin scrapings of dermatitis cases showed sensitivity and resistance to Ketoconozole-138 (86.80%; 13.20%) followed by Itraconozole-122 (76.73%; 23.27%), Fluconozole -96 (63.38%; 36.62%) and Nystatin -73 (45.91%; 54.09%) respectively (Table 5 and Fig.9) Whereas (98) Malassezia isolates from ear swabs were shown sensitivity and resistance to Itraconozole -77 (78.57%; 21.43%) followed by Ketoconazole -64 (65.31%; 34.69%), Fluconozole-48 (48.98%; 51.02%) and Nystatin-29 (48.98%; 70.40%) respectively (Table 6 and Fig.10).

Canine dermatitis is multi infectional, of which majority may be parasitic, bacterial and fungal. During the study, a total of 275 skin scrapings and 189 ear swabs were collected and processed for suspected cases of canines. Out of 275 skin scrapings, 159 samples (57.80%) were found to be fungal followed by bacterial 22.91% (63), parasitic 9.82% (27) and mixed infection 9.45% (26) were recorded. But variation in the occurrence of canine dermatological disorders according to the pathogens were reported by several workers. Singh *et al.,* 2012 reported highest prevalence of parasitic dermatitis (34.82%) followed by bacterial (25%), fungal (18.75%), nonspecific (14.28%) and nutritional (7.14%). Further, Lodh and Das (2014) 28.01%, Ayodhya *et al.,* 2006) (36.53%) and Sharma *et al.,* 2009 (33.33%) (Sharma *et al.,* 2009) were reported the parasitic dermatiits. The prevalence of bacterial dermatitis was reported by Aujla *et al.,* 2000 (31.31%) and Sharma *et al.,* 2008 (28.43%) respectively.

During the study highest prevalence of fungal dermatitis 57.8% was reported in canines. But Sumathi *et al.,* (2009) observed 20.50% of fungal dermatitis in canines in their studies. *Malasezzia pachydermatis* is a commensal of external ear canal and superficial muco-cutaneous sites in dogs. Malassezia dermatitis in dogs is usually a secondary problem due to an underlying skin disease such as allergic disease (canine atopic dermatitis, and flac allergy dermatitis), recurrent bacterial pyoderma and endocrine diseases (hypothyroidism), many predisposing factors viz, increased humidity, presence of skin folds, altered cutaneous P^{H} levels, prolonged antibiotic and cortico steriod therapy may result in the commensal *M .pachydermatitis* becoming a pathogen.

Among bacterial pathogens of canine dermatitis highest prevalence of Staphylococcal species (76.19%) followed by Pseudomonas (23.81%) were recorded during the study. Earlier Alok Kumar Chowdary *et al.,* 2019 reported 92.30% of Staphylococcal species and 10.76% of *Pseudomonas* species and Sousa *et al.,* 2014 reported highest prevalence of 94% staphylococcal species from canine dermatitis cases. Further, several workers reported the various prevalence rates of staphylococcal species from canine dermatitis cases, Griffeth *et al.,* 2008 with 74.0%; Sindha *et al.,* 2015 with 80.0 percent and Chitra *et al.,* 2016 with 59.0% respectively. However, Vijay Kumar *et al.,* 2011; Singh *et al.,* 2012 reported lower rate of staphylococcal prevalence with 13.70% and

25.0% from canine dermatitis cases respectively.

Similarly, *Malassezia pachydermatis* was isolated as predominant pathogen (51.85%) followed by bacterial 42.86% and mixed 5.29% from canine external otitis cases. Bernardo *et al.,* 1998 with highest prevalence of 80.4% and Lusia De Martino *et al.,* 2016 with 67.70% of *Malassezia pachydermatis* was reported in their studies from canine otitis cases. On cultural isolation during the study this higher prevalence of Malassezia infections during the present study might be due to chronic nature of the condition in dogs, hyper pigmentation and other secondary lesions due to scratching and itching habits of the dogs Dorogi *et al.*, 2002. Among yeast, Malasezzia is one of the most common causes of external otitis in dogs Crespo *et al.,* 2002. The predisposing factors for development of Malassezia infection include excessive production of sebum, accumulation of moisture, damage of epidermis, concurrent dermatosis, atopy and bacterial skin infections (Patterson *et al.,* 2002). Malassezia infections could be secondary to other primary diseases like endocrine pathics (hypothyroidism, hyper adrenocorticism and diabetes mellitus), keratinization disorders, immune logic dysfunctions and skin neoplasias Morris (1999).

Among bacterial 79.01% of Staphylococcal species and 20.99% of Pseudomonas species were isolated from canine otitis cases during the study. Earlier Oliveira L.C. De *et al.,* 2008 reported 55.50% of staphylococcus species and 17.0% of pseudomonas species in canine otitis cases. Similarly [46, 20] reported the prevalence of Staphylococcal species with 43.0%, 47.0% respectively and pseudomonas species with 17% &10% respectively. Staphylococcus species found to be major etiological agent of bacterial species causing otitis in canines during the study and it is in agreement with earlier reports (Cole *et al.,* 1998; Nobre *et al.,* 2001).

Antibiotic sensitivity testing in Veterinary practice is an essential tool for treatment. Antimicrobial sensitivity of bacteria and antifungal sensitivity of fungi isolated from different clinical conditions are variable in difficulty geographical areas across the globe. Hence, antimicrobial as well as antifungal agents should be selected on the basis of bacterial and fungal culturing and sensitivity testing in the respective geographical locations to avoid drug resistance.

During the study invitro antibiotic sensitivity of Staphylococcal isolates from canine dermatitis cases revealed higher sensitivity to Enrofloxacin (69.84%) followed by ciprofloxacin (60.32%), Amikacin (41.27%), Pencillin (38.10%) and Gentamycin (33.33%). But higher sensitivity to Gentamycin (98.0%) was observed by Ankita and Gandge, 2018 and Bloom *et al.,* 2014. Whereas, Alok Kumar Chowdary *et al.,* 2019 reported 100% sensitivity to Amoxicillin-Clavulanic acid followed by Cephalexin (96.15%) and lowest sensitivity (84.61%) to Enrofloxacin.

Similarly, during the study invitro susceptibility of Staphylococcal isolates from canine otitis cases revealed higher sensitivity to Enrofloxacin (65.63%) followed by Ciprofloxacin (54.69%), Streptomycin (45.31%) Pencillin (37.50%), Ampicillin (32.81%), Amikacin (28.13%) and Cefrioxome (23.44%). Diren Sigirci *et al.,* 2018 reported 60.10% sensitivity to Enrofloxacin followed by ciprofloxacin 81.90% against Staphylococcal isolates from canine otitis cases.

Pseudomonas isolates from canine dermatitis cases showed higher sensitivity to Gentamycin 73.33% followed by Amikacin-

60%, but canine otitis isolates showed highest sensitivity to Gentamycin (76.48%) followed by Enrofloxacin (69.24%) during the study. Several workers reported variation in the sensitivity of antibiotics against Pseudomonas isolates from canine otitis cases. Turkyilmaz, 2008 Gentamycin (81.0%) and Pencillin (75.0%); Hariharan *et al.*, 2006 Enrofloxacin (82.50%) and Gentamycin (44.40%), Schick *et al.*, 2007 [36] Gentamycin (81.0%) and Enrofloxacin (56.0%) and Keskin *et al.*, 1999 Gentamycin (37.5.0%) and Enrofloxacin (50.0%) respectively.

Malassezia isolates from canine dermatitis cases showed higher sensitivity to Ketoconozole (86.80%) followed by Itraconazole (76.73%) where as Itraconazole (78.57%) followed by Ketoconazole (65.31%) from otitis cases. Several workers Sihelska *et al.*, 2019; Guillot *et al.*, 2003; Miller *et al.*, 2013; Patterson and Frank,2002; Pinchbeck *et al.*, 2002; Weiler *et al.*, 2013; Jacobson (2002) and Peano *et al.*, 2008 . For the treatment of otitis externa in dogs, the combined oto topical preparations with antibacterial, antifungal and anti-inflammatory properties are beneficial. Canine Malassezia dermatitis is showed be treated with systemic azoles especially Ketoconozole, Itraconozole and Fluconazole etc.

The variation in the antibiotic/antifungal susceptibility and resistance of bacterial and fungal isolates from canine dermatitis and otitis cases may be due to indiscriminate usage or frequent usage of antibiotics or antifungal agents without prior testing of antimicrobial/antifungal susceptibility of isolated pathogens and this will vary from one geographical area to another. Further, the lack of detailed prevalence status of etiological agents (either bacterial or fungi) that causes canine dermatitis/otitis in different geographical areas. Hence, prevalence status of etiological agents and their antimicrobial/antifungal susceptibility testing is essential in treating or control of dermatitis and otitis cases in canines.

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