Organisms Recovered from Cases of Canine Pyoderma and their Antibiogram Pattern

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ABSTRACT

Canine pyoderma is one of the most common causes of dermatitis with worldwide occurrence in small animal practice. The condition is diagnosed on the basis of clinical manifestations, isolation and identification of causative organisms by bacteriological cultural examination. A study on 130 clinical cases of canine pyoderma was conducted at the Teaching Veterinary Clinical Complex (TVCC), Veterinary College, Anand during July, 2016 to April, 2017. In the study undertaken, bacteriological culture examination of 116 pus swabs resulted in the recovery of 165 bacterial isolates. Exudate/pus samples were collected and subjected to bacteriological cultural isolation, identification and subsequently *in vitro* antibiotic sensitivity testing. On culture, staphylococci were the most predominantly isolated organisms. Amongst staphylococci, *Staphylococcus pseudintermedius* (49.69%, n=82), a coagulase-positive staphylococci (CPS), was the most predominantly isolated organism, followed by *Staphylococcus aureus* (18.18%, n=30). Moreover, coagulase-negative staphylococci (CNS), *Staphylococcus epidermidis* (3.03%, n=5) and *S. saprophyticus* (0.60%, n=1) were also recovered. The methicillin-resistant staphylococci accounted for 40.07% of the total isolates. Gram negative organisms like *Pseudomonas aeruginosa* (15.15%, n=25), *Klebsiella pneumoniae* (12.12%, n=20) and *Escherichia coli* (0.60%, n=1); and a lone isolate of *Streptococcus* spp. (0.60%) were also isolated majorly in the form of mixed infections. When subjected to *in vitro* antibiotic sensitivity testing the isolates showed highest sensitivity to linezolid followed by enrofloxacin, cephadroxil, clindamycin and amoxicillin-clavulanic acid whereas resistance was exhibited against erythromycin, methicillin and oxacillin.

Keywords: Canine pyoderma, etiology, antibiotic sensitivity, methicillin-resistance, linezolid

In small animal clinics, dermatological disorders constitute a majority of cases and are estimated to range between 12 and 75 per cent as the chief or concurrent owner complaint (Scott and Paradis, 1990; Feijo *et al.*, 1998). "Canine pyoderma", the most common skin diseases of dogs, is the pyogenic bacterial infection of dog's skin. It can be caused by infections, inflammatory reactions, neoplastic conditions or any condition that results in the accumulation of neutrophilic exudate that can be termed as pyoderma. Despite its increasing frequency of occurrence and advancement in clinical diagnostic procedures, many pyodermas are either misdiagnosed or mismanaged depending on availability of diagnostic and therapeutic inputs. Misdiagnosis is often associated with the pleomorphic nature of pyoderma and resultant difficulty in its recognition. The major bacterial agents responsible for canine pyoderma belong to *Staphylococcus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Micrococcus* spp. and *Acinetobacter* spp. while the transient bacteria may include *Bacillus* spp., *Corynebacterium* spp., *Escherichiacoli*, *Proteus mirabilis* and *Pseudomonas* spp. Above all, *Staphylococcus pseudintermedius* of



the Staphylococcus spp. group, is the primary bacterial pathogen of canine skin. Generally, an active involvement of gram negative bacteria (e.g. Pseudomonas aeruginosa, Klebsiella spp., Escherichia coli) is also found in secondary association with the primary coagulasepositive staphylococci. The factors leading to the initiation of pyoderma are obscure. Pyoderma is mainly seen secondary to certain pre-existing diseases like hypersensitivity, ectoparasite infestation, immune competence and endocrinopathies (hypothyroidism). Besides, poor grooming practices as well as injudicious use of corticosteroids may add to the disease process. The lack of knowledge about the significance of various staphylococcal products and host response to staphylococci is emphasized by frequent inability to predict the course of bacterial skin disease in the dog (Fehrer, 1986).

MATERIALS AND METHODS

One hundred thirty dogs with canine pyoderma presented at the Veterinary Clinical Complex (VCC) were considered for the detailed study. The study was undertaken during July, 2016 to April, 2017. Of these, exudate/pus samples were collected from 116 dogs and were further subjected to bacteriological examinations that included cultural isolation on differential media like Blood agar, Baird-Parker agar, MeReSa agar, Eosin-methylene Blue agar and Mac-Conkey agar. The streaking of swabs collected from lesions was carried out in a biosafety cabinet as per the methods described by Koneman et al. (1992). The pathogens were identified morphologically and with the help of biochemical tests like HiStaphTM Rapid Latex agglutination test, ONPG (o-nitrophenyl-β-Dgalactopyranoside) test assay, Novobiocin-sensitivity test and Oxidase test as per the standard procedures. MeReSa agar was used for selective cultural isolation of methicillinresistant S. aureus and S. pseudintermedius. Additionally, to determine methicillin-resistance among the recovered isolates methicillin and oxacillin discs were used. The recovered isolates were subjected to in vitro antibioticsensitivity testing by Kirby-Bauer disc diffusion method.

RESULTS AND DISCUSSION

Dogs harboring dermatological diseases reveal a wide variety of clinical manifestations and recognizing the primary and/or secondary lesions is the most essential feature of the diagnosis. Moreover, the distribution pattern and relevant symptoms like pruritus, inflammation and exudation etc. are useful in the diagnosis of the condition and planning its therapeutic management of it.

In the present study, the lesions pertaining to the cases of acute moist dermatitis had a rapid onset and spread. The lesions were commonly observed on face, neck, tail base and ventral abdomen. Nesbitt (1983) reported lesions of acute moist dermatitis to be multifocal eroded area with an erythematous moist surface that were seen on dorsal back, lateral thighs and/or shoulder which are in agreement with the findings of present study. These observations also concur with the reports of several earlier workers (Muller and Kirk, 1976; Clarke, 2006).

Cases of deep pyoderma showed up multiple lesions on rump, dorsum of back, base of tail, limbs (paws) and ventral abdomen. The lesions were moist with collarettes on the periphery of lesions. The chronic cases studied under the present investigation showed characteristic lesions of thickened skin along with hyper pigmentation, hyperkeratosis and scarring. Cases with pedal folliculitis or furunculosis illustrated folliculitis in the interdigital space, erythema on the interdigital as well as plantar areas, with presence of purulent exudate, matting of hair and inflamed feet. Scott et al. (1995) and White (1989) described pododermatitis is a multifaceted inflammatory disease complex that affects the feet of dogs characterized by red and oedematous tissue with nodular ulcers and serosanguineous or seropurulent exudates. Deeppyoderma was also observed secondary to other skin ailments like dermatophytosis, demodicosis, scabies, tick and flea infestations, hypothyroidism and immune-mediated dermatoses.

In the present study, bacteriological culture examination of 116 pus swabs resulted in the recovery of 165 bacterial isolates. The staphylococcal isolates accounted for 71.15 per cent of total isolates. The role of *Staphylococcus* species has also been emphasized in canine pyoderma (Hajek, 1976; Craig, 2003). *Staphylococcus pseudintermedius* and *Staphylococcus aureus* comprise coagulase-positive staphylococci (CPS) and are considered to be pathogenic to the canine skin. Littlewood *et al.* (1999) and Lautzinhiser *et al.* (2001) in their respective studies reported that coagulase-positive staphylococci were the predominant organisms isolated from cases of canine pyoderma. In the present study, coagulase-positive staphylococci accounted for 67.87 % (n=112) of total isolates, which concurs with the observations of Mhatre (2005) who isolated pathogenic staphylococci from 70.45 per cent cases presented with bacterial infection.

The predominant bacteria recovered from cases of canine pyoderma was Staphylococcus pseudintermedius which accounted for 49.69% (n=82) isolates. Staphylococcus pseudintermedius has been incriminated as an important etiological agent from cases of canine pyoderma attributed to staphylococcal infection (Phillips and Kloos, 1981; Berg et al., 1984). Next in the list was Staphylococcus aureus which was recovered in pure culture in 18.18 per cent of total isolates. Coagulase-negative staphylococci like S. epidermidis (3.03%) and S. saprophticus (0.60%) were also recovered. Gram negative organisms like Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli contributed 15.15%, 12.12% and 0.60% isolates respectively. Isolation of Staphylococcus epidermidis from cases of canine pyoderma has earlier been reported (Nesbitt and Schmitz, 1977; Medleau et al., 1986). Recovery of Pseudomonas aeruginosa isolates in canine pyoderma has been reported to as low as 2.22 and 3 per cent by two groups of workers (Batta et al., 1999; Bes et al., 2002). E.coli and streptococci have earlier been reported as transient organisms in cases of canine pyoderma (Kristensen and Krogh, 1978; Nair, 2004).

In the study under report, some of the staphylococcal species causing canine pyoderma were pronounced to evolve with methicillin-resistance. Of all the isolates recovered on MeReSa agar, 10.76 per cent (n=14) were methicillin-resistant Staphylococcus pseudintermedius (MRSP) and 6.153 per cent (n=8) were methicillin-resistant Staphylococcus aureus (MRSA). On determination of methicillin resistance by using oxacillin and methicillin discs, 23.03 per cent isolates were found to be methicillinresistant Staphylococcus pseudintermedius whereas methicillin-resistant Staphylococcus aureus accounted for 7.87 per cent. Furthermore, one isolate each (0.60%)of Staphylococcus epidermidis and Staphylococcus saprophyticus also exhibited in-vitro resistance. Kohner et al. (2009) concluded in their study that for sensitivity testing, the best phenotypic method to detect mecA geneencoded oxacillin-resistance was by use of oxacillin or methicillin discs followed by disc diffusion test on Mueller-Hinton agar.

The cases of canine pyoderma included in the present study were either the result of monomicrobic infections or mixed infections. The isolates recovered in monomicrobic and mixed infections are shown in Table 1 and 2 respectively.

Table 1: Isolates recovered from monomicrobic infections

Sl. No.	Type of isolate/s	No.
1	Methicillin-resistant <i>Staphylococcus</i> pseudintermedius (MRSP)	30
2	Methicillin-susceptible <i>Staphylococcus</i> pseudintermedius (MSSP)	29
3	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	07
4	Methicillin-susceptible <i>Staphylococcus aureus</i> (MSSA)	10
5	Methicillin-resistant <i>Staphylococcus epidermidis</i> (MRSE)	00
6	Methicillin-susceptible <i>Staphylococcus</i> epidermidis (MSSE)	01
7	Methicillin-resistant <i>Staphylococcus saprophyticus</i> (MRSS)	01
8	Pseudomonas aeruginosa	01

Table 2: Isolates recovered from mixed-infections

SI. No.	Type of isolate/s	No.
	I. Infections caused by two isolates	
1	MRSP + MRSA	1
2	MRSP + S. epidermidis	1
3	MRSP + P. aeruginosa	7
4	MRSP + K. pneumoniae	8
5	MRSA + S. epidermidis	1
6	MRSA + P. aeruginosa	2
7	MRSE + K. pneumoniae	1
8	S. aureus + P. aeruginosa	5
9	S. aureus + K. pneumoniae	1
10	S. epidermidis + P.aeruginosa	1
11	S. pseudintermedius + P. aeruginosa	7
12	S. pseudintermedius + K. pneumoniae	7
13	S. pseudintermedius + Streptococcus spp.	1
14	S. aureus + S. pseudintermedius	2
	II. Infections caused by three isolates	
1	MRSP + MRSA + K. pneumoniae	1
2	MRSP + MRSA + P. aeruginosa	1
3	MRSP + K. pneumoniae + E. coli	1
4	MRSP + K. pneumoniae + P. aeruginosa	1



Bannoehr *et al.* (2007) and Fitzgerald (2009) reported coagulase positive staphylococcal species i.e. *S. pseudintermedius* to be the most commonly isolated causal pathogen. The authors also reported isolation of other staphylococcal organisms e.g. *S. aureus* and non-staphylococcal bacteria (e.g. *Escherichia coli, Pseudomonas* or *Proteus* species) from cases of mixed infections.

In the present study, *in vitro* antibiotic sensitivity test was performed by Kirby-Bauer disc diffusion method (qualitative). A recent study on *in vitro* sensitivity of bacterial isolates recovered from pyoderma in dogs by Reddy *et al.* (2014) revealed that the bacterial isolates showed variable results on *in vitro* disc diffusion test and the authors concluded that the antimicrobial sensitivity test is an essential approach for selection of appropriate antimicrobial agents for treating recurrent pyoderma cases at individual geographic region.

The antibiogram pattern of tested bacterial isolates (Table 3) indicated that all the staphylococci exhibited highest sensitivity towards linezolid. Methcillin-resistant S. pseudintermedius (MRSP) and methicillin-susceptible S. pseudintermedius (MSSP) showed 89.5 per cent and 93.2 per cent sensitivity to linezolid respectively whereas the sensitivity to the same antibiotic exhibited by methicillinresistant S.aureus (MRSA) and methicillin-susceptible S.aureus (MSSA) was 76.9 per cent and 76.47 per cent respectively. Likewise, five isolates of Staphylococcus epidermidis and one of Staphylococcus saprophyticus elicited cent percent susceptibility to linezolid. Gram negative organisms like Pseudomonas aeruginosa, Klebsiella pneumoniae and E. coli showed 72 per cent and 100 per cent sensitivity respectively. Linezolid has been described as an emerging drug in veterinary practice and is used commonly in human beings to treat cases that show methicillin-resistance (Papich, 2012). Frank and Loeffler (2012) suggested that since methicillin-resistant staphylococci show in vitro sensitivity to linezolid, the drug should be used in the therapeutic management of infections caused by such organism.

Besides exhibiting 89.5% sensitivity to linezolid, the methicillin-resistant *Staphylococcus pseudintermedius* exhibited sensitivity (Table 3) in descending order to enrofloxacin, clindamycin, cephalexin, cephadroxil, moxfloxacin, amoxicillin-clavulanic acid, gentamicin; co-

trimoxazole and vancomycin, cefpodoxime, erythromycin, methicillin. On the other hand methicillin-susceptible *S. pseudintermedius* showed highest sensitivity to methicillin and oxacillin, followed by linezolid, cephalexin, clindamycin, enrofloxacin, cephadroxil, amoxicillinclavulanic, moxifloxacin, ampicillin-cloxacillin, cotrimoxazole gentamicin, cefpodoxime and erythromycin.

Further, methicillin-resistant Staphylococcus aureus (MRSA) showed highest sensitivity to linezolid, followed by enrofloxacin, cephadroxil, cephalexin, moxifloxacin, gentamicin, cefpodoxime, whereas complete resistance was exhibited against methicillin, oxacillin and erythromycin. On the contrary, the methicillin-susceptible isolates of Staphylococcus aureus showed maximum sensitivity to methicillin and oxacillin followed by cephadroxil, linezolid, enrofloxacin, cephalexin, clindamycin gentamicin, ampicillin-cloxacillin, amoxicillin-clavulanic acid, cefpodoxime, co-trimoxazole and vancomycin. Mhatre (2005) elucidated that the isolates showed sensitivity to enrofloxacin, cephadroxil, cephalexin, ampicillin-cloxacillin, oxacillin, trimethoprim and sulphadiazine and ampicillin. These findings indicate the variations in antibiogram pattern of isolates and the increasingly developing resistance to antibiotics which is a matter of concern.

In the study under report, coagulase negative staphylococci (CNS) like *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* showed variable range of sensitivity. The only recovered methicillinresistant isolate each of these CNS showed sensitivity to linezolid, enrofloxacin, clindamycin and cephalexin whereas resistance was exhibited to methicillin, oxacillin, erythromycin and co-trimoxazole. Methicillin-susceptible isolatesshowed sensitivity to linezolid, cephalexin, methicillin, oxacillin, enrofloxacinand resistance to clindamycin, cephadroxil, moxifloxacin, co-trimoxazole, ampicillin-cloxacillin and erythromycin.

Pseudomonas aeruginosa isolates elicited highest sensitivity to enrofloxacin, followed by linezolid, methicillin, oxacillin, gentamicin, cephadroxil, amoxicillin-clavulanic acid whereas resistance was exhibited against erythromycin. *Klebsiella pneumoniae* showed highest sensitivity to cephadroxil, followed by methicillin, linezolid, oxacillin, enrofloxacin, gentamicin, vancomycin and moxifloxacin. The only isolate of

SI. No.	Isolate	N0.01				Perc	entage u	of isola	tes sensi	itive to a	antibioti	Percentage of isolates sensitive to antibiotic used in-vitro (%)	n-vitro	(%)			
		isolate	ΓZ	CN	CD	CFR	MET	0X	MO	COT	AX	AMC	CPD	EX	Е	VA	GEN
-					Sta	Staphylococcus pseudintermedius(n=82)	nəsd snə	dinter.	nedius(n	=82)							
	MRSP	38	89.5	39.34	40.9	34.21	0	0	31.57	15.8	21.05	23.68	10.52	84.21	5.2	15.7	23.7
	MSSP	44	93.2	60.41	59.09	43.18	100	100	29.54	18.18	22.72	34.09	13.63	59.09	9.09	11.36	18.18
7						Staphyi	Staphylococcus aureus (n=13)	anreus	s (n=13)								
	MRSA	13	76.9	38.46	15.38	44.45	0	0	23.07	15.38	15.38	15.38	23.07	61.53	0	15.38	23.07
	MSSA	17	76.47	41.17	29.41	69.23	100	100	29.41 17.64	17.64	23.52	23.52	23.52	52.94	0	5.88	29.41
3					*	Staphylococcus epidermidis (n=5)	occus e	piderm	idis (n=:	5)							
	MRSE	1	100	100	0	0	0	0	100	100	0	0	0	100	0	100	25
	MSSE	4	100	50	25	25	100	100	25	25	25	0	0	50	25	0	25
4	MR S. saprophyticus	1	100	0	100	0	0	0	0	0	0	0	0	100	0	0	0
2	Pseudomonas aeruginosa	25	72	48	18	64.7	72	72	16	32	10	56	16	73	0	12	66
9	Klebsiella spp.	20	75	35	45	85	80	70	50	25	30	30	40	70	20	55	60
Г	Streptococcus spp.	1	100	100	0	0	100	100	0	0	0	0	0	100	0	0	0
8	E. coli	1	100	0	100	100	0	0	0	0	0	0	0	100	0	0	100
	Total	165															

Table 3: In vitro Antibiotic Sensitivity Test (ABST) of isolates recovered

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LZ = Linezolid; CN = Cephalexin; CD = Clindamycin; CFR = Cefadroxil; MET = Methicillin; OX = Oxacillin; AMC = Amoxicillin-Clavulanic acid; AX = Ampicillin-Cloxacillin; MO = Moxiftoxacin; CPD = Cefpodoxime; VA = Vancomycin; GEN = Gentamicin; EX = Enroftoxacin; CO T= Co-Trimoxazole.



Streptococcus spp. showed sensitivity to linezolid, cephalexin, methicillin, oxacillin and enrofloxacin. Similarly, the lone isolate of *E.coli* showed sensitivity to linezolid, clindamycin, cephadroxil, enrofloxacin and gentamicin.

Considering the overall efficacy of all the antibacterial drugs used *in vitro* during the furtherance of present study, highest sensitivity by different bacterial isolates was elicited to linezolid, followed by enrofloxacin, cephadroxil, clindamycin and amoxicillin-clavulanic acid, The rest of the antibiotics failed to elicit substantial *in vitro* efficacy against the isolates used under the investigation. Thus, it can be concluded that linezolid can be strongly recommended in cases of canine pyoderma to attain expected therapeutic output.

CONCLUSION

Canine pyoderma is one of the major maladies of canine skin and therefore it should be considered as a matter of concern. Staphylococcus pseudintermedius remains as the most predominant organism causing canine pyoderma followed by Staphylococcus aureus. Use of antibiotics after in vitro sensitivity testing should be prioritized, which decreases the possibility of developing antimicrobial resistance in the organisms and thus results in better therapeutic outcome. Among others, linezolid appears to be the antibiotic to which the causal organisms of pyoderma show high sensitivity. Methicillin-resistance in dogs is progressing with the passage of time and is a cause for concern. Proper diagnosis of ailment by cultural isolation and subsequently appropriate therapeutic regimen are the ways to curb the developing resistance. Dogs infected with MRSA most likely acquire the infection from human. Emergence of MRSP has been noticed over the past ten years and its continuing spread worldwide presents significant clinical challenges.

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CONFLICT OF INTEREST

Authors declare no conflict of interest for this research work.

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