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Isolation of pathogenic bacteria associated with canine dermatitis and therapeutic management

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Abstract
In this study we reported microbiological features and therapeutic management of bacterial dermatitis in canines. Samples were collected from different patients to rule out the etiology and the pattern of antimicrobial sensitivity. Bacterial cultural examination revealed *Staphylococcus aureus* (100%), *Pseudomonas aeruginosa* (70%), *Escherichia coli* (20%) to be involved. Antibiotic sensitivity test (ABST) was carried out for whole samples, suggesting Ceftriaxone, Amoxicillin with sulbactum, Ciprofloxacin to be sensitive and Gentamicin, Erythromycin to be intermediate sensitive.

Keywords: Dermatitis, *Staphylococcus*, *Pseudomonas*, *Escherichia*, Antibiotic sensitivity test, Ceftriaxone

Introduction
Bacterial dermatitis in canine practice is very commonly encountering and sometimes challenging to clinician regarding proper diagnosis and treatment (Muller *et al*, 1989) [1]. A wide range of bacterial organisms including both gram positive, gram negative were isolated from canine dermatitis contributing as primary or secondary pathogens (Swain *et al*., 2002; Seena *et al*., 2005; Reddy *et al*., 2011) [2-4]. Therefore, present study was undertaken to investigate the clinical cases of canine dermatitis with respective to isolation and antimicrobial susceptibility pattern of etiological agents.

Materials and Methods
The study was carried out in ten canine clinical cases showing typical dermatitis clinical signs like intense pruritis, erythema, severe alopecia, papules, pustules, crusts formation over abdomen, face and head regions; unusual odour; exudate from skin lesions. Samples were collected from superficial and deep skin lesions with sterile cotton swab in sterile conditions then subjected to a series of laboratory procedures like staining, inoculation onto different agar media for colony characterization; into broth for ABST. Sample collection and subsequent necessary processing steps followed as prescribed by Quinn *et al.* (1994) [5].

Staining: Smears are prepared directly from swabs and heat fixed for gram staining.

Inoculation: Swabs were touched directly on to solid (to minimize contaminating bacteria) media i.e., Brain Heart Infusion agar (BHI), Mac Conkeys agar, Mannitol Salt Agar (MSA) by and then dipped into Brain Heart Infusion broth. Streak plate method of inoculation was followed on solid media to get individual colonies. Inoculated media were incubated at 37^0C. To select an effective antibiotic, turbidity of inoculated BHI broth indicting bacterial growth, was adjusted with McFarland nephlometer tube no.5 and then ABST was performed on Mueller-Hinton agar (MHA) plates by following Kirby-Bauer disc diffusion method. Commercially available antimicrobial discs (Himedia) were used namely Ciprofloxacillin (5µg), Ceftriaxone+Tazobactum (Intacef-10µg), Gentamycin (10 µg), Amoxycillin+Sulbactum (10 µg), Pencillin G (10 units). After applying discs for each sample on agar plates, kept in incubator at 37^0C.

Bio-Chemical tests
IMViC tests for pink; pale colonies on Mac Conkeys agar; Coagulase test for colonies on MSA, Oxidase test for spindle shaped colonies on BHI were carried out. Catalase test was performed against all types of colonies.

Results

Staining: Mixed bacteria i.e., pink bacilli; purple cocci were appreciated upon gram staining (Fig 1) suggesting that involvement of both gram negative and gram positive bacteria.

Colonial characterization: Details of appearance of different colonies (Fig 2; Fig 3; Fig 4) on different media were given in Table 1.

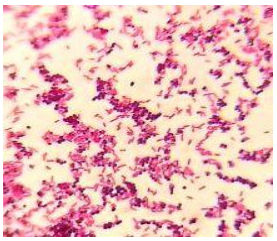


Fig 1: Staining of whole culture smear showing mixed infection i.e, purple cocci; pink bacilli.

Fig 2: Mannitol salt agar with *S. aureus* showing golden coloured; drop of oil paint like colonies.

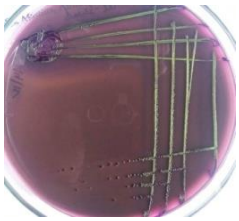


Fig 3: EMB agar with *E. coli* showing green metallic sheen.

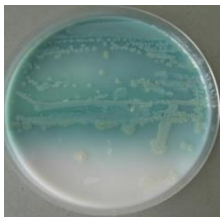


Fig 4: Nutrient agar with *P. aeruginosa* colonies showing spreading type; blue-green colonies.

Table 1: Colony morphology on different agar media.

Name of the agar	Colonial appearance	Indication
Brain Heart Infusion agar	Spindle shape with bluish-green colour, pin point; drop of oil paint type colonies	Mixed bacterial colonies
Mac Conkeys agar	Pink; pale colonies	lactose fermenting; non-lactose fermenting bacteria
Mannitol Salt Agar	Golden-yellow colonies	*Staphylococcus* spp.
Eosin methylene blue (EMB)	Green metallic sheen	*E. coli*

Based on bio-chemical tests results (Table 2) it was confirmed that *E. coli* and *P. aeruginosa* were involved in dermatitis. Golden yellow coloured colonies of MSA agar were found to be positive for coagulase; catalase tests, therefore, confirming the presence of *S. aureus*. Data regarding mixed infections and individual organism wise infections were given in Table 3; Table 4 respectively. Table 4 indicates dominance of *S. aureus* followed by *P. aeruginosa* and *E. coli.*

Table 2: Bio-chemical tests results for colonies isolated on Mac Conkey agar.

Test	Lactose fermented colonies	Non-lactose fermented colonies	Colonies from MSA
Indole	+	-	-
Methyl Red	+	-	+
Voges-Proskauer	-	-	+
Citrate	-	+	+
Nitrate Reduction	+	+	+
Catalase	+	+	+
Coagulase	-	-	+
Oxidase	-	+	-
Pyocyanin	-	+	-

Table 3: Summary of clinical cases with mixed infection.

No. of cases with mixed infections	Organisms isolated	%
2	*S. aureus* + *E. coli* + *P. aeruginosa*	20
5	*S. aureus* + *P. aeruginosa*	50

(From remaining 30% cases only *S. aureus* was isolated.)

Table 4: Individual organisms accounting for infection.

Organisms isolated	No. of samples with particular organism/ Total no. of samples	%
Staphylococcus aureus	10/10	100%
Escherichia coli	2/10	20%
Pseudomonas aeruginosa	7/10	70%

ABST

Isolated bacterial pathogens sensitivity towards various antibiotics were summerized in the following table 5.

Table 5: Antibiotic sensitivity pattern of isolated bacteria.

Bacteria present in sample	Sensitive antibiotic
S. aureus	CTR+, AMS
S. aureus + *E. coli*	CTR+, GEN (Intermediate sensitive), Erythromicin (Intermediate sensitive)
S. aureus + *E. coli* + *P. aeruginosa*	CTR+, Ciprofloxacin, Erythromicin (Intermediate sensitive)

Therapeutic Management

Based on ABST results Intacef (Ceftriaxone+Tazobactum; Intas Animal Health) was given intramuscularly @ 20 mg/kg bd.wt. along with Histanil and oral vitamin supplement for 1 week. From fourth day of treatment satisfactory results were observed i.e, decreased intensity of itching; erythematous lesions. Animal was found to recover completely after 15 days with normal hair coat.

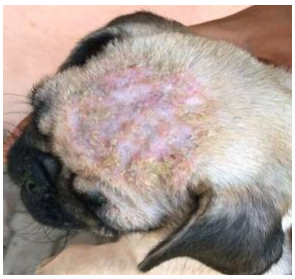


Fig 5.a: Pug showing erythema, alopecia, crusts over head region.

Fig 5.b: German shepherd showing alopecia, crusts over the face.

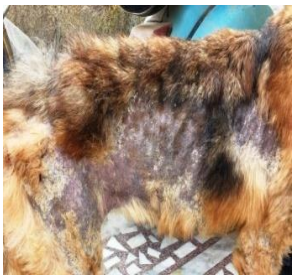
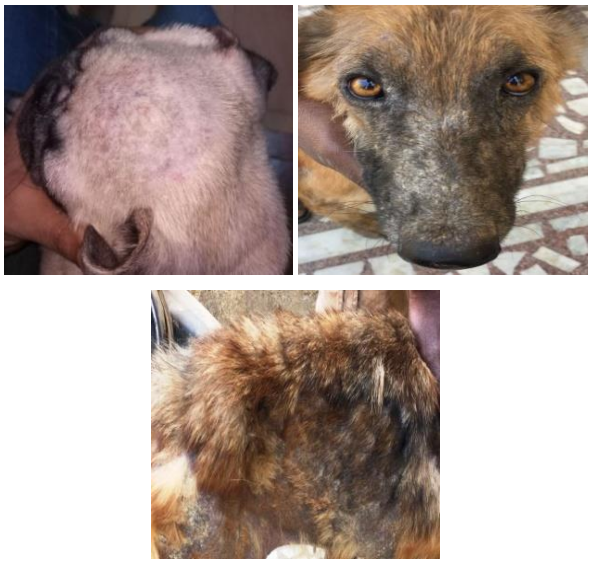


Fig 5.c: German shepherd showing erythema, alopecia, crusts over abdominal region.

Fig 5: Images of patients before treatment



Patients showing recovery from dermatitis i.e, decreasing erythema, restoring normal hair coat and disappearance of crusts compared with Fig 5.

Fig 6: Images of patients after 15 days of post treatment

During this study three different types of bacteria were isolated in different combinations from different samples. Among these, dominance of *S. aureus* might be due to its environment resistance and common inhabitant of skin. This result was in parallel with White *et al.* (1983) [6], Nagase *et al.* (2002) [7]. In general gram negative bacteria can't be isolated from skin lesions but here they were isolated (Reddy *et al.* 2011) [4] and presence of these organisms could be attributed to secondary infection. Results of ABST were in accordance with Rantala *et al.* (2004) [8]; Krishna Prakash (2005) [9]. Therapeutic results were similar as observed by Parida *et al.* (2013) [10].

Conclusion

Staphylococcus spp. was the major pathogen which led to secondary infection with gram negative bacteria. Moreover, Ceftriaxone in combination with Tazobactum was found to be effective against all samples, despite presence of different bacterial combinations.

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