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Staphylococcus pseudintermedius as the Principal Pathogen in Canine Pyoderma: A molecular and phenotypic study in North-Eastern Karnataka

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Abstract
Pyoderma is among the most frequently encountered dermatological disorders in small animal practice, commonly linked with bacterial infections, particularly those caused by *Staphylococci*. The present investigation aimed to determine the major bacterial pathogens responsible for canine pyoderma, identify the predominant *Staphylococcal* species through molecular assays in the North-Eastern Karnataka region. Dogs showing clinical signs of pyoderma presented to the Veterinary Clinical Complex, Veterinary College Bidar, as well as nearby District Polyclinics, were examined, and skin swabs were collected aseptically for bacteriological evaluation. Isolation and identification of bacteria were performed using standard cultural, morphological and biochemical methods. Polymerase Chain Reaction (PCR) assays targeting the *pta* gene (genus-specific) and *nuc* gene (species-specific) were employed to confirm *Staphylococcus spp*. and *Staphylococcus pseudintermedius*, respectively. Of the 118 clinically affected dogs, 86 (73%) samples yielded bacterial isolates. *Staphylococcus spp*. formed the majority (52; 60.46%), followed by *Escherichia spp*. (11; 13%), *Pseudomonas spp*. (9; 11%), *Klebsiella spp*. (8; 9%), and *Proteus spp*. (1; ~1%). Labrador Retrievers showed the highest occurrence (37%), followed by Non-Descript dogs (20%). Pyoderma was more common in young adults aged 6-12 months (38%) and dogs above 24 months (34%), with a higher proportion in males (62%). Molecular confirmation showed that 92% of *Staphylococcal* isolates belonged to *S. pseudintermedius*. The study identifies *S. pseudintermedius* as the key pathogen of canine pyoderma in this region and highlights the need for routine culture and identification to guide appropriate diagnosis, therapy and mitigate the rise of resistant strains.

Keywords: Canine pyoderma, *Staphylococcus pseudintermedius*, *pta*, *nuc*, PCR, North-Eastern Karnataka

Introduction
Skin diseases account for a substantial proportion of clinical presentations in small animal practice, and among these, pyoderma is regarded as one of the most common and therapeutically challenging conditions in dogs [16]. It is characterised by circular patches of hair loss, redness, erythema, papules, pustules, pruritus, alopecia, crust formation and in chronic or severe cases, ulceration and deep fistulous tracts. Bacterial infections of the skin may occur as primary disease or as a consequence of underlying predisposing factors such as allergic dermatitis, ectoparasitism, endocrinopathies, keratinization defects, poor grooming or inappropriate prior antimicrobial therapy [3].
Staphylococci are the principal bacterial agents associated with canine pyoderma worldwide, with *Staphylococcus pseudintermedius* recognized as the most important opportunistic pathogen in dogs [5]. Other bacteria such as *Escherichia spp, Pseudomonas spp, Klebsiella spp, and Proteus spp*. may be recovered either as primary agents in some cases or as components of mixed infections, especially in chronic or deep lesions [13]. In recent years, the emergence of Methicillin-Resistant *S. pseudintermedius* (MRSP) and Methicillin-Resistant *S. aureus* in companion animals has raised significant concern due to therapeutic difficulties and potential zoonotic implications [9, 8]. Molecular diagnostic tools, including PCR assays targeting specific reference and virulence genes, have improved the accuracy and speed of *Staphylococcus* species identification and detection of resistance determinants. [1] Rational

antimicrobial use in dermatological practice requires knowledge of the prevailing aetiological agents and their susceptibility profiles in a given geographical region. Local epidemiological data are particularly important in view of variable patterns in breed predisposition, age distribution, and drug resistance. Information from North-Eastern Karnataka on bacterial causes of canine pyoderma and their antibiogram is limited. Therefore, the present study was conducted to know the bacterial aetiology and characterization by molecular methods.

Materials and Methods

Study area and clinical cases

The study was conducted in the Department of Veterinary Microbiology, Veterinary College, Bidar, under Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU), Bidar, Karnataka, India. Dogs suspected of pyoderma and presented to the Veterinary Clinical Complex, Veterinary College, Bidar, and District Veterinary Polyclinics in Bidar, Kalaburagi, Yadagir, Raichur, Ballari, Vijayanagar and Koppala, formed the basis of the investigation. A total of 118 clinical samples from dogs suspected of pyoderma were selected on the basis of typical dermatological signs such as papules, pustules, epidermal collarettes, erythema, alopecia, pruritus, scaling, crusts, nodules, fistulous tracts, exudation, malodour and ulceration. Samples were collected aseptically using sterile swabs and transported to the lab on ice.

Isolation and phenotypic identification of bacteria

For primary isolation, each swab was inoculated into nutrient broth and incubated at 37 °C for 24 h. Turbid broths were streaked onto nutrient agar to obtain individual colonies. Representative colonies were examined for morphology and Gram-stained to differentiate Gram-positive from Gram-negative bacteria. Selective and differential media were then used to further characterize isolates: Mannitol Salt agar for presumptive *Staphylococcus spp.* Nutrient agar, MacConkey and Eosin Methylene Blue agar for Gram-negative organisms, and Pseudomonas agar for *Pseudomonas spp.* Blood agar plates containing 5% defibrinated sheep blood were employed to observe haemolytic patterns of *Staphylococcal* isolates [4]. Colonies suspected as *Staphylococcus spp.* were examined for Gram-positive cocci morphology, mannitol fermentation on Mannitol Salt Agar and haemolysis on blood agar. *Escherichia spp.* isolates were identified based on characteristic metallic green sheen appearance on EMB agar and confirmed by biochemical reactions such as indole and methyl red positivity. *Klebsiella spp.* isolates were recognized as large, mucoid, lactose-fermenting colonies on MacConkey agar along with Vogues Proskauer and Citrate positive reactions. *Pseudomonas spp.* isolates were identified from non-lactose fermenting colonies on MacConkey agar and bluish green sheen on Pseudomonas agar, oxidase positivity. *Proteus spp*. isolates were recognized by their swarming motility on nutrient agar [17].

Biochemical characterization

Biochemical tests were carried out on purified isolates to confirm identity. Gram-positive cocci were subjected to catalase and tube coagulase tests to distinguish coagulase-positive *Staphylococcus* species from other coagulase positive bacteria. Gram-negative isolates were evaluated using conventional biochemical reactions including catalase, oxidase, indole, methyl red, Vogues-Proskauer, citrate utilization tests (IMViC). Interpretation of results followed standard veterinary microbiology protocols [7, 15].

Molecular characterization of *Staphylococcal* isolates

Staphylococcal isolates were selected for molecular identification. Genomic DNA was extracted from overnight broth cultures using a simple heat lysis method and stored at -20 °C until use. DNA concentration and purity were assessed spectrophotometrically and integrity was checked by agarose gel electrophoresis. PCR was carried out using published primers (Table 01).

Table 1: Primer Sequences for amplification of *pta* and *nuc* genes of *Staphylococcus spp*:

Primer Name	Sequence (5'-3')	Amplicon Size (bp)	Reference
pta-F	GGNAAAGCNACWGAAGAACAA	355	Jimenez-Velasquez *et al*. (2024)
pta-R	CDGAACCNTTHGTWGADAAGC		
nuc-F	AAACACCGAGTAATACGCCG	780	Chitra *et al*. (2015)
nuc-R	TTTAGCGTTCCCAAATGTTCAG		

The genus-level confirmation of *Staphylococcus* isolates was performed by PCR targeting the *pta* (phosphotransacetylase) gene, [10] and species-level identification of *Staphylococcus pseudintermedius* was achieved by amplification of the *nuc* (thermonuclease) gene, [6] according to the reported cycling conditions (Table 02).

Table 2: Reaction Mixture and Cycling conditions for *pta* and *nuc* PCR:

Component	Details
Reaction volume	25 µl
Genomic DNA	100-150 ng
Primer concentration	10 pmol each
Master mix	2 × (Barcode Biosciences, Bengaluru)
Cycling conditions: *pta* gene	Initial denaturation: 95 °C, 3 min; 30 cycles: Denaturation: 95 °C, 30 s; Annealing: 54 °C, 30 s; Extension: 72 °C, 30 s; Final extension: 72 °C, 5 min; Hold: 4 °C, ∞
Cycling conditions: *nuc* gene	Initial denaturation: 94 °C, 3 min; 30 cycles: Denaturation: 94 °C, 30 s; Annealing: 60 °C, 30 s; Extension: 72 °C, 30 s; Final extension: 72 °C, 5 min; Hold: 4 °C, ∞

PCR reactions were prepared using commercial master mix, appropriate primer concentrations and template DNA in a final standard volume. Along with the test samples, a DNA molecular weight marker (100 bp ladder) and no template

control were included for proper validation. Electrophoresis was carried and DNA bands were visualized under UV illumination using a gel documentation system (Gel Doc XR, Bio-Rad, USA), and images were captured for record keeping [18].

Results

Occurrence of canine pyoderma

A total of 118 dogs with clinical signs suggestive of pyoderma were examined during the study period. Affected animals frequently exhibited alopecia, erythema, papules, pustules, epidermal collarettes, scaling, crust formation, nodules, recurrent lesions, hyperpigmentation and varying degrees of pruritus. Lesions ranged from localized to generalized and commonly involved the muzzle, chin, elbows, limbs, inguinal region, rump and interdigital spaces. In deep pyoderma, pain on palpation, foul-smelling purulent discharge, ulceration and formation of fistulous tracts were observed.

Among the cases presented Breed-wise analysis indicated that Labrador Retrievers (37%) constituted the highest proportion of affected dogs, followed by Non-Descript (20%) dogs, Pomeranians (13%) and German Shepherds (10%). Other breeds like Shih-Tzu, Rottweiler, Dachshund, Siberian Husky, Golden Retriever, Beagle, Pug, Doberman and Poodle were represented in lower numbers. Age-wise distribution showed that dogs between 06 to 12 months accounted for the largest share of cases (38%), followed by animals above 24 months of age (34%), while puppies below 6 months had the lowest occurrence. While Sex wise analysis showed that, males (62%) were more affected than females (38%).

Bacterial isolation and phenotypic characterisation

Among the processed, 118 skin swab samples, 86 (73%) yielded bacterial growth, while 32 samples did not yield any bacterial growth under the conditions employed. Of the total positive samples, 52 (60.46%) yielded *Staphylococcus spp.* as predominant bacteria, followed by *Escherichia spp.*11 (13%), *Pseudomonas spp.* 9 (11%), *Klebsiella spp.* 8 (9%) and 1 (≈1%) *Proteus spp.* Mixed infections involving two bacterial species were detected in 5 (4%) samples. District-wise analysis indented recovery of *Staphylococcus* isolates from all study districts. *Staphylococcal* isolates grew well on nutrient agar and Mannitol Salt agar, where many produced pin point colonies with golden yellow pigment due to mannitol fermentation, and appeared as Gram-positive cocci in clusters on microscopy. On blood agar, haemolysis was observed. These isolates were catalase and coagulase positive, supporting their classification as pathogenic *Staphylococcus* species associated with canine pyoderma (Figure 01).

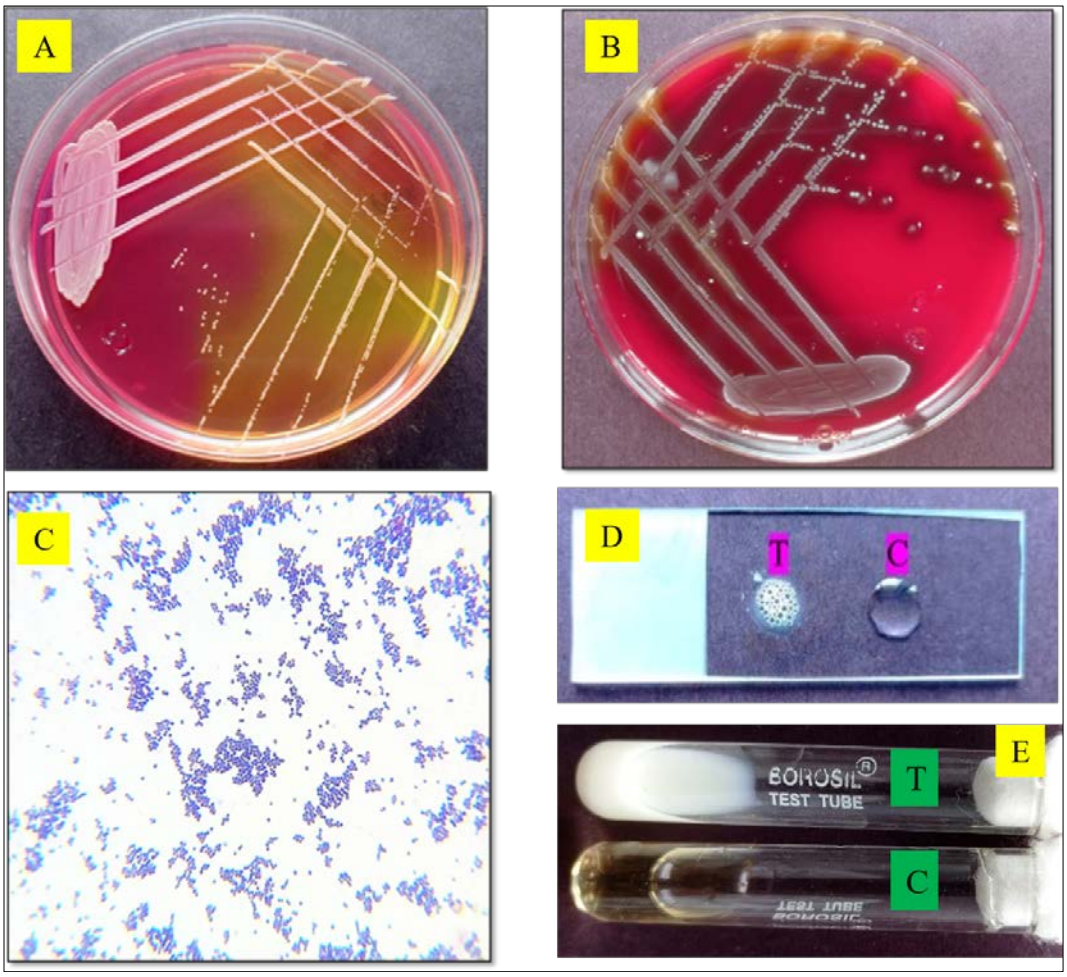

Fig 1: Phenotypic Characterization of *Staphylococcus spp.* A. Growth of *Staphylococcus spp.* on Mannitol salt agar showing pinpoint golden yellow pigmented colonies. B. Growth of *Staphylococcus spp.* on Blood agar medium showing pinpoint haemolytic colonies. C. Gram's staining of *Staphylococcus spp.* showing Gram positive cocci arranged in irregular clusters (Bunches of grapes). D. Catalase test for *Staphylococcus spp.* showing bubble formation. (T: Test sample; C: Control). E. Coagulase test for *Staphylococcus spp.* showing formation of fibrin clump. (T: Test Sample; C: Control)

Among Gram negative isolates that exhibits, pink colonies on MacConkey agar with a Metallic Green Sheen on EMB agar, observed as Gram-negative rods and tested positive for Indole and Methyl red but negative for Voges Proskauer and Citrate, were identified as *Escherichia spp*. Large, mucoid, lactose-fermenting colonies on MacConkey Agar, that appeared as Gram-negative bacilli and tested negative for Indole and Methyl red reaction and tested positive for Voges Proskauer and Citrate, were identified as *Klebsiella spp*. Flat, greenish colonies with a fruity odour on Nutrient agar and Pseudomonas agar, observed as Gram-negative rods and positive for Oxidase, Catalase but negative for Indole, Methyl red and Vogues Proskauer and Citrate positive were identified as *Pseudomonas spp*. Swarming colonies on Nutrient Agar and appearing as Gram-negative rods, Indole negative, Methyl red positive, Voges Proskauer negative and Citrate positive, were identified as *Proteus spp*. The mixed isolates were identified accordingly.

Molecular detection of *Staphylococcus spp. and S. pseudintermedius*: PCR was employed for genus and species-level identification of *Staphylococci* by targeting the *pta* and *nuc* gene respectively (Figure 02 & 03). Out of the 52 *Staphylococcal* isolates, 49 (95%) were confirmed as *Staphylococcus spp.* by *pta*-gene PCR and 45 (92%) were confirmed as *Staphylococcus. pseudintermedius* by *nuc*-gene PCR (Table 03). The findings indicate that *S. pseudintermedius* is the predominant bacterial species involved in pyoderma in dogs of North- Eastern Karnataka Region and other isolates which were not confirmed as *S. pseudintermedius* by this assay, suggesting the presence of other *Staphylococcus* species.

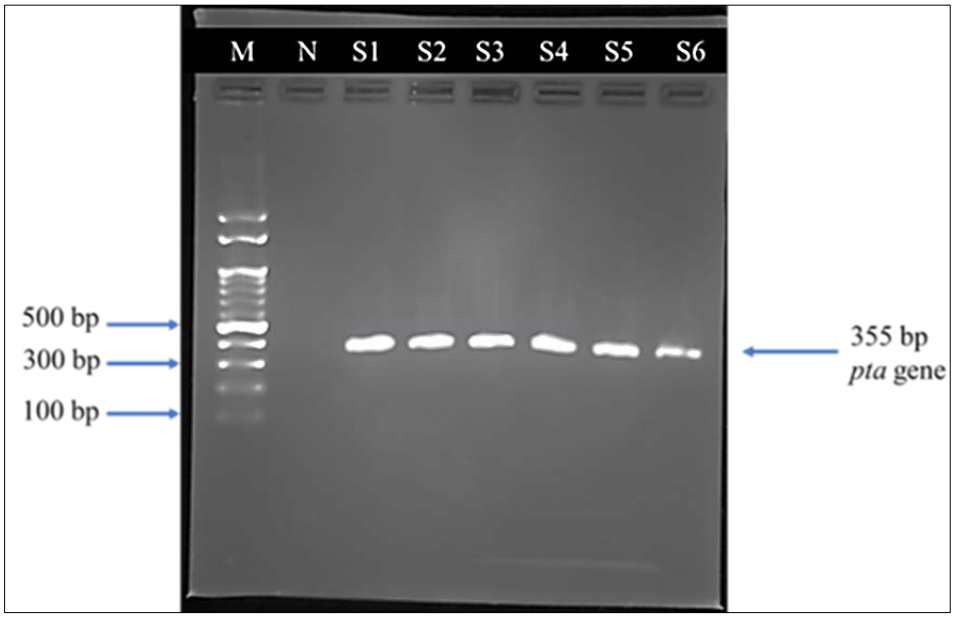

Fig 2: Molecular Characterization of *Staphylococcus spp.* Agarose gel showing PCR amplification products of *pta*-gene (355 bp) of *Staphylococcus spp.* (Lane M: 100 bp DNA Marker, Lane N: No Template Control, Lane S1-S6: Positive samples, showing amplicon of 355 bp *pta*-gene).

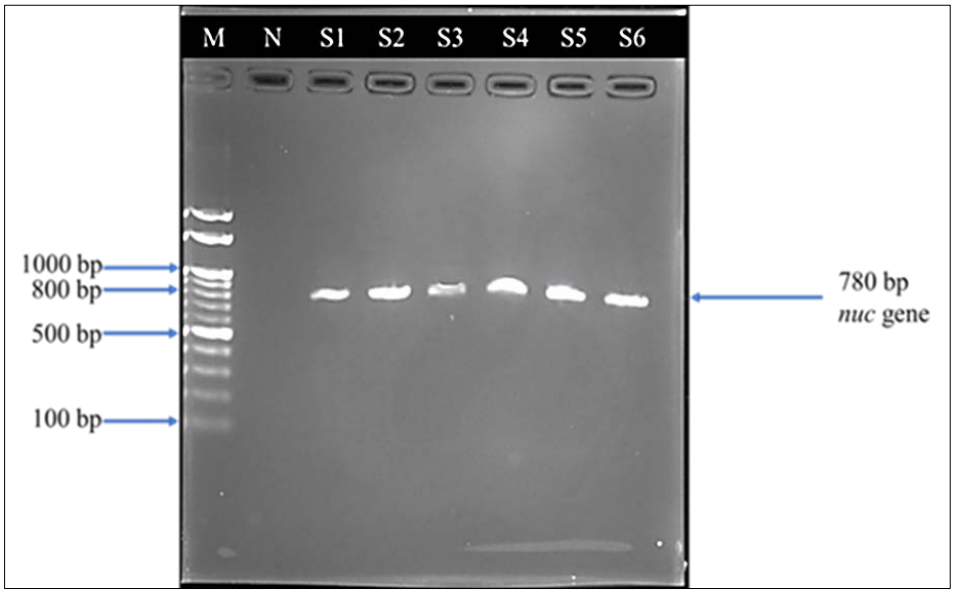

Fig 3: Molecular Characterization of *Staphylococcus pseudintermedius.* Agarose gel showing PCR amplification products of *nuc*-gene (780 bp) of *Staphylococcus pseudintermedius*. (Lane M: 100 bp DNA Marker, Lane N: No Template Control, Lane S1-S6: Positive samples, showing amplicon of 780 bp *nuc*-gene).

Table 3: PCR based detection of *pta* and *nuc* genes in *Staphylococcal* isolates:

Sl. No.	No. of isolates subjected for PCR	Gene targeted	No. of isolates positive by PCR (percentage)
1	52	*pta* gene	49 (95 %)
2	49	*nuc gene*	45 (92 %)

Discussion

The present study confirms that pyoderma constitutes a common dermatological problem in dogs presented to veterinary facilities in North-Eastern Karnataka and reiterates the predominance of *Staphylococcus pseudintermedius* as the principal aetiological agent. The higher occurrence in Labrador Retrievers and other popular companion breeds is in agreement with reports suggesting breed predisposition, possibly linked to genetic, anatomical or management-related factors.[2] The relatively greater involvement of young adult animals and males may reflect behavioural and hormonal influences, environmental exposure and differences in grooming or owner attention between groups [12]. The isolation pattern, with *Staphylococcus spp.* representing the majority of bacterial isolates and organisms such as *Escherichia spp. Klebsiella spp. Pseudomonas spp* and *Proteus spp.* contributing as secondary or opportunistic agents, conforms to the widely recognized role of *Staphylococci* as the main pathogens in canine pyoderma.[5] Recovery of *Pseudomonas spp.* and mixed infections from chronic or deep lesions underscores the importance of appropriate sample collection and laboratory investigation in complicated cases.[11] The finding that a proportion of clinically suspected cases did not yield bacterial growth highlights the possibility of prior antimicrobial use, low bacterial load, fastidious organisms or non-bacterial causes in some dermatological presentations.

Molecular characterization provided robust confirmation of phenotypic identification, demonstrating that most Coagulase-Positive *Staphylococcal* isolates belonged to *S. pseudintermedius*. The use of *pta* and *nuc* gene-based PCR assays enhances diagnostic accuracy and helps to differentiate *S. pseudintermedius* from other closely related species that may not be reliably distinguished by routine biochemical methods alone. Such molecular confirmation is especially important in the context of emerging Methicillin-Resistant *Staphylococci* in companion animals, where species-level identification has implications for epidemiology and infection control [16, 19]. These findings highlight the importance of obtaining appropriate diagnostic samples and tailoring therapy based on laboratory results rather than relying solely on empirical regimens, particularly in recurrent or non-responsive cases. Topical therapy and non-antibiotic measures should be optimized whenever feasible to reduce systemic antimicrobial pressure. At the same time, awareness of the zoonotic potential of *S. pseudintermedius* and other resistant organisms necessitates adherence to hygiene precautions by veterinarians, pet owners and handlers to minimize transmission. [14&20]

Conclusion

The study establishes that *Staphylococcus pseudintermedius* is the predominant bacterial pathogen associated with canine pyoderma in the North-Eastern Karnataka region, with additional contributions from Gram-negative bacteria such as *Escherichia spp. Pseudomonas spp. Klebsiella spp.* and *Proteus spp*. Breed, age and sex wise patterns suggested a higher predisposition in certain breeds, young adult dogs and males. Molecular assays targeting *pta* and *nuc* genes provide reliable confirmation of *Staphylococcal* isolates and support their routine use in reference laboratories. The findings highlight the necessity for routine bacteriological culture and identification in canine pyoderma cases and increased awareness among veterinarians and dog owners regarding the potential zoonotic risk and prudent antibiotic use is essential to prevent further escalation of resistance.

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Ethical Approval

The study was approved by the Institutional Animal Ethics Committee (VCB/IAEC/VMC-44/ 2024-25 Dated: 21.03.2025)

Conflict of Interest

None declared.

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