RESEARCH PAPER

Identification And Categorization Of Pathogenic Bacteria In Dustavrana Vis-À-Vis Chronic Ulcer- An Observational Study

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ABSTRACT

Vrana is described as "Vrana Gaatra Vicoornane, Vranayati iti Vranaha." Which means the break or discontinuity of body tissue is called vrana. When neglected or improperly managed, it may progress into Dustavrana, where "Duṣṭa" denotes a loss of its natural healing tendency. Due to the aggravation of Vata, Pitta, and Kapha, in association with dusta rakta, mamsa, and agantuja bhava, it is considered important for its pathogenesis. Acharya Sushruta mentions the role of krimi in Dustavrana, which has features of putipuyasrava and durgandha. These descriptions have the hallmark features of chronic ulcers, which are infected with pathogenic bacteria. Therefore, identifying and categorizing pathogenic species of bacteria in Dustavrana is of utmost importance in understanding the microbial role in dusti of Vrana. Between ancient Ayurvedic texts and modern microbiological science, a logical connection can be established. It also supports clinical observations of Acharya Sushruta.

OBJECTIVES OF THE STUDY

- *To classify Dustavrana based on their Lakshana as Vataja, Pittaja, Kaphaja.*
- *To identify and categorize the type of Bacteria present in Dustavrana.*

METHODOLOGY:This observational study involved 60 subjects fulfilling the inclusion criteria. Deerghakālanubandhi Vraṇa, ulcers existing for more than two weeks were diagnosed as Dustavrana. Dosha classification was based on predominance, number, and intensity of specific lakshanas. pus samples were collected. One swab underwent Gram staining, and the other was cultured on Blood agar and MacConkey agar, incubated at 37°C for 24–48 hours. Organisms were identified by growth characteristics, Gram staining, microscopy, and biochemical tests, and correlated with Dosha predominance.

RESULTS: The Pearson correlation test revealed a moderate positive correlation between Dosha Pradhana Dustavrana and Organism Identified, with a Pearson correlation coefficient of 0.526 and a p-value less than 0.001, suggesting that the type of Dosha predominance in the wound is significantly associated with the type of microbial colonization. In vata pradhana Dustavrana is predominantly associated with Staphylococcus aureus. In pitta pradhana, Dustavrana is frequently associated with gram-negative bacteria such as Klebsiella, Escherichia coli, and Acinetobacter. Additionally mixed presentation of Staphylococcus aureus with gram-negative bacteria was seen. In kapha pradhana Dustavrana is more frequently associated with Pseudomonas, Proteus, and mixed presentation of Pseudomonas with Staphylococcus aureus.

CONCLUSION: In vata pradhana Dustavrana, Staphylococcus aureus is evident. In pitta pradhana Dustavrana, gram-negative organisms like. Klebsiella, Klebsiella oxytoca, Escherichia coli, and Acinetobacter. Furthermore, polymicrobial patterns, particularly the coexistence of Staphylococcus aureus with Gram-negative isolates, were evident. In kapha pradhana Dustavrana, Pseudomonas and Proteus species were predominantly isolated. Furthermore, characterized by the coexistence of Pseudomonas and Staphylococcus aureus..

Keywords: Rakshith K, Bose MSC, Jalawadi P, Sowmya GS. Identification And Categorization Of Pathogenic Bacteria In Dustavrana Vis-À-Vis Chronic Ulcer- An Observational Study. Int J Drug Deliv Technol. 2026;16(58s): 1374-1386. DOI: 10.25258/ijddt.16.58s.145

Source of support: Nil.

Conflict of interest: Nil.

INTRODUCTION

Ayurveda is a science of life and is believed to have existed as long as the origin of life on this earth, as mentioned in the *Samhita*. Ever since life originated, human beings have been susceptible to injury, which has led them to think about healing from a very early stage of development. In

particular, *Acharya Sushruta* has mentioned various types of *Vrana* and their management [1], which is of prime importance in any surgical practice. Recently, the brilliant progress of surgery in various fields has immensely reduced the incidence of wound infection by decreasing the impediments associated with wound healing to a certain extent. Still, wound management continues to be a matter of speculation.

Chronic ulcers are described under the broad entity of *Dustavrana*, which is characterized as a contaminated, infected, or non-healing ulcer. *Acharya Sushrutha* emphasizes that *Dustavrana* results from the vitiation of *tridosha- vata, pitta, kapha,* along with involvement of *rakta* and *mamsa dhatu*. Each type of ulcer, based on the *dosha* involved, has distinct characteristics and may have different microbial environments.

The global burden of chronic wounds has risen considerably, particularly with the aging population and the increasing prevalence of chronic diseases like diabetes, obesity, and cardiovascular conditions. A 2005 community-based epidemiological study identified the prevalence of chronic ulcers in India to be 4.5 per 1000 of the population, with lower extremity ulcers being the most common. Untreated or inadequately treated acute traumatic wounds are a frequent cause of these chronic ulcers [2]. Among the patients with chronic ulcers evaluated in the highest quality clinical studies, the prevalence of infected wounds is 45%, so about half of chronic ulcers will be infected.[3]

Acharya Sushruta has clearly mentioned the role of *Krimi* in the pathogenesis of *Duṣṭa Vrana*, stating that organisms develop in wounds vitiated by *Dosha* [4]. This concept closely correlates with the role of pathogenic bacteria in chronic non-healing ulcers. Establishing correlations between Ayurvedic clinical features and microbiological profiles may enhance understanding of disease progression and facilitate integrative approaches to wound care.

This study was therefore designed as an observational study to identify and categorize pathogenic bacteria isolated from *Dustavrana*, through microbial culture and analysis. The study aimed to explore associations between *dosha pradhana Dustavrana* and the type of bacteria isolated, thereby attempting to bridge traditional ayurvedic diagnostics with modern microbiological evidence.

OBJECTIVES OF THE STUDY

To classify *Dustavrana* based on their *Lakshana* as *Vataja, Pittaja, Kaphaja*.

To identify and categorize the type of Bacteria present in *Dustavrana.*

MATERIALS AND METHODS

Sample Size – 60 subjects fulfilling the inclusion criteria were selected for the study.

DIAGNOSTIC CRITERIA:

Deerghakalanubandhi vrana (ulcer lasting more than 2 weeks) with two or more of the following *Dustavrana lakshanas* of a particular *Dosha.*

Vataja
Atisamvruta (slightly exposed),
Katina (very hard),
Avasanna (depressed),
Krishna varna (black colour),
Atyartha vedana (severe pain)

Pittaja
Ativivrutha (wide open)
Utsanna (elevated),
Atiushna (increase in local temperature),
Putipuya srava (pus discharge),
Daha (burning sensation),
Paaka (suppuration),
Pidaka (eruptions),
Dushtashonita srava (exuding of vitiated blood)
Amanojna gandha (unpleasant odour)
Rakta and peeta varna (red/yellow colour)
Shopha (swelling/inflammation)

Kaphaja
Atimardava (soft)
Utsangi (hypergranulation),
Kandu (itching)
Shwetha varna (white colour)

INCLUSION CRITERIA:

Subject willing to give consent to participate in this study.
Subjects fulfilling the diagnostic criteria of all genders of age group within 18-85 years.

EXCLUSION CRITERIA:

Subjects with positive HIV and HBsAg.
Ulcer less than 2 weeks duration.
Subjects diagnosed with tubercular ulcer, malignant ulcers, and syphilitic ulcer.

STUDY DESIGN -

It was an observational study.60 subjects who are fulfilling the inclusion criteria were selected for the study. After examination and observation of the chronic ulcer. For diagnosis, *Deerghakālanubandhi Vraṇa* is defined as an ulcer persisting for more than two weeks. A diagnosis of *Doṣhaja Duṣṭa Vraṇa* was established when the chronic ulcer exhibits two or more *Duṣṭa Vraṇa lakṣaṇas* attributable to a specific *Doṣha* (*Vata, Pitta, or Kapha*). For classification into *Vata pradhana, Pitta pradhana, or Kapha pradhana DuṣṭaVraṇa*, the number and intensity of *lakṣaṇas* of one *Doṣha* must be greater than those of the other two *Doṣhas*. A sterile cotton swab was used to collect the pus sample from *Dustavrana* by the Levine technique. Two swabs were collected; one was used for direct microscopic examination after Gram staining, and the other was utilized for culture. Culture was done by the four-streak method on Blood agar & MacConkey agar, and were incubated at 37°C for 24-48 hours. After incubation, the growth in the media was observed for growth characteristics, and later colonies were subjected to Gram staining, microscopic observation, and biochemical tests for identification of the organism. Clinical diagnosis of *Dustavrana* was compared with that of the organism found on growth.

Statistical analysis-

Data was entered in an MS Excel sheet.

Statistical analysis was done by the Pearson correlation test.

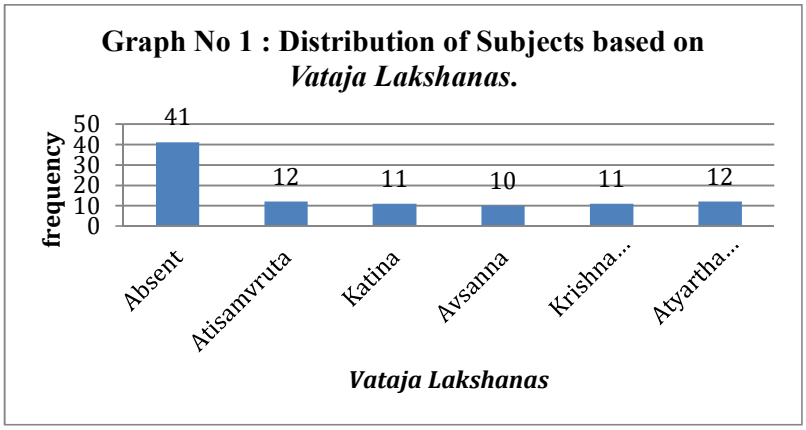
OBSERVATIONS

It was a cross-sectional observational study conducted with a sample size of 60 subjects presenting with *Duṣṭa Vrana* vis-à-vis chronic ulcer. Microbiological evaluation of wound exudates was undertaken to identify and categorize the pathogenic bacteria responsible for the condition. The findings were systematically documented under respective headings. Frequencies and percentages were calculated for categorical variables, and the distribution of bacterial isolates was represented through charts for better visualization of the analyzed data. Furthermore, correlations were drawn between *Doṣhic* involvement and the isolated pathogens to explore possible associations between Ayurvedic diagnostic features and microbiological findings.

***Dustavrana* Lakshanas**

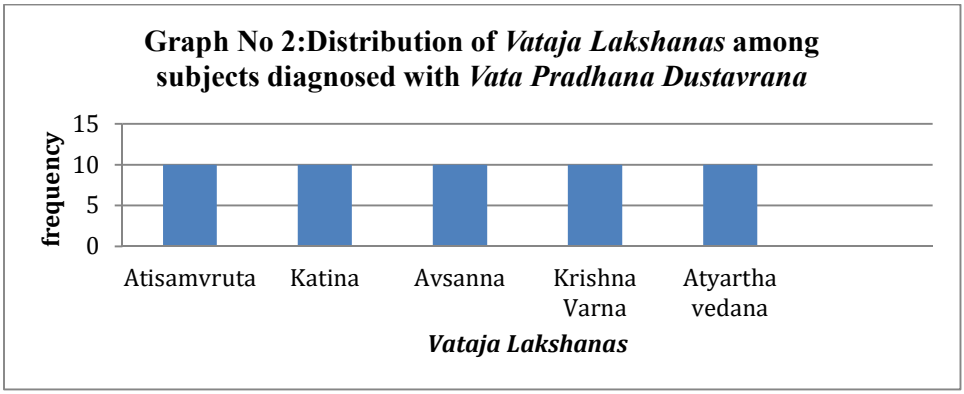
1. *Vataja Lakshanas*: The distribution of subjects based on *Vataja Lakshanas* showed that 41 subjects (68.3%) did not exhibit any *Vataja Lakshanas*. Among those presenting with Vataja features, 12 subjects (20%) showed *Atisamvruta,* 11 subjects (18.3%) had *Katina*, 10 subjects (16.7%) exhibited *Avsanna*, 11 subjects (18.3%) showed *Krishna Varna,* and 12 subjects (20%) showed *Atyartha Vedana*.

Table No 1: Distribution of Subjects based on *Vataja Lakshanas*		
	Frequency	Percentage
Absent	41	68.3%
Atisamvruta	12	20.0%
Katina	11	18.3%
Avsanna	10	16.7%
Krishna Varna	11	18.3%
Atyartha Vedana	12	20.0%



Vata pradhana Dustavrana*:** The distribution of subjects based on *Vataja Lakshanas* among subjects diagnosed with *vata pradhana Dustavrana*. Among those presenting with *Vataja* features, 12 subjects exhibited *Atisamvruta*, 11 subjects had *Katina*, 10 subjects displayed Avsanna, 11 subjects showed Krishna Varna, and 12 subjects presented with *Atyartha* Vedana**.** Among the 10 diagnosed cases of *vata pradhana Dustavrana,** all 10 subjects presented with *atisamvruta, katina, avasanna, Krishna varna,* and *atyartha Vedana*. However, 2 subjects had *atisamvruta*, 1 subject had *katina*, 1 subject had *Krishna varna*, and 2 subjects had *atyartha vedana*, and were diagnosed as having *Pitta* or *Kapha pradhana Dustavrana*.

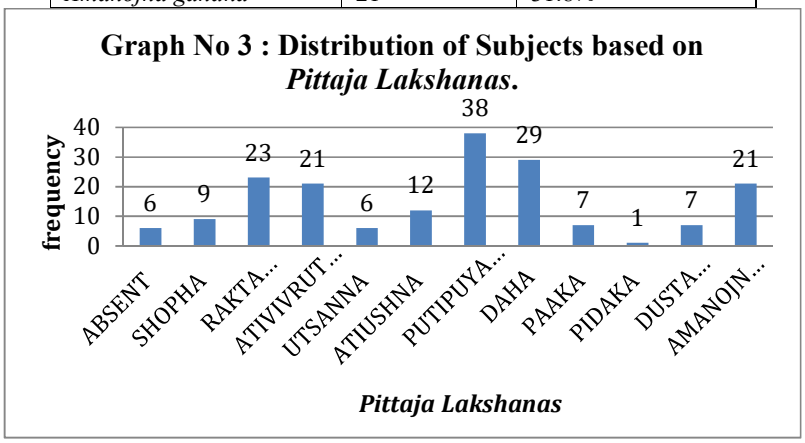
Table No 2: Distribution of *Vataja Lakshanas* among subjects diagnosed *with Vata Pradhana Dustavrana* (total subjects diagnosed with *Vata Pradhana Dustavrana* is 10 cases)	
	Frequency
Atisamvruta	10
Katina	10
Avsanna	10
Krishna Varna	10
Atyartha Vedana	10



2. *Pittaja Lakshanas*: The distribution of subjects based on *Pittaja Lakshanas* showed that 6 subjects (9.1%) did not exhibit any *Pittaja* features. Among the observed *Lakshanas,* 38 subjects (57.6%) presented with *Putipuyasrava*, 29 subjects (43.9%) exhibited *Daha*, and 23 subjects (34.6%) showed *Raktapeetavarna*, and 21 subjects (31.8%) showed *Ativivrutha*, and 21 subjects (31.8%) showed *Amanojna gandha* Other features included *Atiushna* in 12 subjects (18.2%), *Utsanna* in 6 subjects (9.1%), *Paaka* in 7 subjects (10.6%), *Dusta Shonita Srava* in 7 subjects (10.6%), *Shopha* in 9 subjects (13.6%), and *Pidaka* in 1 subject (1.5%).

Table No 3: Distribution of Subjects based on *Pittaja Lakshanas*

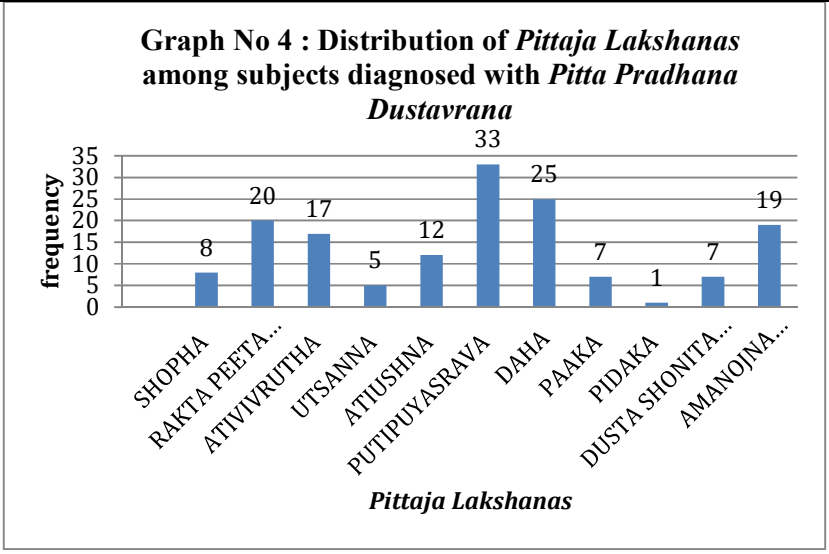
Pittaja Lakshana	**Frequency**	**Percentage (%)**
Absent	6	9.1%
Shopha	9	13.6%
Rakta peeta varna	23	34.6%
Ativivrutha	21	31.8%
Utsanna	6	9.1%
Atiushna	12	18.2%
Putipuyasrava	38	57.6%
Daha	29	43.9%
Paaka	7	10.6%
Pidaka	1	1.5%
Dusta Shonita Srava	7	10.6%
Amanojna gandha	21	31.8%



***Pitta Pradhana Dustavrana*:** The distribution of subjects based on *Pittaja Lakshanas* among subjects diagnosed with *Pitta pradhana Dustavrana*. Among the observed *Lakshanas,* 38 subjects presented with *Putipuyasrava*, 29 subjects exhibited *Daha*, and 23 subjects showed *Raktapeetavarna*, and 21 subjects showed *Ativivrutha* & *Amanojna Gandha*. Other features included *Atiushna* in 12 subjects, *Utsanna* in 6 subjects, *Paaka* in 7 subjects, *Dustashonitasrava* in 7 subjects, *Shopha* in 9 subjects, and *Pidaka* in 1 subject. Among 37 diagnosed *pitta pradhana Dustavrana*, 1 subject presented with *shopha*, 3 subjects presented with *Raktapeetavarna*, 4 subjects presented with *Ativivrutha*, 5 subjects presented with *putipuya srava*,

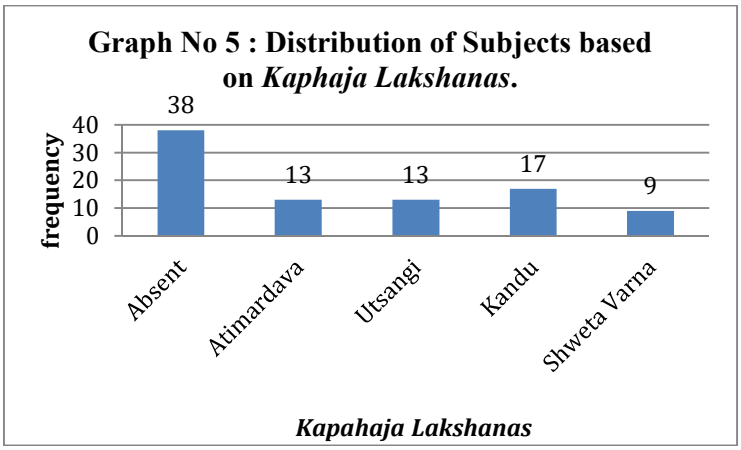
4 subjects presented with *Daha*, 2 subjects presented with *Amanojna Gandha* were diagnosed as *vata* or *kapha pradhana Dustavrana.*

Table No 4: Distribution of *Pittaja Lakshanas* among subjects diagnosed with *Pitta Pradhana Dusta Vrana* (total subjects diagnosed with *pitta pradhana dusta vrana* is 37 cases)	
Pittaja Lakshana	**Frequency**
Shopha	8
Rakta peeta varna	20
Ativivrutha	17
Utsanna	5
Atiushna	12
Putipuyasrava	33
Daha	25
Paaka	7
Pidaka	1
Dusta Shonita Srava	7
Amanojna gandha	19



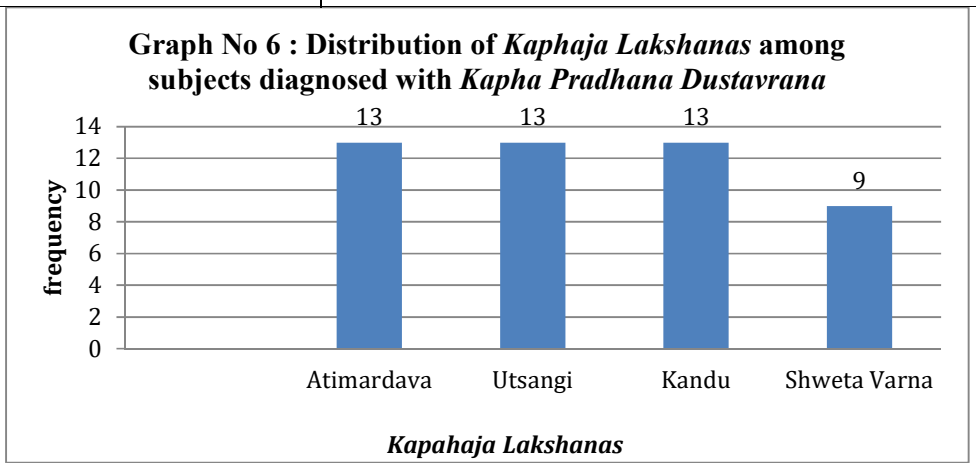
3. Kaphaja Lakshanas: The distribution of subjects based on *Kaphaja Lakshanas* (N = 60) showed that 38 subjects (62.3%) did not exhibit any *Kaphaja* features. Among those presenting with *Kapahaja Lakshanas*, 17 subjects (27.9%) showed *Kandu*, 13 subjects (21.3%) had *Atimardava*, 13 subjects (21.3%) exhibited *Utsangi*, and 9 subjects (14.8%) presented with *Shweta Varna*.

Table No 5: Distribution of Subjects based on *Kaphaja Lakshanas*		
Kaphaja Lakshana	**Frequency**	**Percentage (%)**
Absent	38	62.3%
Atimardava	13	21.3%
Utsangi	13	21.3%
Kandu	17	27.9%
Shweta Varna	9	14.8%



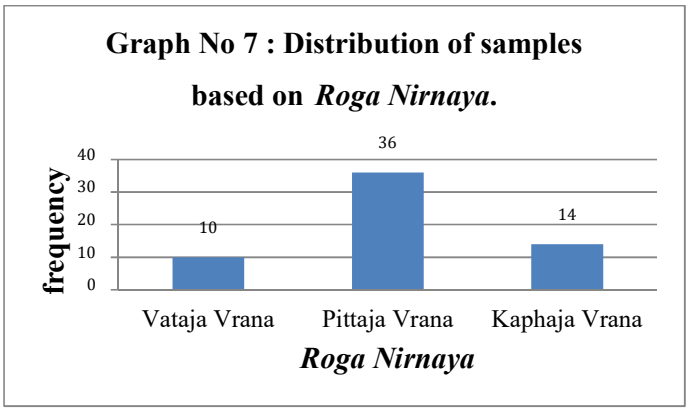
***Kapha pradhana Dustavrana*:** The distribution of subjects based on *Kaphaja Lakshanas* features. Among those presenting with *Kaphaja Lakshanas*, 17 subjects (27.9%) showed *Kandu*, 13 subjects (21.3%) had *Atimardava*, 13 subjects (21.3%) exhibited *Utsangi*, and 9 subjects (14.8%) presented with *Shweta Varna*. Among the 13 diagnosed cases of *Kapha pradhana Dustavrana*, all 13 subjects presented with *Atimardava, Utsangi Kandu*, and 9 subjects presented with *Shweta Varna*. 4 subjects presented with *Kandu* were diagnosed as *pitta or vata pradhana Dustavrana*.

Table No 6: Distribution of *Kaphaja Lakshanas* among subjects diagnosed with *Kapha Pradhana Dustavrana*. (total subjects diagnosed with *kapha pradhana Dustavrana* is 13 cases)	
Kaphaja Lakshana	**Frequency**
Atimardava	13
Utsangi	13
Kandu	13
Shweta Varna	9



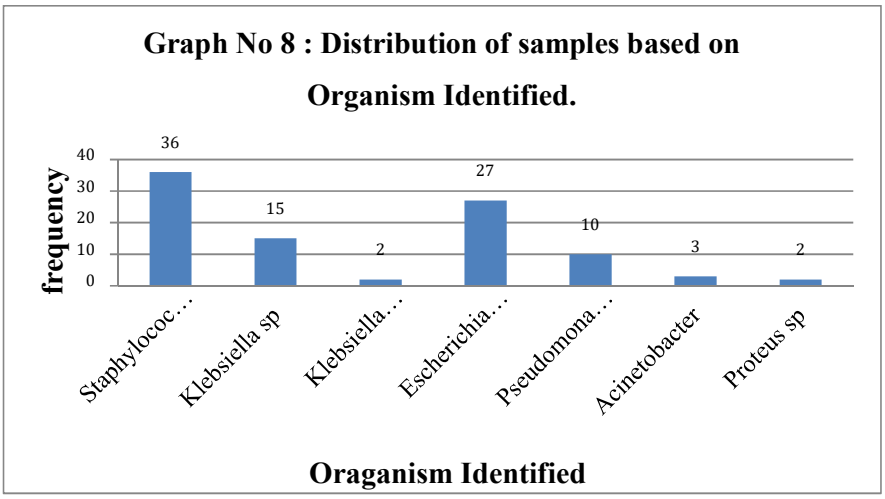
4. *Roga Nirnaya*: The distribution of subjects based on *Roga Nirnaya* (N = 60) showed that 36 subjects (60%) had *Pittaja Vrana*, followed by 14 subjects (23.33%) with *Kaphaja Vrana* and 10 subjects (16.67%) with *Vataja Vrana*.

Table No 7: Distribution of Subjects based on *Roga Nirnaya*		
	Frequency	Percentage
Vataja Vrana	10	16.67%
Pittaja Vrana	36	60.00%
Kaphaja Vrana	14	23.33%
Total	**60**	**100.0%**



5. Organism Identified: The distribution of subjects based on the type of organism isolated (N = 60) showed that 36 isolates (60%) were positive for *Staphylococcus aureus*, 27 isolates (45%) for *Escherichia coli*, 15 isolates (25%) for *Klebsiella species*, 10 isolates (16.7%) for *Pseudomonas species*, 3 isolates (5%) for *Acinetobacter*, 2 isolates (3.3%) for *Klebsiella oxytoca*, and 2 isolates (3.3%) for *Proteus species*.

Table No 8: Distribution of Subjects based on Organism Identified		
Organism	**Frequency (n)**	**Percentage (%)**
Staphylococcus aureus	36	60.0%
Klebsiella species	15	25.0%
Klebsiella oxytoca	2	3.3%
Escherichia coli	27	45.0%
Pseudomonas species	10	16.7%
Acinetobacter	3	5%
Proteus species	2	3.3%



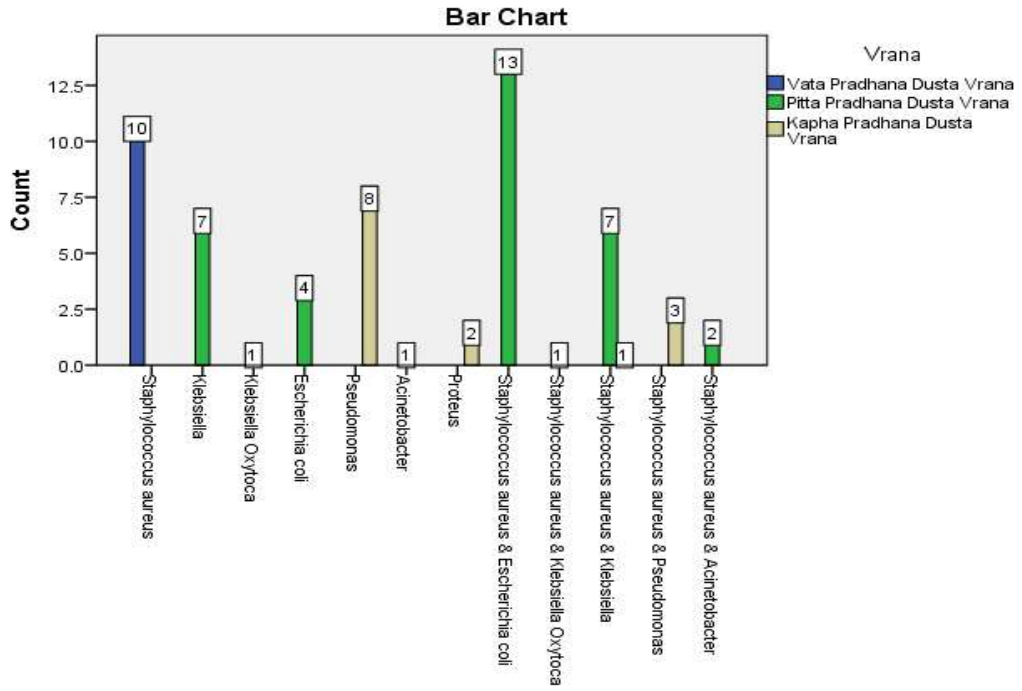
Statistical analysis-

Correlation Analysis between *Dosha Pradhana Dustavrana* and Organism Identified.

Table No. 16 Organism Identified v/s *Vrana* Crosstabulation						
			Vrana			**Total**
			Vata Pradhana Dustavrana	***Pitta Pradhana Dustavrana***	***Kapha Pradhana Dustavrana***	
Organism_	***Staphylococcus aureus***	Count	10	0	0	10
		% within Organism Identified	100.0%	0.0%	0.0%	100.0%
		% within Vrana	100.0%	0.0%	0.0%	16.7%

Identified		% of Total	16.7%	0.0%	0.0%	16.7%
	Klebsiella sp	Count	0	7	0	7
		% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	19.4%	0.0%	11.7%
		% of Total	0.0%	11.7%	0.0%	11.7%
	Klebsiella Oxytoca	Count	0	1	0	1
		% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	2.8%	0.0%	1.7%
		% of Total	0.0%	1.7%	0.0%	1.7%
	Escherichia coli	Count	0	4	0	4
		% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	11.1%	0.0%	6.7%
		% of Total	0.0%	6.7%	0.0%	6.7%
	Pseudomonas sp	Count	0	0	8	8
		% within Organism Identified	0.0%	0.0%	100.0%	100.0%
		% within Vrana	0.0%	0.0%	57.1%	13.3%
		% of Total	0.0%	0.0%	13.3%	13.3%
	Acinetobacter	Count	0	1	0	1
		% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	2.8%	0.0%	1.7%
		% of Total	0.0%	1.7%	0.0%	1.7%
	Proteus sp	Count	0	0	2	2
		% within Organism Identified	0.0%	0.0%	100.0%	100.0%
		% within Vrana	0.0%	0.0%	14.3%	3.3%
		% of Total	0.0%	0.0%	3.3%	3.3%
	Staphylococcus aureus & Escherichia coli	Count	0	13	0	13
		% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	36.1%	0.0%	21.7%
		% of Total	0.0%	21.7%	0.0%	21.7%
	Staphylococcus aureus & Klebsiella Oxytoca	Count	0	1	0	1
		% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	2.8%	0.0%	1.7%
		% of Total	0.0%	1.7%	0.0%	1.7%
	Staphylococcus aureus & Klebsiella sp	Count	0	8	0	8
		% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	13.3%	0.0%	13.3%
		% of Total	0.0%	13.3%	0.0%	13.3%
	Staphylococcus aureus & Pseudomonas sp	Count	0	0	3	3
		% within Organism Identified	0.0%	0.0%	100.0%	100.0%
		% within Vrana	0.0%	0.0%	21.4%	5.0%
		% of Total	0.0%	0.0%	5.0%	5.0%
		Count	0	2	0	2

	Staphylococcus aureus & Acinetobacter	% within Organism Identified	0.0%	100.0%	0.0%	100.0%
		% within Vrana	0.0%	5.6%	0.0%	3.3%
		% of Total	0.0%	3.3%	0.0%	3.3%
Total		Count	10	37	13	60
		% within Organism Identified	16.7%	60.0%	23.3%	100.0%
		% within Vrana	100.0%	100.0%	100.0%	100.0%
		% of Total	16.7%	60.0%	23.3%	100.0%



Correlations			
		Vrana	Organism Identified
Vrana	Pearson Correlation	1	.526**
	Sig. (2-tailed)		.000
	N	60	60
Organism Identified	Pearson Correlation	.526**	1
	Sig. (2-tailed)	.000	
	N	60	60

The analysis of the correlation between *Dosha Pradhana Dustavrana* and Organism Identified demonstrated distinct patterns of microbial distribution across different types of vitiated wounds. Among the *Vata Pradhana Dustavrana* group (n = 10, 16.7% of total subjects), all subjects were exclusively infected with *Staphylococcus aureus*, representing 100% within this organism category.

In the *Pitta Pradhana Dustavrana* group (n = 37, 60.0%), the microbial pattern was more diverse. This group predominantly harbored Gram-negative bacilli such as *Klebsiella species* (n = 7, 19.4% within Vrana) and *Escherichia coli* (n = 4, 11.1% within Vrana). Additionally, mixed infections were notable, including combinations of *Staphylococcus aureus* with *Escherichia coli* (n = 13, 36.1% within Vrana) and *Staphylococcus aureus* with *Klebsiella species* (n = 8, 13.3% within Vrana). Other less frequent organisms in this group included *Klebsiella oxytoca* (n = 1, 2.8%), *Acinetobacter* (n = 2, 5.6%), and various mixed organisms. These findings suggest that Pitta-dominant wounds are more prone to polymicrobial colonization, particularly with both Gram-positive and Gram-negative bacteria.

For the *Kapha Pradhana Dustavrana* group (n = 14, 23.3%), the majority of infections were caused by

Pseudomonas species (n = 8, 57.1% within Vrana), with other less frequent organisms including *Proteus species* (n = 2, 14.3%). *Staphylococcus aureus & Pseudomonas species* (n = 3, 21.4%). These observations indicate that Kapha-dominant wounds are more likely to be associated with opportunistic Gram-negative organisms and mixed infections, reflecting a different microbial ecology compared to *Vata* and *Pitta-dominant* wounds.

The Pearson correlation analysis revealed a moderate positive correlation between *Dosha Pradhana Dustavrana* and Organism Identified ($r = 0.526$, $p < 0.001$), suggesting that the type of *Dosha* predominance in the wound is significantly associated with the type of microbial colonization. This indicates that the microbial pattern in chronic wounds may be influenced by the underlying *Dosha* pathology, with *Vata* dominant wounds favoring *Staphylococcus aureus*, *Pitta* dominant wounds showing polymicrobial tendencies, and *Kapha* dominant wounds being prone to Gram-negative and opportunistic infections.

Discussion

Discussion on observation

1] *Vata pradhana dustavrana lakshana*

The analysis of *vata pradhana dustavrana lakshanas* among subjects reveals that the majority (68.35%). Did not show any features of *vataja dustavrana*. Among the subset of subjects with the presence of *vataja lakshanas*, is 31.7% exhibited one or more *vataja vrana lakshanas. Atisamvruta* (20%), *Katina* (18.3%), *Avasanna* (16.7%), *Krishna Varna* (18.3%), *Atyartha vedana* (20%)

Atisamvruta (20%)- Acharya Dalhana has mentioned *Atisamvruta* as *Atisankuchita* [5], which is indicative of tight, constricted, or non-suppurative lesions. As *vata* predominance is observed, it is characterized by its dryness due to the *Ruksha guna*, which in turn leads to reduced granulation tissue formation, resulting in atrophy and causing a chronic nonhealing ulcer or *Dustavrana.*

Katina (18.3%)- which indicates indurated wound margins and sclerotic changes, which is caused by *vata dosha* predominance [5], having such hardness is an outcome of *Dhatukshaya*. The *Khara guna* of *vata,* which is responsible for roughness and fibrosis of tissue, delays healing. Chronic ulcers often show fibrotic margins due to tissue degeneration caused by vata dosha.

Avasanna (16.7%)- "*avasanna heenmamsa."* [5] Demonstrates depressed or weak tissue tone caused by *vata dosha*, which hampers the *ropana* of *vrana*. Because of the *soshana karma* of *vata*, which causes drying and weakening of the wound bed. And *laghu guna* decreases tissue strength. This results in poor granulation and delayed healing.

Krishna varna (18.3%) - This discoloration indicates impairment in microcirculation or stagnation. As *praspandana* is a quality of *vata dosha*, any impairment results in stagnation of blood, ultimately leading to poor tissue perfusion and gangrene formation, and causing the *Krishna varna*.

Atyartha Vedana (20%)- *Vedana* is a cardinal feature of *vata*. A crucial parameter for chronic ulcers is severe pain. As there is chronic inflammation due to *vata prakopa* [5], it irritates nerve endings, resulting in enhanced pain perception.

Vata pradhana Dustavrana - however, among 10 subjects specifically diagnosed with *vata pradhana Dustavrana*, all classical *Vataja lakshanas- Atisamvruta, Kathina, Avasanna, Krishna varna, and Atyartha Vedana* [5] were present in 100% of cases. This uniform presence of classical *Vataja lakshanas* among *Vata pradhana Dustavrana* cases indicates a strong and clear *Vata* predominance. The findings support the diagnostic accuracy and adherence to classical Ayurvedic criteria in categorizing *Dustavrana* based on *dosha* predominance. However, 2 subjects had *Atisamvruta*, 1 subject had *Katina*, 1 subject had *Krishna varna*, and 2 subjects had *Atyartha vedana*, and were diagnosed as having *Pitta* or *Kapha pradhana Dustavrana*. This highlights the mixed *doṣha* involvement commonly seen in chronic ulcers. This overlap reflects the dynamic nature of *doṣha* interaction in chronic non-healing wounds, where secondary *doṣhas* may manifest alongside the predominant *doṣha.*

2] *Pitta pradhana dustavrana lakshana*

The analysis of *Pitta pradhana dustavrana lakshana* among subjects reveals that only a fraction of subjects (9.1%) did not exhibit any *Pittaja* features. Among the subset of subjects with the presence of *pittaja lakshanas*, one or more *pittaja dustavrana lakshanas*. Among observed *lakshanas, Putipuyasrava* (57.6%), *Rakta peeta varna* (34.6%), *Daha* (43.9%), *Ativivrutha* (31.8%), *Atiushna* (18.2%), *Utsanna* (9.1%), *Paaka* (10.6%), *Dustashonitasrava* (10.6%), *shopha* (13.6%), and *Pidaka* (1.5%). This indicates that *putipuyasrava* and *daha* are more commonly observed *Pittaja lakshanas* among subjects.

Putipuyasrava (57.6%)- foul-smelling purulent discharge is a hallmark feature of *Pittaja vrana*. *Tikshna guna* of *pitta* causes tissue breakdown, *ushna* guna facilitates tissue metabolism, leading to tissue liquefaction, and *pachaka karma* leads to tissue degradation, creating a conducive atmosphere for the growth of pathogenic bacterial species. This high prevalence of *putipuyasrava* [5] highlights pitta predominance, which reflects an active inflammatory and suppurative phase, directly contributing toward chronicity.

Rakta peeta varna (34.6%)- this signifies the involvement of *rakta dhatu*. With *asharaya aharayi bhava,* along with *pitta dosha* [5], it imparts reddish-yellowish color to the ulcer. The discoloration corresponds to erythema, hyperemia, and slough formation in chronic wounds, reflecting active inflammation and impaired microcirculation.

Daha (43.9%), *Ati-usna* (18.2%)- it's the most defining *Pittaja lakshana, usna guna* does increase the local temperature due to inflammatory hyperthermia. *Teekshna guna* penetrates deeper tissues, causing worsening of the burning sensation.

*Ativivrutha (*31.8%)- progression of ulcer margins due to extensive tissue breakdown. As healing fails due to chronic inflammation of tissues causes breakdown at the edges. *Teekshna Guna* leads to the destruction of viable tissue at margins, leading to the widening of margins. An ulcer will be exposed to the external environment, causing contamination by pathogenic bacteria.

Utsanna (9.1%)- raised ulcer margins, indicating impaired hypergranulation. When *pachana karma* is impaired, it causes the growth of excessive, unhealthy tissue.
Paka (10.6%)- suppuration is a classical feature of an ulcer. Impaired *pachaka karma* facilitates liquefaction & putrefaction of tissues. This provides an appropriate environment for bacterial growth.
Dustashonitasrava (10.65%)- discharge of vitiated blood suggesting *pitta & rakta* involvement. Inflammatory bleeding is seen in a chronic ulcer.
Shopha (13.6%)-swelling which is caused by fluid build-up in tissues because of increased permeability of blood vessels.
Pidaka (1.5%)- papular eruptions are seen around the ulcer area, which are induced due to inflammation. This micro suppuration is caused due to the *ushna* and *teekshna guna* of *pitta.*
Pitta pradhana Dustavrana - Among the 37 subjects diagnosed with *Pitta Pradhāna Duṣta Vraṇa.* 8 subjects presented with *shopha*, 20 subjects presented with *Raktapeetavarna*, 17 subjects presented with *Ativivrutha*, 5 subjects presented with *Utsanna,* 12 subjects presented with *Atisuhna,* 33 subjects presented with *Putipuyasrava*, 25 subjects presented with *Daha*, 7 subjects presented with *Paka*, 1 subject presented with *Pidaka*, 7 subjects presented with *Dustadhonitasrava* and 19 subjects presented with *Amanojna Gandha*. A subset of *Pittaja lakṣaṇas* was also observed in subjects ultimately diagnosed as *Vata or Kapha Pradhana Duṣta Vraṇa*. Specifically, *Shopha* was seen in 1 subject, *RaktaPeetaVarṇa* in 3 subjects, *Ativivṛtta* in 4 subjects*, Putipuya Srava* in 5 subjects*, Daha* in 4 subjects, and *Amanojna Gandha* in 2 subjects. This overlap of *lakshanas* indicates the presence of mixed *doṣha* involvement in chronic ulcers, reinforcing the Ayurvedic concept that *dusta vraṇa* is often *tridoṣhaja* in nature, with one *dosha* predominating clinically.
3] *Kapha pradhana dustavrana lakshanas*-
The analysis of *Kapha pradhana dustaVrana Lakshanas* among subjects reveals that 62.3% did not exhibit any *kapha*-dominant *vrana* features, suggesting *Kaphaja Vrana Lakshanas* are not predominant. However, among the subset of subjects who did manifest *Kaphaja lakshanas*, the most common was *Kandu* (27.9%), followed by *Atimardava* (21.3%), *Utsang*i (21.3%), and *Shweta varna* (14.8%).
Kandu (27.9%)- Itching is the defining feature of *kapha dustavrana lakshana*.[5] Due to *Snigdha* and *picchila guna*, which promote excessive moisture in the ulcer. This results in microbial colonization. Thus, leading to chronic non-healing ulcers.
Atimardava and *utsangi* (21.3%)- *Atimardava* indicated a soft, flaccid wound bed due to excess *Sneha guna* [5], causing excess fluid retention. Due to impaired lymphatic clearance, chronic inflammatory edema causes the formation of unhealthy granulation tissue.
Utsangi [5] is induration/swelling. Because of the growth of unhealthy granulation tissue, which causes abnormal induration and swelling around the ulcer, leading to thick, dense tissues.
Shweta Varna (14.8%)- *Shweta Varna* is whitish in coloration, which is a classical sign of *Kapha dosha* involvement [5]. This indicated poor tissue perfusion or reduced vascularity.
Kapha pradhana Dustavrana- Among the 13 subjects diagnosed with *Kapha Pradhana Duṣta Vraṇa*, all subjects (100%) presented with *Atimardava, Utsangi,* and *Kandu*, while *Shweta Varṇa* was observed in 9 subjects. This uniform presence of classical *Kaphaja lakṣhaṇas* supports the reliability of the diagnostic criteria used for *Kapha Pradhana Duṣta Vraṇa*. Additionally, *Kandu* was observed in four subjects who were diagnosed as *Vata* or *Pitta Pradhana Duṣta Vraṇa,* suggesting overlapping *doṣha* features and mixed *doṣha* involvement in chronic ulcers.
4] *Roga Nirnaya*-
The majority of subjects were diagnosed with *Pitta pradhana Dustavrana* (60%), followed by *Kapha pradhana Dustavrana* (23.33%) and *Vata pradhana Dustavrana* (16.67%).
Pitta pradhana Dustavrana may be correlated with chronic ulcers characterized by inflammation, burning sensation, redness, pus discharge, and pain, features which are commonly observed in chronic venous ulcers and diabetic foot ulcers, where local inflammation, microbial colonization, and ischemia coexist.
Kapha pradhana Dustavrana can be correlated with chronic ulcers exhibiting excessive slough, edema, excessive granulation tissue, and itching, features which represent impaired lymphatic drainage, venous stasis, and biofilm formation. These are chronic ulcers associated with metabolic disorders like diabetes and obesity.
Vata pradhana Dustavrana corresponds to an ulcer marked by dryness, irregular margins, and deep tissue involvement. These features are often seen in ischemic ulcers and pressure ulcers, where poor perfusion and tissue necrosis play a major role.
Microbiology
5] Organism Identified-
Staphylococcus aureus was the most frequently isolated organism, identified in 60% of subjects, followed by *Escherichia coli* in 45%. Other Gram-negative organisms, such as *Klebsiella species* (25%), *Pseudomonas species* (16.7%), *Acinetobacter* (5%), *Klebsiella oxytoca* (3.3%), and *Proteus species* (3.3%) were also isolated.
Staphylococcus aureus is widely recognized as the most prevalent pathogen in both acute and chronic ulcers [6]. The high isolation rate of *Escherichia coli* bacilli suggests prolonged wound chronicity and environmental contamination [7]. Isolation of *Pseudomonas species* in 16.7% of subjects is clinically significant, as this organism is strongly associated with chronic non-healing wounds and biofilm formation [8]. Similarly, *Klebsiella, proteus and Acinetobacter species* are opportunistic pathogens frequently encountered in chronic ulcers, particularly in hospitalized patients or with compromised immunity.

Discussion on results-

The study was performed on 60 subjects; among them, 10 subjects were diagnosed as *Vata Pradhana Dustavrana*, 37 subjects were diagnosed as *Pitta Pradhana Dustavrana*,

and 13 subjects were diagnosed as *Kapha Pradhana Dustavrana*.

Among the *Vata Pradhana Dustavrana* group, all subjects were exclusively infected with *Staphylococcus aureus*, representing 100% within this organism category. No other organisms were identified in this group, indicating a strong association between *Vata*-dominant wounds and *Staphylococcus aureus* colonization. In this ulcer are often characterized by dryness and tissue ischemia. As *Staphylococcus aureus* is a common skin commensal that readily colonizes such wounds and can persist due to its ability to adhere to damaged tissue, and as there is no wound moisture, the growth of gram-negative organisms is not favorable [9].

Among the *Pitta Pradhana Dustavrana* group, the microbial pattern was more diverse. This group predominantly harbored Gram-negative bacilli such as *Klebsiella species* in 7 cases and *Escherichia coli* in 4 cases. Additionally, mixed infections were notable, including combinations of *Staphylococcus aureus* with *Escherichia coli* in 13 cases and *Staphylococcus aureus* with *Klebsiella species* in 8 cases. *Staphylococcus aureus* with *Klebsiella oxytoca* in 1 case, *Staphylococcus aureus* with *Acinetobacter* in 2 cases. Other less frequent organisms in this group included *Klebsiella oxytoca* in 1 case and *Acinetobacter* in 1 case. The predominance of gram-negative bacilli and the high frequency of polymicrobial infections involving both gram-positive cocci and gram-negative bacilli. which are often associated with increased local temperature, tissue breakdown, exudation, and suppuration. Such an environment supports the growth of Gram-negative organisms.[10] This pattern aligns with the inflammatory nature of *Pitta-pradhana Dustavrana.*

Among the *Kapha Pradhana Dustavrana* group, the majority of infections were caused by *Pseudomonas species* in 8 cases, with other less frequent organisms, including *Proteus species* in 2 cases and mixed presentation of *Pseudomonas species* & *Staphylococcus aureus* in 3 cases. These observations indicate that *Kapha*-dominant wounds are more likely to be associated with opportunistic Gram-negative organisms and mixed infections. *Pseudomonas species* [11] and *Proteus species* [12] thrive in moist, exudative, and poorly oxygenated environments, such as long-standing, heavily exudating chronic wounds. These conditions promote colonization of these opportunistic bacteria.

CONCLUSION

Based on the present study following conclusions were drawn

In *vata pradhana dustavrana,* characterized by *Atisamvruta, Katina, Avsanna, Krishna Varna & Atyartha Vedana*, *Staphylococcus aureus* is evident.

In *pitta pradhana dustavrana,* characterized by *Putipuyasrava, Daha, Raktapeetavarna, & Ativivrutha,* gram-negative organisms like *Klebsiella species*, *Klebsiella oxytoca, Escherichia coli, and Acinetobacter.* Furthermore, polymicrobial patterns, particularly the coexistence of *Staphylococcus aureus* with Gram-negative isolates, were evident.

In *kapha pradhana dustavrana,* characterized by *Kandu, Atimardava & Utsangi, Pseudomonas* and *Proteus species* were predominantly isolated. Along with the coexistence of *Pseudomonas species* and *Staphylococcus aureus*.

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