A PROSPECTIVE COMPARATIVE TWO ARM STUDY OF THE ROLE OF ORAL PROBIOTICS IN THE SURGICAL WOUND HEALING – A STUDY OF 100 CASES

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ABSTRACT

Background: The process of wound healing is well coordinated and involves a complex interplay of cellular and molecular events. Any interruptions in this process of wound healing may result into complications like delayed wound healing, higher infection rate and impaired tissue regeneration. This further leads to longer hospital stay, dampened quality of life, and higher healthcare expenses.

Skin microbiome provides protection from external threats and keeps a robust homeostasis. Surgical procedures disrupts the skin continuity which marks the beginning of process of wound healing. The phases of wound healing are hemostasis, inflammation, proliferation, and remodeling. Severe wound site infections by endogenous flora and antibiotics resistant pathogens, hinders the process of wound healing. Globally reported rates of surgical site infection varies from 2.5% to 41.9%. Escherichia coli, Staphylococci, and Enterococci are the predominant pathogens identified as causes in postoperative wounds.[1]

Surgical site wound care has many challenges with multiple comorbidities and varied wound healing environments. Universal use of antimicrobials as preoperative prophylactic have decreased postoperative wound infections rates. Antibiotic resistance is Achille's heel in the mechanism of wound healing. Developing the new and efficient antibiotics to combat the resistant pathogens has been stalled because of economic and regulatory measures. This necessitates for the search of the alternative measures like probiotics, bacteriocins, and nanoparticles for combating wound healing obstacles.[1]

Aims: A Prospective comparative study of effect of oral probiotics on surgical wound healing with and without the use of oral probiotics.

Methods: A prospective comparative study was conducted in a total of 100 patients, divided in two groups of 50 each –Group A and Group B, by random allocation.

GROUP A: Oral probiotics were given as adjuvant therapy for 5 days after being operated (From Day 1 to Day 5) along with antibiotics.

GROUP B: Only antibiotics was given.

The wounds were assessed by applying Southampton Scoring system on Day 1, Day 3, Day 5, Day 7, and on the day of stitch removal.
Thereafter, the patients were followed up on outpatient basis once a week for 1 month from the day of surgery.
The study includes quantitative discrete data (prospective comparative study). At the end of study, the data was compiled, presented, illustrated in suitable tables and graphs and analyzed using appropriate statistical tests (SPSS Software).

De Simone Formulation[11]:
Lactobacillus:
Lactobacillus acidophilus DSM 24735
Lactobacillus plantarum DSM 24730
Lactobacillus paracasei DSM 24733
Lactobacillus delbrueckii spp. bulgaricus DSM 24734

Bifidobacterium:
Bifidobacterium longum DSM 24736
Bifidobacterium breve DSM 24732
Bifidobacterium infantis DSM 24737

Streptococcus:
Streptococcus thermophilus DSM 24731

Results: The Southampton Score which measure wound healing, showed no statistically significant differences between the two groups at Days 1, 3, 5, 7, and at the time of stitch removal. This suggests that addition of the oral probiotics did not significantly impact the wound healing process as measured by this scale.

Conclusion: The use of oral probiotics as an adjuvant therapy did not show significant improvements in wound healing as measured by the Southampton Score. The study demonstrates that the use of oral probiotics is safe, with no adverse effects or complications. However, conclusion of this study does not provide strong evidence to support the routine use of oral probiotics as an adjuvant therapy for improving surgical wound healing.

Keywords: Surgical wound healing, Oral probiotics, De Simone Formulation, Southampton Score

INTRODUCTION

Wound healing maintains the homeostasis with outer protective barrier of the body. Surgical wound healing is a very much complicated process that can be impaired by infections and can therefore have a significant economic and social impact. Moreover, the overuse of antibiotics has resulted in drug resistant bacteria and their efficacy has been blunted. So, the need for alternative antimicrobial agents is very urgent. The new approaches on wound dressings employs the new therapeutic agents, such as probiotics.[1]

Skin microbiome barrier provides protection from outside threats and maintains homeostasis. With surgical procedures, skin continuity disrupted which marks the beginning of the wound healing process.Wound healing phases are hemostasis, inflammation, proliferation, and remodeling. Severe wound site infections caused by endogenous flora, antibiotics resistant pathogens, hinders the process of wound healing. Globally, reported rates of SSI varies between

2.5% to 41.9%. *Escherichia coli*, *Staphylococci*, and *Enterococci* are the predominant pathogens identified as causes in postoperative infected wounds .[1]

Surgical site wound care has many challenges, with multiple comorbidities and varied wound healing environment. The universal use of antimicrobials for preoperative prophylactic have decreased postoperative wound infections rates, Antibiotic resistance is Achille's heel in the surgical wound healing process. Developing new efficient antibiotics to combat the resistant pathogens has been stalled due to economic and regulatory measures. This necessitates for the search of the alternative measures like probiotics, bacteriocins, and nanoparticles for combating wound healing obstacles, [1]

The evidences suggests that gut microbiota is an essential factor of the "microbiome–gut–brain axis", and transmits critical signals to brain via the vagus nerve. Supplements like probiotics may enhance microbiome environment via the upregulation of the neuropeptide hormone oxytocin. This hormone alteration have a regulatory role on hypothalamus and pituitary gland, thus influencing mammalian homeostasis. Their effects on physical and mental health are speculated to be very crucial.[1][2][3]

Probiotics are the nonpathogenic microorganisms extracted naturally from different sources like dairy foods. The rationale for the use of probiotics for medical purposes is based on a hypothesis that oral or topical probiotics use may replenish the depleted human microbiome. They are promising biomaterials which exert a broad range of positive effects on human body against pathogens, from the GI diseases to atopic dermatitis, by stimulating the body immune response or directly competing out the pathogens. Probiotics primarily effects the phase of inflammation, which plays a very significant role in wound healing. Probiotics applied topically or systematically, have shown in recent studies on humans and animals a clear-cut benefit in the wound healing, through oxytocin mediated effects on inflammatory responses.[1][2]

Wound healing benefit of the probiotics is based on competition with pathogens for the adhesion and the utilization of nutrients and growth factors. Thus probiotics also known as immunobiotics prevents the microbial colony formation and modifies host immune response. The probiotics also generate low molecular weight substances like lactic acid and bacteriocins. Probiotics immunomodulates by the activation of the transcriptional pathways and T-cell activation via the cytokines. Additionally some elements i.e., bacterial components or its byproducts activates some specific receptors in immune system in initiating inflammatory reation.The wound healing mechanism is also based on the theory of the beneficial actions of the probiotics in the gastrointestinal tract where the bacterial host interactions with the epithelial cells, regulatory T lymphocytes, and the dendritic cells. All of these cumulative actions of probiotics mentioned above may reduce the bacterial load at the wound site, and regulate inflammatory cell infiltration and promotes the wound healing.[1]

MATERIALS AND METHODS

Methodical entry of records of admitted and operated patients were done.A prospective comparative study was conducted in a total of 100 patients divided in two equal groups of 50 each – A and B, by random allocation. The study was conducted after approval from Institutional Ethical Committee and Thesis Committee. A written informed consent was taken from all the patients undergoing intake of oral probiotics (De Simone Formulation) after being operated.

GROUP A: Oral probiotics was given as adjuvant therapy for 5 days after being operated (From Day 1 to Day 5) along with antibiotics.

GROUP B: Only antibiotics was given.

The wound was assessed and Southampton Scoring was done on Day 1, Day 3, Day 5, Day 7, and on the day of stitch removal.

Thereafter, patients were followed up on outpatient basis once a week for 1 month from the day of surgery.

De Simone Formulation [11]:
Lactobacillus:
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Bifidobacterium breve DSM 24732
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SOUTHAMPTON SCORE:
Grade 0—Normal healing

Grade I—Normal healing with mild bruising or erythema A—Some bruising
B—Considerable bruising C—Mild erythema

Grade II—Erythema plus other signs of inflammation A—At 1 point
B—Around sutures C—Along wound D—Around wound

Grade III—Clear or haemoserous discharge A-At 1 point only (<2 cm)
B-Along wound (>2 cm)
C-Large volume
D-Prolonged (>3 days)

Grade IV—Pus
A—At 1 point only (<2 cm) B—Along wound (>2 cm)

Grade V—Deep or severe wound infection with or without tissue breakdown; hematoma requiring aspiration.

Oral probiotics (De Simone Formulation) was administered once daily for a period of 5 days (Day 1 to Day 5) after being operated. It was taken with glass of normal water. It was taken on empty stomach in the morning shortly before food.[12]

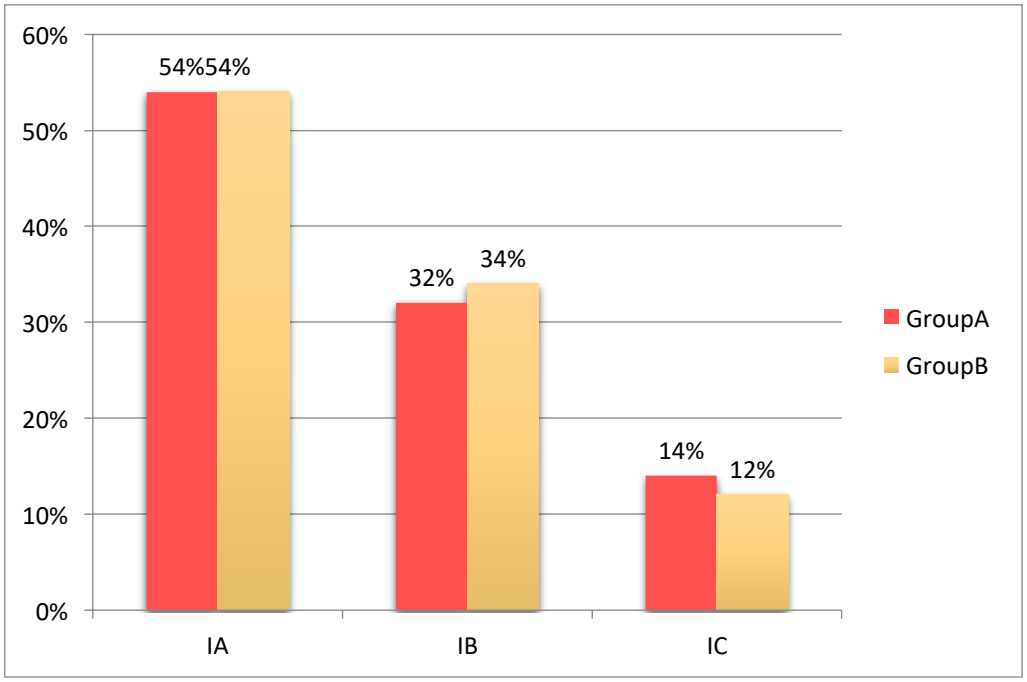
OBSERVATION AND RESULTS

The Southampton Scoring system, which measure wound healing, showed no statistically significant differences between the two groups at Days 1, 3, 5, 7, and at the time of stitch removal. This suggests that the addition of oral probiotics did not significantly impact the wound healing process as measured by this scale.

Table 1: Comparison of Southampton Score at Day 1 Between Group A and Group B

Southampton Score at Day 1		Group A	Group B	P value
IA	N	27	27	0.948
	Percentage	54.0%	54.0%	
IB	N	16	17	
	Percentage	32.0%	34.0%	
IC	N	7	6	
	Percentage	14.0%	12.0%	
Total	N	50	50	
	Percentage	100.0%	100.0%	

Graph 1: Comparison of Southampton Score at Day 1 Between Group A and Group B

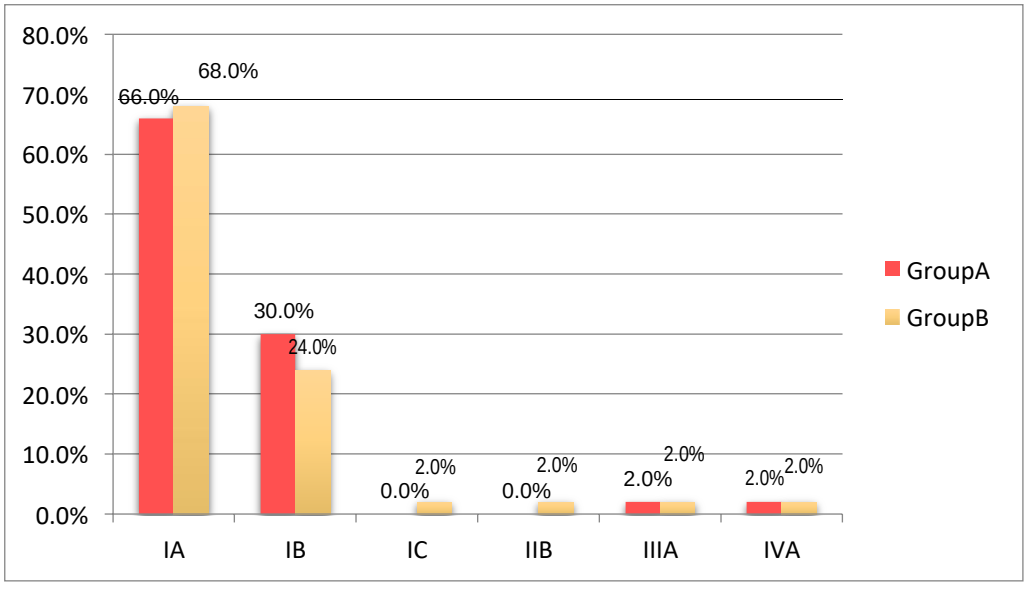


In Group A, 54% (27 participants) scored IA, 32% (16 participants) scored IB, and 14% (7 participants) scored IC. Similarly, in Group B, 54% (27 participants) scored IA, 34% (17 participants) scored IB, and 12% (6 participants) scored IC. The p-value of 0.948 indicates that there is no statistically significant difference between the two groups in terms of their Southampton Score distribution at Day 1.

Table 2: Comparison of Southampton Score at Day 3 Between Group A and Group B

Southampton Score at Day 3		Group		Total
		Group A	Group B	
IA	N	33	34	
	Percentage	66.0%	68.0%	
IB	N	15	12	
	Percentage	30.0%	24.0%	
IC	N	0	1	
	Percentage	0.0%	2.0%	0.799
IIB	N	0	1	
	Percentage	0.0%	2.0%	
IIIA	N	1	1	
	Percentage	2.0%	2.0%	
IVA	N	1	1	
	Percentage	2.0%	2.0%	
Total	N	50	50	
	Percentage	100.0%	100.0%	

Graph 2: Comparison of Southampton Score at Day 3 Between Group A and Group B

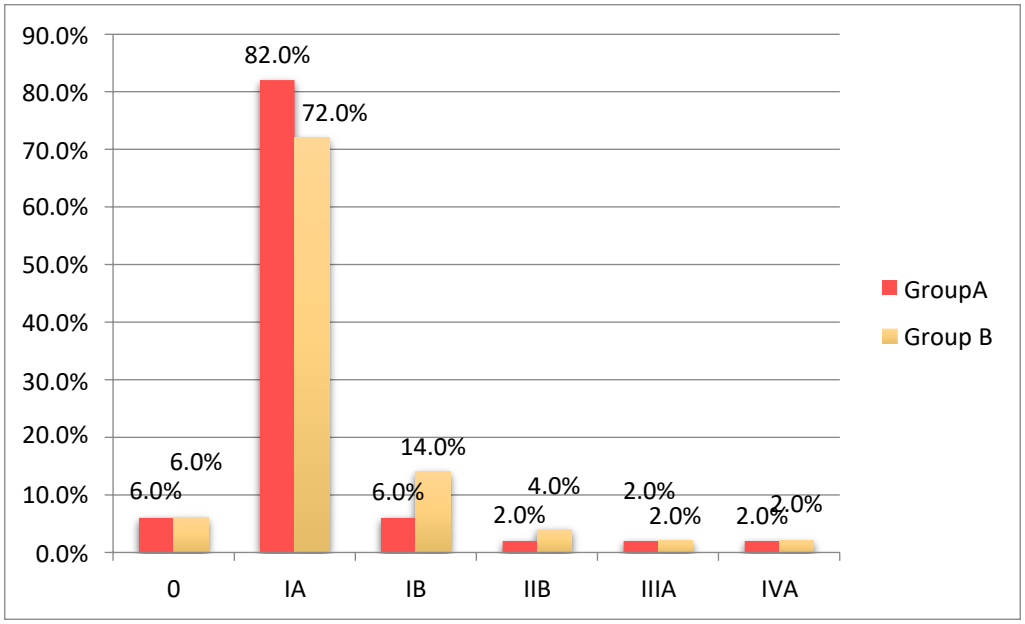


This table compares the Southampton Score at Day 3 between Group A and Group B. In Group A, 66% scored IA, 30% IB, and 2% each for IIIA and IVA, with no scores in IC or IIB. Group B showed a similar distribution: 68% IA, 24% IB, 2% each for IC, IIB, IIIA, and IVA. The p-value of 0.799 indicates no statistically significant difference between the groups' score distributions at Day 3.

Table 3: Comparison of Southampton Score at Day 5 Between Group A and Group B

Southampton Score at Day 5		Group		Total
		Group A	Group B	
0	N	3	3	
	Percentage	6.0%	6.0%	
IA	N	41	36	
	Percentage	82.0%	72.0%	
IB	N	3	7	
	Percentage	6.0%	14.0%	0.812
IIB	N	1	2	
	Percentage	2.0%	4.0%	
IIIA	N	1	1	
	Percentage	2.0%	2.0%	
IVA	N	1	1	
	Percentage	2.0%	2.0%	
Total	N	50	50	
	Percentage	100.0%	100.0%	

Graph 3: Comparison of Southampton Score at Day 5 Between Group A and Group B

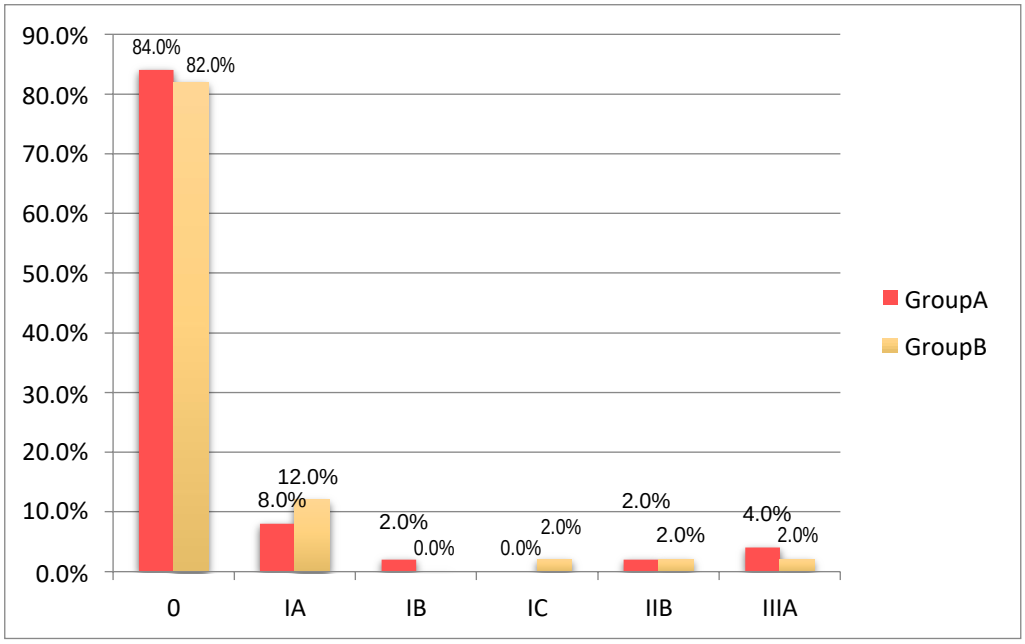


In Group A, 6% (3 participants) scored 0, 82% (41 participants) scored IA, 6% (3 participants) scored IB, 2% (1 participant) scored IIB, 2% (1 participant) scored IIIA, and 2% (1 participant) scored IVA. Group B showed a similar distribution: 6% (3 participants) scored 0, 72% (36 participants) scored IA, 14% (7 participants) scored IB, 4% (2 participants) scored IIB, 2% (1 participant) scored IIIA, and 2% (1 participant) scored IVA. The p-value of 0.812 indicates no statistically significant difference between the two groups' score distributions at Day 5.

Table 4: Comparison of Southampton Score at Day 7 Between Group A and Group B

Southampton Score at Day 7		Group		Total
		Group A	Group B	
0	N	42	41	0.739
	Percentage	84.0%	82.0%	
IA	N	4	6	
	Percentage	8.0%	12.0%	
IB	N	1	0	
	Percentage	2.0%	0.0%	
IC	N	0	1	
	Percentage	0.0%	2.0%	
IIB	N	1	1	
	Percentage	2.0%	2.0%	
IIIA	N	2	1	
	Percentage	4.0%	2.0%	
Total	N	50	50	
	Percentage	100.0%	100.0%	

Graph 4: Comparison of Southampton Score at Day 7 Between Group A and Group B

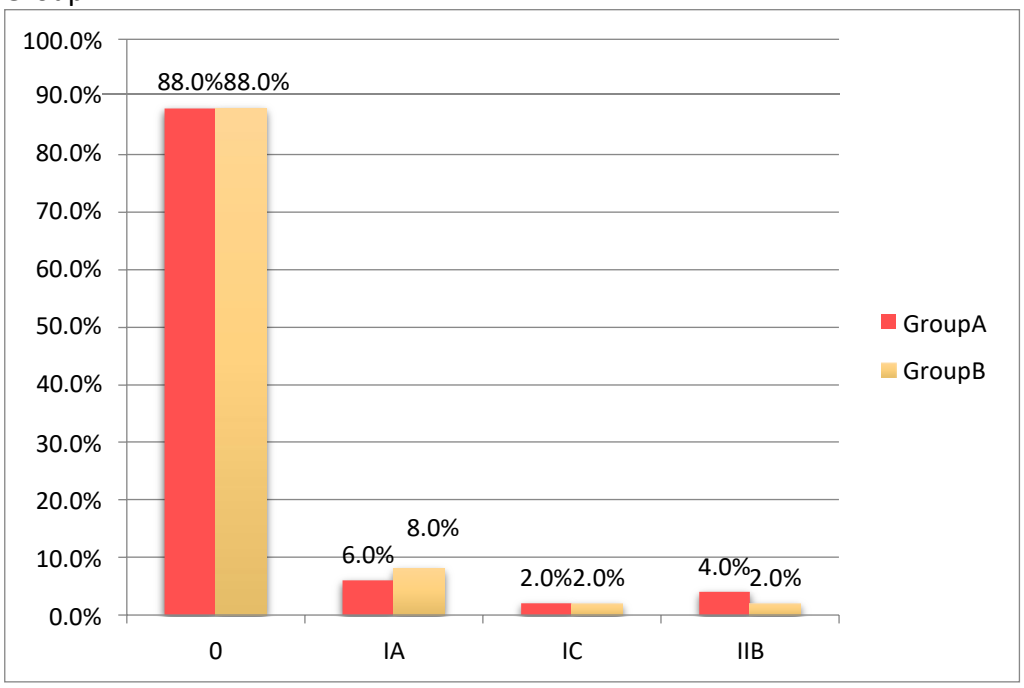


In Group A, 84% (42 participants) scored 0, 8% (4 participants) scored IA, 2% (1 participant) each scored IB and IIB, and 4% (2 participants) scored IIIA. Group B showed a similar distribution: 82% (41 participants) scored 0, 12% (6 participants) scored IA, 2% (1 participant) each scored IC, IIB, and IIIA. The p-value of 0.739 indicates no statistically significant difference between the groups' score distributions at Day 7.

Table 5: Comparison of Southampton Score at Day of Stitch Removal Between Group A and Group B

Southampton Score at Day of Stitch Removal		Group A	Group B	P value
0	N	44	44	0.924
	Percentage	88.0%	88.0%	
IA	N	3	4	
	Percentage	6.0%	8.0%	
IC	N	1	1	
	Percentage	2.0%	2.0%	
IIB	N	2	1	
	Percentage	4.0%	2.0%	
Total	N	50	50	
	Percentage	100.0%	100.0%	

Graph 5: Comparison of Southampton Score at Day of Stitch Removal Between Group A and Group B



In both Group A and Group B, 88% (44 participants) achieved a score of 0, indicating optimal recovery. In Group A, 6% (3 participants) scored IA, 2% (1 participant) scored IC, and 4% (2 participants) scored IIB. Similarly, in Group B, 8% (4 participants) scored IA, 2% (1 participant) scored IC, and 2% (1 participant) scored IIB. The p-value of 0.924 indicates no statistically significant difference between the two groups' score distributions at the time of stitch removal.

DISCUSSION

This prospective comparative study investigated the effects of oral probiotics on surgical wound healing in 100 patients, divided equally into two groups A and B. The study's findings provide valuable insight into the potential role of probiotics in post-surgical care and contribute to the growing body of research.

In the present study the ages are categorized into four groups: ≤30, 31-40, 41-50, and 51-60 years old. Both groups have identical representations in the youngest (≤30) and oldest (51-60) categories, with 10 participants (20%) and 15 participants (30%) respectively. The main differences lie in the middle age ranges: Group A has a higher proportion of participants aged 31-40 (38% vs 22% in Group B), while Group B has more participants aged 41-50 (28% vs 12% in Group A). The age distribution between Group A (probiotic group) and Group B (control group) was not significantly different (p=0.149). Kotzampassi et al. in their study on probiotics in colorectal surgery patients reported a mean age of 69.5 ± 14.2 years in the probiotic group and 68.0 ± 12.6 years in the placebo group. This indicates an older study population compared to our study. Liu et al. in their meta-analysis of probiotics in colorectal surgery, included studies with mean ages ranging from 45.6 to 70.3 years. Yang et al. (2016), studying probiotics in cesarean section patients, reported a much younger population with a mean age of 28.6 ± 3.2 years in the probiotic group and 29.1 ± 3.5 years in the control group.[4]

In our study, Group A comprised 60% females and 40% males, while Group B had an equal 50-50 split. The difference was not statistically significant (p=0.422).

The present study included a wide variety of surgical procedures, with open cholecystectomy being the most common (40% in Group A, 28% in Group B } Right Inguinal Hernioplasty is the second most common in Group A (14%), while Excision of Lipoma is second in Group B (14%). Group A shows a wider variety of procedures, including some not present in Group B (e.g., Bilateral Jaboulay's procedure, Excision of Breast Lump). Group B has a higher incidence of Left Inguinal Hernioplasty (10% vs 2% in Group A) and some procedures not found in Group A (e.g. Feeding Jejunostomy, Splenectomy). The distribution reveals that while there's overlap in many procedures, there are notable differences in the frequency and types of surgeries between the two groups. This variation could be due to random allocation, differences in patient needs, or other factors not specified in the table. The diversity of procedures, ranging from minor excisions to major surgeries like nephrectomies, indicates a broad spectrum of surgical interventions across both groups. Many probiotic studies in surgical patients focus on specific types of surgeries. Liu et al. and Kotzampassi et al. focused solely on colorectal surgeries, Rayes et al. studied liver transplant patients and Yang et al. focused on cesarean sections.[5][6]

In the present study the wounds are categorized into two types: Clean and Clean Contaminated. In Group A, 26 patients (52%) had Clean wounds, while 24 patients (48%) had Clean Contaminated wounds. Group B showed a slightly higher proportion of clean wounds with 31 patients (62%), and consequently, a lower proportion of Clean Contaminated wounds with 19 patients (38%). Despite this apparent difference, the p-value of 0.419 indicates that variation in wound classification between the two groups is not statistically significant at the conventional 0.05 level. Kotzampassi et al. focused on colorectal surgeries, which are typically classified as clean-contaminated. Their study didn't include clean wounds, limiting direct comparison. Rayes

et al. studying liver transplant patients, dealt with clean-contaminated surgeries exclusively and Yang et al. focusing on cesarean sections, worked with clean- contaminated wounds. Our study's inclusion of both clean and clean-contaminated wounds provides a broader perspective, potentially making the results more generalizable across different types of surgeries.[7][8]

The present study employed the Southampton Scoring System (SSS) to evaluate wound healing, a validated tool for assessing surgical site infections.This scoring system provides a standardized method for tracking wound healing progress over time. Our results showed no significant differences in Southampton scores between the probiotic and control groups at any time point (Day 1, 3, 5, 7, and day of stitch removal). By the day of stitch removal, 88% of patients in both groups achieved a score of 0, indicating optimal recovery. These findings contrast with some previous studies that have reported beneficial effects of probiotics on wound healing for instance Peral et al. , studying burns, reported faster re-epithelialization with topical probiotics (8.7 days) compared to silver sulfadiazine (10.1 days). Huseini et al. reported a significant reduction in ulcer size in diabetic foot ulcers treated with probiotics (mean reduction 33.2 cm^2) compared to placebo (mean reduction 2.8 cm^2) after 12 weeks. Our study's lack of significant difference contrasts with these positive findings, possibly due to differences in probiotic administration route, wound type, or assessment method.[9][10]

Our study reported no adverse effects or complications in either group, with satisfactory wound healing in all cases. While our study did not demonstrate significant benefits of oral probiotics on surgical wound healing, it contributes valuable data to the ongoing discussion about the role of probiotics in surgical care. The contrasting results in the literature suggest that the effects of probiotics may be context-dependent, varying based on factors such as the type of surgery, patient characteristics, probiotic strains used, and administration protocols. Our study provides important insights into the use of probiotics in a diverse surgical population. While we did not observe significant effects on wound healing, the safety of probiotic administration was confirmed. Based on these results, there isn't strong evidence to recommend the routine use of oral probiotics as an adjuvant therapy for improving surgical wound healing.

CONCLUSION

This prospective comparative study aimed to evaluate the effects of oral probiotics on surgical wound healing. The study included 100 patients divided into two equal groups: Group A received oral probiotics (De Simone Formulation) as adjuvant therapy for 5 days post-surgery along with standard antibiotic treatment, while Group B received only standard antibiotic treatment.

The key findings of this study are:

1. There were no significant differences between the two groups in terms of age distribution, gender, type of surgery, or wound classification, indicating comparable baseline characteristics.
2. The Southampton Scores, which measure wound healing, showed no statistically significant differences between the two groups at Days 1, 3, 5, 7, and at the time of stitch removal. This suggests that the addition of oral probiotics did not significantly impact the wound healing process as measured by this scale.
3. Both groups showed similar rates of wound swab culture and sensitivity tests, with no significant difference in the need for additional wound investigation.
4. No adverse effects or complications were reported in either group, and wound healing was satisfactory for all participants in both groups.

In conclusion, while the use of oral probiotics as an adjuvant therapy did not show significant improvements in wound healing as measured by the Southampton Score, it did appear to influence certain hematological and biochemical parameters. The clinical significance of these differences requires further investigation. The study demonstrates that the use of oral probiotics is safe, with no adverse effects or complications reported. However, based on the primary outcome measures of wound healing, this study does not provide convincing evidence to support the routine use of oral probiotics as an adjuvant therapy for improving surgical wound healing. Probiotics may have systemic effects beyond the gastrointestinal tract, potentially influencing immune function, blood clotting mechanisms, and metabolism. However, the clinical relevance of these changes in the context of wound healing or overall patient health is not clear from this study alone.

LIMITATIONS

1. This study is only a clinical study.
2. This study is done on a relatively small group which limits the statistical power to detect significant differences between groups.
3. This study doesn't include humoral, cellular, histological and other parameters.

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